VNIVERSITAT DE VALÈNCIA Facultat de Medicina i Odontología

Departamento de Fisiología

TESIS DOCTORAL
POR COMPENDIO DE PUBLICACIONES

Efectos del ejercicio físico y la administración de alopurinol sobre biomarcadores musculares y cardiovasculares. El ejercicio físico como promotor de longevidad.

Presentada por:
Fabián Sanchis Gomar

Dirigida por:
Prof. D. José Viña Ribes
Prof. Dña. Mari Carmen Gómez Cabrera
Dra. Dña. Gloria Olaso González

Facultad de Medicina y Odontología

Departamento de Fisiología

Prof. **D. José Viña Ribes,** Catedrático del Departamento de Fisiología de la Facultad de Medicina y Odontología de la Universidad de Valencia.

Prof. **Dña. Mari Carmen Gómez Cabrera,** Profesora Titular del Departamento de Fisiología de la Facultad de Medicina y Odontología de la Universidad de Valencia.

Dra. **Dña. Gloria Olaso González,** Doctora en Químicas por la Universidad de Valencia y Técnico Superior de Investigación en el Departamento de Fisiología de la Facultad de Medicina y Odontología de la Universidad de Valencia

CERTIFICAN:

Que D. Fabián Sanchis Gomar, Diplomado en Fisioterapia y Máster en Investigación y Uso Racional del Medicamento por la Universidad de Valencia, ha realizado bajo su dirección para la obtención del título de Doctor la presente tesis titulada:

Efectos del ejercicio físico y la administración de alopurinol sobre biomarcadores musculares y cardiovasculares. El ejercicio físico como promotor de longevidad.

Y para que conste a los efectos oportunos, firman la presente certificación.

Fdo. D. José Viña Ribes

Fdo. Dña. Mari Carmen Gómez Cabrera

Fdo. Dña. Gloria Olaso González

A todas las personas que participaron e hicieron posible este proyecto, muchas gracias por vuestro apoyo y enseñanzas recibidas.

To everyone who participated and made this project possible, thank you very much for your support and teachings.

A tutti coloro che hanno partecipato e reso possibile questo progetto va il mio ringraziamento per il sostegno e gli insegnamenti ricevuti.

ÍNDICE DEL CONTENIDO

ÍNDICE DEL CONTENIDO

3. RESUMEN

4. APÉNDICES

APÉNDICE 2: Copia de los trabajos publicados

Sanchis-Gomar F, Pareja-Galeano H, Gomez-Cabrera MC, Candel J, Lippi G, Mann GE, Viña J. Allopurinol prevents skeletal and cardiac muscle damage in professional soccer players. *Scand J Med Sci Sports*. 2013 (bajo revisión)

Sanchis-Gomar F, Bonaguri C, Aloe R, Pareja-Galeano H, Martinez-Bello V, Mari Gomez-Cabrera MC, Candel J, Viña J, Lippi G. Effects of acute exercise and xanthine oxidase inhibition on novel cardiovascular biomarkers. *Transl Res*. 2013; 162(2):102-9.

Sanchis-Gomar F, Bonaguri C, Pareja-Galeano H, Gomez-Cabrera MC, Candel J, Viña J, Lippi G. Effects of acute exercise and allopurinol administration on soluble urokinase plasminogen activator receptor (suPAR). *Clin Lab*. 2013; 59(1-2):207-10.

Lippi G, Schena F, Salvagno GL, Sanchis-Gomar F, Guidi GC. Serum copeptin and mid-region pro-adrenomedullin (MR-proADM) after an ultra-marathon. *J Clin Lab Anal*. 2013.

Lippi G, Sanchis-Gomar F, Salvagno GL, Aloe R, Schena F, Guidi GC. Variation of serum and urinary neutrophil gelatinase associated lipocalin (NGAL) after strenuous physical exercise. *Clin Chem Lab Med*. 2012; 14;50(9):1585-9.

Sanchis-Gomar F, Olaso-Gonzalez G, Corella D, Gomez-Cabrera MC, Vina J. Increased average longevity among the "Tour de France" cyclists. *Int J Sports Med*. 2011; 32(8):644-7.

Viña J, Borras C, Sanchis-Gomar F, Martinez-Bello VE, Olaso-Gonzalez G, Gambini J, Ingles M, Gomez-Cabrera MC. Pharmacological Properties of Physical Exercise in The Elderly. *Curr Pharm Des*. 2013.

Sanchis-Gomar F. The skeletal muscle–metabolism axis in prostate-cancer therapy. *N Engl J Med*. 2012; 367(23):2257-8.

Sanchis-Gomar F, Gómez-Cabrera MC, Viña J. The loss of muscle mass and sarcopenia: non hormonal intervention. *Exp Gerontol*. 2011; 46(12):967-9.

Vina J, Sanchis-Gomar F, Martinez-Bello V, Gomez-Cabrera MC. Exercise acts as a drug; the pharmacological benefits of exercise. *Br J Pharmacol*. 2012;167(1):1-12.

Gomez-Cabrera MC, Sanchis-Gomar F, Garcia-Valles R, Pareja-Galeano H, Gambini J, Borras C, Viña J. Mitochondria as sources and targets of damage in cellular aging. *Clin Chem Lab Med*. 2012; 50(8):1287-95.

Viña J, Gomez-Cabrera MC, Borras C, Froio T, Sanchis-Gomar F, Martinez-Bello VE, Pallardo FV. Mitochondrial biogenesis in exercise and in ageing. *Adv Drug Deliv Rev*. 2009; 30;61(14):1369-74.

Derbré F, Gomez-Cabrera MC, Nascimento AL, Sanchis-Gomar F, Martinez-Bello VE, Tresguerres JA, Fuentes T, Gratas-Delamarche A, Monsalve M, Viña J. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1α to exercise training. *Age (Dordr)*. 2012; 34(3):669-79.

ABREVIATURAS

ABREVIATURAS

ACSM: Colegio Americano de Medicina del Deporte

ADH: Vasopresina u hormona antidiurética

ADM: Adrenomedulina

ADP: Adenosín difosfato

AICAR: 5-aminoimidazol-4-carboxamida-1-beta-D-ribofuranosido

ALT: Alanina aminotransferasa

AMPK: Quinasa activada por monofosfato de adenina

ARAII: Antagonista del receptor de la angiotensina II

AST: Aspartato aminotransferasa

AT1: Angiotensina II de tipo 1

ATP: Adenosín trifosfato

BDNF: Factor neurotrófico derivado del cerebro

CaMK: Quinasa dependiente de Ca+2/calmodulina

CK: Creatin quinasa

CK-MB: Isoforma cardíaca de la creatin quinasa

COI: Comité Olímpico Internacional

Cys-C: Cistatina C

DNA: Ácido desoxirribonucleico

EGFR: Tasa estimada de filtración glomerular

EMEA: Agencia Europea de Medicamentos

eNOS: Óxido nítrico sintasa endotelial

EO: Estrés oxidativo

FDA: Food and Drugs Administration

GDF15: Factor de diferenciación de crecimiento 15

GDNF: Factor neurotrófico derivado de la glía

GFT: Tasa de filtración glomerular

GGT: Gamma-glutamil transferasa

GPX: Glutatión peroxidasa

HDL: Lipoproteína de alta densidad

hGH: Hormona de crecimiento

HIF-1: Factor inducido por hipoxia 1

Hs-TnT: Troponina T de alta sensibilidad

HSF: Factor de choque térmico

IL-6: Interleucina 6

IL-18: Interleucina 18

IGF: Factor de crecimiento insulínico

iNOS: Óxido nítrico sintasa inducible

JNK: quinasa c-Jun NH_2-terminal

KIM-1: Molécula de enfermedad renal 1

KO: Knock out - eliminado

L-FABP: Proteínas hepáticas de unión a ácidos grasos

LDH: Lactato deshidrogenasa

LRA: Lesión renal aguda

MAPK: Proteína quinasa activada por mitógenos

MCP: Péptido quimiotáctico de monocitos

MDA: Malondialdehido

MET: Tarea equivalente metabólica

MnSOD: Manganeso superóxido dismutasa

MR-proADM: Región media de la proadrenomedulina

mTOR: Diana de rapamicina en células de mamífero

NAD^+: Nicotinamida adenina dinucleótido

NF-κB: Factor nuclear Kappa B

NGAL: Lipocalina asociada con la gelatinasa de neutrófilos

NRF-1: Factor respiratorio nuclear 1

NRF-2: Factor respiratorio nuclear 2

OMS: Organización Mundial de la Salud

PCR: Proteína C reactiva

PCT: Procalcitonina

PGC-1α: Coactivador-1α del receptor-γ activado por la proliferación del peroxisoma

PGC-1β: Coactivador-1 β del receptor-γ activado por la proliferación del peroxisoma

PlGF: Factor de crecimiento placentario

PPARγ: Receptor-γ activado por la proliferación del peroxisoma

PPARδ: Receptor- δ activado por la proliferación del peroxisoma

preproADM: Preproadrenomedulina

PRX: Peroxirredoxinas

RLs: Radicales libres

RNA: ácido ribonucleico

ROS: Especies reactivas del oxígeno

RTK: Receptor de tirosina quinasa

SARM: Moduladores selectivos del receptor de andrógenos

SESNs: Sestrinas

sNGAL: Lipocalina asociada con la gelatinasa de neutrófilos-sérica

suPAR: Receptor del activador del plasminógeno soluble en uroquinasa

sVEGFR-1/sFLT-1: Receptor del factor de crecimiento endotelial vascular-1

T3: Triyodotironina

TdF: Tour de France

TFAM: Factor de transcripción mitocondrial A

TGF-β: Factores de crecimiento transformante β

TORC1: Complejo diana de la rapamicina 1

uNGAL: Lipocalina asociada con la gelatinasa de neutrófilos-urinaria

VEGF: Factor de crecimiento del endotelio vascular

VO_{2max}: Consumo máximo de oxígeno

WT: Wild type - animal tipo salvaje

XDH: Xantina deshidrogenada

XO: Xantina oxidasa

XOR: Xantina óxido-reductasa

SINOPSIS

En esta sinopsis pretendemos dar una visión global de la presente tesis por compendio de publicaciones, con una estructura argumental lógica, dividiéndola en siete puntos fundamentales:

1.- El ejercicio físico agotador produce radicales libres que están implicados en el daño muscular y renal en deportes individuales (maratón, ciclismo, halterofilia). Hace algo más de medio siglo que se comprobó que el músculo esquelético contiene radicales libres [1]. Existen evidencias de que la actividad muscular intensa puede desembocar en un estrés oxidativo que se manifiesta en un aumento en la oxidación del glutatión, oxidación de proteínas, oxidación del DNA y lipoperoxidación [2,3]. En el laboratorio del Dr. Viña se demostró en 1992 que el ejercicio físico cuando se realiza hasta el agotamiento causa estrés oxidativo. En este sentido encontramos una correlación lineal entre el cociente glutatión oxidado y reducido y el cociente lactato-piruvato [4]. Este trabajo dio paso a una serie de publicaciones en las que, utilizando distintos modelos experimentales, estos resultados han sido confirmados [5,6].

2.- Existen diversas fuentes de radicales libres en el músculo esquelético. Nuestro grupo de investigación ha estudiado durante años el papel de la enzima xantina oxidasa (XO). La XO utiliza el oxígeno molecular lo que implica un aumento de la producción de radical superóxido [7]. Este incremento podría estar relacionado con el daño muscular asociado al ejercicio físico agotador. El alopurinol es un inhibidor conocido de la XO. En experimentos tanto en humanos [8,9] como en animales [10] nuestro grupo de investigación ha demostrado que el alopurinol previene la

oxidación del glutatión, oxidación de proteínas y la lipoperoxidación asociada al agotamiento. En unos experimentos que llevamos a cabo en ciclistas profesionales, durante dos ediciones consecutivas del Tour de Francia, encontramos que la administración oral de una dosis diaria de 300 mg de alopurinol previno el aumento en la actividad de la creatin quinasa y de la aspartato amino transferasa en plasma (marcadores de daño muscular) únicamente en la etapa en la que todos los miembros del equipo se ejercitaron a la máxima intensidad durante un tiempo superior a 1 hora [3]. Del mismo modo encontramos un aumento en los niveles plasmáticos de MDA en todos los participantes al acabar la carrera, sin embargo este incremento fue significativamente mayor en el grupo placebo cuando se comparó con el grupo alopurinol. Estos resultados sugieren que la XO está implicada en el daño muscular asociado a la realización de ejercicio físico agotador.

3.- Tras lo anteriormente expuesto, nos planteamos estudiar el papel de la enzima XO en el daño oxidativo, músculo-esquelético y cardiovascular en un deporte de equipo (fútbol) mediante la determinación de diversos biomarcadores clásicos de daño oxidativo, muscular y cardiaco como MDA, CK, LDH, AST, mioglobina, CK-MB y Hs-TnT [11], así como testar las modificaciones que se producen en nuevos biomarcadores cardiovasculares como son: copeptina, MD-proADM, GDF15, PIGF, sVEGFR1/sFLT1 y suPAR [12,13], antes y después de un partido.

4.- El ejercicio agotador produce daño tisular, y esto lo hemos querido comprobar evaluando el efecto del ejercicio físico de muy larga duración

sobre nuevos biomarcadores de daño cardiovascular y renal en atletas antes y después de una ultramaratón de 100 Km [14,15].

5.- Sin embargo, el entrenamiento deportivo se asocia a efectos beneficiosos, favoreciendo el envejecimiento saludable y la longevidad, aparte de otros muchos efectos sobre la salud [16,17]. Para clarificar estos hechos hemos analizado las implicaciones del ejercicio físico de alta intensidad sobre la longevidad en sujetos entrenados, utilizando como modelo los ciclistas participantes en el Tour de Francia entre los años 1930-1964 [18].

6.- Por otra parte, una de las bases del envejecimiento saludable es el mantenimiento de la mitocondriogénesis en el músculo-esquelético, lo que se asocia con la prevención de la sarcopenia [19-21] y justifica la prescripción del ejercicio en sujetos de edad avanzada [22].

7.- Finalmente, aunque el ejercicio se asocia al mantenimiento de la mitocondriogénesis en jóvenes, en viejos no encontramos una buena respuesta de PGC-1 [23], por lo que consideramos que si el ejercicio se empieza en edades avanzadas, cuando el sujeto/animal ya es mayor, se pierden parte de sus efectos beneficiosos. Es por esto por lo que hemos dedicado el último bloque de la presente tesis doctoral al estudio del papel del entrenamiento aeróbico en la inducción de la cascada de la biogénesis mitocondrial y sus implicaciones en el envejecimiento músculo-esquelético.

TESIS POR COMPENDIO DE PUBLICACIONES

1. TESIS COMO COMPENDIO DE TRABAJOS PREVIAMENTE PUBLICADOS

La presente tesis doctoral, de acuerdo con el informe correspondiente, autorizado por los Directores de Tesis y el Órgano Responsable del Programa de Doctorado, se presenta como un compendio de trabajos previamente publicados. Las referencias completas de los artículos que constituyen el cuerpo de la tesis y en las cuales el doctorando aparece como primer autor son:

1. ***Sanchis-Gomar F***, Pareja-Galeano H, Gómez-Cabrera MC, Candel J, Lippi G, Mann GE, Viña J. Allopurinol prevents skeletal and cardiac muscle damage in professional soccer players. *Scand J Med Sci Sports*. 2013 (bajo revisión)

2. ***Sanchis-Gomar F,*** Bonaguri C, Aloe R, Pareja-Galeano H, Martinez-Bello V, Gomez-Cabrera MC, Candel J, Viña J, Lippi G. Effects of acute exercise and xanthine oxidase inhibition on novel cardiovascular biomarkers. *Transl Res*. 2013; 162(2):102-9.

3. ***Sanchis-Gomar F,*** Bonaguri C, Pareja-Galeano H, Gomez-Cabrera MC, Candel J, Viña J, Lippi G. Effects of acute exercise and allopurinol administration on soluble urokinase plasminogen activator receptor (suPAR). *Clin Lab*. 2013; 59(1-2):207-10.

4. Sanchis-Gomar F, Olaso-Gonzalez G, Corella D, Gómez-Cabrera MC, Vina J. Increased average longevity among the "Tour de France" cyclists. *Int J Sports Med*. 2011; 32(8):644-7.

5. ***Sanchis-Gomar F***. The skeletal muscle–metabolism axis in prostate-cancer therapy. *N Engl J Med.* 2012; 367(23):2257-8.

6. Sanchis-Gomar F, Gómez-Cabrera MC, Viña J. The loss of muscle mass and sarcopenia: non hormonal intervention. *Exp Gerontol*. 2011;46(12):967-9.

Asimismo, se considera oportuno incluir en la presente Tesis con el fin de complementar su contenido los siguientes artículos en los cuales el doctorando participa como coautor:

7. Lippi G, Schena F, Salvagno GL, ***Sanchis-Gomar F***, Guidi GC. Serum copeptin and mid-region pro-adrenomedullin (MR-proADM) after an ultra-marathon. *J Clin Lab Anal*. 2013.

8. Lippi G, ***Sanchis-Gomar F***, Salvagno GL, Aloe R, Schena F, Guidi GC. Variation of serum and urinary neutrophil gelatinase associated lipocalin (NGAL) after strenuous physical exercise. *Clin Chem Lab Med*. 2012; 14; 50(9):1585-9.

9. Viña J, Borras C, ***Sanchis-Gomar F***, Martinez-Bello VE, Olaso-Gonzalez G, Gambini J, Ingles M, Gomez-Cabrera MC. Pharmacological Properties of Physical Exercise in The Elderly. *Curr Pharm Des*. 2013.

10. Vina J, ***Sanchis-Gomar F***, Martinez-Bello V, Gomez-Cabrera MC. Exercise acts as a drug; the pharmacological benefits of exercise. *Br J Pharmacol.* 2012; 167(1):1-12.

11. Gomez-Cabrera MC, ***Sanchis-Gomar F***, Garcia-Valles R, Pareja-Galeano H, Gambini J, Borras C, Viña J. Mitochondria as sources and targets of damage in cellular aging. *Clin Chem Lab Med*. 2012; 50(8):1287-95.

12. Viña J, Gomez-Cabrera MC, Borras C, Froio T, ***Sanchis-Gomar F***, Martinez-Bello VE, Pallardo FV. Mitochondrial biogenesis in exercise and in ageing. *Adv Drug Deliv Rev*. 2009; 30;61(14):1369-74.

13. Derbré F, Gomez-Cabrera MC, Nascimento AL, ***Sanchis-Gomar F***, Martinez-Bello VE, Tresguerres JA, Fuentes T, Gratas-Delamarche A, Monsalve M, Viña J. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1α to exercise training. *Age (Dordr).* 2012; 34(3):669-79.

INTRODUCCIÓN

2. INTRODUCCIÓN

2.1. EJERCICIO FÍSICO Y ADMINISTRACIÓN DE ALOPURINOL SOBRE BIOMARCADORES MUSCULARES Y CARDIOVASCULARES.

Hace algo más de medio siglo que se comprobó que el músculo-esquelético produce radicales libres (RLs) [1]. Existen evidencias de que la actividad muscular intensa puede desembocar en un estrés oxidativo (EO) que se manifiesta en un aumento en la oxidación del glutatión así como en la oxidación de proteínas, lípidos y DNA [2,3]. En 1992 se demostró en nuestro laboratorio que el ejercicio físico, cuando se realiza hasta el agotamiento, causa EO. En este sentido encontramos una correlación lineal entre el cociente glutatión oxidado y reducido y el cociente lactato-piruvato [4]. Este trabajo dio paso a una serie de publicaciones en las que dichos resultados, utilizando distintos modelos experimentales, se confirmaron [5,6].

Existen diversas fuentes de RLs en el músculo-esquelético. Nuestro grupo de investigación ha estudiado durante años el papel de la enzima xantina oxidasa (XO). La XO y la xantina deshidrogenada (XDH) son isoenzimas de la xantina óxido-reductasa (XOR). La XO se encuentra en las células del músculo liso de la pared de los vasos y en las células endoteliales en el músculo-esquelético. La conversión de la XDH a XO es posible vía proteasas vasculares. La hipoxantina se forma en el músculo durante el ejercicio físico intenso o en las últimas fases del ejercicio físico de muy larga duración. Al ser tan permeable atraviesa la membrana celular con gran facilidad. La hipoxantina es el sustrato de la XOR. La XOR cataliza el paso de hipoxantina a xantina y de ésta a ácido úrico. Mientras que la XDH transfiere de forma preferente los electrones resultantes de la oxidación al NAD, la XO utiliza el oxígeno molecular lo que implica un aumento de la

producción de radical superóxido [7]. Este incremento podría estar relacionado con el daño muscular asociado al ejercicio físico agotador.

El alopurinol es un análogo estructural de la base púrica hipoxantina y un inhibidor conocido de la XO. En experimentos tanto en humanos [8,9] como en animales [10] nuestro grupo de investigación ha demostrado que el alopurinol previene la oxidación del glutatión, oxidación de proteínas y la lipoperoxidación asociada al agotamiento. Del mismo modo y en unos experimentos que llevamos a cabo en ciclistas profesionales, durante dos ediciones consecutivas del Tour de Francia (TdF), encontramos que la administración oral de una dosis diaria de 300 mg de alopurinol previno el aumento en la actividad de la creatin quinasa (CK) y de la aspartato amino transferasa (AST) en plasma (marcadores de daño muscular) en la etapa en la que todos los miembros del equipo se ejercitaron a la máxima intensidad durante un tiempo superior a 1 hora [3] (Ver Figura 1). Del mismo modo encontramos un aumento en los niveles plasmáticos de MDA en todos los participantes al acabar la carrera, sin embargo este incremento fue significativamente mayor en el grupo placebo cuando se comparó con el grupo alopurinol. Estos resultados sugieren que la XO está implicada en el daño muscular asociado a la realización de ejercicio físico hasta el agotamiento.

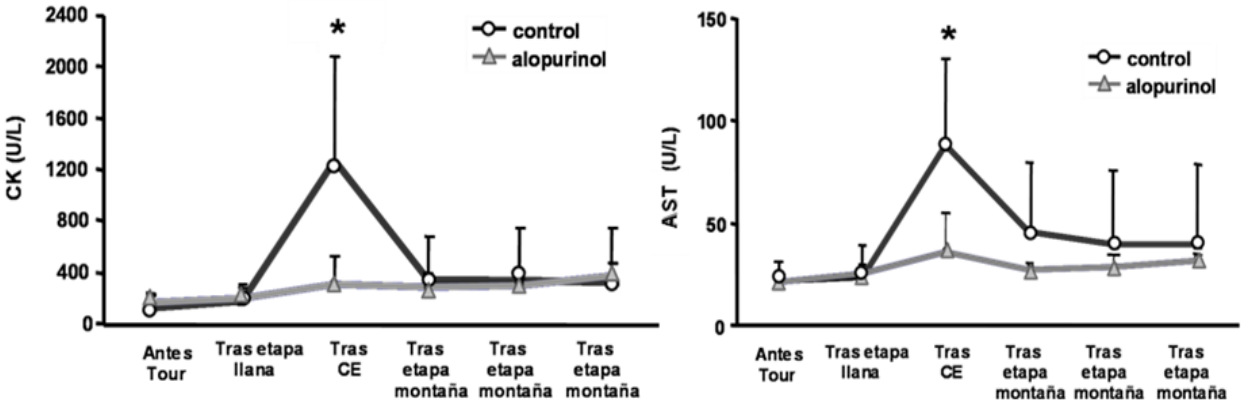


Figura 1. *Valores de las medias de actividad CK y GOT. Grupo control (n=5), grupo alopurinol (n=4). La comparación de las medias se realizó utilizando un análisis de varianza de medidas repetidas. El valor de la p se determinó utilizando el test de Scheffe de comparaciones múltiples. La significación estadística se expresa como (*) p=0,02 frente a grupo alopurinol. CE: Contrarreloj por equipos.*

Estos datos los hemos confirmado en un estudio posterior en el que trabajamos con corredores de maratón que participaron en la Maratón de Valencia. En este caso encontramos un aumento significativo en los niveles de MDA plasmático tras la realización de la prueba y que fue prevenida con la administración de alopurinol [24]. Además, nuestro grupo ha demostrado muy recientemente que la inhibición de la actividad XO por medio de la administración de alopurinol previene la atrofia muscular al inhibir la vía p38 MAPK-MAFbx, y por tanto puede tener beneficios clínicos como son combatir la atrofia muscular en pacientes encamados, sarcopénicos o caquéxicos [25].

El ejercicio físico, sobre todo el excéntrico, puede llegar a provocar daño muscular [26,27]. Una excesiva tensión en el sarcómero es la principal causa de la lesión muscular por una disrupción de las membranas que permite la hidrólisis de proteínas estructurales, provocando la desestructuración miofibrilar que se observa habitualmente [28]. Posteriormente aparece el fenómeno de la inflamación que ayuda a degradar y reparar el tejido. El deporte del fútbol presenta un elevado componente excéntrico durante su práctica. Dada la elevada acumulación de partidos en aquellos equipos que disputan varias competiciones durante el año (Liga Española, Ligas Europeas, Copa del Rey etc.) el daño muscular sufrido por los jugadores puede tener consecuencias muy negativas sobre la incidencia de lesiones entre los futbolistas de los primeros equipos.

El ejercicio físico se recomienda para prevenir una amplio número de enfermedades crónicas tales como enfermedades cardiovasculares, cáncer, osteoporosis o diabetes [17]. También se ha demostrado que el ejercicio de resistencia mejora la esperanza de vida [18,29]. Sin embargo, como hemos comentado anteriormente, el ejercicio físico intenso puede generar RLs, vía XO, que pueden causar daño muscular [4].

Para contrarrestar estas lesiones oxidativas potenciales, la administración de alopurinol puede ser una estrategia eficaz para prevenir el daño muscular y la

peroxidación lipídica como se demostró en ciclistas y en maratonianos [3,24]. Algunos deportes como el ciclismo o las carreras de larga distancia (por ejemplo, maratón y ultra-maratón) se han asociado con un aumento post-ejercicio de biomarcadores de daño músculo-esquelético, cardíaco y hepático, incluyendo las troponinas cardíacas, AST, alanina aminotransferasa (ALT), gamma-glutamil transferasa (GGT), lactato deshidrogenasa (LDH) y CK, entre otras [30-36], así como con hallazgos radiológicos sugestivos de fibrosis y daño miocárdico [37]. Como se ha dicho anteriormente, existen varias fuentes celulares de producción de especies reactivas del oxígeno (ROS) en el músculo-esquelético, incluyendo la XO. La XOR es una enzima intracelular que participa en el catabolismo de la purina, que cataliza la reducción de hipoxantina y xantina en ácido úrico [38]. El ejercicio físico agotador o agudo provoca un aumento en la generación de ROS, y por lo tanto de EO en el músculo-esquelético y en otros órganos, que finalmente resulta en una lesión celular [2,39,40]. Por lo tanto, el EO está claramente implicado en la fisiopatología de la remodelación e insuficiencia cardíaca, así como en el daño del músculo-esquelético inducido por el ejercicio agotador [41]. El alopurinol es un inhibidor de la XOR muy conocido y que se usa frecuentemente en la práctica clínica [42], y como ha descrito nuestro grupo en estudios previos, un prometedor fármaco para prevenir el daño oxidativo muscular durante el ejercicio físico agotador [2,24].

Asimismo, se ha demostrado que el ejercicio y el entrenamiento pueden desencadenar cambios fisiopatológicos en las concentraciones séricas y urinarias de una variedad de parámetros de laboratorio y su evaluación permite controlar el daño a un nivel tisular específico (es decir, hígado, riñón, músculo-esquelético y corazón). Este aspecto ha incrementado inherentemente el uso de los biomarcadores de lesión tisular tradicionales por los médicos deportivos e investigadores del campo del deporte durante las últimas décadas [43]. Más allá, las nuevas iniciativas en la investigación deportiva también tienen por objeto

acelerar la búsqueda de biomarcadores innovadores y prometedores que nos permitan un mejor seguimiento del entrenamiento y del rendimiento deportivo, el diagnóstico de las lesiones relacionadas con el deporte, la predicción del sobreentrenamiento, e incluso la identificación de la época más adecuada para volver a la competición después de una lesión.

2.1.1. El sistema hipoxantina/ xantina oxidasa

La XOR es una enzima descrita originalmente como una aldehído oxidasa en 1902 [44]. Esta enzima está ampliamente distribuida entre seres vivos de distinta complejidad. En las distintas especies cataliza la hidroxilación de una amplia gama de sustratos como purinas, pirimidinas, pterinas y aldehídos. La XDH es capaz de utilizar tanto el NAD+ como el oxígeno como aceptor de electrones, aunque tiene preferencia por el primero. La XO sólo es capaz de utilizar el oxígeno como aceptor de electrones. Es la enzima encargada de la degradación de las purinas como se muestra en la Figura 2.

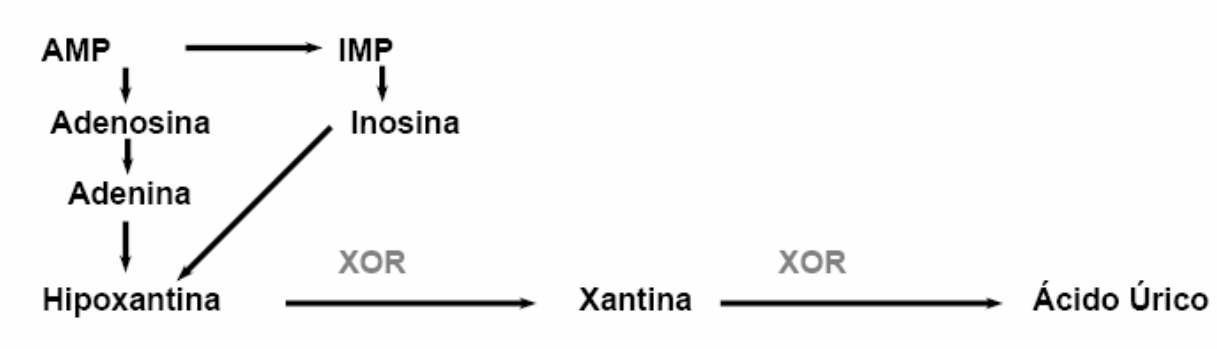


Figura 2. *Esquema de degradación de las purinas*

2.1.2. Xantina Oxidasa (XO)

Pese a que varios grupos de investigación, como hemos comentado en el apartado anterior, han señalado a la mitocondria como principal fuente generadora de ROS en el ejercicio, en estas observaciones yace un problema conceptual. El radical

superóxido producido por los músculos en contracción puede ser detectado en el espacio extracelular [45] y en el compartimento vascular [46]. El anión superóxido generado en la mitocondria es muy poco probable que pueda ser medido fuera de la célula.

Esto supondría que especies reactivas y cargadas eléctricamente, escapasen a los sistemas antioxidantes en la matriz mitocondrial y difundiesen a través de la membrana mitocondrial interna y externa, citosol y sarcolema sin implicarse en ninguna reacción química. La difusión a través del endotelio capilar en el compartimento vascular todavía parece menos probable [47]. La XO representa una fuente alternativa de ROS con soporte experimental. En músculo esquelético, la XO se localiza principalmente en el endotelio vascular [48]. La administración de inhibidores de la enzima disminuye la liberación de radical superóxido en el espacio vascular en los músculos en contracción [49] y parcialmente inhibe la fatiga *in vivo* [50]. En oposición a lo que ocurre con la mitocondria que genera RLs en un estado basal, las ROS derivadas de la XO cobran importancia en cuatro procesos: en la respuesta inflamatoria al ejercicio físico excéntrico, en el ejercicio físico de alta intensidad, en el ejercicio prolongado [51], o en el daño causado por los procesos de isquemia-reperfusión [52].

La XO fue inicialmente identificada como una fuente potencial de RLs en el citosol de la célula muscular [53]. Sin embargo, estudios posteriores utilizando anticuerpos monoclonales para la XDH/XO revelan inmunoreactividad en las células del músculo liso de la pared de los vasos a la vez que en células endoteliales [54] y descartan su presencia en el interior de la célula muscular.

La hipoxantina se forma en el músculo durante el ejercicio físico intenso y existe un incremento marcado de esta purina del músculo en la sangre [55]. La cantidad de hipoxantina que se acumula en sangre depende principalmente de la intensidad del ejercicio. Los niveles más elevados en plasma se han encontrado tras el ejercicio físico agotador [56]. La formación de hipoxantina parece estar asociada

con la acumulación de IMP en el músculo, lo que a su vez está relacionado con la intensidad del ejercicio y con su duración [57]. En estos últimos trabajos se sugiere que los nucleótidos son degradados cuando la resíntesis del ATP está limitada debido a los bajos niveles de glucógeno muscular. Con respecto al ácido úrico, las observaciones publicadas sobre la ausencia significativa de liberación del ácido úrico por el músculo pueden ser el resultado de una baja sensibilidad en los métodos de detección [58,59]. En ratas sí que se ha encontrado acumulación de ácido úrico tras estimulación eléctrica [60].

Existe una correlación lineal entre el pico de hipoxantina y el de ácido úrico en plasma tras un ejercicio físico agotador [61]. Esta observación indica que la concentración plasmática de hipoxantina es importante en el flujo de la vía XDH/XO, ya que un elevado nivel de hipoxantina en plasma supondría un aumento en la producción de radical superóxido en el caso en el que la conversión a XO hubiese sucedido. En 1981 el grupo de Granger demostró que el tratamiento del intestino de felino con superóxido dismutasa previo al proceso de isquemia atenuaba el daño durante la subsiguiente reperfusión, lo que apuntaba hacia el radical superóxido como responsable del daño tisular [62]. Estos autores propusieron que la fase de isquemia producía una conversión de las XDH a XO y una degradación de los nucleótidos de adenina a hipoxantina. De esta forma con la reintroducción del oxígeno molecular durante la reperfusión, una considerable cantidad de radical superóxido, podría ser generada en la reacción catalizada por la XO.

2.1.3. Alopurinol

El alopurinol [1H-pirazolo (3,4-d)pirimidina-4-ol] es un análogo estructural de la base púrica natural hipoxantina (peso molecular 136.11) y actúa sobre el catabolismo de las purinas sin modificar su biosíntesis. Reduce la producción de ácido úrico al inhibir las reacciones bioquímicas que conducen a su formación. El

alopurinol actúa como un inhibidor de la XOR, la enzima responsable de la conversión de hipoxantina a xantina y de xantina a ácido úrico el producto final de catabolismo de las purinas en el hombre. La inhibición de la enzima XO por este fármaco es efectiva tanto *in vivo* como *in vitro* [63]. El alopurinol inhibe la XO formando un complejo reversible con el molibdeno e interfiriendo así la interacción de las purinas con la enzima, de forma que no puede realizarse la oxidación de éstas [64]. Es absorbido por el tracto intestinal en un 90% aproximadamente y metabolizado a aloxantina (oxipurinol) que también es un inhibidor de la XO. El alopurinol y el oxipurinol son eliminados por los riñones; por lo tanto, los cambios en la función renal tienen un efecto profundo en la dosificación (Ver Figura 3).

Como resultado de la inhibición de la XO, en los pacientes tratados con alopurinol se han detectado unos niveles de xantina + hipoxantina de 0,3 a 0,4 mg/dl en comparación con los niveles normales de aproximadamente 0,15 mg/dl. El valor máximo detectado, de 0.9 mg/dl de estas oxipurinas después de dosis muy altas de alopurinol están muy por encima de la saturación (> 7 mg/dl).

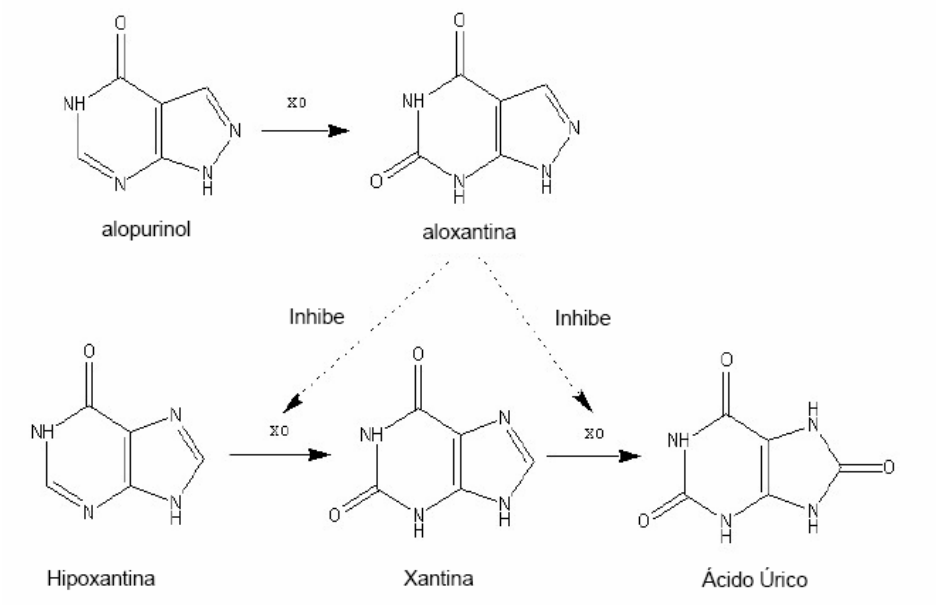


***Figura 3**. Inhibición de la XO por el alopurinol*

2.2. EJERCICIO FÍSICO AGOTADOR Y DE LARGA DURACIÓN SOBRE EL SISTEMA CARDIOVASCULAR Y RENAL.

Es bien sabido que el ejercicio físico confiere grandes beneficios para un estilo de vida saludable, así como que es muy útil en la creciente batalla contra la obesidad, enfermedades cardiovasculares y otros trastornos crónicos, tales como la diabetes, osteoporosis, disminución de la función cognitiva y cáncer [65]. Recientemente, el Comité Olímpico Internacional (COI) ha aprobado recomendaciones defendiendo que todos los gobiernos deben fomentar la práctica de ejercicio físico y la promoción de la salud [65]. A pesar de que parece evidente que el ejercicio físico de alta intensidad en población no entrenada y/o que padezca algún tipo de patología podría estar contraindicado y resultar poco saludable e incluso perjudicial, sigue existiendo controversia sobre si el ejercicio físico extenuante y físicamente exigente podría determinar efectos biológicos a corto y largo plazo que podrían a su vez ser contraproducentes para la salud [66]. Varias modalidades deportivas como el ciclismo y las carreras de larga distancia (por ejemplo, maratón y ultramaratón) se han asociado con un aumento notable en biomarcadores de lesión cardiaca [30,31,33,67], así como con los hallazgos radiológicos sugestivos de necrosis miocárdica y fibrosis [37]. El EO está claramente implicado en la fisiopatología de la remodelación y la insuficiencia cardíaca, así como en el daño muscular inducido por el ejercicio físico agotador [41].

Por otra parte, es bien conocido que el flujo sanguineo renal es de alrededor de 1.2 L/min en reposo, que es aproximadamente el 20% del gasto cardíaco [68]. La actividad física induce cambios hemodinámicos profundos. El aumento del flujo sanguíneo muscular durante el ejercicio causa, de hecho, una disminución dramática de la circulación esplácnica y renal, lo que parece ser directamente proporcional a la intensidad y la duración del esfuerzo físico (por ejemplo, en el ejercicio intenso el flujo sanguíneo renal podría caer hasta un 25% del valor de

reposo) [68,69]. Varios mecanismos se han postulado para explicar la perturbación en la hemodinámica renal en deportistas, incluyendo un aumento en la actividad del sistema nervioso simpático, así como una mayor actividad de dos vasoconstrictores importantes, tales como la angiotensina II y vasopresina [70]. Estos cambios se ven compensados por un mecanismo de autorregulación local, que tiene como objetivo preservar la tasa de filtración glomerular (GFT). El aumento de la fracción de filtración resultante, que puede ser dos veces mayor que la de reposo, limita parcialmente el paso de metabolitos o sustancias a través de los glomérulos, reduciendo el grado de proteinuria inducida por el ejercicio [71]. Una proteinuria transitoria post-ejercicio es un hecho común en los deportistas, y parece estar directamente relacionada con la intensidad del ejercicio [71].

Esto se debe principalmente a un mecanismo combinado de un aumento del aclaramiento de proteínas plasmáticas debido a un aumento de la permeabilidad glomerular, y una inhibición parcial de la reabsorción tubular [70]. Que una reducción prolongada e intensa del flujo sanguíneo renal esté relacionada con una lesión del parénquima sigue sin demostrarse y la clarificación de este problema está limitada por la falta de un biomarcador fiable de daño renal. Históricamente, los criterios diagnósticos para la lesión renal aguda (LRA) se han basado fundamentalmente en las variaciones agudas de creatinina sérica o la producción de orina. Aunque el parámetro anterior sigue siendo el método de referencia para el diagnóstico de insuficiencia renal [72], su evaluación en el ajuste de la LRA tiene varios inconvenientes tales como su dependencia de la edad, género, estado nutricional, fármacos, y/o masa muscular, así como un valor predictivo positivo subóptimo en pacientes con azotemia prerrenal. El valor predictivo negativo de la creatinina sérica también está limitado por la gran "reserva renal", ya que una variación significativa de la creatinina sérica se hace evidente cuando se pierde más de la mitad de la tasa de filtración glomerular [73,74], de manera que el diagnóstico de la LRA se puede retrasar sustancialmente por su cinética enlentecida. Por lo

tanto, la isquemia renal inducida por un ejercicio aeróbico vigoroso podría causar daño a nivel del tejido renal.

Una gran cantidad de proteinas séricas y urinarias han sido recientemente propuestas y estudiadas como potenciales marcadores de la LRA, incluyendo entre ellas la lipocalina asociada con la gelatinasa de neutrófilos (NGAL), cistatina-C (Cys-C), interleucina-18 (IL-18), molecula de enfermedad renal-1 (KIM-1), péptido quimiotáctico de monocitos (MCP-1), netrina-1 y proteínas hepáticas de unión a ácidos grasos (L-FABP). Entre todos ellos, de la que mejor evidencia clínica se tiene hasta el momento es de NGAL [73,75].

2.2.1. Biomarcadores cardiovasculares emergentes

2.2.1.1. Copeptina

La copeptina es la porción terminal de la provasopresina, siendo un glicopéptido de 39 aminoácidos producido durante la escisión de la vasopresina u hormona antidiurética (ADH) [76]. La ADH es liberada por la neurohipófisis para mantener el equilibrio hídrico por el riñón, y por lo tanto contribuye a la homeostasis osmótica y cardiovascular. Los niveles séricos de copeptina se correlacionan con los niveles individuales de estrés [77], mientras que la combinación de la copeptina con troponinas cardíacas en pacientes con dolor torácico puede mejorar el diagnóstico temprano del síndrome coronario agudo [78-82]. Además, la copeptina proporciona información pronóstica fiable en pacientes después de un infarto agudo de miocardio o en la insuficiencia cardíaca crónica [83-88]. Niveles elevados de este biomarcador también pueden predecir un riesgo aumentado de sufrir diabetes independientemente de los factores de riesgo convencionales [89]. El valor pronóstico de la copeptina ha sido bien establecido en otras enfermedades graves, incluyendo sepsis, neumonía, infecciones de las vías respiratorias inferiores y accidentes cerebrovasculares [90-93].

2.2.1.2. Región media de la proadrenomedulina (MR-proADM)

La adrenomedulina (ADM) es un péptido vasoactivo de 52 aminoácidos con un puente disulfuro interno que juega un papel importante en la microcirculación y en la disfunción endotelial. Su precursor, la preproadrenomedulina (preproADM), es transcripcionalmente inducida por la insulina, la hipoxia, y varios estímulos inflamatorios. Después de una serie de transformaciones post-traduccionales, se generan dos péptidos amidados con actividad biológica: la ADM, y el péptido aminoterminal de la proadrenomedulina (PAMP). La PAMP se degrada en dos péptidos: la parte de la región media de la ADM (MR-proADM 45–92) y la parte terminal de la molécula (proADM 153–185) [94]. La parte de la región media de la proADM (MR-proADM) es más estable que la propia ADM y por lo tanto más indicada para el uso en la práctica clínica y su determinación en muestras almacenadas [95]. Evidencias recientes muestran que la MR-proADM es un predictor independiente de eventos en pacientes con enfermedad arterial coronaria sintomática [96], eventos cardiovasculares [97], insuficiencia cardiac crónica [98], infarto [99], y otras enfermedades severas como son amiloidosis [100], sepsis [101], neumonía adquirida en la comunidad [102,103], y disnea aguda [104-106]. Además, se ha observado que MR-proADM se asocia con el VO_2 pico independiente de la función pulmonar en pacientes con enfermedad pulmonar crónica [107]. Sin embargo, la función fisiológica de la MR-proADM está aún bajo investigación. Aunque tanto la copeptina como la MR-proADM están surgiendo como biomarcadores cardiovasculares, poco se sabe sobre la influencia del ejercicio físico en su concentración.

2.2.1.3. Factor de diferenciación de crecimiento 15 (GDF15)

El GDF15 es un miembro perteneciente a la superfamilia de los factores de crecimiento transformante β (TGF-β). Varios estudios han demostrado que la inflamación o la enfermedad regulan al alza la expresión de GDF15 a nivel

cardíaco [108,109]. Por otra parte, se ha demostrado que este nuevo biomarcador está asociado con trastornos vasculares tales como la aterosclerosis, hipertensión, isquemia e insuficiencia cardíaca [110], lo que sugiere que puede ser un buen marcador de estrés a nivel de los cardiomiocitos.

2.2.1.4. Receptor del factor de crecimiento endotelial vascular-1 (sVEGFR-1/sFLT-1) y factor de crecimiento placentario (PlGF)

En diferentes estudios animales y humanos se ha demostrado que la expresión de VEGF aumenta a nivel muscular después del ejercicio físico agudo [111-113]. PlGF es un miembro de la familia de los factores de crecimiento del endotelio vascular que se expresa en varios tipos de células inflamatorias y actúa como un mediador crucial en el reclutamiento de células madre hematopoyéticas y en etapas tardías de la angiogénesis [114]. PIGF media la arteriogénesis principalmente en respuesta a la isquemia o daño tisular [115]. El sVEGFR-1/sFLT-1 es un receptor de tirosina quinasa (RTK) específico para los factores angiogénicos de VEGF (VEGF-A), PlGF, y VEGF-B [116]. Los niveles del VEGFR1/sFLT-1 cambian en diferentes patologías [117-119], y tiene valor pronóstico [120]. También se ha sugerido que los niveles del sVEGFR1/sFLT-1 están aumentados junto con los de VEGF en condiciones experimentales y patológicas [121]. Sin embargo, muy poco se conoce acerca de la regulación de ambos por el ejercicio físico.

2.2.1.5. Receptor del activador del plasminógeno soluble en uroquinasa (suPAR)

El suPAR es una proteína circulante con un peso molecular comprendido entre 20 y 50 kDa, dependiendo del grado de glicosilación y la fragmentación proteolítica [122,123]. SuPAR está presente en sangre en concentraciones muy bajas en condiciones fisiológicas, mientras que el aumento de los niveles plasmáticos refleja de forma fiable la activación inmune y por lo tanto se puede considerar un valioso

marcador para varias enfermedades infecciosas [122,123]. Esta proteína es expresada por varias células inmunorreguladoras, incluyendo neutrófilos, células T activadas y macrófagos [124], por lo que se observan niveles elevados siempre en asociación con la inflamación, en la progresión de la enfermedad y en el desenlace fatal [125,126]. Se ha demostrado que el suPAR puede predecir el cáncer, las enfermedades cardiovasculares, la diabetes y la mortalidad en la población general [127], mientras que los niveles sistémicos de suPAR se correlacionan positivamente con los marcadores de disfunción orgánica y de la gravedad de una enfermedad [128].

2.2.1.6. Lipocalina asociada con la gelatinasa de neutrófilos (NGAL)

La NGAL es una proteina de fase aguda producida en gran parte por el túbulo renal y también está regulada por otras condiciones patológicas, como en el cáncer de páncreas y de recto, la diabetes, la aterosclerosis y el infarto de miocardio [73,129,130]. Los miembros de la familia de la lipocalina también pueden ser sobreexpresados por un aumento de ROS [131], que pueden ser a su vez generadas por el ejercicio físico agotador o agudo [40].

2.3. EJERCICIO FÍSICO, SALUD Y LONGEVIDAD

2.3.1. Ejercicio, movimiento y salud: Definiciones

La promoción de la salud es la ciencia y el arte de ayudar a la gente a cambiar su estilo de vida para avanzar hacia un estado de salud óptima [132]. La Organización Mundial de la Salud (OMS) define la salud como "El completo bienestar físico, mental y social, y no sólo la ausencia de afecciones o enfermedades". La forma física se define como el estado fisiológico de bienestar que permite a uno mismo satisfacer las actividades de la vida diaria (salud relacionada con la aptitud física) o que proporcione la base para un adecuado rendimiento deportivo (relacionada con el rendimiento físico), o ambos. Aunque somos conscientes de que hay una clara diferencia entre los términos actividad física ("cualquier movimiento corporal") y ejercicio físico ("una parte de la actividad física que se caracteriza por un entrenamiento planificado e intencionado") [133], utilizamos estos dos conceptos como sinónimos ya que en multitud de estudios se emplean indistintamente.

2.3.2. Antecedentes históricos

La hipótesis de que la actividad física promueve la salud y la longevidad no es nueva. Ya se encontró en antiguos registros chinos que datan de 2500 AC la recomendación de ejercicio organizado para la promoción de la salud [134,135]. En tiempos Greco-Romanos, hace 2500 años, Hipócrates (460–370 AC) y posteriormente Galeno (129–210 DC), reconocieron la necesidad de promover y prescribir ejercicio físico para obtener beneficios relacionados con la salud, y la necesidad de proporcionar atención médica para el deportista [136]. En este sentido, el gran filósofo Platón (427–347 AC), dijo: " La falta de actividad física destruye la buena condición de todo ser humano, mientras que el movimiento y el ejercicio físico metódico lo guarda y lo conserva " [137].

La simple comparación de hombres con diferentes ocupaciones proporcionó la primera evidencia empírica de que la actividad física se asocia con la salud. Los primeros estudios que demuestran una relación significativa inversa entre la actividad física y la enfermedad coronaria fueron las realizadas en conductores de autobús londinenses por Morris y colaboradores a principios de la década de los 50 [138]. Estos autores encontraron que los conductores de autobús físicamente más activos tenían una menor probabilidad de padecer una enfermedad coronaria frente a los conductores menos activos. Su comparación posterior con los carteros y empleados postales menos activos produjo resultados similares [139]. Estos trabajos fueron seguidos por los de Paffenbarger y colaboradores en la década de los 70 en los que evaluaron el aumento en el riesgo relativo de muerte por cualquier causa y por enfermedades específicas asociadas con la inactividad física [140,141].

2.3.3. El ejercicio físico es bueno para la salud

El ejercicio es una de las terapias más frecuentemente prescritas tanto en la salud como en la enfermedad. Existen pruebas irrefutables que muestran los efectos beneficiosos del ejercicio, tanto para prevenir como para tratar diversas enfermedades. Diversas investigaciones han demostrado que los hombres y mujeres que realizan mayores niveles de actividad física y con una mejor condición física tienen un menor riesgo relativo de muerte (entre un 20% y un 35%) [142,143].

Investigaciones recientes sugieren que modestos incrementos en el gasto energético mediante la actividad física (~1000 kcal por semana) o un incremento en la forma física de 1MET (Equivalente Metabólico) está asociada con una disminución en la mortalidad de aproximadamente el 20% [144]. Mujeres de mediana edad físicamente inactivas (menos de una hora de ejercicio a la semana) experimentan un incremento del 52% en la mortalidad por cualquier causa, doblan la mortalidad

por causas cardiovasculares e incrementan un 29% la mortalidad por cáncer cuando se compara con mujeres físicamente activas [145].

Por tanto, hay una clara evidencia de que la práctica de actividad física regular produce efectos beneficiosos para la salud y reduce el riesgo de muerte prematura por cualquier causa y por enfermedad cardiovascular, en particular entre sujetos asintomáticos.

Los beneficios de la actividad física son evidentes, no sólo en personas sanas, sino también en enfermos. Estudios observacionales y aleatorizados han demostrado que la actividad física regular contribuye al tratamiento de varias enfermedades crónicas [146,147]. Hay evidencias suficientes para prescribir ejercicio físico en la prevención primaria y secundaria de enfermedades pulmonares y cardiovasculares (enfermedad coronaria, enfermedad pulmonar obstructiva crónica, hipertensión, claudicación intermitente), trastornos metabólicos (diabetes tipo 2, dislipidemia, obesidad, resistencia a la insulina), cáncer óseo y enfermedades articulares (artritis reumatoide, fibromialgia, síndrome de fatiga crónica, osteoporosis), y depresión [147,148].

También se ha demostrado que el ejercicio induce un marcado aumento en los niveles plasmáticos y musculares de folistatina e irisina [149-151]. Los cambios de expresión en los niveles de folistatina, tanto en el músculo como en la grasa de ratas diabéticas puede ser modulada por ejercicio físico [152]. Las concentraciones de irisina aumentan significativamente después del ejercicio en ratones y en humanos, y los niveles de irisina circulantes se correlacionan con los niveles de ARN mensajero de irisina en el tejido muscular [151]. Todos estos hechos ponen de relieve la importancia del ejercicio con respecto a la sobreexpresión de miocinas y, por lo tanto, con respecto a la prevención y el tratamiento de la sarcopenia y la fragilidad. En este sentido, nuestro grupo también ha propuesto el ejercicio físico para prevenir la sarcopenia inducida por el tratamiento antiandrogénico en el cáncer de próstata [16]. Por lo tanto, y a pesar de que tal vez no sea viable para

algunos pacientes, los médicos deben recomendar encarecidamente el ejercicio o simplemente la actividad física como una terapia ayudante siempre que sea posible [16].

Sin embargo, aunque el ejercicio es un buen remedio para todas estas enfermedades, como con cualquier otro fármaco, la dosis (volumen e intensidad del ejercicio), la frecuencia de administración (sesiones semanales), tipo (ejercicio aeróbico frente al anaeróbico), efectos sistémicos y psicoactivos, y contraindicaciones y efectos secundarios del ejercicio se deben tener en cuenta para lograr el mejor resultado clínico posible. Por ejemplo, tanto el entrenamiento aeróbico como el anaeróbico han demostrado ser beneficiosos para el control de la diabetes, sin embargo, el entrenamiento anaeróbico puede tener mayores beneficios para el control glucémico que el entrenamiento aeróbico [153].

2.3.4. La dosificación del ejercicio

La dosificación es importante en medicina clínica. Todos los medicamentos que se comercializan requieren datos sobre su eficacia y seguridad [154]. Se sabe que existe una cantidad mínima de actividad física para obtener beneficios saludables. Estos beneficios se incrementan con el aumento de la cantidad de ejercicio, pero más allá de un cierto nivel, los efectos adversos son mayores que los beneficios [154]. Sin embargo, a diferencia de los fármacos químicos, la dosis mínima, la respuesta a la dosis, y la dosis segura de actividad física no se conocen bien [154].

Existe un debate contínuo sobre cuánto, qué tipo, con qué frecuencia, qué intensidad y durante cuánto tiempo se debe practicar actividad física. Esto es importante para formular recomendaciones de salud pública [155]. Es difícil llegar a alguna conclusión resumiendo la información disponible obtenida a través de diferentes estudios ya que los investigadores lo han medido en diferentes intensidades de ejercicio y han clasificado la actividad física de acuerdo a diferentes dosis, siendo entonces difíciles de comparar [154]. A lo largo de los

años, diferentes grupos de expertos, basándose en las mejores evidencias científicas disponibles, han propuesto diferentes recomendaciones y directrices para la práctica de actividad física (Ver Tabla 1).

	Physical Activity Recommendations			
	Intensity	Minutes	Frequency	Reference
1970s-1980s	Vigorous exercise (e.g. running)	Twenty minutes/day	3 times/week	[156]
1990s	Moderate exercise (eg, brisk walking)	Thirty minutes/day	Most days of the week	[157,158]
2000s	Moderate exercise	Sixty minutes/day	3 times/week	[154]
2010 (Healthy adults ages 18-45)	Moderate exercise	Thirty minutes/day (150 per week)	Most days of the week (5 days/week)	[159]
	Vigorous exercise	75 minutes/week		[159]

Tabla 1. *Evolución histórica en las recomendaciones de la cantidad de actividad física a realizar y pautas a seguir [17].*

Los niveles de intensidad del ejercicio físico pueden expresarse en relación con el consumo de oxígeno (VO_2) o con la frecuencia cardíaca [160]. Actividades de intensidad moderada son aquellas en las que los ritmos cardíaco y respiratorio aumentan, pero, aún así, es posible hablar con comodidad. Esto ocurre alrededor de 4-6 METs. Caminar a paso acelerado a 3 mph (80,4 m x min^{-1}) es una de esas actividades. En las actividades físicas intensas las frecuencias cardíaca y respiratoria son mayores, y la conversación es más dificultosa (alrededor de 6-8 MET) [160], por ejemplo trotar. Se ha demostrado que la práctica de ejercicio físico en incluso el 50% de lo recomendado (setenta y dos minutos de ejercicio moderado a la semana) parece suficiente para proporcionar alguna mejora en la forma física. Sin embargo, esta baja dosis de ejercicio no mejora factores de riesgo cardiovascular (presión arterial, perfil lipídico y peso) [161]. De hecho, para

muchas personas, hasta sesenta minutos de actividad física todos los días son más adecuados cuando el control del peso es el objetivo principal [154]. Por lo tanto, las relaciones dosis-respuesta entre la actividad física y los diferentes efectos sobre la salud son diferentes. La evaluación de la cantidad mínima de actividad física (dosis baja) necesaria para lograr sus efectos beneficiosos ha sido objeto de una investigación intensa. Wen y colaboradores han descubierto recientemente que quince minutos al día o noventa minutos por semana de ejercicio de intensidad moderada es beneficioso en términos de esperanza de vida, incluso para sujetos con riesgo cardiovascular [162].

2.3.5. Ejercicio físico agotador y longevidad

Aunque los beneficios de la actividad física realizada en el tiempo libre están bien documentados, la asociación entre la práctica de ejercicio vigoroso y la mortalidad/longevidad de los deportistas de élite no está clara [163]. Durante siglos, la creencia general ha sido que el ejercicio agotador y competitivo es perjudicial para la salud y disminuye la esperanza de vida [164]. Por ejemplo, en 1968, Moorstein [165] observó que todos los miembros del equipo de remo de Harvard (año 1948) habían fallecido a una edad temprana por enfermedades cardíacas, aunque más tarde se demostró que no había sido así ya que los propios remeros confirmaron que estaban vivos. Posteriormente se demostró que la participación en deportes de resistencia de alta competición aumenta la esperanza de vida, encontrándose que la esperanza de vida de remeros fue mayor que la de sus controles no entrenados [166,167]. Karvonen y colaboradores encontraron que esquiadores finlandeses de alto nivel (nacidos entre 1845–1910) vivieron de 2,8 a 4,3 años más que la población general masculina. Contrariamente a la mayoría de los estudios de esta época, Polednak [168] informó de evidencias en contra de los efectos beneficiosos del ejercicio físico extenuante. Él encontró diferencias en la longevidad y mortalidad cardiovascular en función de la participación en eventos

atléticos universitarios. Además, en un estudio reciente con animales se ha observado que el ejercicio físico de resistencia realizado de forma vigorosa puede, en algunos casos, promover remodelaciones cardíacas con efectos adversos, pudiendo ser el sustrato de arritmias cardíacas [169]. La incidencia de muerte cardíaca súbita entre atletas jóvenes (estimada entre 1–3 por 100.000 personas al año), es mayor que entre no atletas, e incluso puede ser que esté todavía infraestimada [170]. Sin embargo, se ha visto que la causa más común de muerte súbita en atletas jóvenes es debida a cardiopatías subyacentes heredadas, como las miocardiopatías, las anomalías coronarias congénitas y canalopatías [171]. La mayoría de los datos extraidos de estudios realizados en humanos apoyan la noción de que la prescripción de ejercicio físico aeróbico vigoroso, practicado de forma regular, puede ser una herramienta útil y con una respuesta dosis-efecto para mejorar el estado de salud general y la longevidad de la población general [164,172]. Por lo tanto, parece existir una relación dosis-respuesta, de manera que las personas con mayores niveles de actividad y condición física tienen un menor riesgo de muerte prematura [147].

El nivel de entrenamiento es un factor muy relevante en la prescripción del ejercicio en cuanto a "dosis". Se han encontrado evidencias en individuos entrenados que muestran que el aumento en las dosis de ejercicio tiene consecuencias positivas para la salud [18,164]. Mittleman y colaboradores, encontraron que el ejercicio físico intenso puede provocar infartos agudos de miocardio, sobre todo en personas sedentarias [173]. Resultados del mismo grupo mostraron que los hombres físicamente menos activos que relizaron actividad física vigorosa tenían más probabilidades de tener un infarto de miocardio durante la práctica del ejercicio frente a hombres físicamente más activos [174].

En el tratamiento farmacológico de muchas enfermedades, los médicos comienzan normalmente con una dosis mínima efectiva. A continuación, si el paciente no responde, esta dosis inicial puede ajustarse de manera ascendente hasta una dosis

máxima, más allá del cual los efectos adversos del fármaco son inaceptables para seguir el tratamiento [154]. Del mismo modo, la intensidad del entrenamiento aeróbico debe ser también valorada en personas sanas [160]. Se ha demostrado que las personas desentrenadas pueden obtener mejoras significativas en la forma física con una intensidad baja de entrenamiento, mientras que las personas con una mejor forma física necesitan una mayor intensidad de ejercicio para lograr mejoras [175]. Por lo tanto, los individuos con buena forma física que han cumplido con los niveles de ejercicio recomendados durante al menos seis meses pueden obtener beneficios adicionales para la salud al realizar 300 minutos/semana o más de actividad aeróbica de intensidad moderada, o 150 minutos/semana o más de actividad aeróbica de intensidad vigorosa, o una combinación equivalente de actividad aeróbica de intensidad moderada y vigorosa [134,159]. Estas dosis relativamente bajas son, obviamente, no aplicables a los deportistas profesionales de alto nivel que realizan ejercicio en dosis mucho más altas.

Las directrices mencionadas anteriormente son generalmente apropiadas para jóvenes y adultos de mediana edad. Pero, al igual que con los medicamentos, se deben tomar consideraciones especiales cuando se prescribe ejercicio a personas con necesidades específicas, tales como ancianos, niños, mujeres embarazadas, pacientes con sobrepeso u obesidad, y pacientes con enfermedades crónicas [160]. Por ejemplo, se ha demostrado que actividades físicas vigorosas no son esenciales para la reducción del riesgo cardiovascular en hombres de más de sesenta años. La actividad física regular es suficiente para lograr una reducción significativa de la mortalidad en esta población. Por lo tanto, el mayor beneficio para la salud se obtiene con un ejercicio moderado y sostenido, por encima del cual no parece haber ningún beneficio adicional para la salud en hombres mayores [176,177].

En cuanto a la "dosis" de ejercicio, si debe llevarse a cabo de forma continua, o en dos o más tandas acumuladas, las evidencias disponibles sugieren que, al menos para la forma física, tanto el ejercicio contínuo o acumulado de la misma duración

total confieren beneficios similares [178]. Por ejemplo, se ha demostrado que de cinco a ocho tandas acumuladas de dos minutos de subir escaleras en el transcurso de un día confieren beneficios para la salud, incluyendo incrementos en la forma física en comparación con los controles no ejercitados [179].

Aunque la actividad física es beneficiosa para la salud, con o sin pérdida de peso, los adultos que tienen dificultades para mantener un peso normal y los adultos con mayor riesgo de padecer enfermedades cardiovasculares o diabetes tipo 2 pueden beneficiarse de incrementar gradualmente los niveles de actividad recomendados para los adultos sanos hasta alcanzar las recomendaciones para los individuos entrenados [159].

2.3.6. Adaptaciones sistémicas al ejercicio físico

Las adaptaciones inducidas por el ejercicio son especialmente evidentes en el sistema cardiorespiratorio, aparato locomotor, la composición corporal y el metabolismo [147,180]. Además, los beneficios documentados del ejercicio físico para la salud también incluyen la disminución de los síntomas de la depresión y la ansiedad, entre otros [181].

El músculo-esquelético es la principal diana del ejercicio físico. Las modificaciones a nivel músculo-esquelético son cruciales para la mejora en la resistencia y eficiencia metabólica [182]. Las fibras musculares se clasifican comúnmente como tipo I de contracción lenta o fibras oxidativas, con un alto contenido mitocondrial y tipo II de contracción rápida o fibras glicolíticas, que tienen menor cantidad de mitocondrias. Los ejercicios de resistencia inducen un aumento en la biogénesis mitocondrial, un cambio en la distribución de fibras glicolíticas a oxidativas, y un aumento en la oxidación de ácidos grasos que en última instancia conduce a un aumento en la capacidad aeróbica y previene la aparición de enfermedades tales como la obesidad, la diabetes tipo 2 o enfermedades cardiovasculares [183,184].

Se ha demostrado que el ejercicio físico regular puede reducir la adiposidad abdominal y mejorar el control de peso [147], mejorar los perfiles lipoproteicos (por ejemplo, reducir los niveles de triglicéridos, aumentar la lipoproteína de alta densidad y disminuir los niveles de lipoproteína de baja densidad), mejorar la homeostasis de la glucosa y la sensibilidad a la insulina, reducir la presión arterial, mejorar el tono autonómico, reducir la inflamación sistémica, disminuir la coagulación sanguínea, mejorar el flujo sanguíneo coronario, aumentar la función cardiaca y mejorar la función endotelial [147].

La actividad física regular también se asocia con un mejor bienestar psicológico (por ejemplo, reduciendo el estrés, la ansiedad y la depresión) [185]. Los efectos beneficiosos del ejercicio sobre la función cognitiva son bien conocidos [186]. El mecanismo por el que el ejercicio produce estos efectos no está del todo claro pero parece estar asociado con un aumento en la expresión de factores neurotróficos en algunas áreas del cerebro. El aumento en la expresión de estos factores se relaciona con una mejor memoria y función cognitiva. El factor neurotrófico derivado del cerebro (BDNF) puede mejorar la supervivencia y la diferenciación de las neuronas, y se ha demostrado que el ejercicio voluntario lo aumenta [187]. El bienestar psicológico es especialmente importante para la prevención y tratamiento de enfermedades cardiovasculares, pero también tiene implicaciones significativas en la prevención y el tratamiento de otras enfermedades crónicas como la diabetes, la osteoporosis, la hipertensión, la obesidad, el cáncer y la depresión [147]. Se ha demostrado que la actividad física resulta en adaptaciones específicas que afectan a estados individuales en todas estas enfermedades. Por ejemplo, son de gran importancia las adaptaciones que afectan a la homeostasis de la glucosa en la diabetes tipo 2, incluyendo aumentos en las actividades de la glucógeno sintasa y de la hexoquinasa, aumento en la expresión de GLUT-4 y mejora de la densidad capilar del músculo [188].

El ejercicio físico produce una reducción significativa en las tasas de cáncer (especialmente de colon y mama) [148,189]. Las posibles explicaciones incluyen la reducción de las reservas de grasa, aumento del gasto energético compensando una dieta alta en grasas, cambios relacionados con la actividad en los niveles de hormonas sexuales, función inmune, insulina y factores de crecimiento similares a la insulina, generación de RLs, y los efectos directos sobre la biología de las células tumorales [190].

La mayoría de los mecanismos propuestos se han discutido en el contexto de las adaptaciones crónicas a la actividad física regular. Sin embargo, se ha demostrado que sesiones de ejercicio aisladas también provocan cambios transitorios y beneficiosos para enfermedades crónicas [191]. Muchas de las adaptaciones al ejercicio se producen por una única sesión que provoca cambios celulares a nivel de genes dando lugar a efectos acumulativos del entrenamiento. El efecto agudo del ejercicio provoca una reducción pasajera en las concentraciones de triglicéridos, aumenta los niveles de lipoproteína de alta densidad (HDL), disminuye la presión sanguínea, reduce la resistencia a la insulina y mejora el control glucémico [191]. Estos cambios agudos evidencian el importante papel que las sesiones individuales de ejercicio tienen sobre la salud. Así pues, las dosis únicas de ejercicio tienen también un impacto importante sobre la salud. La Figura 4 resume los efectos beneficiosos del ejercicio físico.

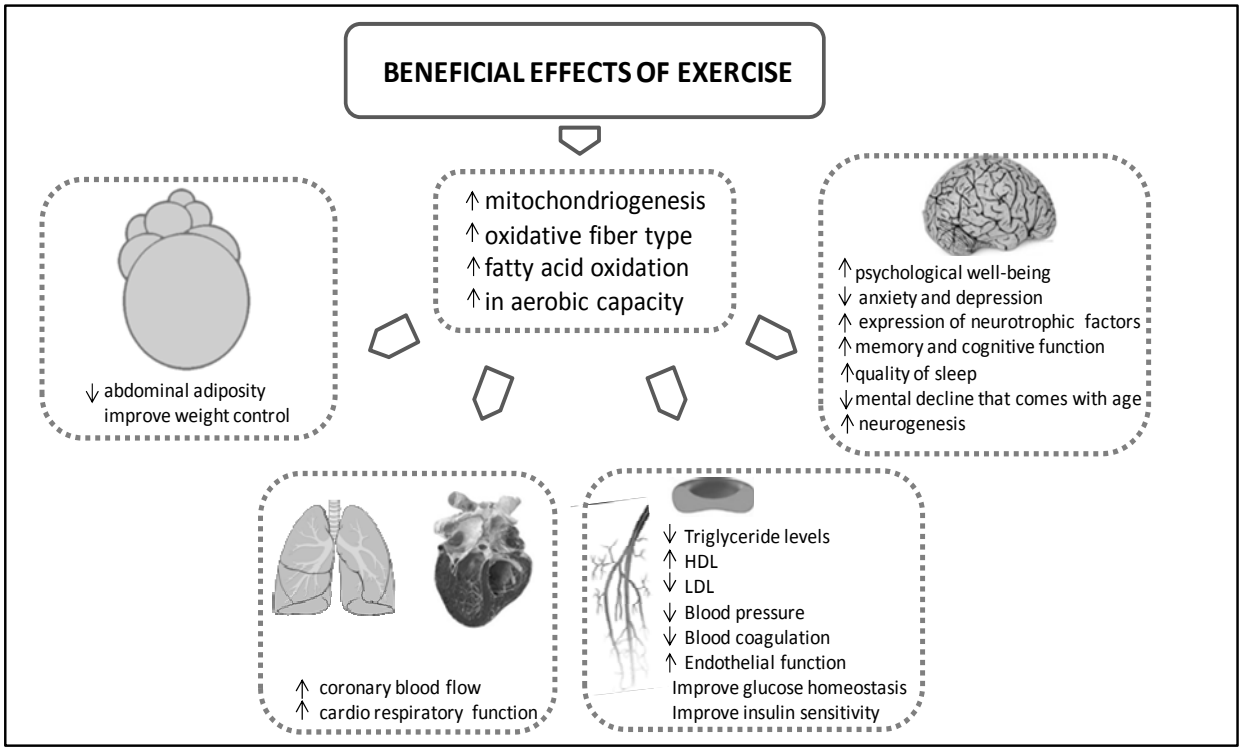


Figura 4. *Beneficios del ejercicio físico sobre la salud. Efectos sobre órganos y tejidos [17].*

2.3.7. Vías de señalización reguladas por el ejercicio en el músculo-esquelético

La regulación de las funciones celulares por el ejercicio dependen de muchos estímulos: alteraciones en las concentraciones de ciertos metabolitos, un cambio en el ratio ATP/ADP, cambios en la concentración intracelular de Ca^{+2}, en el pH intracelular, y activaciones de las vías de señalización sensibles al EO [192,193]. Dilucidar los mecanismos de señalización molecular que permiten responder al estímulo contráctil al músculo-esquelético y que median las adaptaciones inducidas por el ejercicio físico es de gran importancia [192]. Se ha establecido claramente que el ejercicio físico puede activar las vías de las proteínas quinasas activadas por mitógenos (MAPKs), incluyendo ERK1/2 [194], p38 [195], y la quinasa c-Jun N-terminal (JNK) [194]. También puede aumentar la actividad de la proteína quinasa activada por AMP, Akt, y de la quinasa p70 S6 [192] (Ver Figura 5). La señalización regulada por Ca^{+2} en el músculo-esquelético también ha sido

ampliamente demostrada. Además de activar la contracción del músculo a través del sistema troponina, el Ca^{+2} también está involucrado en la regulación de proteínas intracelulares importantes como son la proteína quinasa C, la calcineurina, y la calmodulina quinasa que median la transducción de la señal celular [196].

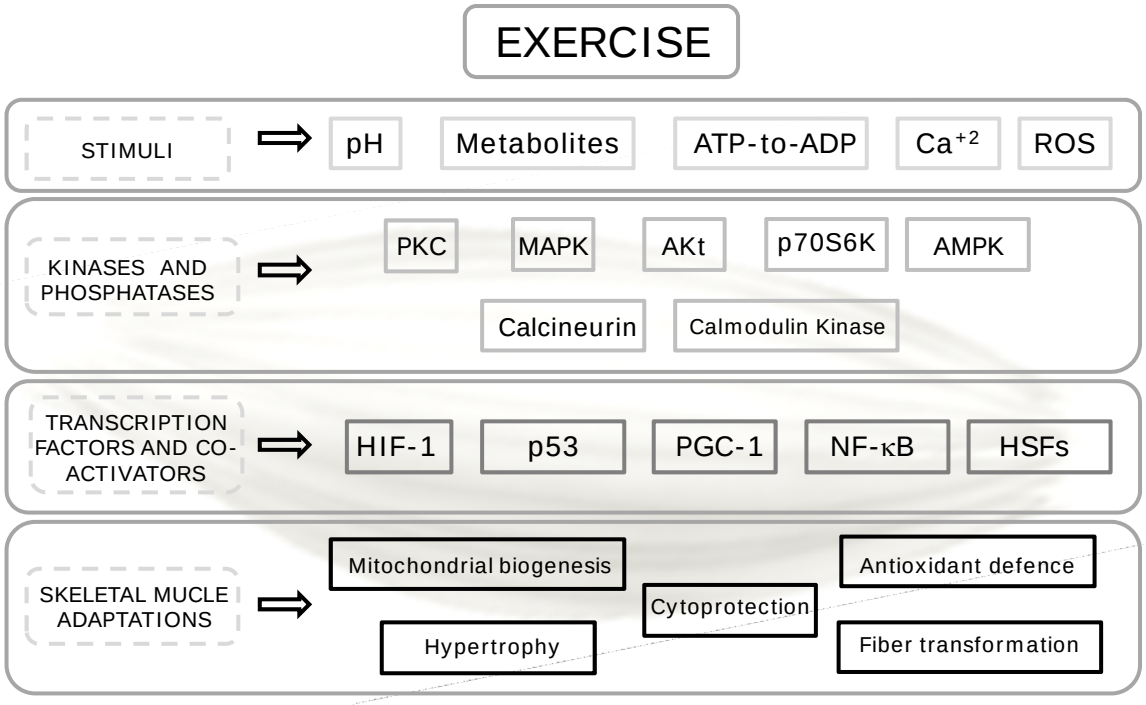


Figura 5. *Vías de señalización reguladas por el ejercicio físico en el músculo-esquelético [17].*

Más recientemente se ha observado que niveles bajos y moderados de ROS también juegan múltiples funciones de regulación célular tales como el control de la expresión génica, la regulación de las vías de señalización celular y la modulación de la producción de fuerza muscular [197]. El papel de las ROS en las adaptaciones inducidas por el ejercicio físico en el músculo-esquelético se ha estudiado en profundidad [198,199]. La idea de que las ROS tienen efectos nocivos ha estado muy arraigada en la comunidad científica durante los últimos treinta años. Sin embargo, existe una creciente evidencia de que la presencia continua de

bajas concentraciones de RLs es capaz de inducir la expresión de enzimas antioxidantes y otros mecanismos de defensa. En este contexto, los RLs pueden ser vistos más como beneficiosos que como perjudiciales, ya que actúan como señales para mejorar las defensas cuando las células están expuestas a altos niveles de estos radicales. En animales que realizaron ejercicio (entrenamiento crónico) se ha observado un menor daño oxidativo después de realizar ejercicio agotador cuando se compararon con animales no entrenados [198]. Esto se debe en gran parte a la regulación de enzimas antioxidantes endógenas como son la glutatión peroxidasa, la manganeso superóxido dismutasa (MnSOD), y la γ-glutamilcisteina sintetasa [200]. Una de las principales conclusiones que se pueden extraer de estos resultados es que el ejercicio en sí es un antioxidante, ya que el entrenamiento aumenta la expresión de enzimas antioxidantes [199]. También ha sido demostrado que la suplementación con sustancias antioxidantes previene la inducción de la biogénesis mitocondrial, de reguladores moleculares de la sensibilidad a la insulina y de la defensa antioxidante endógena inducida por el ejercicio físico [40,201]. Por lo tanto, las ROS inducidas por el ejercicio físico actúan como señales ya que su disminución previene la activación de importantes vías de señalización que provocan adaptaciones celulares de gran útilidad.

Debido a las importantes implicaciones en casi todas las funciones biológicas de las ROS, es difícil definir todas las rutas y genes que se ven afectados por la señalización redox durante el ejercicio. Las siguientes son algunas de las vías de señalización más relevantes moduladas por el ejercicio: el coactivador-1α y β del receptor-γ activado por la proliferación del peroxisoma (PGC-1α y PGC-1β) [40,201], p53 [202], el factor inducido por hipoxia 1 (HIF-1) [203], el factor de choque térmico (HSF) [204], el factor nuclear Kappa B (NF-κB) y las vías de señalización de las MAPKs [193,195]. Varias adaptaciones importantes en el músculo-esquelético como son la biogénesis mitocondrial, la defensa antioxidante, la hipertrofia, la citoprotección y la transformación de las fibras se regulan

principalmente por estas vías. Por lo tanto, su regulación debe ser estrechamente controlada [205].

2.3.8. Ejercicio físico, un fármaco psicoactivo

El impacto de la práctica de ejercicio físico en la función cerebral se ha estudiado ampliamente. A principios de los años 80 se demostró que el ejercicio causa un marcado incremento en beta-endorfinas sanguíneas [206,207]. Concentraciones séricas elevadas de beta-endorfina inducidas por el ejercicio se han relacionado con varios cambios psicológicos y fisiológicos, incluyendo cambios en el estado de ánimo y en el estado de euforia inducido por el ejercicio, alteraciones en la percepción del dolor y respuestas a numerosas hormonas de estrés (hormona de crecimiento, ACTH, prolactina, catecolaminas y cortisol) [208].

El ejercicio físico puede influir favorablemente la función cognitiva [209,210]. Diferentes investigaciones han mostrado que el ejercicio mejora la capacidad de aprendizaje y la memoria [211], mejora la calidad del sueño, contrarresta el deterioro mental como consecuencia de la edad [212], facilita la recuperación funcional producida por lesiones cerebrales [213] y la depresión [214,215]. El ejercicio físico es un estímulo muy potente para la inducción de neurogénesis en el giro dentado adulto [211] que puede contribuir a la remodelación de los circuitos sinápticos hipocampales y a aumentar la función cognitiva. También puede mitigar las consecuencias de la exposición aguda a diferentes tipos de estrés psicológico [210]. Las alteraciones inducidas por el ejercicio en los sistemas serotoninérgico y noerepinefrinérgico pueden explicar estas respuestas [210]. La mayoría de los efectos positivos del ejercicio, como se ha mencionado anteriormente, se han relacionado con la inducción, en diferentes áreas del cerebro, de proteínas neurotróficas, incluyendo BDNF, factor neurotrófico derivado de la glía (GDNF), y factor de crecimiento insulínico (IGF). Que las respuestas metabólicas cerebrales a la actividad física se extienden más allá de las regiones que participan

específicamente en el control motor, sensorial o cardiovascular no está todavía claro [210]. Se han observado incrementos transitorios en el uso local de la glucosa cerebral y en el flujo sanguíneo cerebral en las diferentes áreas del cerebro en respuesta a la carrera agotadora en tapiz rodante tanto en ratas como en humanos [216]. Se ha demostrado también que la velocidad de descarga de una selección de células piramidales del hipocampo aumenta a medida que aumenta la velocidad de carrera [217]. Además, el ejercicio aumenta la capacidad metabólica en el cortex motor así como en el estriado [218].

Los efectos psicoactivos del ejercicio que acabamos de mencionar no están libres de riesgos. En la literatura se encuentran comportamientos patológicos entre los usuarios de gimnasios [219]. Al igual que en los pacientes con trastornos de alimentación, las personas físicamente activas suelen preocuparse por su cuerpo, ponen especial atención en sus patrones de alimentación, muestran adicción al ejercicio, y tienen un rasgo de personalidad perfeccionista [220]. Este desorden de la imagen corporal se ha bautizado como anorexia inversa, vigorexia o dismorfia muscular [219]. Basándonos en una revisión en la que incluyen una gran variedad de estudios sobre la adicción al ejercicio, se ha estimado que su prevalencia en la población general es de alrededor de un 3%. Entre algunos grupos, como los corredores de ultramaratón, los culturistas y los estudiantes de ciencias del deporte, el porcentaje es aún mayor [220,221].

2.3.9. Contraindicaciones del ejercicio físico

Aunque el sistema cardiopulmonar se beneficia significativamente de la actividad física hay algunas contraindicaciones cuando el ejercicio es llevado a cabo por pacientes que padecen enfermedades cardíacas y/o pulmonares. Pedersen y Saltin [148] revisaron las posibles contraindicaciones del ejercicio físico en muchas de las enfermedades en las cuales ha mostrado efectos beneficiosos. Por ejemplo y por lo que respecta a enfermedades cardíacas, el ejercicio físico está contraindicado hasta

que el paciente se haya mantenido estable durante al menos cinco días. Por otra parte, la disnea en reposo, la estenosis aórtica, pericarditis, miocarditis y endocarditis, la fiebre y la hipertensión severa son contraindicaciones para la práctica de ejercicio físico [148]. Black y colaboradores fueron los primeros que encontraron que el ejercicio físico vigoroso puede causar una lesión aguda a nivel de placas coronarias, lo que lleva a la oclusión de arterias coronarias [222]. Sin embargo, años más tarde se encontró que aunque el riesgo de parada cardíaca aumentó transitoriamente durante una sola sesión de ejercicio físico vigoroso, el ejercicio vigoroso habitual se asocia con una disminución global de este riesgo [223,224]. No hay contraindicaciones absolutas para el ejercicio muy moderado en pacientes con enfermedad pulmonar obstructiva crónica [148]. No obstante, en los pacientes con asma se recomienda detener de forma inmediata el entrenamiento cuando se produce una exacerbación aguda. En los casos de infección, se recomienda una pausa en el entrenamiento hasta que el paciente esté asintomático durante al menos un día, pudiendo entonces reanudar el entrenamiento lentamente [148].

En cuanto a enfermedades músculares, óseas y articulares, por ejemplo, la osteoartritis y la artritis reumatoide, el ejercicio está contraindicado en casos de inflamación articular aguda, si el dolor empeora después del entrenamiento, y en casos de pericarditis y pleuritis [148]. El entrenamiento en pacientes con osteoporosis debe incluir actividades con un bajo riesgo de caída [148].

En pacientes con cáncer tratados con quimioterapia o radioterapia la práctica de ejercicio físico está contraindicado cuando la concentración de leucocitos cae por debajo de 0.5 x 10^9/L, hemoglobina por debajo de 10g/ dL, plaquetas por debajo de 20 x 10^9/L y temperatura corporal por encima de 38 ºC. Los pacientes con metástasis óseas no deben realizar entrenamiento de fuerza con carga elevada. En casos de infección, se recomienda detener el entrenamiento de forma inmediata hasta que el paciente permanezca asintomático durante al menos un día, pudiendo

entonces reanudar lentamente el entrenamiento [148]. Una de las principales preocupaciones es si la práctica de ejercicio influye sobre los efectos anticancerígenos de la terapia citotóxica convencional. La posible interacción entre el ejercicio y la eficacia de la quimioterapia es biológicamente plausible. De hecho, estudios preclínicos previos han informado tanto de un posible efecto inhibidor [225] así como de un posible efecto estimulador [226] del entrenamiento de resistencia en el crecimiento del tumor mamario así como su progresión, aunque otros han informado de que no hay ninguna asociación [227].

En pacientes diabéticos (tanto tipo I como tipo II), el ejercicio debe ser suspendido si la glucosa en sangre es > 2.5 g/L con cetonuria o si la glucosa en sangre es > 3.0 g/L. En los pacientes con hipertensión y retinopatía proliferativa activa, el entrenamiento de alta intensidad o el entrenamiento que implica maniobras de Valsalva o similares debe evitarse. Los pacientes con neuropatía o con úlceras incipientes en el pie deben abstenerse de realizar actividades que impliquen el impacto del propio peso del cuerpo sobre el cojinete plantar.

En enfermedades relacionadas con el síndrome metabólico, tales como resistencia a la insulina, la dislipemia y la obesidad, no existen contraindicaciones generales, pero el entrenamiento debe tener en cuenta las comorbilidades [148]. Finalmente, los pacientes hipertensos con una presión arterial > 180/105 deben comenzar un tratamiento farmacológico antes de iniciar la actividad física regular (contraindicación relativa) [228]. No hay evidencias de un mayor riesgo de muerte súbita o de accidente cerebrovascular en personas con hipertensión físicamente activas [229]. El ACSM recomienda precaución al realizar ejercicio dinámico muy intenso o entrenamiento de fuerza con pesas y pesos elevados. Los pacientes con hipertrofia cardiaca izquierda deben ser especialmente cautelosos con el entrenamiento de fuerza intenso. Los pacientes con enfermedad coronaria deberían evitar la práctica ejercicio intenso de corta duración.

Es bien sabido que las contracciones musculares excéntricas provocan un daño estructural en las células musculares o reacciones inflamatorias en el propio músculo, evidenciado por ejemplo por un incremento en las actividades plasmáticas de enzimas citosólicos [26]. La severidad del daño y la extensión de las molestias se agrava con el tiempo y puede durar varios días. Los efectos perjudiciales de la actividad muscular excéntrica pueden afectar a las sesiones de ejercicio posteriores debido al dolor muscular residual, la restricción de movimiento y la capacidad reducida para hacer ejercicio a una intensidad que puede ser beneficiosa para el deportista [230]. Por lo tanto, se debe tener precaución con los programas de ejercicio que incluyen contracciones excéntricas, especialmente en practicantes recreativos o de edad avanzada.

Por lo que respecta a las personas mayores, en muchas ocasiones conviven con enfermedades crónicas, trastornos geriátricos cognitivos, visuales, auditivos y disminuciones en la reserva fisiológica [22]. Esta población tiene una mayor susceptibilidad a las enfermedades relacionadas con la edad, como son el cáncer, las enfermedades cardiovasculares y neurodegenerativas y la diabetes [22]. Aunque tanto el sistema cardiovascular como el pulmonar se benefician significativamente de la actividad física, también existen contraindicaciones cuando el ejercicio se realiza por personas mayores con afectación cardíaca o pulmonar [231]. La incidencia de insuficiencia cardíaca aumenta con la edad, debido en gran parte al desarrollo de factores de riesgo como la hipertensión y la enfermedad arterial coronaria [232]. Las exacerbaciones agudas de la enfermedad pulmonar obstructiva crónica se asocian con mayores índices de mortalidad y hospitalización, especialmente en pacientes de edad avanzada [233]. Con el envejecimiento, hay una reducción significativa en la formación ósea. La pérdida ósea relacionada con la edad también se observa en varones [234]. Por tanto, el entrenamiento de los pacientes con osteoporosis debe incluir actividades con bajo riesgo de caída [148].

Por lo tanto, el ejercicio físico debe ser prescrito con mucha precaución y de forma individualizada en esta población.

2.3.10. Miméticos del ejercicio

A pesar de la clara evidencia que muestra la poderosa influencia del ejercicio sobre la salud, la falta de actividad física sigue siendo un problema grave y urgente de salud pública. Los avances tecnológicos y los incentivos económicos fomentan la inactividad física [235]. Por otra parte, el ejercicio de resistencia también puede ser inaccesible para muchas personas al no poderse practicar debido a las limitaciones físicas o, como se mencionó en la sección anterior, a los efectos secundarios. Este hecho estimula la búsqueda de sustancias miméticas del ejercicio (o "píldoras del ejercicio") que imitan los efectos del ejercicio físico, y por lo tanto, ha sido objeto de importantes investigaciones en las últimas décadas [236].

El ejercicio físico de resistencia mejora el rendimiento mediante la activación de varias vías que inducen cambios genéticos, particularmente en el músculo-esquelético, aumentando el metabolismo aeróbico y la vascularización para en última instancia mejorar el rendimiento [237]. La quinasa activada por monofosfato de adenosina (AMPK) se activa por el ejercicio y es el interruptor fundamental para el cambio a miofibras aeróbicas mediado por el ejercicio en el músculo-esquelético [238]. La AMPK estimula las vías catabólicas y suprime las vías anabólicas en un esfuerzo para restaurar los niveles celulares de ATP y se activa en el músculo-esquelético tanto por el ejercicio físico agudo como por el crónico [182,239]. Se ha publicado recientemente que la biomolécula 5-aminoimidazol-4-carboxamida-1-beta-D-ribofuranosido (AICAR) puede imitar el efecto del ejercicio aumentando en contenido de GLUT-4 y la actividad hexoquinasa, incrementando el contenido mitocondrial a nivel muscular, así como reestableciendo el contenido de glucógeno [240]. La biogénesis mitocondrial es activada por AMPK [241]. Esto puede explicarse porque la AMPK está presente en

un complejo transcripcional con el receptor-δ activado por la proliferación del peroxisoma (PPARδ) donde puede potenciar la actividad del receptor a través de la interacción directa proteína-proteína y/o mediante la fosforilación y la activación de coactivadores, tales como PGC-1α [242]. Puigserver y colaboradores [243] identificaron a PGC-1α como el regulador maestro de la mitocondriogénesis tisular en mamíferos. Koves y colaboradores [244] observaron que PGC-1α media la remodelación metabólica de los miocitos, mimetiza el ejercicio y revierte la ineficiencia mitocondrial inducida por lípidos. Por lo tanto, el entrenamiento y el tipo de fibra oxidativo se asocian con un aumento de la expresión de PGC-1α [245]. La sobreexpresión de PGC-1α en ratones induce cambios dramáticos en el músculo esquelético, tales como el aumento de la biogénesis mitocondrial y la remodelación de las fibras musculares [246]. Además, PGC-1α puede ser activado por la vía de señalización de Ca^{+2} en la que están implicadas la calcineurina y la quinasa dependiente de Ca^{+2}/calmodulina (CaMK) y por la MAPK p38 [247].

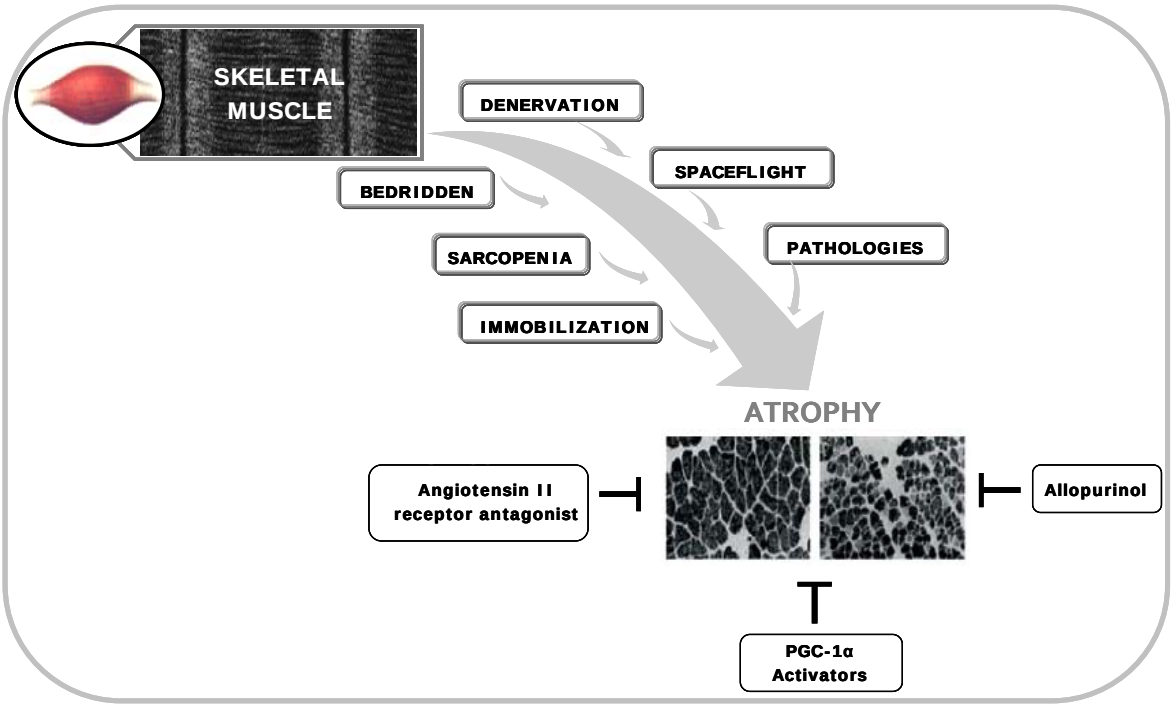


Figura 6. *Posibles intervenciones médicas no hormonales para prevenir la pérdida de masa muscular y la sarcopenia [20].*

La modulación de los niveles de PGC-1α en el músculo-esquelético es crucial para la prevención y el tratamiento de las enfermedades relacionadas con la edad [248]. En consecuencia, recientemente hemos propuesto varias intervenciones farmacológicas, no hormonales, para prevenir la pérdida de masa muscular y la sarcopenia, tales como activadores de PGC-1α, antagonistas del receptor de angiotensina II y el alopurinol (Ver Figura 6) [20].

Por otra parte, la activación o la sobreexpresión del factor de transcripción PPARδ en el músculo-esquelético también resulta en un incremento en la biogénesis mitocondrial y en la proporción de fibras musculares oxidativas [240]. Esto produce un aumento en la resistencia aeróbica y protege frente a la obesidad y la diabetes tipo 2 inducidas por la dieta. Lo contrario también es cierto: en animales KO para PPARδ se ha observado una pérdida dependiente de la edad de fibras musculares oxidativas, de resistencia y de sensibilidad a la insulina [249]. Así pues, también se han identificado y propuesto como fármacos miméticos del ejercicio de resistencia varios agonistas potentes y selectivos de PPARδ tales como GW1516 [240].

El resveratrol ha sido también considerado como un mimético del ejercicio. Dosis elevadas de resveratrol mejoran la resistencia física [250]. La enzima deacetilasa Sir2 y sus homólogo en mamíferos SIRT1 han sido identificadas como sus posibles dianas principales. El resveratrol también activa AMPK en cultivos celulares y se ha propuesto para prevenir la disfunción mitocondrial [251] y la atrofia muscular asociada a la descarga mecánica [252]. En cambio, se ha demostrado muy recientemente en un modelo animal que, mientras que el ejercicio físico incrementa la actividad/contenido de proteínas oxidativas, DNA mitocondrial y proteínas angiogénicas a nivel músculo-esquelético y reguladas por PGC-1α, el resveratrol no tiene ningún efecto adicional [253]. Tampoco se observó ningún efecto cuando únicamente se administró resveratrol sin la combinación con ejercicio [253]. Este mismo grupo también ha demostrado en sujetos sometidos a un entrenamiento de

alta intensidad y suplementados con resveratrol, no sólo que la suplementación con resveratrol no produjo ningún efecto sobre los niveles de SIRT1, sino que además la suplementación con resveratrol puede mitigar los efectos beneficiosos que el entrenamiento induce sobre el sistema cardiovascular [254].

La remodelación muscular a través del ejercicio físico es extremadamente compleja, con muchas enzimas de señalización, factores de transcripción, transportadores y chaperonas implicados [255]. Por lo tanto, hay muchas dianas disponibles para imitar las adaptaciones del músculo-esquelético inducidas por el ejercicio físico, y en definitiva, el ejercicio de resistencia induce un mayor potencial oxidativo y la reprogramación de las fibras musculares. Sin embargo, el ejercicio está relacionado con otras múltiples adaptaciones fisiológicas que afectan a la gran mayoría de los órganos. Parece prematuro concluir que todas estas moléculas o sustancias son miméticas del ejercicio hasta que se investiguen los efectos en otros órganos. La aplicabilidad de estos compuestos en la actualidad es limitada y es por ello que se sigue investigando en este campo.

El ejercicio físico puede ser tan beneficioso para la salud que debería ser considerado como un medicamento. Al igual que con cualquier otro medicamento, la dosificación es muy importante. De lo contrario, puede producir efectos secundarios desfavorables.

Algunos de los efectos favorables del ejercicio se aplican a la población general. Entre los más destacados son su papel en la prevención de enfermedades y en la promoción de una longevidad saludable (Ver Figura 4). Pero el ejercicio también puede ser considerado como tratamiento de las enfermedades establecidas tales como la depresión, la diabetes o las enfermedades cardiovasculares.

2.4. LA BIOGÉNESIS MITOCONDRIAL EN EL ENVEJECIMIENTO Y SU INDUCCIÓN POR EL EJERCICIO FÍSICO.

Las mitocondrias son uno de los orgánulos celulares más ampliamente estudiados. Su función clásica es la de producir energía a través de la cadena respiratoria. Sin embargo, muchas más funciones han ido emergiendo y en particular las relacionadas con el papel de estos orgánulos en la señalización celular. Por ejemplo, las mitocondrias generan señales que son esenciales para el inicio de la ruta mitocondrial de la apoptosis. Ésta se desencada por la liberación de citocromo c en condiciones adecuadas, lo que conduce a la activación de la caspasa-3 y finalmente a la muerte celular [256]. Más recientemente, varios grupos de investigación han conseguido evidenciar científicamente que proteínas, que hace algunos años se consideraban únicamente citosólicas, penetran en las mitocondrias y controlan sus funciones. Una de ellas, y muy importante, es la MAPK denominada JNK, que penetra en la mitocondria y regula la enzima crítica piruvato deshidrogenasa por fosforilación [257,258]. Muy recientemente, se ha demostrado que la telomerasa también se acumula en las mitocondrias y de hecho sirve como un antioxidante en este orgánulo [259]. Por lo tanto, la importancia de las mitocondrias en la función celular continúa siendo objeto de estudio.

Las mitocondrias están involucradas en el ejercicio físico y el envejecimiento [260]. El hecho de que el entrenamiento físico, en particular el entrenamiento aeróbico, es un estímulo muy claro para activar la mitocondriogénesis está fuera de toda duda. En animales o en sujetos que entrenan se ha observado que poseen un mayor número de mitocondrias con un aumento ulterior de la capacidad de utilización de oxígeno [183,260,261].

Las mitocondrias son también muy importantes en el envejecimiento. De hecho, la teoría de los RLs del envejecimiento fue postulada por primera vez en 1956 por Harman [262] junto con los hallazgos de Boveris y Sies en el laboratorio de Britton

Chance. Estos autores observaron que el 2% de oxígeno consumido por las mitocondrias en estado 4 se convierte en peróxido de hidrógeno [263]. Esto llevó a Denham Harman a postular que las mitocondrias pueden ser críticas en la generación de radicales libre responsables del daño asociado al envejecimiento. Esta teoría fue perfeccionada por Jaime Miquel, que en los años 70 reformuló la teoría de los RLs del envejecimiento. Dos contribuciones cruciales de Miquel fueron: que subraya la importancia del ADN mitocondrial como una diana de los oxidantes producidos durante el envejecimiento y señala que la mitocondriogénesis podría verse afectada en el mismo [264]. La mayoría de los estudios realizados por Jaime Miquel en los Estados Unidos durante los años 70 eran básicamente histológicos [265]. Más tarde en los años 90 el grupo del Profesor Viña demostró que en el envejecimiento las mitocondrias están dañadas [266,267]. Esto fue comprobado de forma casi simultánea por el grupo de Bruce Ames [268]. Por otra parte, se encontró que el daño oxidativo al ADN mitocondrial está incrementado en el envejecimiento y esto podría evitarse mediante la administración de suplementos antioxidantes [269]. Así que la predicción inicial de la teoría de los RLs del envejecimiento, es decir, que el ADN mitocondrial es una diana clave de los daños asociados con el envejecimiento fue confirmado experimentalmente. Sin embargo, la predicción de que el envejecimiento estuviese asociado con la renovación mitocondrial a nivel celular llevó mucho más tiempo. La razón principal fue que era (y sigue siendo) difícil evaluar la cantidad de mitocondrias en una célula, en particular de una manera dinámica, es decir, si las intervenciones aceleran o disminuyen la velocidad de mitocondriogénesis. Para resolver este problema, fue necesario elucidar la vía mitocondriogénica.

2.4.1. La vía mitocondriogénica

Las mitocondrias se producen continuamente en la célula, y de hecho las mitocondrias viejas o dañadas también se eliminan de forma continua en los

compartimentos celulares. Por tanto, es fundamental entender los mecanismos por los que se sintetizan las mitocondrias.

De suma importancia en esta vía fue el descubrimiento de PGC-1α, teniendo una variedad de funciones muy amplia. Por ejemplo, es un regulador de la defensa antioxidante. En esta situación, PGC-1α es capaz de controlar la respuesta celular al EO [270]. PGC-1α activa la producción del factor respiratorio nuclear 1 (NRF-1) y del factor respiratorio nuclear 2 (NRF-2). Estos factores están implicados en la mitocondriogénesis. De hecho, estos dos factores, y de manera más importante el NRF-1, son potentes estimuladores de la expresión del factor de transcripción mitocondrial A (TFAM), que a su vez es un potente estimulador de la duplicación del ADN mitocondrial [243]. Por lo tanto, cuando se requiere activar la mitocondriogénesis, PGC-1α se activa, y esto conduce a una activación de NRF-1, un aumento posterior en la síntesis de TFAM y un aumento de la duplicación del ADN mitocondrial. Esto a su vez conduce a un aumento del número de copias de ADN presente en la célula y, finalmente, a la duplicación de las mitocondrias.

Por lo tanto, ahora somos capaces de diseccionar todo el itinerario mitocondriogénico en nuestros intentos de estudiar la velocidad de síntesis de la mitocondria y su regulación en las diferentes condiciones fisiológicas (Ver Figura 7)

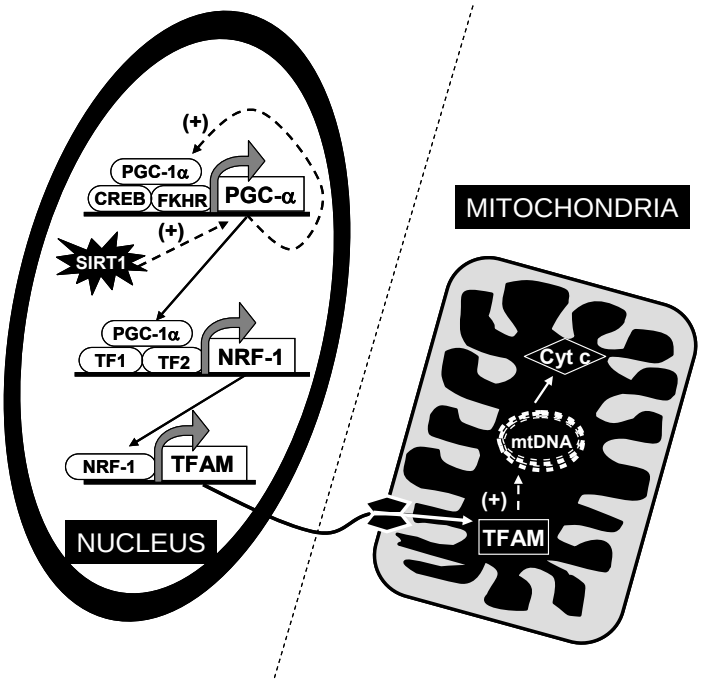


Figura 7. *Representación esquemática de la regulación de la síntesis mitocondrial [19].*

2.4.2. La mitocondriogénesis está alterada en el envejecimiento causando acumulación de daño en la mitocondria

Para estudiar la pérdida de estímulos mitocondriogénicos en el envejecimiento se puede esperar que para promover la síntesis de estas microorganelas podemos utilizar intervenciones como por ejemplo el ejercicio (véase más adelante). Por lo tanto, estudiamos la actividad de la vía mitocondriogénica mediante la medición de la expresión de PGC-1α, de NRF-1 [243] y finalmente estimamos la cantidad de mitocondrias determinando la expresión de citocromo C. El problema de la síntesis de mitocondrias en el envejecimiento ya ha sido experimentalmente abordado por el grupo de Gadaleta en Bari desde hace más de una década [271]. Actualmente, hemos sido capaces de diseccionar la vía mitocondriogénica y determinar la expresión de los diversos componentes de esta vía. Para ello hemos utilizado el tejido cardíaco, ya que principalmente contiene células post-mitóticas y es de gran importancia en el envejecimiento. La Figura 8 (Panel A) muestra la expresión de PGC- 1α en los corazones de los animales jóvenes y viejos. Como se

puede observar, la expresión en animales viejos es significativamente inferior a la de los jóvenes. De hecho, en los animales viejos nos encontramos con una expresión de PGC- 1α aproximadamente un tercio inferior si la comparamos con la de los animales jóvenes. Por lo que respecta a NRF-1, el corazón de los animales viejos expresa aproximadamente un 25 % menos de NRF-1 en comparación con el de los jóvenes (Figura 8; Panel B). La diferencia es altamente significativa. Así pues, ambos factores estudiados que activan críticamente la síntesis de mitocondrias en miocitos están muy disminuidos en el envejecimiento. Por lo tanto, un resultado esperado sería que las proteínas mitocondriales fuesen significativamente inferiores en el corazón de los animales viejos, en comparación con los animales jóvenes, y esto es de hecho lo que hemos observado. Los niveles de citocromo c fueron significativamente inferiores en los animales viejos cuando lo comparamos con los jóvenes (Ver Figura 8; Panel C). Por lo tanto, nuestros resultados indican que los factores reguladores de la mitocondriogénesis, así como sus proteínas constituyentes, son significativamente inferiores en los animales viejos cuando los comparamos con los jóvenes. Por otra parte, el hecho de que haya significativamente menos mitocondrias en un tejido que tiene que ser muy activo, y tan aeróbico como el corazón, nos proporciona las bases para comprender, al menos en parte, la cardiomiopatía senil.

Por supuesto, el hecho de que el entrenamiento aeróbico es tan dependiente de la mitocondriogénesis, nos dió una base común para tratar de entender los dos mecanismos, la afectación de la vía mitocondriogénica en el envejecimiento y su posible prevención mediante el ejercicio. En siguientes apartados se hará referencia a los experimentos sobre el efecto del entrenamiento aeróbico sobre la mitocondriogénesis.

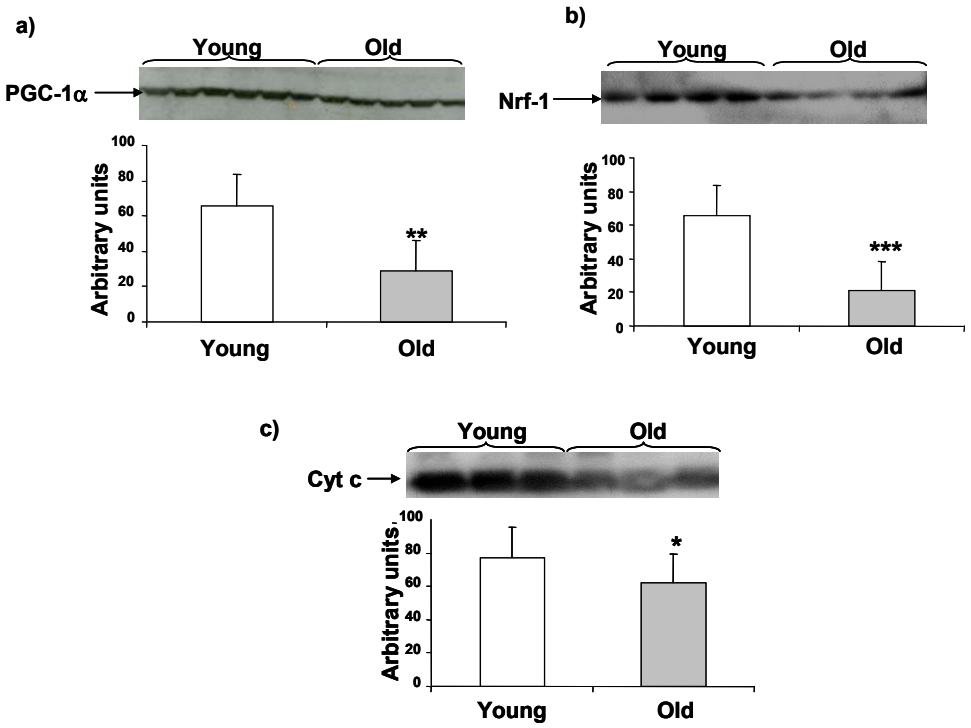


Figura 8. *La mitocondrogénesis cardíaca está dañada en el envejecimiento. Panel A: expresión de PGC- 1α en corazones de animales jóvenes y viejos. La expresión en animales viejos fue significativamente inferior a la de los jóvenes. Panel B: expresión de NRF-1 en corazones de animales jóvenes y viejos. De nuevo, la expresión en animales viejos fue significativamente inferior a la de los jóvenes. Panel C: expresión de citocromo c en corazones de animales jóvenes y viejos. Los niveles de citocromo c fueron significativamente inferiores en los animales viejos cuando lo comparamos con los jóvenes [19].*

2.4.3. Una biogénesis mitocondrial disminuida está asociada con la falta de reactividad de PGC-1α

Los mecanismos que controlan la pérdida de masa muscular son muy relevantes en medicina debido a que la contracción muscular está involucrada en la prevención de enfermedades crónicas. Por lo tanto, la comprensión de la vía de señalización que regula la masa muscular puede proporcionar posibles dianas terapéuticas [272]. El envejecimiento muscular es un componente clave en el aumento de la fragilidad [273]. La fragilidad es un síndrome altamente prevalente con el aumento de edad y se compone de una disminución de la reserva y la resistencia al estrés celular, debilidad, disminución de la actividad, pérdida de peso, y mayor vulnerabilidad a los efectos adversos para la salud [274]. Uno de los principales componentes de la fragilidad es la sarcopenia. Éste es un síndrome caracterizado por la pérdida

progresiva y generalizada de la masa y la fuerza del músculo-esquelético y con un riesgo de resultados adversos tales como la discapacidad física, la mala calidad de vida e incluso la muerte [275,276]. La activación de la mitocondriogénesis es crítica para prevenir el envejecimiento en el músculo-esquelético. Un músculo funcional que no ha perdido la capacidad de sintetizar mitocondrias sanas es un factor muy importante en la prevención de la fragilidad [277,278]. Como se muestra en la Figura 9, el envejecimiento se ha asociado, en el músculo-esquelético, con reducciones en la actividad de fosforilación oxidativa mitocondrial [279], mutaciones en el DNA mitocondrial [280], reducciones en el contenido de DNA mitocondrial [281], disminución de la actividad de la cadena de transporte electrónico mitocondrial [282], señalización apoptótica alterada y un aumento en la liberación mitocondrial de RLs [283]. Varias estrategias se han desarrollado para estimular la biogénesis mitocondrial. Entre ellas se han propuesto diferentes compuestos para mejorar el control de la respiración mitocondrial o estimular la biogénesis mitocondrial como por ejemplo la pirroloquinolina quinona, resveratrol, genisteína, hidroxitirosol, GW1516, AICAR y la quercetina [240,250,284]. En estudios *in vivo* e *in vitro* se ha demostrado que los niveles de PGC-1α estimulan la proliferación mitocondrial en el músculo-esquelético [285]. Un aumento en los niveles de PGC-1α en el músculo-esquelético evita la pérdida de masa muscular mediante la reducción de la autofagia, de la degradación por el proteasoma y de la apoptosis [286]. La autofagia se utiliza para definir el reciclaje controlado y la degradación de las estructuras intracelulares (orgánulos disfuncionales y los agregados de proteínas) para reponer las reservas de nutrientes y asegurar la integridad de la célula y su supervivencia [287]. La acumulación de mitocondrias no funcionales en individuos de edad avanzada podría ser contrarrestada eliminando las mitocondrias dañadas por un proceso autofágico llamado "mitofagia" [288].

En un estudio realizado por el grupo de Henriette Pilegaard se ha demostrado que se requiere PGC-1α para conseguir los efectos beneficiosos del ejercicio moderado en edades avanzadas para mantener el metabolismo mitocondrial y la capacidad antioxidante [289]. Estos estudios sugieren que la modulación de los niveles de PGC-1α en el músculo-esquelético representa una vía para la prevención y el tratamiento de los trastornos relacionados con la edad.

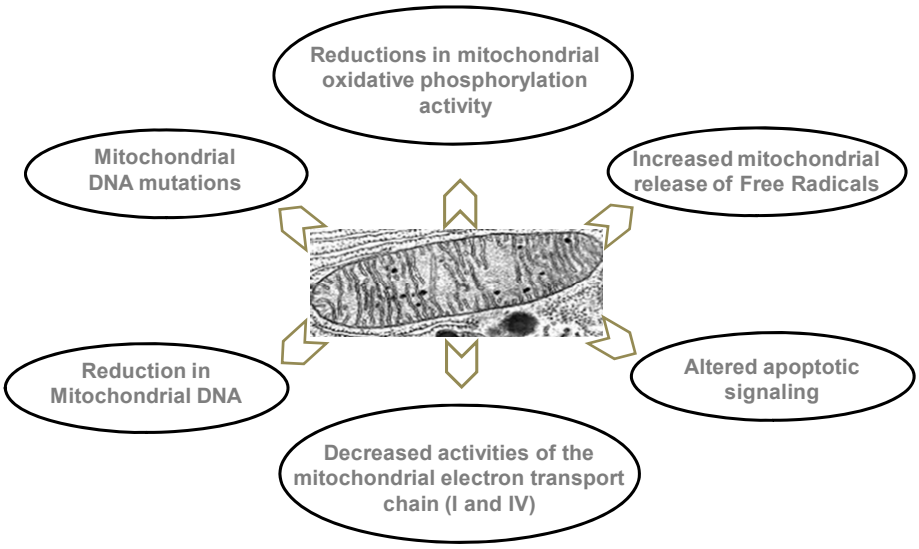


Figura 9. *Alteraciones mitocondriales asociadas al envejecimiento en el músculo-esquelético [21].*

2.4.4. Las mitocondrias están dañadas en el proceso de envejecimiento

En 1996, nuestro grupo estudió la participación de las mitocondrias en el envejecimiento utilizando células hepáticas totales y mitocondrias no aisladas [267]. Hasta entonces, el daño mitocondrial que conduce al envejecimiento sólo se había probado con mitocondrias aisladas. Posteriormente, otros grupos de investigación mostraron cambios relacionados con la edad en los sistemas de transporte y la función respiratoria mitocondrial utilizando este mismo enfoque experimental [290,291]. Determinamos la tasa de gluconeogénesis y de cetogénesis, que dependen críticamente de la función mitocondrial, en el hígado de animales viejos y jóvenes. También se determinó el tamaño mitocondrial, la

producción de peróxido y el potencial de membrana en las células hepáticas totales usando citometría de flujo [267]. Nuestros resultados mostraron, por primera vez en células intactas, una correlación entre el deterioro asociado a la edad del metabolismo celular y los cambios específicos en la función mitocondrial y en su morfología. Esto fue confirmado casi simultáneamente por el grupo de Bruce Ames [268]. También se observó que la actividad respiratoria mitocondrial no sólo disminuye con la edad en el hígado, sino también en otros tejidos tales como el músculo y el cerebro.

2.4.5. La mitocondria como una fuente de radicales libres

Las mitocondrias han sido reconocidas clásicamente como los orgánulos que producen la energía necesaria para los procesos endergónicos de la vida celular a través de la cadena respiratoria, pero también se consideran como la fuente celular generadora de RLs más importante, como principal reguladora de los RLs y acciones tóxicas, y como una fuente de moléculas de señalización que regulan el ciclo celular, la proliferación y la apoptosis [292]. La aparición de los RLs en los procesos biológicos es ampliamente aceptada [1]. Más del 95% de todo el oxígeno que respiramos se somete a una reducción para producir agua en la cadena de transporte electrónico mitocondrial. La citocromo oxidasa es el aceptor terminal de electrones en la cadena y debe liberar sus equivalentes reductores para permitir el transporte de electrones continuado y la producción de ATP [292]. Aunque la cadena de transporte electrónico mitocondrial es un sistema muy eficiente, predispone a cada portador de electrones a reacciones secundarias con oxígeno molecular. Si un átomo/molécula contiene uno o más electrones desapareados se le conoce como un "radical libre" [293]. Los principales RLs que se generan en las células son el anión superóxido ($O_2^{\bullet -}$) y el óxido nítrico ($NO^{\bullet}$) [294]. La generación mitocondrial de estos RLs, así como la de peróxido de hidrógeno (H_2O_2) y peroxinitrito ($ONOO^{\bullet -}$), representa la mayor fuente intracelular de generación de

ROS bajo condiciones fisiológicas [292]. Las mitocondrias parecen ser (cuantitativamente) el sitio celular más importante de producción de $O_2^{\bullet-}$ y H_2O_2 en mamíferos, y la concentración en estado estacionario de $O_2^{\bullet-}$ en la matriz mitocondrial es de aproximadamente 5 a 10 veces más alta que en el citosol y el núcleo [292]. Por lo tanto, los componentes mitocondriales están expuestos a un alto flujo de RLs. Éstos causan daños a los componentes mitocondriales e inician procesos de degradación. El término EO fue acuñado por primera vez por Helmut Sies en 1985 como "una alteración en el equilibrio prooxidante-antioxidante a favor del primero" [295,296]. Aunque esta definición se ha usado ampliamente durante más de dos décadas, es probable que la definición de EO sufra modificaciones en el futuro. En un esfuerzo para afinar la definición de EO, Dean Jones ha propuesto que este término debe volver a definirse como "una interrupción de la señalización y el control redox"[297].

2.4.6. Los radicales libres como teoría del envejecimiento: ¿es necesaria una reformulación?

Las reacciones tóxicas asociadas al EO constituyen el dogma central de la teoría de los RLs del envejecimiento. El envejecimiento se asocia con una pérdida total de la función a nivel de todo el organismo que tiene su origen en el deterioro celular. Bernard Strehler define el envejecimiento a través de cuatro postulados: El envejecimiento es universal, debe ser intrínseco, progresivo y perjudicial [298] (ver Figura 10).

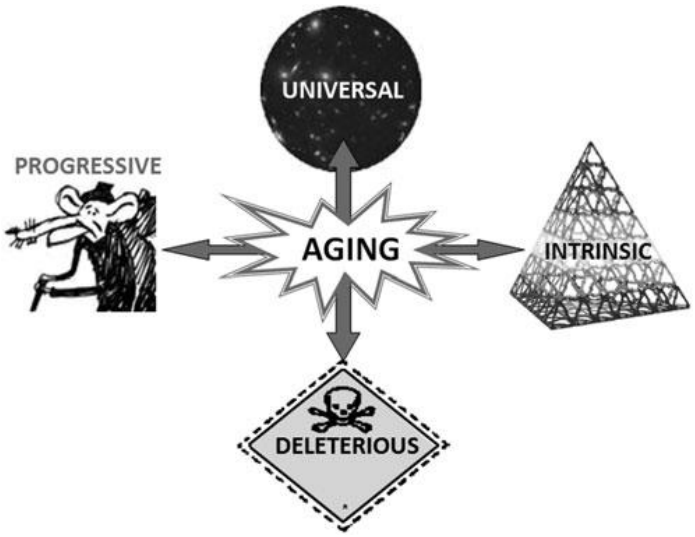


Figura 10. *Características del envejecimiento [299].*

El envejecimiento provoca una pérdida en la capacidad de mantener el medio interno de la persona cuando se enfrenta a cambios en el ambiente externo. Por lo tanto, el individuo pierde la capacidad de mantener la homeostasis y casi todas las funciones fisiológicas pierden eficacia con la edad [300].

Las mitocondrias requieren el reciclaje continuo a lo largo de la vida útil y son particularmente susceptibles a daño, ya que son el principal mecanismo bioenergético y fuente de EO celular [301]. La teoría de los RLs del envejecimiento es una de las teorías más prominentes para explicar el envejecimiento. La teoría mitocondrial del envejecimiento [302], ha sido estudiada en varios laboratorios y hay muchos artículos publicados que la apoyan. Por ejemplo, datos de nuestro propio grupo muestran que las mitocondrias de animales viejos producen más ROS que las de animales jóvenes [303,304]. Por otra parte, existe una relación inversa entre la producción de peróxido mitocondrial y la longevidad en mamíferos [305,306]. Estos resultados apoyan la hipótesis de que la tasa de generación de oxidantes por las mitocondrias es un factor crítico en el envejecimiento [307]. De hecho, la tasa de generación de peróxido aumenta con la edad. Corbisier y Remacle postularon que las mitocondrias están implicadas en la

degeneración celular, y lo estudiaron mediante la microinyección de mitocondrias aisladas de fibroblastos de ratas viejas en células de animales jóvenes. Estos autores encontraron que las células que habían sido inyectadas con mitocondrias "viejas" entraban en senescencia rápidamente [308]. Así pues, la generación de RLs por las mitocondrias continua a lo largo de la vida provoca un EO crónico que juega un papel crítico en el envejecimiento. Por tanto, la tasa de producción de ROS en la mitocondria es un determinante clave de la esperanza de vida, al menos □□□□□□□□□□ï□□□□□□in embargo, la teoría mitocondrial del Sin embargo, la teoría mitocondrial del envejecimiento ha sido cuestionada recientemente, existiendo una gran controversia al respecto. Esta teoría ha proporcionado un marco teórico para una enorme cantidad de trabajos que han conducido a importantes avances en la comprensión del envejecimiento. Hasta el cambio de siglo, la teoría recibió abundante apoyo a través de observaciones procedentes de campos tan dispares como la fisiología comparativa o la biología molecular. Como se ha dicho anteriormente, existen trabajos de muchos laboratorios que apoyan la teoría, por ejemplo, muestran que la sobreexpresión de enzimas antioxidantes produce incrementos en los años de vida útil [309-313]. Sin embargo, otros laboratorios han demostrado que, en algunos casos, un aumento del EO no produce un aumento de la longevidad [193,314-316] (Ver Figura 11). El descubrimiento de que los RLs no sólo pueden causar daño molecular a las células, sino que también sirven como señales, ha dado lugar a la propuesta de que actúan como moduladores de procesos fisiológicos. Por ejemplo, las ROS estimulan adaptaciones fisiológicas al ejercicio físico.

Un golpe crítico a la teoría mitocondrial del envejecimiento vino de estudios epidemiológicos que muestran que la suplementación antioxidante no bajó la incidencia de muchas enfermedades asociadas con la edad, aumentando incluso el riesgo de muerte. Por otra parte, evidencias moleculares recientes han demostrado que el aumento de la generación de ROS, en algunos casos, aumenta la longevidad

[299]. Por lo tanto, los gerontólogos interesados en la biología de los RLs se encuentran en una encrucijada y nuevos conocimientos al respecto son necesarios para clarificar el papel de las ROS en el proceso de envejecimiento [299].

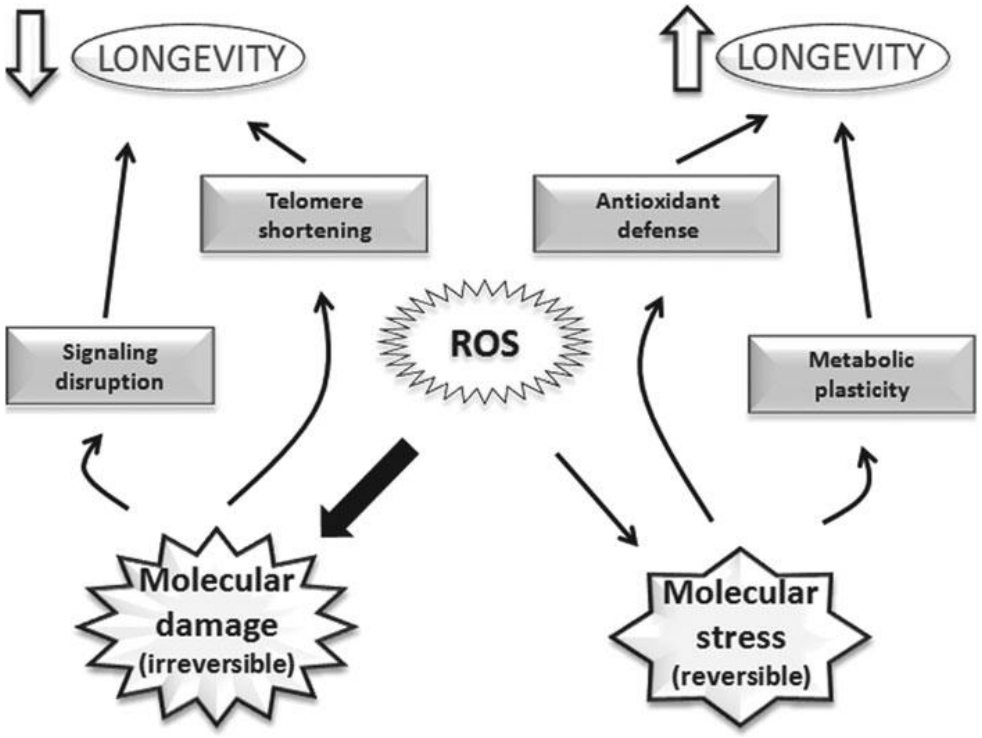


Figura 11. *La espada de doble filo de los radicales libres. Tienen efectos horméticos. Cuando los radicales causan agresiones graves sobre las biomoléculas provocan daño (es decir, alteraciones irreversibles), mientras que cuando la agresión es leve, hay un estrés y éste puede tener efectos de señalización, así como los efectos hormeticos ya mencionados [299].*

2.4.7. La mitocondriogénesis en el ejercicio físico está controlada por ROS

El hecho de que PGC-1α es un regulador maestro de la respuesta celular al EO [317] y por lo tanto puede ser un buen sensor para la respuesta celular a los RLs nos llevó a postular la idea de que los RLs generados en el ejercicio podrían ser señales para aumentar la mitocondriogénesis [40]. De hecho, el trabajo pionero llevado a cabo por Kelvin Davies y desarrollado en el laboratorio de Lester Packer ya sugirieron esta idea. Cuando estos autores informaron de la primera evidencia inequívoca de que los RLs se producen por el músculo en el ejercicio físico, sugirieron que la mitocondriogénesis podría ser un resultado de la acción de estos

radicales [39]. Sin embargo, a principios de los años 80 la idea no podía ser probada experimentalmente. Retomamos esta idea casi veinte años después, y estudiamos el papel de los oxidantes y antioxidantes en la mitocondriogénesis asociada con el ejercicio físico.

Nuestros estudios previos habían demostrado que la XO es una de las fuentes más importantes de generación de ROS asociados con el ejercicio físico agotador. Evaluamos esta hipótesis de forma experimental en animales [2,10] y en humanos [3,24]. Administramos alopurinol a animales sometidos a ejercicio físico y determinamos la activación de vías de señalización que participan en las adaptaciones musculares al ejercicio físico (MAPKs y NF-kB) [2,193].

La Figura 12 muestra que el alopurinol o la vitamina C (que contrarresta las ROS por diferentes mecanismos) son capaces de prevenir la activación de MAPKinasas y de NF-kB inducidas por el ejercicio en el músculo-esquelético y por lo tanto la posterior regulación de la expresión génica de MnSOD, óxido nítrico sintasa inducible (iNOS) y óxido nítrico sintasa endotelial (eNOS). Pero los efectos que presentamos en la Figura 12 podrían deberse al alopurinol en sí y no a sus efectos antioxidantes. Se confirmó que la vitamina C, que actúa por un mecanismo diferente al alopurinol, tuvo los mismos efectos sobre la ruta de NF-kB-MAPKs.

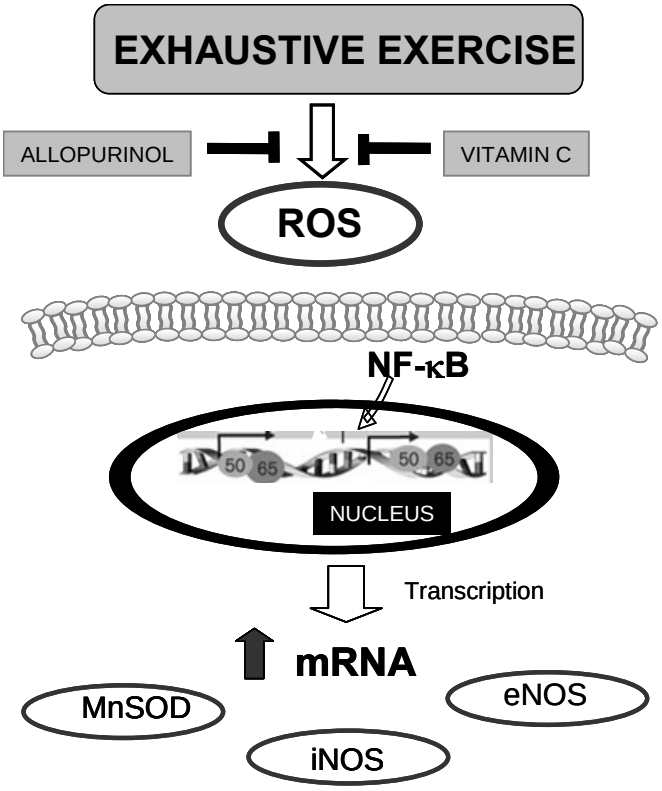


Figura 12. *Prevención, por la administración de alopurinol y de vitamina C, de la activación de NF-kB inducida por el ejercicio en el músculo-esquelético y la posterior regulación de la expresión génica de MnSOD, iNOS y eNOS [19].*

La Figura 13 muestra un claro efecto de la vitamina C en la disminución de la respuesta mitocondriogénica al ejercicio físico (PGC-1α, NRF-1, TFAM y citocromo C). Tanto la transcripción de RNA como la expresión de proteína disminuyen cuando los animales son tratados con vitamina C. Por lo tanto, la inhibición del efecto de los antioxidantes es capaz de obstaculizar la respuesta mitocondriogénica inducida por el ejercicio físico [40].

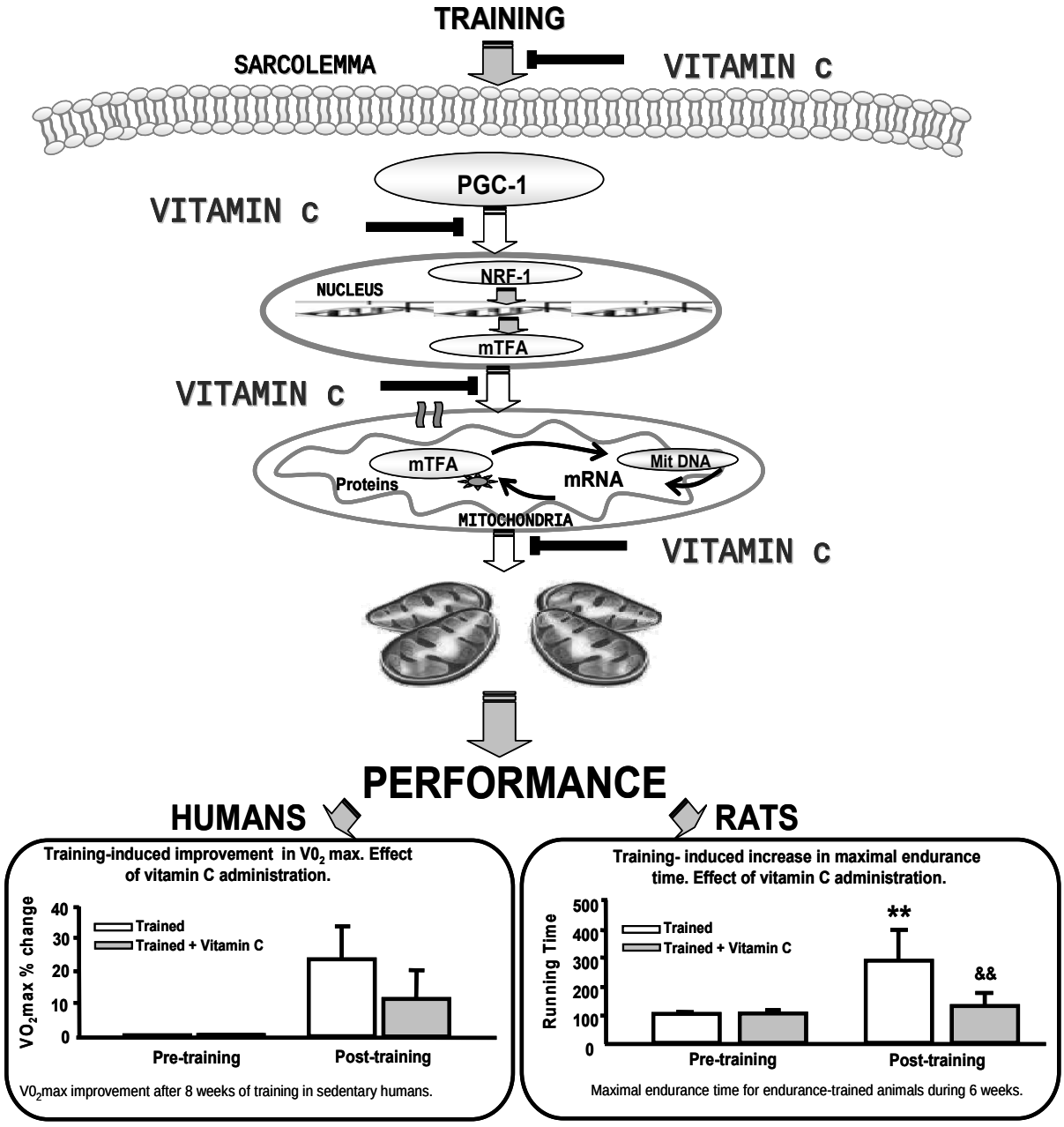


Figura 13. *Resumen del papel de las ROS en la señalización de las adaptaciones celulares durante el entrenamiento y sus efectos sobre el rendimiento deportivo [19].*

2.4.8. Especies reactivas del oxígeno, antioxidantes y rendimiento

El hecho de que la mitocondriogénesis esté inhibida tanto por la administración de alopurinol como de vitamina C nos llevó a pensar que el tratamiento con antioxidantes durante el entrenamiento podría no ser ventajoso para la eficacia del entrenamiento. Esta era una cuestión importante ya que más del 40% de la población que hace ejercicio físico en los Estados Unidos y en Europa toman regularmente suplementos antioxidantes [318]. Dado que una mitocondriogénesis activa es imprescindible durante el entrenamiento aeróbico, el tratamiento antioxidante podría dificultar la eficacia de dicho entrenamiento. Un estudio ciego (animales) y un estudio doble ciego (humanos) indican que la administración de antioxidantes reduce la eficiencia del entrenamiento [40]. La Figura 13 (paneles de abajo) indica que, efectivamente, el aumento en el tiempo de carrera hasta el agotamiento de las ratas, después de ocho semanas de entrenamiento, fue mucho menos pronunciado cuando a las ratas se les administró vitamina C cuando lo comparamos con los controles [40]. Del mismo modo, la administración de 1 gramo de vitamina C al día en el estudio con humanos redujo el incremento en el $VO_{2máx}$ que se produce después del entrenamiento aeróbico [40]. Por lo tanto, nuestros estudios teóricos sobre la activación de la vía mitocondriogénica inducida por el ejercico nos llevó a una conclusión práctica y esto es que no se debe administrar antioxidantes a los atletas cuando están entrenando. Así pues, una pregunta permanece sin respuesta: ¿debemos dar suplementos antioxidantes a cualquier atleta?. Nuestra visión actual es que la suplementación con antioxidantes puede ser útil en el ejercicio muy agotador y que puede causar daño muscular (es decir, en competición). Sin embargo, no se debe dar antioxidantes cuando los atletas están entrenando o preparándose para la competición, ya que puede obstaculizar las adaptaciones beneficiosas inducidas por el entrenamiento [40].

Estos resultados se han confirmado y ampliado recientemente para el efecto saludable del ejercicio (y no sólo para el rendimiento) por Michael Ristow y

colaboradores [201]. Ellos han demostrado que el EO inducido por el ejercicio mejora la resistencia a la insulina, causa una respuesta adaptativa y mejora la capacidad de defensa antioxidante endógena. La suplementación con antioxidantes (vitamina C y E) se opone a los efectos de promoción de la salud inducidos por el ejercicio físico en los seres humanos [201].

En resumen, se recomienda la administración de antioxidantes en competición, pero no durante los periodos de entrenamiento, en los que se necesitan las ROS producidas en el ejercicio para lograr adaptaciones musculares máximas.

Nuestros estudios corroboran la idea de que las ROS no sólo no son perjudiciales para las células, sino que tienen una importancia crítica como señales celulares para adaptarse a diversas demandas fisiológicas [319,320].

RESUMEN: OBJETIVOS DE LA INVESTIGACIÓN

3. RESUMEN

A. OBJETIVOS DE LA INVESTIGACIÓN

En base a lo expuesto anteriormente, los *OBJETIVOS* que se acometen en la presente tesis doctoral son:

Objetivo nº 1.- Determinar el papel de la enzima XO en el daño oxidativo, músculo-esquelético y cardiovascular mediante la valoración de diversos biomarcadores clásicos en sangre tales como: MDA, CK, LDH, AST, mioglobina, CK-MB y Hs-TnT, así como de nuevos biomarcadores de daño cardiovascular: copeptina, MD-proADM, GDF15, PIGF, sVEGFR1/sFLT1 y suPAR, en jugadores de fútbol profesional antes y después de un partido.

Objetivo nº 2.- Determinar el efecto del ejercicio físico de muy larga duración sobre nuevos biomarcadores de daño cardiovascular y renal en atletas antes y después de una ultramaratón de 100 Km.

Objetivo nº 3.- Analizar las implicaciones del ejercicio físico de alta intensidad sobre la longevidad en sujetos entrenados utilizando como modelo los ciclistas participantes en el Tour de Francia entre los años 1930-1964.

Objetivo nº 4.- Determinar el papel del entrenamiento aeróbico en la inducción de la cascada de la biogénesis mitocondrial y sus implicaciones en el envejecimiento músculo-esquelético.

RESUMEN: DISEÑOS EXPERIMENTALES

B. DISEÑOS EXPERIMENTALES DE LOS ESTUDIOS REALIZADOS SEGÚN OBJETIVOS

OBJETIVO 1: Determinar el papel de la enzima XO en el daño oxidativo, músculo-esquelético y cardiovascular mediante la valoración de diversos biomarcadores clásicos en sangre, así como de nuevos biomarcadores de daño cardiovascular, en jugadores de fútbol profesional antes y después de un partido.

Doce jugadores del Valencia Club de Fútbol (Edad: 25±2 años; Peso: 75,0±8,2 Kg; Altura: 1,80±0,1 m; Masa corporal magra: 36,2±3,0 Kg; Masa corporal grasa: 8,1±1,0 Kg) fueron divididos en dos grupos experimentales. Una dosis oral de 300 mg de alopurinol se administró a seis jugadores cuatro horas antes de un partido de Primera División de la Liga de Fútbol Española (grupo alopurinol). Los otros 6 jugadores recibieron placebo (celulosa – grupo placebo) (Ver tabla 2). El partido fue el Sevilla C.F. – Valencia C.F. que se jugó a las 22 horas y durante la segunda mitad del partido se cambiaron dos jugadores. Estos jugadores también se incluyeron en el estudio, mientras que el portero fue excluido. A los jugadores se les ofreció bebidas *ad libitum* (sin vitaminas antioxidantes añadidas) durante el partido para evitar la deshidratación. Se les solicitó el consentimiento informado por escrito antes de la participación en el estudio. El alopurinol no se encuentra en la lista de sustancias prohibidas por la Agencia Mundial Antidopaje (AMA) o de la *Fédération Internationale de Football Association* (FIFA).

El estudio cumple con la Declaración de la Asociación Médica Mundial de Helsinki y los principios éticos para las investigaciones médicas en seres humanos. El protocolo experimental fue aprobado por el Comité de Ética de Investigación Clínica del Hospital Clínico Universitario de Valencia.

L	M	X	J	V	S	D
			Extracción de sangre basal.		Alopurinol (300 mg/día) a los titulares del partido y reservas **DÍA DEL PARTIDO**	A las 12 horas extracción de sangre post-partido

Tabla 2. *Esquema del diseño experimental*

Extracción, recogida y conservación de muestras

Muestras de sangre venosa se obtuvieron antes del partido (Basal) y doce horas más tarde (Post-partido). Todas las muestras de sangre se recogieron en tubos de vacío, a la misma hora y en las mismas condiciones (temperatura y humedad), por punción de una vena superficial de la fosa antecubital después de que los sujetos hubiesen permanecido sentados durante 10 min. La extracción de sangre se realizó en ayunas. Ninguna muestra fue descartada debido a la flebotomía insatisfactoria, dificultad en la localización de un acceso venoso, la falta de sentido, hemólisis o lipemia visible.

Se recogieron un total de 2 muestras de sangre de un volumen aproximado de 11 mL distribuidos en 3 tubos Vacutainer: 1) 3,5 mL se recogieron en tubo seco. Todos estos tubos se dejaron en posición vertical durante 45 minutos a temperatura ambiente (20ºC) para permitir la coagulación completa antes de la centrifugación [321]. El suero se separó luego por centrifugación a 1500 x*g* durante 15 min a temperatura ambiente, se almacenó en alícuotas y se mantuvo congelado a -20ºC hasta la medición. 2) 3,5 mL se recogieron en tubos con EDTA. 3) 3,5 mL se recogieron en tubos con heparina. Del mismo modo, el plasma se separó luego por centrifugación a 1500 x*g* durante 15 min a temperatura ambiente, se almacenó en alícuotas y se mantuvo congelado a -20ºC hasta la medición.

En resumen, las extracciones de sangre se procesaron siguiendo los siguientes protocolos:

1.- Tubo seco o con heparina para medir CK, CK-MB, LDH GOT, GPT, GGT, Myo, Hs-TnT, NT-proBNP, CoP, MR-proADM, GDF15, sVEGFR-1/sFLT-1, PlGF, suPAR:

1.1. Extraer 3,5 ml de sangre

1.2. Centrifugar 1500 x*g* durante 15 minutos.

1.3. Recoger suero, alicuotar en al menos 5 eppendorfs

1.4. Colocar en hielo y congelar a -20ºC

2.- Para medir MDA y grupos carbonilos en proteínas plasmáticas:

2.1. Extraer 3,5 ml de sangre en tubo con EDTA.

2.2. Centrifugar a 1500 x*g* durante 15 minutos.

2.3. Recoger plasma, alicuotar en al menos 5 eppendorfs

2.4. Colocar en hielo y congelar a -20ºC

Determinaciones

Todas las alícuotas de suero se descongelaron al mismo tiempo. Las concentraciones de ácido úrico en suero, CK, CK-MB, LDH, GOT, GPT, GGT, Myo, Hs-TnT, NT-proBNP, CoP; MR-proADM; GDF15; sVEGFR-1/sFLT-1; PlGF; suPAR; MDA (Ver Tabla 3) fueron analizados por duplicado inmediatamente después de la descongelación. Los detalles de los métodos utilizados para realizar cada una de las determinaciones se pueden encontrar más detalladamente en las correspondientes publicaciones [11-13](Ver Apéndice 2).

Análisis estadístico

Se utilizó un análisis de la varianza de dos vías con medidas repetidas para determinar la diferencia entre los dos parámetros durante el experimento (Basal frente a Post-partido). Cuando se encontró una diferencia, se realizaron comparaciones múltiples con la prueba *post hoc* de Tukey. El análisis estadístico se

realizó con programa Analyse-it para Microsoft Excel (Analyse-it Software Ltd, Leeds, Reino Unido).

BIOMARCADORES	DESCRIPCIÓN	ESPECIFICIDAD
CK	Creatin quinasa	Muscular
CK-MB	Creatin quinasa – isoforma cardíaca	Muscular-cardíaco
LDH	Lactato deshidrogenasa	Muscular
AST	Aspartato aminotransferasa	Muscular
ALT	Alanina aminotransferasa	Hepático
GGT	Gamma-glutamil transferasa	Hepático
Myo	Mioglobina	Muscular
Hs-TnT	Toponina T de alta sensibilidad	Muscular-cardíaco
NT-proBNP	Fracción amino terminal del péptido natriurético cerebral	Muscular-cardíaco
CoP	Copeptina	Cardíaco
MR-proADM	Región media de la proadrenomedulina	Cardíaco
GDF15	Factor de diferenciación de crecimiento 15	Cardíaco y renal
sVEGFR-1/sFLT-1	Receptor del factor de crecimiento endotelial vascular-1	Inflamación y angiogenesis
PlGF	Factor de crecimiento placentario	Inflamación y angiogenesis
suPAR	Receptor del activador del plasminógeno soluble en uroquinasa	Inflamación
MDA	Malondialdehido	Peroxidación lipídica

Tabla 3. *Lista de biomarcadores que se midieron en las muestras séricas de los jugadores con sus correspondientes especificidades.*

OBJETIVO 2: Determinar el efecto del ejercicio físico de muy larga duración sobre nuevos biomarcadores de daño cardiovascular y renal en atletas antes y después de una ultramaratón de 100 Km.

La población de estudio consistió en 16 atletas varones de raza caucásica entrenados (edad media: 42 años. Rango de edad: 34 - 52 años), que hicieron un entrenamiento específico de resistencia de entre 3 a 10 años (media de entrenamiento 240 ± 32 min/semana; consumo máximo de oxígenoVO_{2max}: 65 ± 2 ml/kg/min). Todos los atletas corrieron un ultramaratón de 60 km equipados con un monitor de frecuencia cardíaca (HR) a 80 ± 4% del $VO_{2\ máx}$ (el % se calculó de acuerdo a la relación VO_2/HR determinados con el test incremental). Ninguno de los atletas padecía enfermedades agudas o crónicas, o informó la ingesta de medicamentos, incluyendo antioxidantes o nicotina.

El ejercicio vigoroso se evitó 36-48 h antes de la prueba. La carrera se inició a las 8:00 y se llevó a cabo en un recorrido ondulado y exigente, en un día parcialmente nublado y lluvioso, con una temperatura de entre 6°C y 8°C, y una humedad del 54% al 87 %. A los atletas se les ofrecieron bebidas *ad libitum* durante la carrera para evitar la deshidratación excesiva.

Dos muestras de sangre y orina fueron recogidas. Una después de un período de ayuno de 8 horas, 20 minutos antes de iniciar el calentamiento ("pre-ejercicio"), y otra dentro de los 10 minutos después de la finalización de la carrera ("post-ejercicio"). La sangre se recogió en tubos de vacío sin aditivos (Becton - Dickinson, Oxford, Reino Unido). La orina y el suero se almacenaron en alícuotas y se mantuvieron congeladas a -20ºC hasta la medición. Todos los sujetos dieron su consentimiento informado, y el estudio se llevó a cabo de conformidad con la Declaración de Helsinki y en los términos de la legislación local pertinente.

Determinaciones

Todas las muestras de suero y orina se descongelaron al mismo tiempo. Las concentraciones de EGFR, creatinina sérica y urinaria, albúmina, NGAL sérico y urinario, copeptina y MR-proADM fueron analizados por duplicado inmediatamente después de la descongelación. Los detalles de los métodos utilizados para realizar cada una de las determinaciones se pueden encontrar más detalladamente en las correspondientes publicaciones [14,322](Ver Apéndice 2).

Análisis estadístico

Los tests de Wilcoxon y de $\chi 2$ (para variables categóricas) se utilizaron para evaluar la importancia de las variaciones inducidas por el ejercicio. Los datos con una distribución no normal se normalizaron usando una transformación logarítmica antes del análisis. El análisis estadístico se realizó a través del programa Analyse-it para el software Excel (Analyse-it Software, Leeds, Reino Unido) y el nivel de significación estadística se estableció en $p<0,05$. Los datos se muestran como media y rango intercuartil (IQR).

OBJETIVO 3: Analizar las implicaciones del ejercicio físico de alta intensidad sobre la longevidad en sujetos entrenados utilizando como modelo los ciclistas participantes en el Tour de Francia entre los años 1930-1964.

De los 1.318 participantes que han participado en el Tour de Francia (TdF) entre los años 1930 y 1964, sólo 1.229 corredores se consideraron para nuestro estudio por haber obtenido su fecha de nacimiento y defunción. Los ciclistas que no completaron todas las etapas del TdF se excluyeron. Otros criterios de exclusión para este estudio fueron: ciclistas que hubiesen participado en el TdF antes de 1930 o después de 1964 y de haber nacido en un país que hubiese contribuido con menos de 100 participantes durante este período. De estos 1.229 ciclistas, todos hombres, 834 procedían de Francia (n = 465), Italia (n = 196) y Bélgica (n = 173), lo que representa el 68 % de los participantes en el TdF en los años estudiados. El resto procedía de 21 países diferentes (véase Figura 14), cada una representada por sólo un pequeño número de ciclistas. Para simplificar, nos centramos en los tres países con el mayor número de participantes en el TdF y comparamos su longevidad con la de la población media en sus respectivos países. Además, estos países tienen un registro demográfico de la población desde el siglo XIX, lo que nos permitió la comparación de las tasas de supervivencia de los ciclistas participantes en el TdF con la población general. Sólo datos muy dispersos se pueden obtener en la literatura sobre los ciclistas que participaron en el TdF antes de 1930. Por supuesto, de los que participaron en años posteriores a 1964, muchos todavía están vivos y la tasa de supervivencia no se puede calcular. Esto nos deja con una amplia muestra de 834 ciclistas (de un total posible de 1.318) que constituyen una muestra representativa de los participantes en el TdF. Las tasas de supervivencia para los participantes en el TdF entre 1930 y 1964 corresponden a los años de nacimiento entre 1892 y 1942. Esto se comparó con la de la población general, es decir, los hombres nacidos entre 1892 y 1942 (los años en los que nacieron los ciclistas

estudiados). Nuestros datos provienen de tres sitios web electrónicos oficiales (www.letour.fr, www.cyclingarchives.com, www.memoire-du-cyclisme.net), que contienen información detallada acerca de cada ciclista que haya tomado parte en el TdF, incluyendo las fechas de nacimiento y muerte. La edad media de muerte para hombres de la población general en los países de origen se calculó a partir de datos obtenidos de la base de datos de mortalidad humana (www.mortality.org).

Las fechas de nacimiento y muerte, así como el porcentaje de supervivientes para cada edad, a 31 de diciembre de 2007, se registraron para el cálculo de la curva, tanto para la población general como para los ciclistas. El porcentaje de supervivencia de los ciclistas nacidos en cada año (1930-1964) se representó y se comparó con los valores calculados para la población general y para los grupos de edad correspondientes de Francia, Italia y Bélgica.

La variable denominada "porcentaje de supervivientes" se define de la siguiente manera: número de personas nacidas en un año dado que estaban vivos a 31 de diciembre 2007 dividido por el número de personas nacidas en ese año. La variable "Edad" se calcula como: 2007 - año de nacimiento. Lo mismo se aplica para los participantes en el TdF.

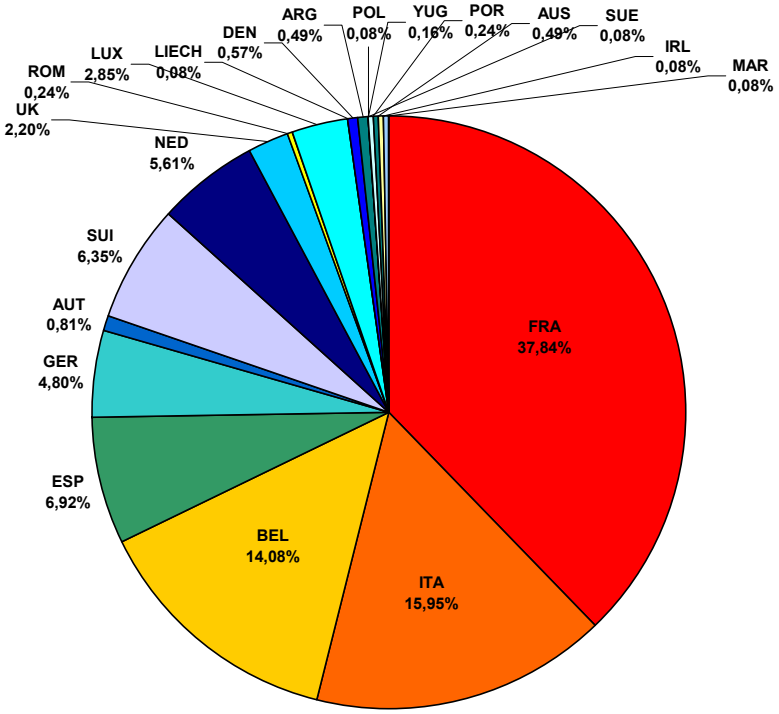


Figura 14. *Distribución por países de los ciclistas que participaron en el TdF entre los años 1930 y 1964. ARG: Argentina; AUS: Australia; AUT: Austria; BEL: Bélgica; DEN: Dinamarca; ENG: Inglaterra; ESP: España; FRA: Francia; GER: Alemania; ITA: Italia; IRL: Irlanda; LIE: Liechtenstein; LUX: Luxemburgo; MAR: Marruecos; NED: Holanda; POL: Polonia; POR: Portugal; ROM: Rumanía; SWE: Suecia; SUI: Suiza; YUG: Yugoslavia. Argelia no está representado porque solo hay un corredor [18].*

Análisis estadístico

Se realizó un estudio de casos y controles. El análisis estadístico se realizó con el software para Windows SPSS (Chicago, IL, USA; versión 17.0). Se ajustaron las curvas de regresión polinómica para cada población y se midieron las áreas bajo la curva. La significación estadística de la diferencia entre las áreas se calculó por el estadístico z. El test no paramétrico Mann-Whitney U se aplicó para comparar el porcentaje medio de supervivencia para cada población. El nivel de significación estadística se estableció en $p < 0,05$.

OBJETIVO 4: Determinar el papel del entrenamiento aeróbico en la inducción de la cascada de la biogénesis mitocondrial y sus implicaciones en el envejecimiento músculo-esquelético.

Ratas

Para los experimentos de ejercicio veinticuatro ratas Wistar macho se dividieron aleatoriamente en cuatro grupos experimentales: jóvenes no entrenadas (n = 6), jóvenes entrenadas (n = 6), viejas no entrenadas (n = 6) y viejas entrenadas (n = 6). Para los experimentos de inducción de frío dieciséis ratas Wistar macho se dividieron aleatoriamente en cuatro grupos experimentales: jóvenes control (n = 4), jóvenes expuestas al frío (n = 4), viejas control (n = 4), viejas expuestas al frío (n = 4). Para los experimentos con hormona tiroidea, dieciséis ratas Wistar macho se dividieron aleatoriamente en cuatro grupos experimentales: jóvenes control (n = 4), jóvenes tratadas con triyodotironina − T3 − (n = 4), viejas control (n = 4), viejas tratadas con T3 (n = 4). En todos los modelos experimentales las ratas viejas eran de 24 meses de edad y las jóvenes eran de 3 meses de edad.

Ratones

La generación y el fenotipo de los ratones KO PGC-1α se han descrito anteriormente [323]. Veintisiete ratones macho (14 WT y 13 KO de PGC-1α) fueron divididos aleatoriamente en cuatro grupos experimentales: WT no entrenados (n = 6), WT entrenados (n = 8), KO de PGC-1α no entrenados (n = 7) y KO de PGC-1α entrenados (n = 6). Los animales fueron proporcionados por el Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC, Madrid. España). Los animales tenían de 5 a 6 meses de edad al inicio del protocolo experimental.

Todos los animales fueron alimentados con una dieta de laboratorio *ad libitum* (Global 2014l dieta; Teklad Harlan, Madison, WI) y se mantuvieron a 23°C bajo

un ciclo de luz/oscuridad de 12h/12h. El protocolo experimental fue aprobado por el Comité de Ética en Investigación Animal de la Facultad de Medicina de la Universidad de Valencia.

Protocolos de entrenamiento

Ratas jóvenes y viejas se sometieron a entrenamientos de resistencia (5 días/semana) en una tapiz rodante (Modelo 1050 LS Exer3/6; Columbus Instruments, Columbus, OH) al 75% $VO_{2máx}$ [324,325]. Durante todo el experimento, el grado de inclinación de la cinta fue del 15% para las ratas jóvenes y del 5% para las ratas viejas. Seguimos una modificación del protocolo de Davies *et al*. [326]. La sesión de entrenamiento del primer día fue de 25 y 15 minutos de duración para las ratas entrenadas jóvenes y viejas, respectivamente. La duración de cada período de entrenamiento se incrementó en 5 min cada día, de manera que el último día de la tercera semana, las ratas corrían a una velocidad de desplazamiento de 30 m/min (jóvenes) y 15 m/min (viejas) durante 1 hora. Los grupos no entrenados se ejercitaron a la misma velocidad durante sólo 10 min cada 3 días durante las 3 semanas. Se evaluó la capacidad de resistencia, antes y después del período de entrenamiento, mediante un test de carrera hasta el agotamiento a 26,8 m/min y con una inclinación del tapiz del 15% para las ratas jóvenes, y a 18 m/min y con una inclinación del tapiz del 5% para las ratas viejas [39].

Los ratones KO de PGC-1α y WT fueron asignados al azar a un grupo entrenamiento o control. Los grupos de entrenamiento completaron 4 semanas de entrenamiento en tapiz rodante 5 veces por semana y aumentando progresivamente hasta los 60 minutos a 20 m/min (10% de inclinación del tapiz) al final de la segunda semana. Se evaluó la capacidad de resistencia, antes y después del período de entrenamiento, mediante un test de carrera hasta el agotamiento a 20 m/min y con una inclinación del tapiz del 10%. Después de los tests, los animales estuvieron en reposo durante 48 horas antes de ser sacrificados.

Protocolo de exposición al frío

Después de un periodo de aclimatación (1 semana), ratas Wistar macho jóvenes y viejas fueron aleatoriamente divididas en dos grupos: animales expuestos al frío (4ºC ± 1°C durante 24 horas) y animales control (24ºC ± 1°C) [243]. Los músculos fueron extraídos inmediatamente tras finalizar la exposición al frío.

Tratamiento con triyodotironina (T3)

A ratas macho Wistar jóvenes y viejas se les inyectó intraperitonealmente T3 (0.4 mg/kg) o vehículo (0,9% NaCl-propilenglicol; 40:60 vol/vol). Los músculos fueron extraídos 6 horas después de las inyecciones [327]. Los músculos gastrocnemio y sóleo fueron retirados rápidamente, congelados inmediatamente y conservados a -80°C. Todos los animales fueron sacrificados mediante una sobredosis de pentobarbital sódico.

Determinaciones

1. Mediante Western Blotting se determinaron las concentraciones de: PGC-1α, NRF-1, citocromo C y α-Actina.
2. Mediante el kit de oxidación de proteínas 'OxyBlot' (Intergen) se determinó la carbonilación de proteínas como se ha descrito en anteriores estudios [328].

Los detalles de los métodos utilizados para realizar cada una de las determinaciones se pueden encontrar más detalladamente en las correspondientes publicaciones [23] (Ver Apéndice 2).

Análisis estadístico

Los resultados se expresan como media ± desviación estándar (SD). La normalidad de la distribución se comprobó mediante la prueba de Kolmogorov, y la homogeneidad de varianza fue evaluada con el método de Levene. Para la

capacidad de resistencia, se realizó un análisis de medidas repetidas de 2 factores. Para el análisis del entrenamiento se realizaron medidas repetidas (antes del entrenamiento en comparación con después), el segundo factor fue el estado de los animales (control o entrenamiento). El principal efecto del entrenamiento fue evaluado con la prueba *post hoc* de Newmann-Keuls. Para el análisis de los Western Blot, se utilizó un análisis de la varianza de dos vías (ANOVA) y la prueba de Bonferroni *post hoc* (Sigma Stats, versión 3.11). Los resultados se consideraron estadísticamente significativos para $p < 0,05$.

RESUMEN: RESULTADOS OBTENIDOS

C. RESUMEN DE LOS RESULTADOS OBTENIDOS

1. Estudio del papel de la enzima XO en el daño oxidativo, músculo-esquelético y cardiovascular mediante la valoración de diversos biomarcadores sanguíneos.

Las lesiones en el fútbol, tanto a nivel profesional como amateur, causan una enorme carga para la sociedad en términos de sufrimiento para los propios participantes y también en términos económicos. Debido a que el alopurinol es un fármaco seguro, se podría considerar la posibilidad de que los jugadores tomasen alopurinol para prevenir el daño a nivel músculo-esquelético y cardíaco. Por lo tanto, este estudio no solo aborda un problema específico de fisiología deportiva, sino también un problema de carácter general. El hecho de que en la última década los jugadores profesionales han comenzado a jugar tres partidos a la semana (a diferencia de antes, cuando sólo jugaban uno cada fin de semana) le da más fuerza a la posible acción preventiva del alopurinol en esta población. Por este motivo hemos estudiado el efecto del alopurinol en la prevención del daño músculo-esquelético y cardiovascular en futbolistas profesionales.

1.1 Efecto de la administración de alopurinol sobre marcadores de daño muscular en jugadores de fútbol.

Los jugadores que recibieron placebo mostraron un aumento significativo en la actividad sérica de CK, LDH y AST, y en los niveles séricos de mioglobina 12 horas después del partido. El alopurinol inhibió los aumentos de CK, AST y la mioglobina, y en parte, los de LDH inducidos por el ejercicio (Ver Tabla 4).

1.2. Efecto de la administración de alopurinol sobre marcadores de daño cardíaco en jugadores de fútbol.

Los niveles séricos de CK-MB y Hs-TnT fueron evaluados como marcadores específicos de lesión miocárdica. CK-MB y Hs-TnT aumentaron significativamente en el grupo placebo, pero no en el grupo tratado con alopurinol. No se observaron diferencias significativas entre los grupos placebo y alopurinol tras el partido (Ver Tabla 4).

1.3. Efectos del alopurinol sobre los marcadores de daño hepático en jugadores de fútbol

La actividad ALT sérica se midió como biomarcador de daño hepático. La actividad de dicha enzima aumentó significativamente después del partido tanto en el grupo placebo como alopurinol, poniendo de relieve la falta de efecto del alopurinol en la actividad de la ALT a nivel hepático (Ver Tabla 4).

1.4. Efecto de la administración de alopurinol sobre el ácido úrico sérico y la peroxidación lípidica plasmática en jugadores de fútbol.

Para establecer el papel de los RLs derivados de la XO inducidos por el ejercicio físico en el daño tisular, determinamos los niveles séricos de ácido úrico en los jugadores. Los jugadores a los que se les administró alopurinol, pero no en los del grupo placebo, mostraron una disminución significativa en los niveles séricos de ácido úrico 12 horas después del partido (Ver Tabla 6). Anteriormente ya observamos que deportistas de alto nivel a los que se les administró alopurinol, pero no en los del grupo placebo, exhibieron una disminución significativa de RLs derivados de la XO implicados en la peroxidación lipídica [3]. Del mismo modo, en este estudio también observamos diferencias significativas en los niveles de peroxidación lipídica plasmática determinada como MDA entre los grupos placebo

y alopurinol tras el partido (Ver Tabla 4). El alopurinol previene la peroxidación lipídica inducida por el ejercicio físico como reflejan los valores de MDA.

Para excluir la posibilidad de que los cambios en los biomarcadores se debieran a una hemoconcentración, medimos las concentraciones séricas de sodio, calcio y GGT. Como no hubo diferencias significativas entre los grupos placebo y tratado con alopurinol (resultados no mostrados), llegamos a la conclusión de que los cambios observados en los biomarcadores no son una consecuencia de la hemoconcentración.

1.5. Variables antropométricas y perfiles de actividad física en los jugadores de fútbol.

Los siguientes datos de actividad se midieron durante el partido: la distancia total recorrida, la distancia recorrida a alta intensidad, y la distancia cubierta en carreras cortas. No observamos ninguna diferencia significativa entre el grupo placebo y el grupo tratado con alopurinol. Asimismo, tampoco se encontraron diferencias en las siguientes variables antropométricas: peso, talla, masa grasa y masa muscular, entre los distintos grupos experimentales (Ver Tabla 5).

	Placebo			Allopurinol		
	Basal	Post-match	Δ	Basal	Post-match	Δ
Skeletal muscle						
CK (U/L)	229 ± 123	1287 ± 1092$^{\#\#}$	1103	199 ± 218	592 ± 536*	379
LDH (U/L)	199 ± 36	449 ± 69$^{\#\#\#}$	180	200 ± 42	353 ± 58$^{\$\$\$,**}$	151
AST (U/L)	22 ± 3	55 ± 36$^{\#\#}$	21	22 ± 6	30 ± 9*	6
Myoglobin (ng/mL)	33.3 ± 5.3	121.8 ± 98.5$^{\#\#}$	88.0	36.2 ± 31.2	63.6 ± 18.4*	15.8
Cardiac muscle						
CK-MB (μg/L)	2.4 ± 1.6	9.3 ± 9.8$^{\#}$	6.5	1.9 ± 1.9	4.3 ± 3.7	2.2
Hs-TnT (ng/L)	5.5 ± 2.5	14.1 ± 3.4$^{\#}$	8.6	4.8 ± 3.8	9.5 ± 5.8*	6.6
Liver						
ALT (U/L)	7 ± 3	19 ± 4$^{\#\#\#}$	11	8 ± 2	22 ± 4$^{\$\$\$}$	13
Oxidative damage						
MDA (nmol/mL)	0.6 ± 0.4	0.9 ± 0.3	0.3	0.6 ± 0.3	0.6 ± 0.3*	0.0

ALT (alanine aminotransferase), AST (aspartate aminotransferase), CK (creatine kinase), CK-MB (creatine kinase myocardic isoenzyme), Hs-TnT (high sensitive troponin T), LDH (lactate dehydrogenase), MDA (malondialdehyde). Values as means ± SD (n=6 placebo; n=6 allopurinol). Δ is defined as the difference between post-match and basal. Ratio of effect is calculated as the Δ (placebo) / Δ (allopurinol).
$^{*}p<0.05$ and $^{**}p<0.01$ between placebo and allopurinol groups in post-match;
$^{\$}p<0.05$ and $^{\$\$\$}p<0.001$ in allopurinol group between basal and post-match;
$^{\#}p<0.05$, $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$ in placebo group between basal and post-match

Tabla 4. *Parámetros bioquímicos de daño muscular, cardíaco, hepático y oxidativo en jugadores de fútbol. Efectos de la administración de alopurinol [11].*

		Placebo	Allopurinol
Anthropometric variables			
Age (years)		26.3 ± 3.4	25.1 ± 2.2
Weight (Kilograms)		78.9 ± 8.9	71.8 ± 6.3
Height (meters)		1.8 ± 0.1	1.8± 0.1
Muscle mass (Kilograms)		37.5 ± 3.3	35.0 ± 2.6
Fat mass (Kilograms)		8.8± 1.5	7.6 ± 1.1
Physical activity profiles			
Total distance (m)	1st half	5508.3 ± 524.2	5321.6 ± 444.2
	2nd half	5235.8 ± 1195.4	5258.6 ± 523.0
Distance covered at HI (21Km/h-24Km/h)	1st half	349.3 ± 153.0	194.4 ± 81.6
	2nd half	359.0 ± 91.4	298.0 ± 126.1
Distance covered in sprint (>24Km/h) (m)	1st half	197.0 ± 98.9	69.0 ± 43.8
	2nd half	160.8 ± 37.3	169.2 ± 110.2

HI: High Intensity; m: meters. Values as means ± SD (n=6 placebo; n=6 allopurinol).

***Tabla 5**. Variables antropométricas y perfiles de actividad física de los jugadores durante un partido [11].*

1.6. Efecto de la administración de alopurinol sobre nuevos biomarcadores de daño cardiovascular en jugadores de fútbol

La concentración de copeptina mostró una disminución tras el partido en el grupo placebo, aunque ésta no fue significativa. Sin embargo, en el grupo alopurinol observamos un aumento, aunque tampoco fue significativo, después del partido. No se observaron diferencias significativas entre los grupos placebo y alopurinol al inicio del estudio, mientras que la diferencia entre los grupos placebo y alopurinol después del partido fue estadísticamente significativa (Ver Tabla 6).

Los niveles de MR-proADM séricos aumentaron significativamente en el grupo placebo después del partido ($p = 0,02$). Del mismo modo, también aumentó en el grupo alopurinol, aunque esta variación no fue estadísticamente significativa. No se observaron diferencias significativas entre los grupos placebo y alopurinol en condiciones basales o tras el partido (Ver Tabla 6).

Los niveles séricos de GDF15 aumentaron significativamente tanto en el grupo placebo como en el grupo de alopurinol tras el partido. No se observaron diferencias significativas entre los grupos placebo y alopurinol, tanto a nivel basal como después del partido (Ver Tabla 6).

Por lo que respecta a la concentración sérica de PlGF y VEGFR1/Flt-1, no se observaron cambios estadísticamente significativos antes o después del partido en ninguno de los grupos experimentales. Tampoco se observaron diferencias si comparamos al grupo placebo con el grupo alopurinol, ni antes ni después del partido (Ver Tabla 6).

	Placebo		Alopurinol	
	Basal	Post-match	Basal	Post-match
Uric acid (mg/dL)	5.2±0.6	5.3±0.8	5.1±1.2	3.8±0.7$
Copeptin (pmol/L)	9.8 ± 6.2	6.9 ± 2.3	9.4 ± 3.6	12.2 ± 4.2*
MR-proADM (nmol/L)	0.26 ± 0.10	0.36 ± 0.06#	0.29 ± 0.06	0.35 ± 0.05
GDF15 (pg/dL)	227.0 ± 56.6	299.2 ± 70.6#	239.8 ± 73.4	313.1 ± 83.7$
PlGF (pg/dL)	2.62 ± 0.20	2.51 ± 0.30	2.81 ± 0.30	2.79 ± 0.30
sVEGFR1/sFLT-1 (pg/dL)	55.5 ± 9.3	62.12 ± 13.0	56.4 ± 11.9	65.8 ± 8.8

Tabla 6. *La tabla muestra las tendencias de los biomarcadores séricos en los futbolistas que recibieron placebo o alopurinol antes y después del partido. Los valores se muestran como medias ± SD (n=6 placebo, n=6 alopurinol). * p<0,05 entre los grupos placebo y alopurinol post-partido; $ p<0,05 entre basal y post-partido en el grupo alopurinol; # p<0,05 entre basal y post-partido en el grupo placebo.*

1.7. Efecto de la administración de alopurinol sobre los niveles séricos de suPAR en jugadores de fútbol.

La figura 15 muestra los niveles séricos de suPAR antes y después del partido en ambos grupos (placebo y alopurinol). La concentración de suPAR aumentó de 2,0 ± 0,3 a 2,3 ± 0,4 ng/mL en el grupo placebo (p = 0,14) y de 1,8 ± 0,5 a 2,0 ± 0,4 ng/mL en el grupo alopurinol (p = 0,20).

No se encontraron cambios estadísticamente significativos antes o después del partido en ninguno de los grupos experimentales. Además, no se observaron diferencias entre los niveles basales de SuPAR de ambos grupos (2,0 ± 0,3 frente a 1,8 ± 0,5; p = 0,20) o entre los niveles post-partido (2,3 ± 0,4 frente a 2,0 ± 0,4; p = 0,18).

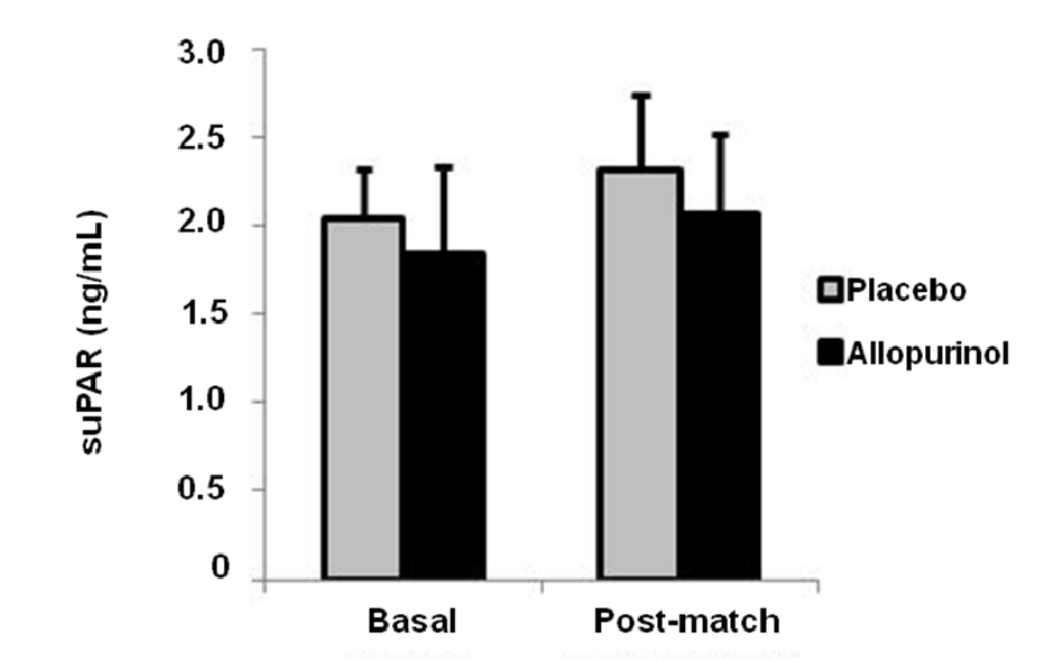


Figura 15. *Niveles de suPAR en jugadores de fútbol profesional antes y después de un partido de fútbol. Los atletas también se dividieron en los que tomaron una dosis oral de 300 mg de alopurinol (n = 6) o placebo (n = 6) [13] .*

2. Estudio del efecto del ejercicio físico de alta intensidad (ultramaratón) sobre diferentes biomarcadores de daño cardiovascular y renal en sujetos entrenados.

Todos los atletas que formaron parte de este estudio completaron con éxito la ultramaratón, sin síntomas clínicamente significativos. La concentración de creatinina aumentó significativamente en un 31% (IQR: 13-58%) después de la carrera, mientras que la tasa estimada de filtración glomerular (EGFR) se redujo en el mismo grado. Las concentraciones séricas de copeptina y MR-proADM aumentaron notablemente después de los 60 km de carrera, 6,4-veces (IQR: 2,7-10,4) y 2,3-veces (1,8-2,6) (Ver Tabla 7), respectivamente. Se observó una correlación altamente significativa entre el aumento delta (es decir, la relación entre el valor post- y el valor pre-carrera) de creatinina y MR-proADM (r = -0,680, p = 0,004), pero no entre creatinina y copeptina (r = -0,064, p = 0,81) (Ver Figuras 16 y 17).

	Pre-run	**Post-run**	**P**
Creatinine (μmol/L)	68 (60-76)	92 (72-114)	<0.001
EGFR (mL/min/1.73 m^2)	111 (98-127)	80 (61-106)	<0.001
Copeptin (pmol/L)	5.9 (5.0-10.3)	40.6 (20.3-100.2)	<0.001
MR-proADM (nmol/L)	0.29 (0.27-0.34)	0.68 (0.51-0.80)	<0.001

Tabla 7. *Variación de la creatinina sérica, tasa estimada de filtración glomerular (EGFR), copeptina y MR-proADM después de una ultramaratón de 60 km (n = 16) [15].*

Finalmente, el porcentaje de sujetos que muestran valores por encima del límite del rango de referencia superior antes de la ultramaratón fue del 0%, tanto para copeptina como para MR-proADM, pero aumentó después del ejercicio en un 81% y un 63%, respectivamente.

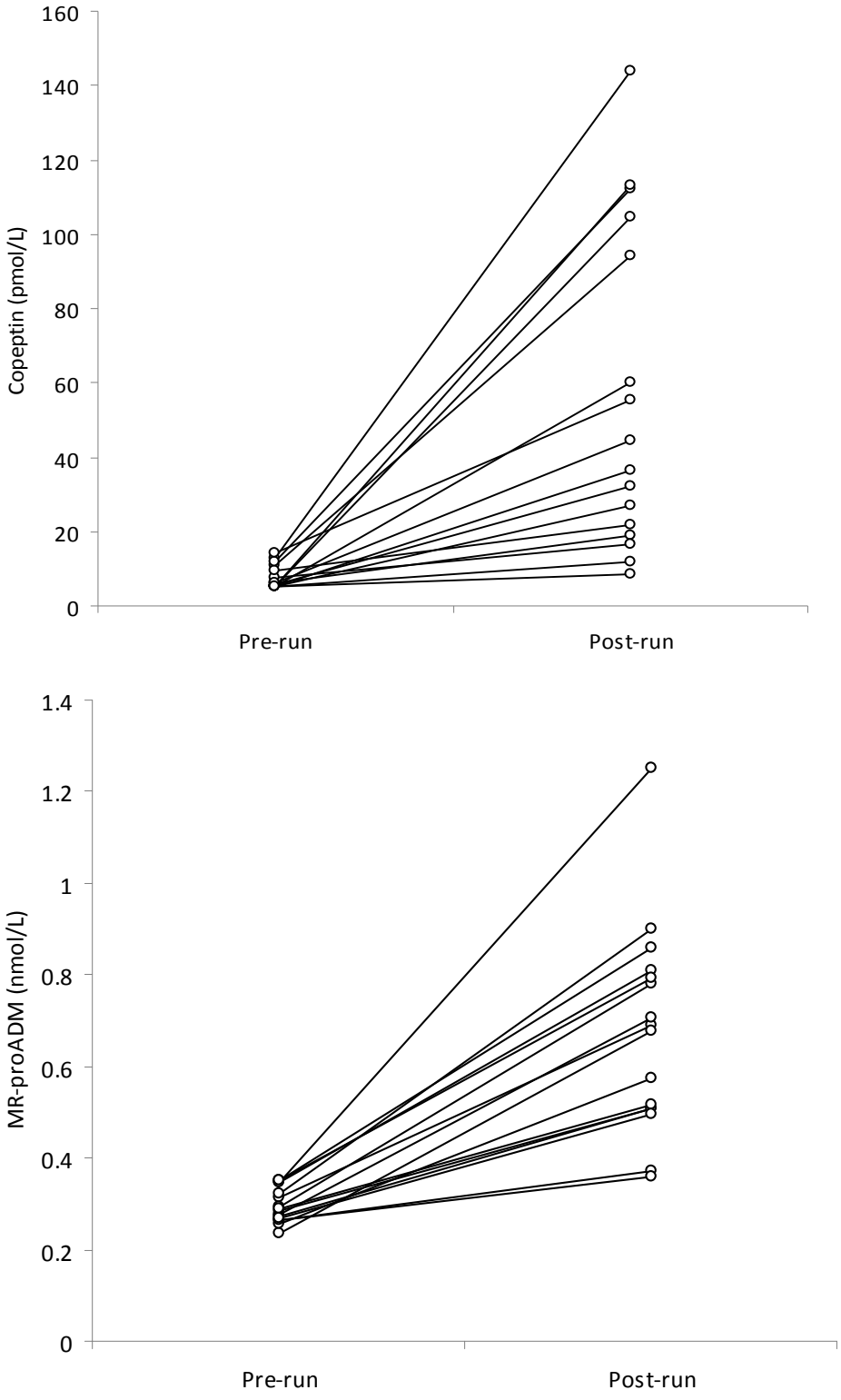
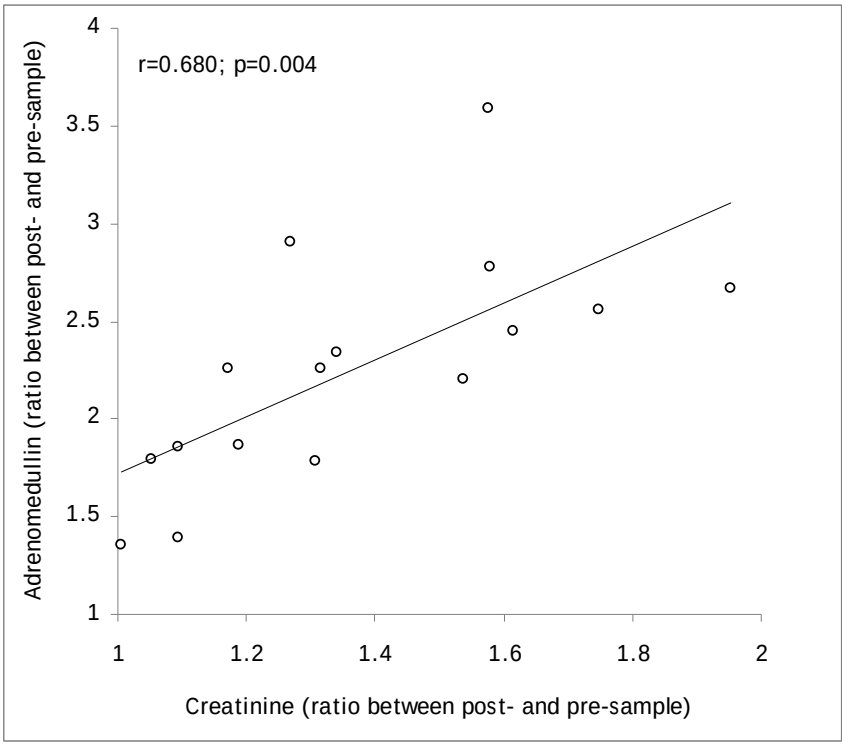


Figura 16. *Variación individual de la copeptina y MR-proADM después de una ultramaratón de 60 km (n = 16) [15].*



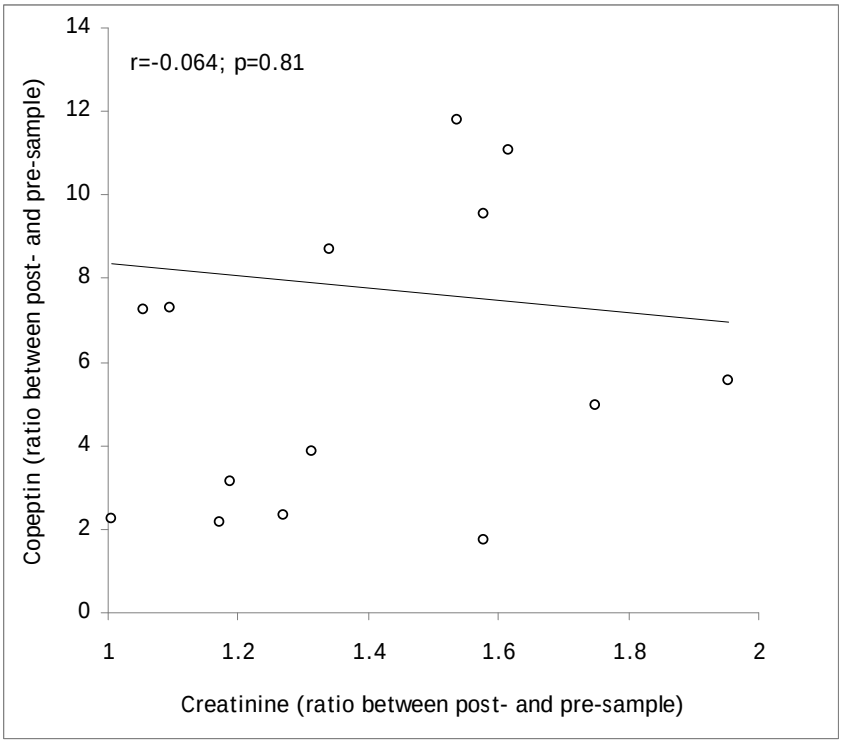


Figura 17. *Análisis de regresión lineal y el coeficiente de correlación de Spearman (r) entre el aumento delta (es decir, la relación entre el valor post- y pre-competición) de la creatinina y copeptina, y de creatinina y MR-proADM [15].*

Los cambios de volumen plasmático después de la carrera, calculados como pre- (4,51 g/L; IQR: 4,31-4,70 g/L) y post-ejercicio (4,80 g/L; IQR: 4,63-4,96 g/L; p < 001) a partir de la variación de la concentración de albúmina, fue de un 5,6 % (IQR: 2,2%-7,0%). Básicamente, todos los parámetros de la prueba mostraron una variación sustancial después del ejercicio. De acuerdo con resultados previos [329-332], la creatinina sérica y urinaria aumentó en un 38 % (IQR: 14% - 66%) y un 78% (IQR: 21%-158%), respectivamente. El EGFR disminuyó en un 31% (IQR: 14%-44%). sNGAL, uNGAL y la relación uNGAL/creatinina aumentaron 1,6 veces (IQR: 1,3 a 2,0 veces), 7,7 veces (IQR: 1,9 a 37,3 veces) y 2,9 veces (IQR: 1,6 a 25,8 veces), respectivamente (Ver Tabla 8). No se observaron valores anormales de EGFR (es decir, < 60 ml/min/1,73 m^2) en ninguno de los atletas pre-ejercicio y en cinco atletas (29 %; p = 0,015) después del ejercicio. En seis de los 16 atletas (38%) , el aumento agudo de la creatinina sérica reunió los criterios de la *Acute Kidney Injury Network* (AKIN) para considerar una enfermedad renal aguda (Estadio 1) , que se define como el 50% de aumento en la creatinina sérica [333]. En cuanto a los valores de NGAL, se observaron niveles superiores al nivel de corte del test (es decir, 200 ng/ml) pre-ejercicio en un atleta para sNGAL y en ninguno para uNGAL, así como en uno para sNGAL (es decir, 6% vs 6 %, p = ns) y dos para uNGAL (es decir, 0 % vs 12 %, p = 0,144) después del ejercicio (Ver Figura 18).

Curiosamente, no hemos podido observar ninguna relación significativa entre las concentraciones antes del ejercicio para sNGAL y uNGAL (r = - 0,147; p = 0,574), así como en la relación sNGAL y uNGAL/creatinina antes del ejercicio (r = -0,110; p = 0,673). Del mismo modo, no se encontró relación significativa entre los valores de sNGAL y uNGAL después del ejercicio (r = 0,085; p = 0,746) ni entre sNGAL y la relación uNGAL/creatinina después del ejercicio (r = 0,192; p = 0,460)

No se encontró un correlación significativa en la variación post- ejercicio de NGAL expresada como una relación entre los valores post- y pre-ejercicio

(sNGAL frente a uNGAL; r = 0,025; p = 0,925 y sNGAL frente a la relación uNGAL/ creatinina; r = - 0,016; p = 0,950). Por último, se encontró una correlación altamente significativa entre los cambios pre- y post-ejercicio de la creatinina sérica y sNGAL (r = 0,813; p < 0,001), pero no entre los cambios pre- y post- ejercicio de la creatinina sérica y uNGAL (r = 0,248; p = 0,338) o la relación uNGAL/creatinina (r = 0,254; p = 0,325).

	Pre-exercise	**Post-exercise**	**p-Value**
Serum creatinine (μmol/L)	68 (58-76)	98 (76-118)	<0.001
Urinary creatinine (μmol/L)	106 (76-142)	168 (138-257)	<0.001
eGFR (mL/min/1.73 m^2)	112 (97-133)	75 (57-99)	<0.001
Serum NGAL (ng/mL)	105 (86-158)	196 (139-290)	<0.001
Urinary NGAL (ng/mL)	4.4 (0.5-33.9)	35.6 (12.5-86.3)	<0.001
Urinary NGAL to creatinine ratio	0.05 (0.01-0.26)	0.17 (0.06-0.60)	<0.001

Tabla 8.*Variación de los niveles de creatinina, EGFR y NGAL después de una ultramaratón de 60 km (n = 16) [14].*

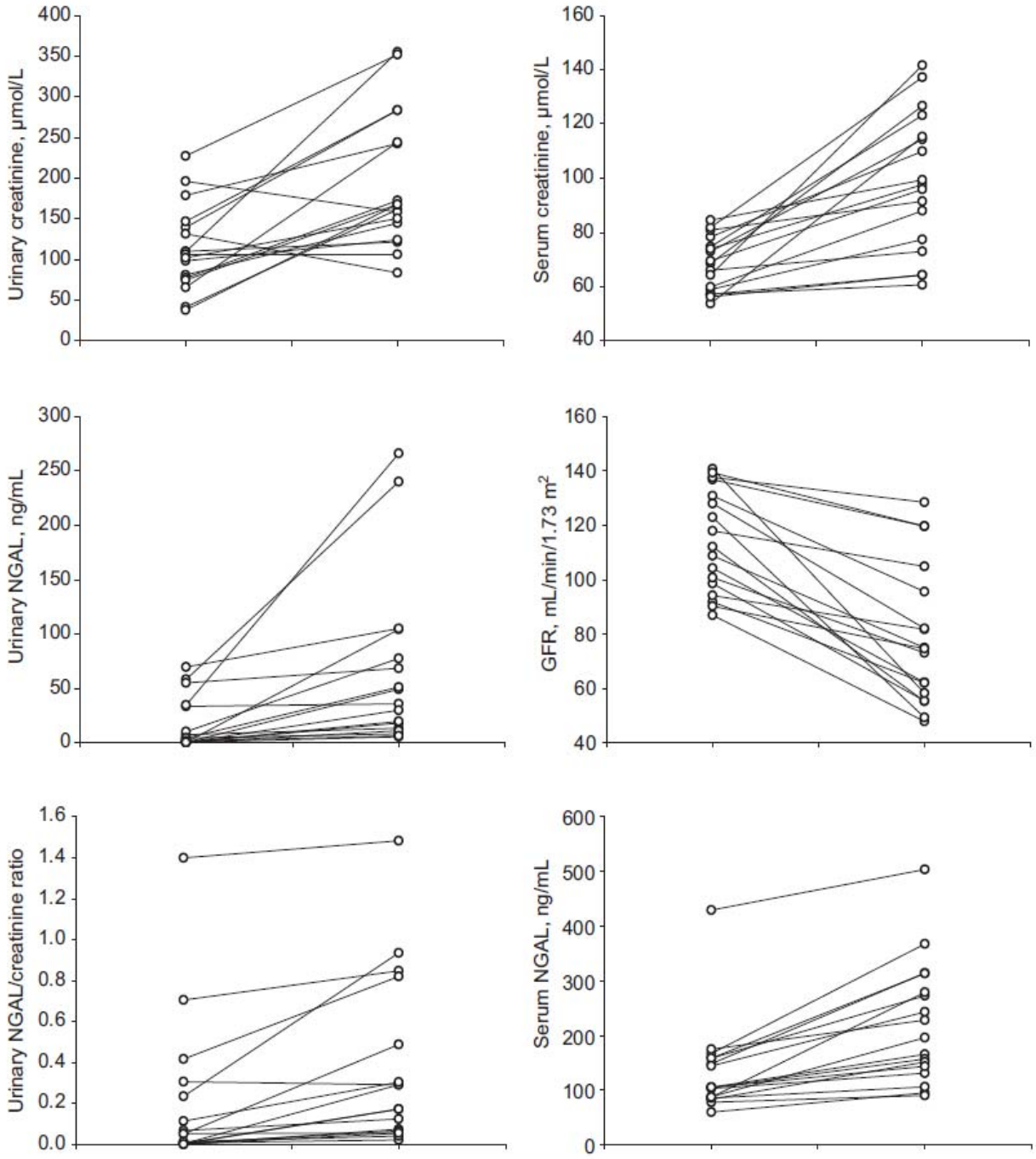


Figura 18. *Variaciones individuales de los niveles de creatinina, EGFR y NGAL después de una ultramaratón de 60 km (n = 16) [14].*

3. Estudio de las implicaciones del ejercicio físico de alta intensidad sobre la longevidad en sujetos entrenados.

Como hemos visto anteriormente, el ejercicio físico extenuante o de alta intensidad produce un incremento en ciertos marcadores de daño muscular y cardíaco. Así pues, mientras que los beneficios de la actividad física moderada practicada de forma regular se han demostrado claramente, existe una importante controversia sobre los posibles efectos adversos del ejercicio físico de alta intensidad practicado de forma regular. En este contexto, el efecto de este tipo de ejercicio sobre la longevidad de los deportistas de élite es de especial interés, aunque los datos que tenemos en la actualidad son escasos. El Tour de Francia (TdF) es sin duda una de las competiciones deportivas fisiológicamente más exigentes [334]. El esfuerzo realizado se ha comparado con hacer una maratón varios días a la semana durante casi tres semanas [335] (Ver Tabla 9), mientras que la altura total de las subidas se puede comparar con subir tres veces el Everest. Con la finalidad de comprobar este fenómeno hemos analizado la longevidad de los ciclistas que participaron en el TdF entre los años 1930-1964.

	TOURS 1930-1964
Average of total kilometers performed per tour	4537.7±238.5 (Km)
Average total time employed per tour	138.0±16.2 (h)
Average of completed tours per cyclist	2.4±2.0 (times)
Average age of cyclists who competed in the tours	27.3±3.6 (years)
Average speed (all tours)	33.1±2.7 (Km/h)

Tabla 9. *Algunas características del Tour de Francia celebrados entre 1930 y 1964 [18].*

La Figura 19 muestra que la longevidad de los participantes en el TdF es significativamente mayor que la de la población general (al comparar el área bajo la curva correspondiente a los participantes TdF con la de la población general; p <0,05). La media de supervivencia entre 65 y 115 años fue del 39,1% en los participantes del Tour, mientras que para la población general fue del 21,5%. La edad a la que el 50% de la población general murió fue de 73,5 frente a 81,5 años en los participantes del TdF, es decir, un 11% mayor (Ver Figura 19).

Hay que tener en cuenta que los valores en el eje de ordenadas son el porcentaje de participantes vivos. Por ejemplo, de los 5 sujetos que nacieron en 1900 y que compitieron en el TdF entre 1930 y 1964, 2 estaban vivos el 31 de Diciembre de 2007 (40%). Teniendo en cuenta todas las edades estudiadas, el porcentaje medio de supervivencia de los participantes en el TdF (área bajo la curva) fue de un 17% superior a la de la población general.

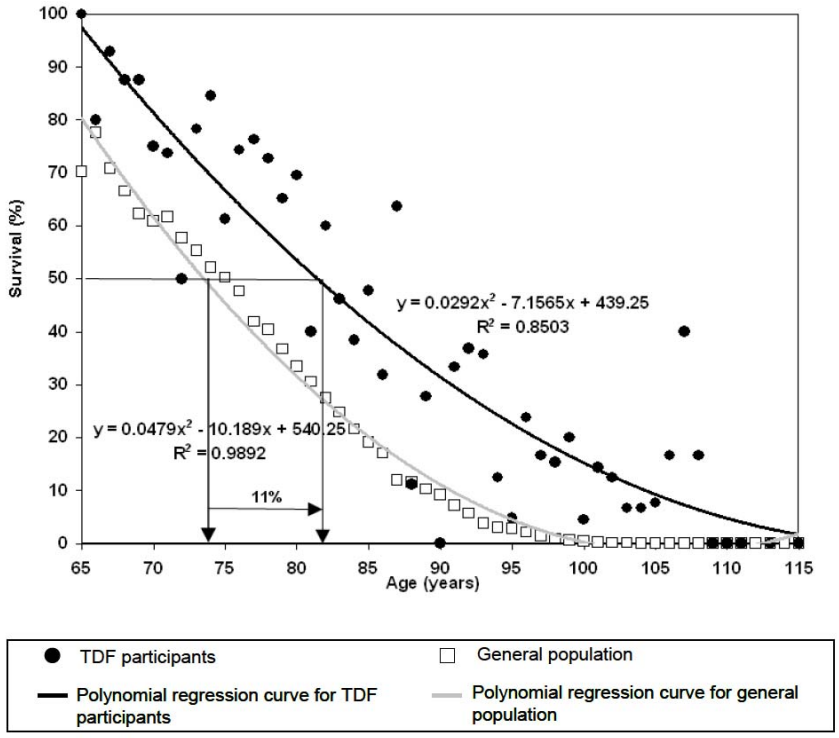


Figura 19. *Porcentaje de supervivencia relacionada con la edad de los participantes en el TdF y la población general. Se incluyeron los sujetos nacidos entre 1892 y 1942. La vida media de los participantes en el TdF es mayor (p = 0,004; 17,5%) que la población general del mismo país en el que los ciclistas nacieron. La edad media en la que el 50% de la población murió fue de 73,5 frente a 81,5 años en los participantes del TdF, es decir, un 11% superior [18].*

4. Estudio sobre el papel del entrenamiento aeróbico en la inducción de la cascada de la biogénesis mitocondrial y sus implicaciones en el envejecimiento músculo-esquelético.

Uno de los mecanismos por los que el entrenamiento puede ejercer efectos beneficiosos sobre la salud de los deportistas es el de la inducción de la mitocondriogénesis. En este trabajo nos planteamos su estudio tanto en animales jóvenes como viejos y KO de PGC-1α.

4.1. Efecto del envejecimiento o la supresión de PGC-1α sobre la resistencia aeróbica en respuesta al entrenamiento.

El tiempo de carrera hasta el agotamiento durante un test de resistencia fue aproximadamente un 63% más bajo en las ratas viejas que en las jóvenes (Ver Tabla 10a). Del mismo modo, el tiempo de carrera hasta el agotamiento fue aproximadamente un 65-70% inferior en los ratones KO de PGC-1α que en los animales WT (Ver Tabla 10b). Estos resultados concuerdan con estudios previos que demostraron que la capacidad de resistencia es menor en animales KO de PGC-1α que en WT [336]. La Tabla 10a también muestra que la intensidad y duración del programa de entrenamiento seguido por los animales jóvenes y viejos fue suficiente para inducir una mejora significativa en la resistencia aeróbica (~200% y ~135%, respectivamente). Asimismo, el protocolo de entrenamiento aumentó la capacidad de resistencia tanto en los ratones WT como en los KO de PGC-1α (~284% y ~173%, respectivamente) (Ver Tabla 10b). No se puede descartar que las diferencias encontradas en la prueba de resistencia entre las ratas jóvenes y viejas puedan explicarse por las diferentes duraciones de los protocolos de entrenamiento, aunque esto es poco probable. Ambos grupos mejoraron su capacidad de resistencia después de un protocolo de entrenamiento de intensidad

similar (~75% de su VO_{2max}). Las diferentes duraciones de los entrenamientos podrían explicar, en parte, algunos de nuestros resultados.

	Untrained	Trained	Untrained	Trained
Endurance capacity (min)[a]	Young rats		Aged rats	
Before	36.8±4.6	42.0±12.1	13.2±4.8	14.7±5.8
After 3 weeks	38.5±4.0	115.7±18.2*, **	19.7±7.5	46.3±6.5*, **
Endurance capacity (min)[b]	Wild-type mice		PGC-1α KO mice	
Before	37.0±24.5	39.3±26.2	12.0±6.9	13.2±8.8
After 4 weeks	41.3±27.8	158.8±53.3***, ****,*****	21.2±3.6	58.0±16.4***, ****

Table 10. *Mejora en la resistencia aeróbica inducida por el entrenamiento en los animales. a) Media (± DE) de los resultados de la resistencia aeróbica antes y después del entrenamiento de resistencia en ratas jóvenes y viejas. Veinticuatro ratas Wistar macho fueron divididas aleatoriamente en cuatro grupos experimentales: jóvenes no entrenadas (n = 6), jóvenes entrenadas (n = 6), viejas no entrenadas (n = 6), y viejas entrenadas (n = 6). b) Media (± DE) de los resultados de la prueba de resistencia máxima antes y después del entrenamiento de resistencia en ratones WT y KO de PGC-1α. Veintisiete ratones macho se dividieron al azar en cuatro grupos experimentales: WT no entrenados (n = 6), WT entrenados (n = 8), KO de PGC-1α no entrenados (n = 7), y KO de PGC-1α entrenados (n = 6) [23].*
** p <0,05 en comparación con los valores anteriores al entrenamiento*
*** p <0,05 en comparación con los grupos no entrenados*
**** p <0,05 en comparación con los valores anteriores al entrenamiento*
***** p <0,05 en comparación con el grupo no entrenado*
****** p <0,05 en comparación con los ratones KO de PGC-1α entrenados*

4.2. Mitocondriogénesis muscular en ratones KO de PGC-1α y en ratones viejos.

La Figura 20 muestra que la mitocondriogénesis muscular se deteriora considerablemente en los ratones deficientes en PGC-1α. El panel A muestra que el entrenamiento provocó un aumento en el contenido de PGC-1α en ratones WT.

Como era de esperar, no encontramos ninguna presencia de la proteína PGC-1α en los animales sedentarios o en los animales KO de PGC-1α entrenados. Aunque la banda correspondiente a la proteína PGC-1α estaba ausente en nuestros ratones KO (92 kDa), una banda débil, que es probable que sea PGC-1β, apareció en el Western Blotting a más de 100 kDa.

La Figura 20 (Panel B) muestra que el entrenamiento aumentó los niveles de NRF-1, un intermediario crítico de la vía mitocondriogénica, en los animales WT, pero

no en los KO de PGC-1α. También observamos que los animales KO de PGC-1α sedentarios tienen considerablemente menos NRF-1 que los controles.

El contenido mitocondrial se puede medir directamente, usando estimaciones morfométricas del volumen organular en relación con el total de volumen celular. Más comúnmente, se estima por el cambio en la actividad máxima, medida en condiciones óptimas *in vitro*, de una "enzima marcadora" típica como es la citrato sintasa, o por el cambio en el contenido de una sola proteína como es la citocromo C [337,338]. Varios autores han utilizado la citocromo C como un marcador de masa mitochondrial [285,289,336], y ésta es la metodología que nosotros hemos utilizado. El entrenamiento provocó un aumento en el contenido de citocromo C en los ratones WT, pero no en los ratones KO de PGC-1α. El contenido de citocromo C en los animales KO de PGC-1α también fue menor que en los WT (Panel C).

En resumen, la deficiencia de PGC-1α empeoró la activación de la vía mitocondriogénica en respuesta al entrenamiento. En ratas viejas encontramos resultados muy similares, es decir, una pérdida en la capacidad de aumento de los niveles de PGC-1α, NRF-1 y citocromo C inducidos por el ejercicio (Figura 20; Paneles D, E y F).

La idea principal de este estudio es que el envejecimiento se asemeja a la deficiencia de PGC-1α en términos de falta de respuesta al entrenamiento. En efecto, los animales jóvenes mostraron un aumento en los niveles de proteína de PGC-1α después del entrenamiento que no se producen en los animales viejos (Figura 20; Panel D).

En el panel E demostramos que el contenido de NRF-1 muscular de las ratas jóvenes aumentó tras el entrenamiento. Este efecto se pierde cuando se estudia en animales viejos.

Finalmente, el panel F muestra que el entrenamiento aumenta el contenido de citocromo C en el músculo de las ratas jóvenes, pero de nuevo este efecto también se perdió en ratas viejas.

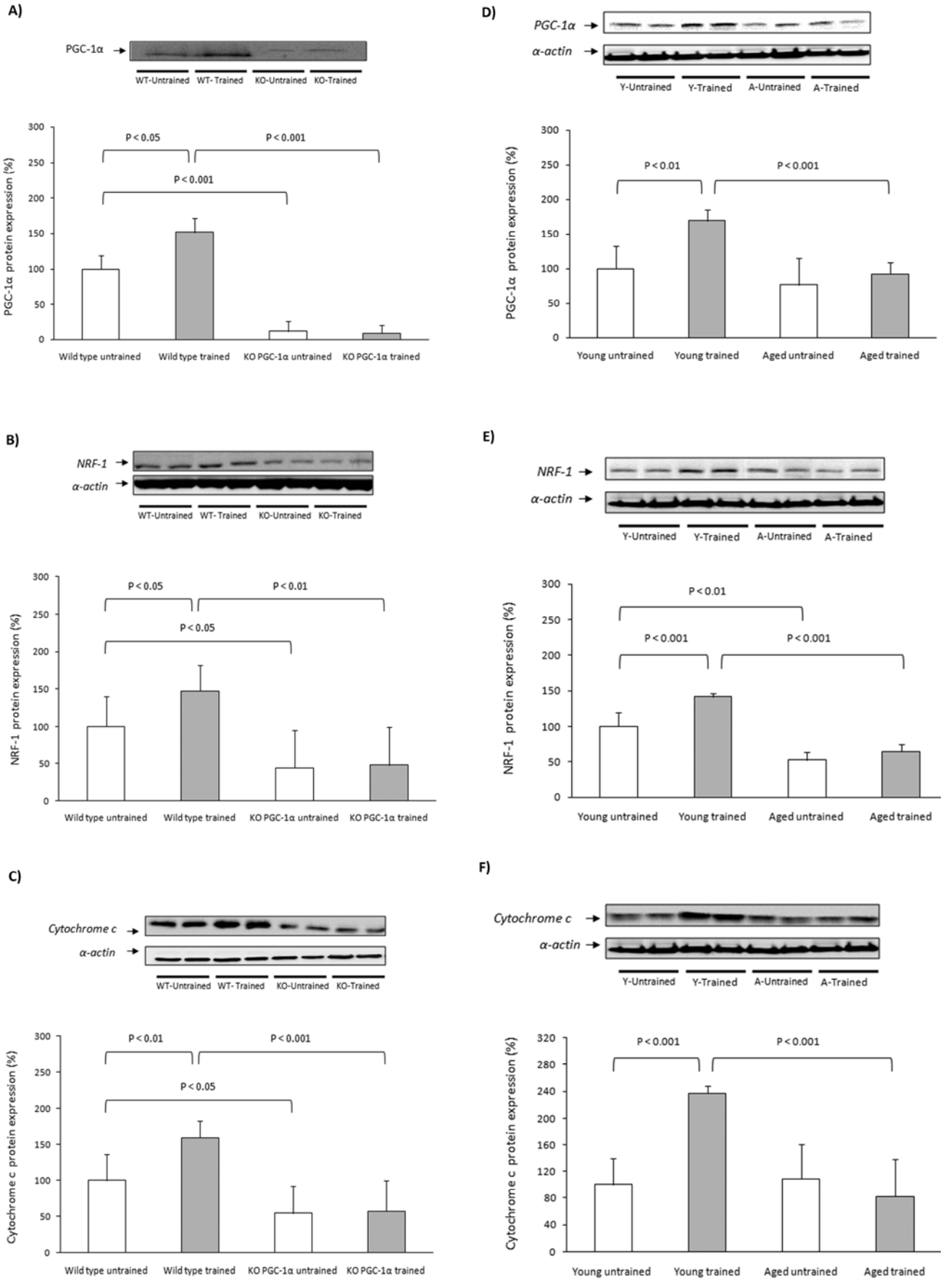

A)
PGC-1α
WT-Untrained
WT- Trained
KO-Untrained
KO-Trained
PGC-1α protein expression (%)
P < 0.05
P < 0.001
P < 0.001
Wild type untrained
Wild type trained
KO PGC-1α untrained
KO PGC-1α trained
B)
NRF-1
α-actin
NRF-1 protein expression (%)
P < 0.05
P < 0.01
P < 0.05
C)
Cytochrome c
α-actin
Cytochrome c protein expression (%)
P < 0.01
P < 0.001
P < 0.05
D)
PGC-1α
α-actin
Y-Untrained
Y-Trained
A-Untrained
A-Trained
PGC-1α protein expression (%)
P < 0.01
P < 0.001
Young untrained
Young trained
Aged untrained
Aged trained
E)
NRF-1
α-actin
NRF-1 protein expression (%)
P < 0.01
P < 0.001
P < 0.001
F)
Cytochrome c
α-actin
Cytochrome c protein expression (%)
P < 0.001
P < 0.001

Figura 20. *Activación inducida por el ejercicio de la vía de la biogénesis mitocondrial en el músculo esquelético. Análisis de Western Blotting para detectar PGC-1α (A y D), NRF-1 (B y E), y citocromo C (C y F) en ratones KO de PGC-1α y ratas viejas. Veintisiete ratones macho se dividieron al azar en cuatro grupos experimentales: WT no entrenado (n = 6), WT entrenado (n = 8), KO de PGC-1α no entrenado (n = 7), y KO de PGC-1α entrenado(n = 6). Veinticuatro ratas Wistar macho fueron divididas aleatoriamente en cuatro grupos experimentales: jóvenes no entrenadas (n = 6), jóvenes entrenadas (n = 6), viejas no entrenadas (n = 6), y viejas entrenadas (n = 6). Se muestran blots representativos. Para el análisis densitométrico de los resultados, los valores se muestran como la media (± SD). El contenido de α-actina, un marcador usado como control de carga en el músculo esquelético, se determinó en todos los grupos experimentales [23].*

4.3. Estrés oxidativo y entrenamiento en animales viejos y KO de PGC-1α.

Como se observa en la Figura 21, muchas de las adaptaciones del músculo esquelético al entrenamiento en los animales viejos son similares a las encontradas en los animales KO de PGC-1α. Se midió el efecto del ejercicio físico sobre el estado de oxidación de las proteínas del músculo esquelético en los animales jóvenes y viejos, y en los animales WT y KO de PGC-1α. En la Figura 21 se muestra la oxidación de proteínas en los ratones KO de PGC-1α (Panel A) y en ratas viejas (Panel B). Los animales KO de PGC-1α muestran un aumento en la oxidación de proteínas en reposo (Panel A). Lo mismo ocurre en animales viejos (Panel B). No se observaron efectos del entrenamiento en ningún grupo experimental.

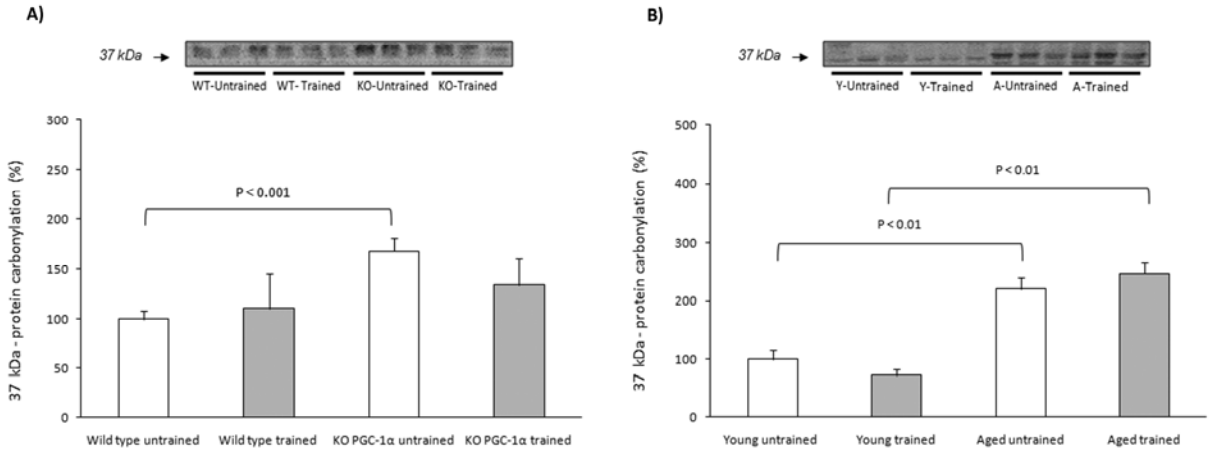


Figura 21. *Oxidación de proteínas musculares en animales entrenados y no entrenados. Análisis de Western blot para detectar proteínas carboniladas (37 kDa). Se muestran experimentos representativos. Para el análisis densitométrico de los resultados de los valores se muestran como la media (± SD) de: A) WT no entrenado (n = 6), WT entrenado (n = 8), KO de PGC-1α no entrenado (n = 7), y KO de PGC-1α entrenado (n = 6); B) joven no entrenado (n = 6), joven entrenado (n = 6), viejo no entrenado (n = 6), y viejo entrenado (n = 6) [23].*

4.4. Falta de activación de PGC-1α por la exposición al frío o por tratamiento con hormona tiroidea en animales viejos.

Los experimentos descritos anteriormente muestran que el músculo de los animales viejos no sobreexpresaron PGC-1α en respuesta al entrenamiento, haciéndonos pensar que el envejecimiento podría resultar en una falta de capacidad de respuesta de PGC-1α a otros estímulos fisiológicos. Dos de los estimuladores claves de PGC-1α son la hormona tiroidea [327] y la exposición al frío [243]. La Figura 22 (Panel A) muestra que los animales jóvenes, cuando se expusieron al frío, sobreexpresaron hasta 3 veces los niveles de PGC-1α, mientras que los animales viejos no lo hicieron. Lo mismo sucede con la administración de triyodotironina (T3) (Panel B).Nuestros resultados muestran que el músculo de ratas viejas presenta una marcada pérdida en la activación mitocondriogénica y que esto puede ser debido a la falta de inducción de PGC-1α [226]. Encontramos una sorprendente similitud entre la respuesta al entrenamiento en ratones KO de PGC-1α y en ratas viejas. En ratas jóvenes, PGC-1α se activó en el músculo esquelético no sólo por el entrenamiento, sino también por la exposición al frío o T3.

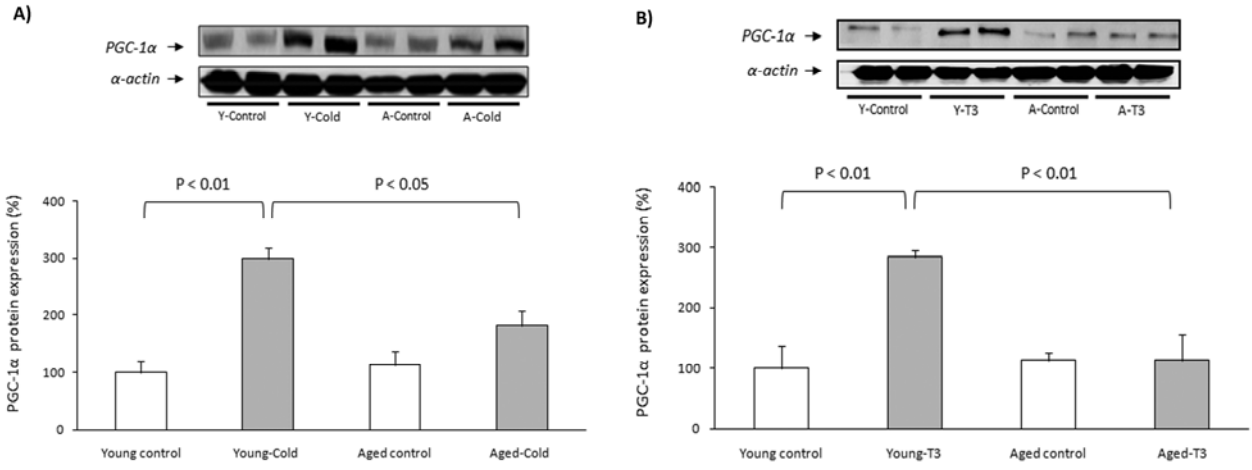


Figura 22. *Niveles de PGC-1α en el músculo esquelético de los animales expuestos al frío (4 ± 1°C) o tratados con triyodotironina (0,4 mg × kg^{-1}). Se muestran experimentos representativos. Para el análisis densitométrico de los resultados los valores se muestran como la media (± SD) de: A) Joven control (n = 4), joven expuesto a frío (n = 4), viejo control (n = 4), y viejo expuesto al frío (n = 4); B) Joven control (n = 4), joven tratado con T3 (n = 4), viejo control (n = 4), viejo tratado con T3 (n = 4). El contenido de α-actina, un marcador usado como control de carga en el músculo esquelético, se determinó en todos los grupos experimentales [23].*

Presentamos aquí que hay una falta de expresión de PGC-1α en el músculo esquelético de rata en respuesta al entrenamiento asociada a la edad o a cualquiera de los otros estímulos probados (Ver Figura 23).

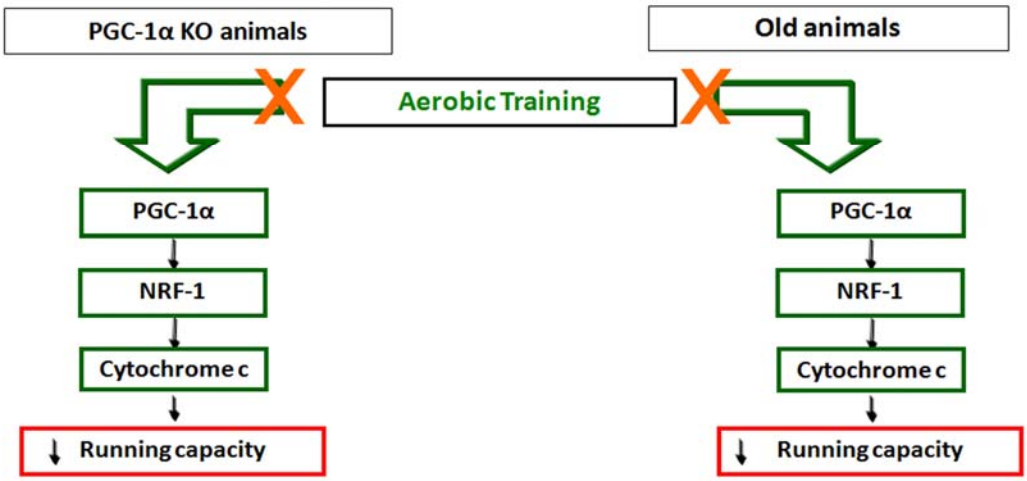


Figura 23. *Interpretación esquemática de los resultados con proposición de mecanismo. PGC-1α no es funcional en el músculo esquelético de animales viejos y puede estar implicado en la disminución de la biogénesis mitocondrial durante el envejecimiento [23].*

RESUMEN: DISCUSIÓN

D. DISCUSIÓN

1.- Estudio del efecto de la administración de alopurinol sobre el daño músculo-esquelético y cardiovascular inducido por ejercicio físico de alta intensidad en sujetos entrenados.

El ejercicio excéntrico puede provocar daño, debilidad y dolor muscular [339]. En algunos deportes como el fútbol, las microlesiones causadas por el ejercicio excéntrico pueden progresar a peor, pudiendo dar lugar a lesiones graves como resultado de las elevadas demandas musculares asociadas a la actividad competitiva [339]. Posteriormente aparece el fenómeno de la inflamación que ayuda a degradar y reparar el tejido. Dada la elevada acumulación de partidos en aquellos equipos que disputan varias competiciones durante el año (Liga Española, Ligas Europeas, Copa del Rey etc.) el daño muscular sufrido por los jugadores de fútbol puede tener consecuencias muy negativas sobre la incidencia de lesiones entre los futbolistas de los primeros equipos. La prevención y el tratamiento eficaz de lesiones en el fútbol son un desafío continuo para la medicina deportiva y los entrenadores [340]. Un buen enfoque para prevenir lesiones musculares en el fútbol puede ser contrarrestar el daño muscular causado por las contracciones repetidas realizadas tanto en el entrenamiento como en la competición, sin que ello afecte negativamente al rendimiento del jugador. El papel de alopurinol como un antioxidante en el ejercicio ha sido ampliamente estudiado por nuestro grupo [2,3,341,342] y otros equipos de investigación [343,344]. Todos estos estudios se han dirigido a determinar la implicación de la XO como una fuente de radicales libres en el músculo-esquelético. Así pues, se ha demostrado que la XO, enzima que genera RLs involucrados en el daño inducido por isquemia-reperfusión [345], causa daño muscular asociado con el ejercicio físico agotador [3,10,24]. El hallazgo nuevo de esta investigación es que el alopurinol previene el daño

músculo-esquelético y cardiovascular inducido por el ejercicio físico de alta intensidad en futbolistas de alto nivel.

El partido indujo un aumento significativo en los marcadores séricos de daño músculo-esquelético (CK, LDH, AST y mioglobina) que se previno eficazmente mediante la administración de alopurinol. A pesar de que los niveles séricos de estos marcadores dependen de edad, género, raza, masa muscular y actividad física [346], no se encontraron diferencias en estos parámetros entre los grupos alopurinol y placebo antes del partido. Se midió el daño oxidativo a lípidos mediante la determinación de un indicador fiable de lipoperoxidación, el MDA [8]. Como observamos anteriormente en ciclistas profesionales, la administración de alopurinol previene la peroxidación lipídica inducida por el ejercicio en jugadores de fútbol profesional [3].

No se encontraron diferencias en el rendimiento de los grupos durante el partido. De hecho, no se observaron diferencias entre los jugadores pertenecientes al grupo placebo y los tratados con alopurinol en su perfil de actividad basado en la distancia total cubierta, la distancia recorrida a alta intensidad y la distancia recorrida en carreras cortas. Estos hallazgos son consistentes con los de nuestro estudio anterior sobre los beneficios de la administración de alopurinol en ciclistas participantes en el TdF [3].

Se ha publicado recientemente que el alopurinol prolonga el tiempo total de ejercicio en pacientes con angina de pecho estable [347]. Sin embargo, nosotros no observamos ningún efecto ergogénico del alopurinol, probablemente debido a que este estudio no fue diseñado para identificar tal efecto, sino más bien los efectos beneficiosos del alopurinol para la integridad del músculo-esquelético.

Existen evidencias indiscutibles que muestran los efectos beneficiosos del ejercicio para prevenir y tratar enfermedades tales como las enfermedades cardiovasculares, osteoporosis y cáncer. Como se ha expuesto en la introducción de esta tesis, recientemente hemos introducido el concepto de que el ejercicio físico se comporta

como un fármaco [231,348]. Sin embargo, el entrenamiento de resistencia intenso y de larga duración favorece la remodelación y la susceptibilidad cardíaca específica a arritmias ventriculares [169,349]. El ejercicio físico intenso puede generar isquemia transitoria, estrés miocárdico y disfunción ventricular diastólica izquierda, induciendo a menudo un aumento en los parámetros bioquímicos analizados habitualmente en el diagnóstico de enfermedades cardíacas [350-352]. En nuestro estudio encontramos un incremento significativo en marcadores séricos de daño cardíaco, CK-MB y Hs-TnT, tras el partido, lo cual pudo ser prevenido mediante la administración de alopurinol.

El aumento de biomarcadores cardíacos relacionado con el ejercicio ha sido ampliamente descrito, pero no se ha encontrado una explicación fisiopatológica definitiva [43]. Parece plausible que estos incrementos no reflejen un daño miocárdico de elevada relevancia clínica, sino más bien el inicio de una lesión reversible de los cardiomiocitos [33,43]. Los efectos beneficiosos del tratamiento con alopurinol en diferentes patologías cardiovasculares han sido demostrados. En la insuficiencia cardíaca crónica, el tratamiento con alopurinol a largo plazo mejora la hemodinámica ventricular izquierda y también evita la remodelación del ventrículo izquierdo. Estos efectos a largo plazo son, al menos parcialmente, causados por una reducción transitoria de los RLs a nivel miocárdico por la administración de alopurinol [353]. El alopurinol es también un fármaco anti-isquémico útil y seguro para los pacientes con angina estable crónica [347]. Existe una asociación causal entre la hiperuricemia y el riesgo cardiovascular [354]. Niveles elevados de ácido úrico se han asociado con un aumento del riesgo de eventos cardiovasculares, enfermedades coronarias y accidentes cerebrovasculares [355]. También se ha demostrado que el ácido úrico tiene un valor predictivo de mortalidad asociada a la insuficiencia cardíaca crónica [356]. Así pues, nuestro hallazgo principal es que el alopurinol, un fármaco ampliamente utilizado en la práctica clínica, evita el aumento de marcadores de daño músculo-esquelético y

cardíaco asociado con la práctica del fútbol profesional. En este sentido, nuestro grupo también ha demostrado que la inhibición de la actividad XO por medio de la administración de alopurinol previene la atrofia muscular al inhibir la vía p38 MAPK-MAFbx, y por tanto también puede tener beneficios clínicos como son combatir la atrofia muscular en pacientes encamados, sarcopénicos o caquéxicos [25].

La importancia de estos hallazgos es que, a pesar de que lo hayamos estudiado en futbolistas profesionales altamente entrenados, estos resultados pueden aplicarse también a jugadores de fútbol de nivel inferior ya que si bien la intensidad del ejercicio es menor, los deportistas están menos entrenados. Las lesiones en el mundo del fútbol, tanto a nivel profesional como amateur, causan una enorme carga para la sociedad en términos de sufrimiento para los participantes así como también en términos económicos. Debido a que el alopurinol es un fármaco seguro, se podría considerar administrar alopurinol a los jugadores para prevenir el daño tanto músculo-esquelético como cardíaco. El hecho de que los jugadores profesionales en la última década hayan comenzado a jugar tres partidos a la semana (a diferencia de antes, cuando solo jugaban uno cada fin de semana) le da más fuerza a la acción preventiva del alopurinol en esta población. Por lo tanto, la administración de alopurinol es una forma segura y económica para minimizar no sólo el daño muscular, sino también el daño cardíaco en los jugadores de fútbol de élite.

En la segunda parte de este estudio hemos evaluado la variación de nuevos biomarcadores de daño cardiovascular antes y después de un partido de fútbol profesional y el efecto de la administración de alopurinol. Un gran número de biomarcadores cardiovasculares convencionales e innovadores son considerados como indicadores prometedores del nivel de daño inducido por el ejercicio. Los biomarcadores son parámetros fundamentales para la evaluación del impacto de las diferentes intensidades y regímenes de ejercicio en medicina deportiva, cardiología

y bioquímica clínica. En este contexto, la identificación de nuevos biomarcadores con una sensibilidad asecuada es esencial. El objetivo de nuestro estudio fue evaluar los niveles de copeptina, MR-proADM, GDF15, sVEGFR-1/sFLT-1 y PlGF como biomarcadores de daño inducido por el ejercicio utilizando muestras de suero de jugadores de fútbol profesional. Por otra parte, también se investigó si la administración de alopurinol puede afectar los niveles circulantes de estos biomarcadores mediante la inhibición de la actividad XO.

Dos estudios recientes han evaluado los niveles de copeptina en relación con el estado de hidratación en ultramaratonianos. Hew-Butler y colaboradores encontraron que los niveles de copeptina aumentan significativamente durante y al finalizar carreras de distancias extremas. Los autores encontraron correlaciones significativas entre los niveles de copeptina y el porcentaje de cambio de volumen plasmático [357]. Del mismo modo, Bürge y colaboradores observaron que la concentración plasmática de copeptina aumentó, en casi doce veces, en 50 sujetos varones tras una ultramaratón de 100 km, y que existe una correlación entre los cambios en la copeptina y en los niveles de sodio sérico [358]. En nuestro estudio, no hemos podido encontrar ninguna variación significativa en la concentración de copeptina, aunque este hallazgo puede explicarse por el protocolo experimental diferente que se siguió con respecto a la intensidad y la duración del ejercicio (un partido de fútbol frente a una carrera de 100 km). Además, las muestras se recogieron a las doce horas de haberse jugado el partido, mientras que en los dos estudios referenciados en los que que si que se encontraron cambios, las muestras fueron recogidas inmediatamente después del ejercicio. Se ha demostrado recientemente que la copeptina tiene una vida media plasmática relativamente corta de entre 23 y 47 minutos [359]. Por lo que no encontramos una explicación al hecho de que la administración de alopurinol aumente la concentración sérica de copeptina tras el partido.

Hasta donde nosotros sabemos, no existen estudios que hayan investigado la variación de MR-proADM después del ejercicio. Normalmente, el incremento en los niveles de adrenomedulina se asocia a un daño a nivel endotelial [94]. En ciertas condiciones, el aumento de la concentración de adrenomedulina tiene una función hormonal, causando una disminución general de la resistencia vascular y una caída en la presión arterial [94]. En nuestro estudio observamos un aumento significativo en los niveles séricos de MR-proADM en el grupo placebo después del partido. La administración de alopurinol fue eficaz en la prevención de los incrementos inducidos por el ejercicio en los niveles séricos de MR-proADM. Independientemente de las causas subyacentes del incremento de MR-proADM, el hallazgo de que la actividad de XO está involucrada en la variación de este marcador es interesante y también pueden tener algunas implicaciones y aplicaciones clínicas (por ejemplo, el seguimiento de los pacientes con hiperuricemia). Por lo tanto, la concentración de MR-proADM aumentó notablemente después del ejercicio agudo de alta intensidad. Dado que hemos comprobado la hipótesis de que este incremento en los niveles de MR-proADM ha sido provocado por el ejercicio, dicho incremento debe descartarse antes de utilizar este biomarcador para la evaluación del riesgo cardiovascular o de lesiones miocárdicas. También vale la pena mencionar que su aumento puede desempeñar un papel fisiopatológico, pudiendo ejercer efectos biológicos favorables o desfavorables que podrán ser aclarados una vez que se haya establecido el papel definitivo de esta hormona.

Elevados niveles de GDF15 se asocian con cardiopatía hipertrófica [360]. Sin embargo, la práctica de ejercicio moderado (es decir, tres veces a la semana, una hora, durante más de seis meses) no afectó los niveles circulantes de GDF15 en pacientes con enfermedad coronaria estable [361]. GDF15 aumenta en la insuficiencia cardíaca con fracción de eyección normal y en la insuficiencia cardíaca sistólica [362]. Además, se asocia de forma independiente con un

deterioro en la capacidad de ejercicio y en la calidad de vida. Por otra parte, se ha visto que la utilidad diagnóstica de GDF15 es al menos tan buena como la de NT-proBNP, y la combinación de estos marcadores puede mejorar la exactitud diagnóstica de los péptidos natriuréticos en pacientes con insuficiencia cardíaca [362]. Tchou y colaboradores observaron incrementos significativos en las concentraciones séricas circulantes de GDF15 después de una ultramaratón [363]. Nosotros observamos un aumento significativo en los niveles séricos de GDF15 después del partido, tanto en el grupo placebo como en el grupo alopurinol. La administración de alopurinol no afectó a las concentraciones séricas de GDF15, lo que nos lleva a la conclusión de que el metabolismo de GDF15 es independiente de la administración de alopurinol, y por lo tanto, de la actividad de XO.

Por lo que respecta a sVEGFR-1/sFLT-1, Bailey y colaboradores observaron que el ejercicio aumenta sus niveles circulantes en voluntarios sanos [364]. sVEGFR-1/sFLT-1 actúa como un inhibidor del factor de crecimiento del endotelio vascular (VEGF) endógeno, por lo que puede disminuir funcionalmente los niveles plasmáticos del VEGF libre. También se ha descrito una correlación positiva significativa entre el porcentaje de aumento de los niveles plasmáticos de sVEGFR-1/sFLT-1 y el consumo máximo de oxígeno durante el ejercicio [364]. Sin embargo, Kivelä y colaboradores no observaron cambios en la expresión de VEGFR-1 en el músculo-esquelético, ni en ratones sanos ni diabéticos después del ejercicio [365]. En nuestro estudio no hemos podido observar un aumento significativo de la concentración de sVEGFR-1/sFLT-1 ni de PlGF después del partido en ninguno de los grupos. Esto nos lleva a sugerir que estos biomarcadores son prácticamente insensibles al ejercicio físico, al menos en nuestras condiciones experimentales de intensidad y duración del ejercicio. También hemos demostrado que la administración de alopurinol no modifica los niveles séricos de sVEGFR-1/sFLT-1 ni los de PlGF, lo que sugiere que el metabolismo de estos dos biomarcadores no se ve influenciado por la actividad de la XO.

Este es el primer estudio sobre los efectos del ejercicio de alta intensidad y la administración de alopurinol en varios biomarcadores cardiovasculares innovadores en futbolistas profesionales. No obstante el número limitado de sujetos estudiados, la tendencia incremental post-ejercicio de los niveles séricos de MR-proADM y GDF15 da fe de que el metabolismo de éstos está claramente influenciado por el ejercicio, que de ese modo representa una fuente potencial de variabilidad biológica en su evaluación clínica. Por otro lado, su capacidad de respuesta al ejercicio agudo abre un nuevo escenario para su uso clínico, por ejemplo, para el seguimiento de las respuestas biológicas después de las pruebas de ejercicio de evaluación cardiopulmonar. Finalmente, más investigación es necesaria para confirmar el sorprendente efecto de la administración de alopurinol en valores de copeptina en individuos sanos.

La influencia potencial de la variabilidad biológica es uno de los problemas más críticos en la validación clínica y la evaluación rutinaria de nuevos biomarcadores diagnósticos. El ejercicio físico, en particular, tiene una importante influencia en los biomarcadores inflamatorios más ampliamente utilizados, incluyendo la procalcitonina (PCT), proteína C reactiva (PCR) y la interleucina-6 (IL-6). Recientemente hemos demostrado que los niveles séricos de PCT pueden aumentar hasta 4 veces después del ejercicio físico [366]. De forma análoga, se reportó que las concentraciones de IL-6 y CRP aumentaron notablemente después una carrera a pie, hasta 16 y 28 veces, respectivamente [367]. El ejercicio físico, por lo tanto, debe ser considerado como un factor de confusión potencial en la determinación de estos marcadores, lo que puede afectar considerablemente al rendimiento diagnóstico de la PCT, PCR y la IL-6.

suPAR es el único marcador de riesgo que se comporta como una "alarma maestra" para un estado de riesgo en pacientes diabéticos o con cáncer así como con enfermedad renal, cardiovascular, infecciosa, inflamatoria o autoinmune. Un aumento de los niveles séricos de suPAR evidencia una condición crítica que

representa un aumento del riesgo de mortalidad, mientras que la disminución de los niveles de suPAR es un índice valioso de la mejoría de estos pacientes y de la eficacia de la intervención terapéutica [124-127]. Los resultados de nuestro estudio, que muestran claramente que los niveles séricos de suPAR no están influenciados ni por el ejercicio físico ni por la administración de alopurinol, tienen implicaciones clínicas importantes. En primer lugar, la medición de suPAR (frente a PCT, PCR e IL-6) es muy fiable, independientemente del ejercicio físico realizado por el paciente. También cabe destacar que el alopurinol se utiliza cada vez más en pacientes con diversos tipos de lesiones tisulares y vasculares, como son el síndrome coronario agudo, la insuficiencia cardíaca crónica, enfermedades inflamatorias, shock séptico [368], quemaduras, heridas [369] y otras formas de infecciones localizadas [370], donde la PCT, la PCR y la IL-6 se controlan habitualmente. La evidencia de que ni el ejercicio físico ni el alopurinol modifican los niveles séricos de suPAR permitiría su uso de manera fiable en pacientes tratados con inhibidores de la XO o que realizan ejercicio físico.

2.- Estudio del efecto del ejercicio físico de muy larga duración sobre nuevos biomarcadores de daño cardiovascular y renal en atletas antes y después de una ultramaratón.

Aunque en el estudio comentado anteriormente no encontramos cambios en los niveles de copeptina asociados a la práctica del fútbol, nos planteamos estudiar sus posibles modificaciones en ultramaratonianos. Maeder y colaboradores originalmente estudiaron a 161 pacientes que fueron sometidos a una prueba de esfuerzo cardiopulmonar en cicloergómetro limitada por los síntomas (la media de tiempo de ejercicio fue de 6,3 min e incrementos de 10 a 25 W/min) [371], y se observó un aumento significativo en la concentración de copeptina cuando compararon los niveles en reposo y tras la finalización de la prueba. Curiosamente, los incrementos en los niveles de copeptina se han asociado con el nivel de ejercicio y con el uso de ciertos medicamentos en pacientes con enfermedad coronaria o que hayan sufrido un infarto agudo de miocardio precedente. Hew-Butler y colaboradores reportaron que los niveles de copeptina aumentaron significativamente durante y al final de una carrera de larga distancia a pesar de la disminución plasmática de sodio, lo que sugiere la existencia de una estimulación no osmótica de la vasopresina durante el ejercicio físico vigoroso [357]. Por otro lado, Bürge y colaboradores también investigaron a 50 ultramaratonianos varones que realizaron una ultramaratón de 100 km y encontraron que la creatinina sérica aumentó en casi un 30% tras el ejercicio [358], mientras que los niveles plasmáticos de copeptina se incrementaron notablemente en casi 12 veces, y por lo tanto a niveles superiores a los observados en nuestro estudio (es decir, 6,4 veces) [372]. Curiosamente, el cambio en los niveles de copeptina no se asoció con el número de micciones, la excreción de la orina o de la ingesta de líquidos durante la carrera. Esto es consistente con nuestros resultados, mostrando que el aumento de copeptina post-ejercicio no está relacionado con el de la creatinina sérica, lo que

sugiere que la variación de este marcador no está relacionado con la función renal y/o la homeostasis de líquidos, pero podría ser atribuible a otros factores que activan el eje hipotálamo-hipofisario, como por ejemplo el estrés [77,90]. La vasopresina se sintetiza esencialmente en el hipotálamo en respuesta a una hiperosmolaridad o a una hipovolemia arterial, pero también se libera sustancialmente en respuesta al estrés, incluyendo el ejercicio vigoroso [90].

La asociación significativa entre la variación de la creatinina sérica y de MR-proADM sugiere que la función renal y la hipovolemia pueden haber contribuido en parte a dicha variación. En ciertas condiciones un aumento de la adrenomedulina plasmática tiene, de hecho, una función hormonal, causando una disminución general de la resistencia vascular y una caída en la presión arterial [94], mientras que los efectos hormonales de la vasopresina incluyen vasoconstricción, estimulación de la liberación de la hormona adrenocorticotropa, activación prolongada de los receptores de vasopresina, que median los efectos antidiuréticos e hipertrofia de miocardio [83]. En este mismo grupo de atletas hemos medido previamente los niveles de troponina I, aumentando éstos significativamente después de la prueba. Sin embargo, el punto de corte diagnóstico fue superado en una minoría de los casos (20%) y el aumento global fue limitado, siendo por lo tanto compatible con cambios transitorios y reversibles de isquemia miocárdica en lugar de poderse atribuir a necrosis del cardiomiocito [67]. La variación tanto de copeptina como de MR-proADM es debida a un estrés cardíaco provocado por el ejercicio vigoroso, en lugar de a los efectos necróticos, incluyendo daño miocárdico.

Independientemente de las causas subyacentes que apoyan el aumento tanto de copeptina como de MR-proADM, los resultados de este estudio tienen implicaciones clínicas. De acuerdo con estudios anteriores, la concentración de copeptina, así como la de MR-proADM, se incrementaron notablemente después de carreras de larga distancia. Por lo tanto, estos datos deben ser considerados antes

de usar estos marcadores biológicos para la evaluación del riesgo cardiovascular, lesión de miocardio o daño celular.

La evidencia de que una carrera de 60 km provoca un aumento clínicamente significativo no solo de las troponinas cardíacas [67], sino también de copeptina y de MR-proADM, plantea varias dudas sobre si el ejercicio físico agotador podría considerarse globalmente beneficioso o incluso seguro, sobre todo en población no entrenada o no apta. También vale la pena mencionar que el aumento de ambos péptidos puede desempeñar un papel fisiopatológico, pudiendo producir efectos negativos para la salud. La copeptina y la MR-proADM deben ser considerados como biomarcadores innovadores adecuados para la aplicación clínica y en medicina del deporte, ya que pueden ser utilizados con seguridad para descartar daño celular en pacientes que hayan sido sometidos a ejercicio físico agotador recientemente. Aunque se necesitan más estudios para determinar el papel exacto de estos péptidos, nuestros resultados abren la posibilidad de utilizar estas señales periféricas como posibles biomarcadores de daño inducido por el ejercicio y el estrés.

Por otra parte, es bien sabido que el ejercicio físico extenuante, sobre todo pruebas de maratón, puede estar asociado con varias complicaciones agudas, incluyendo un daño significativo en el músculo-esquelético, proceso normalmente conocido como "rabdomiólisis de esfuerzo" [30,373], problemas gastrointestinales debido a la disminución del flujo sanguíneo mesentérico [374], así como de eventos cardíacos y trombosis [352]. Aunque los mismos mecanismos patológicos que pueden desencadenar el daño gastrointestinal y cardíaco (por ejemplo, desequilibrio hemodinámico, inflamación o estrés oxidativo) también pueden afectar al riñón, se sabe menos sobre el desarrollo de insuficiencia renal aguda (IRA) potencial después del ejercicio físico de resistencia extenuante. La IRA puede ser una grave consecuencia de la isquemia renal debido a una dramática reducción del flujo sanguíneo renal [375], pero sólo hay dos estudios que han investigado los cambios

en los marcadores renales después de carreras de larga distancia. McCullough y colaboradores evaluaron la concentración sérica de creatinina y Cys-C, y urinaria de NGAL y KIM-1 en 25 atletas (13 mujeres y 12 hombres) después de una maratón [376]. A diferencia de nuestro estudio, los valores basales fueron recogidos cuatro semanas antes de la maratón. También se midieron los valores máximos de todos los biomarcadores inmediatamente después de la carrera, aunque sólo midieron las concentraciones de uNGAL mientras que no midieron los niveles de sNGAL. En analogía con nuestro estudio, casi el 40% de los corredores (frente al 38% de nuestro estudio) experimentaron un aumento transitorio de la creatinina sérica que cumplía con los criterios de una IRA. Asimismo se observó una elevación paralela de los niveles séricos de Cys-C, de uNGAL y los niveles urinarios de KIM-1. Concretamente, la concentración de uNGAL aumentó de 8,2 ± 4,0 a 47,0 ± 28,6 ng/ml, lo cual es comparable al aumento observado en nuestro estudio (4,4 a 35,6 ng/ml). Spyropoulos y colaboradores investigaron la variación de lipocalina-2 (es decir, un miembro secretado de la familia de proteínas de la lipocalina que parece ser eficaz en la inhibición de la producción de eritrocitos) en atletas sanos después de una carrera de fondo. De acuerdo con nuestros hallazgos, se alcanzó un notable aumento de las concentraciones de lipocalina-2 inmediatamente después de la carrera, lo que llevó a estos autores a sugerir que la inflamación inducida por el ejercicio puede ser un importante modulador de la producción de lipocalinas [377].Los resultados de nuestro estudio están de acuerdo con los de McCullough y colaboradores, aunque presentaron otros aspectos interesantes. En primer lugar, hemos demostrado por primera vez que, junto con uNGAL, también la concentración sérica de este biomarcador renal (sNGAL) aumenta dramáticamente después de carreras de larga distancia.

Curiosamente, el aumento sérico fue más homogéneo (por ejemplo, las variaciones séricas estuvieron comprendidas entre 1.3 y 2.0 veces, mientras que en orina estuvieron comprendidas entre 1.9 y 37.3 veces). La falta de correlación entre

sNGAL y uNGAL, ya sea solo o uNGAL teniendo en cuenta la relación con la creatinina urinaria en todas las condiciones evaluadas, mostraron que sNGAL y uNGAL siguen una cinética y un metabolismo diferente, en el caso de una insuficiencia renal de esfuerzo. La correlación altamente significativa observada entre la variación aguda de la creatinina sérica y sNGAL, así como la síntesis y liberación de esta proteína por fuentes que no sean el riñón (por ejemplo, neutrófilos, médula ósea, próstata, glándulas salivares, estómago, colon, tráquea, pulmón e hígado) [378] parece atestiguar que el incremento sérico observado en nuestro estudio podría ser en gran medida independiente de la insuficiencia renal, y más bien atribuible a la inflamación inducida por el ejercicio (por ejemplo, la producción de leucocitos o disminución de la filtración glomerular) [377,379]. Por el contrario, los diferentes patrones de incrementos observados en uNGAL harían la valoración urinaria de este marcador biológico más independiente de las variaciones de creatinina y de las fuentes extrarrenales, y por lo tanto más fiable para monitorizar la afectación renal en este y otros tipos de deterioro renal. Curiosamente, un atleta mostró unos niveles muy altos de sNGAL (pero no de uNGAL) en situación basal (429 ng/ml), que aumentó aún más después del ejercicio, hasta 503 ng/ml. Aunque la fuente de estos datos anómalos no se pueda establecer definitivamente, este sujeto también exhibió leucocitosis antes del ejercicio ($9{,}2 \times 10^3/\mu L$, 70% de neutrófilos), mientras que el recuento de leucocitos era normal en todos los otros atletas ($<9{,}0 \times 10^3/\mu$ l).

Por lo tanto, es muy posible que este aumento de sNGAL al inicio del estudio pudiera haber sido provocado por causas extrarrenales, muy probablemente por el aumento de la liberación por parte de los neutrófilos. Independientemente de los mecanismos que subyacen a nuestros hallazgos, es bien sabido que cuando el flujo de sangre renal disminuye drásticamente y se vuelve inadecuado para cubrir las demandas metabólicas, se producen una serie de cambios fisiopatológicos, que en

última instancia pueden dar lugar a inflamación, muerte celular y disfunción del tejido [375].

El ejercicio físico induce cambios importantes en la hemodinámica renal y la excreción de proteínas. El flujo plasmático renal disminuye durante el ejercicio en relación con la intensidad del ejercicio y el flujo sanguíneo renal puede caer hasta un 25% del valor de reposo cuando se realiza un ejercicio extenuante [68]. Además, se ha demostrado que el esfuerzo físico agudo induce un aumento de ROS y de especies reactivas del nitrógeno (RONS), pudiendo desencadenar ambas un estrés oxidativo [380]. Esto apoyaría el incremento en sNGAL ya que la sobreexpresión mediada por ROS de miembros de la familia de la lipocalina ya ha sido claramente demostrada [131]. La obstrucción intraluminal debida a una hematuria de origen glomerular causada por el esfuerzo intenso y prolongado se ha reportado en ocasiones en los corredores de maratón, pudiendo ser otra causa alternativa [381,382]. Sin embargo, nosotros no observamos hematuria macroscópica en nuestros atletas, y por tanto es poco probable que este mecanismo haya contribuido sustancialmente en nuestro caso. El desarrollo de hemoconcentración extrema no se puede considerar como una explicación fiable a nuestros hallazgos ya que a los atletas se les ofreció bebida *ad libitum* durante la carrera para prevenir una deshidratación excesiva. Por otra parte, el cambio del volumen plasmático (hemoconcentración), se calculó a partir de la variación de la concentración de albúmina después del ejercicio (4,80 frente a 4,51 g/L; aumento medio + 5,6 %), que sólo en parte explicaría el aumento de NGAL sérico (+ 1,6 veces) y urinario (+ 7,7 veces). Por lo tanto, todos estos factores que apoyan la evidencia de que el ejercicio físico intenso aumenta la concentración de NGAL y creatinina como consecuencia de los mecanismos de adaptación inducidos, tales como la reducción del flujo sanguíneo renal, el aumento en el daño oxidativo celular, el aumento en la excreción de proteínas y la inflamación. Así pues, el ejercicio de alta intensidad podría causar algún tipo de deterioro en el tejido renal.

3.- Estudio del efecto del ejercicio físico de alta intensidad sobre la longevidad en sujetos entrenados.

Bajos niveles de actividad física (2.5 h/semana a una intensidad moderada) reducen la mortalidad en un 19%. El aumento de éste a una sesión de 1h 7 días a la semana (7 h/semana) podría aumentar el beneficio en un 24% [383]. Los niveles de actividad de los ciclistas profesionales son de aproximadamente 30 h/semana de entrenamiento a una intensidad de moderada a alta [384]. El efecto de este nivel de ejercicio sobre los niveles de mortalidad ha sido estudiado de forma muy escasa. En nuestro estudio demostramos que los ciclistas profesionales exhiben una mayor longevidad.

En nuestra opinión, los médicos, los profesionales sanitarios y la población en general no deben tener la impresión de que el ejercicio físico intenso y/o competiciones deportivas aeróbicas de alto nivel tienen efectos nocivos, son malos para la salud, y/o acortan la vida en sujetos entrenados. Recientemente se ha demostrado que la actividad física no vigorosa reduce el riesgo de mortalidad por cualquier causa [383]. En otro estudio, Chakravarty y colaboradores han llegado a la conclusión de que el ejercicio vigoroso en personas de mediana edad y en edades avanzadas se asocia con una reducción de la discapacidad en la vejez y un beneficio notable sobre la supervivencia [385]. Este estudio demuestra que niveles incluso más elevados de actividad también aumentan la longevidad. Tenemos que tener en cuenta que los datos de nuestro estudio se limitan a 1964, que es tal vez previo a que se utilizaran algunas sustancias dopantes y/o peligrosas de las que se pudiesen estar utilizando en la actualidad, como por ejemplo, esteroides anabólicos, dopaje sanguíneo, etc… Por otra parte, los ciclistas de resistencia pueden ser una población seleccionada porque las personas con mala salud son menos propensas a convertirse en ciclistas profesionales, y por lo tanto éstos también son más propensos a tener hábitos más saludables que la población general

[386]. Sin embargo, mientras este trabajo estaba en proceso, un estudio publicado por Ruiz y colaboradores mostró que la asociación entre el ejercicio aeróbico vigoroso, realizado por atletas de élite, y el aumento de la esperanza de vida no está sesgada por una selección genética [387]. Sus resultados indicaron que los deportistas de alto nivel tienen características genotípicas relacionadas con las enfermedades similares a las observadas en los sujetos no atletas [387]. Sin embargo, otros factores del estilo de vida también pueden contribuir al aumento de la longevidad media entre los participantes del TdF. Parece ser que los ex atletas fuman menos, consumen menos alcohol y tienen una dieta más saludable que la población general [388]. Además, se ha demostrado que los ex atletas son físicamente más activos en edades avanzadas en comparación con la población general [389]. Nuestros resultados están de acuerdo con estudios anteriores en los que se ha demostrado que la mejora de la capacidad cardiorrespiratoria se asocia con un menor riesgo de mortalidad por todas las causas [180].

En nuestra opinión, el factor crítico para obtener el mayor beneficio es estar en buena forma física. Muchos pacientes y médicos están confundidos acerca de la cantidad de ejercicio necesaria para mejorar la salud [161]. Se ha demostrado que la práctica de ejercicio físico en incluso el 50% de lo recomendado (setenta y dos minutos de ejercicio moderado a la semana) parece suficiente para proporcionar alguna mejora en la forma física. Sin embargo, esta baja dosis de ejercicio no mejora factores de riesgo cardiovascular (presión arterial, perfil lipídico y peso) [161]. De hecho, para muchas personas, hasta sesenta minutos de actividad física todos los días son más adecuados cuando el control del peso es el objetivo principal [154]. Por lo tanto, las relaciones dosis-respuesta entre la actividad física y los diferentes efectos sobre la salud son diferentes. La evaluación de la cantidad mínima de actividad física (dosis baja) necesaria para lograr sus efectos beneficiosos ha sido objeto de una investigación intensa. Wen y colaboradores han descubierto recientemente que quince minutos al día o noventa minutos por semana

de ejercicio de intensidad moderada es beneficioso en términos de esperanza de vida, incluso para sujetos con riesgo cardiovascular [162].

Somos conscientes de algunas limitaciones inevitables de este estudio con ciclistas. Por ejemplo, no tenemos información sobre comorbilidades o causas de muerte de la población estudiada. Del mismo modo, el análisis más convencional para estimar la longevidad de una muestra de la población es mediante una curva de supervivencia, y concretamente una curva de Kaplan-Meier, pero en nuestro estudio no la pudimos utilizar debido a que ciertas variables de la población control no pudieron ser determinadas, por ejemplo las migraciones humanas no pudieron ser excluidas de nuestro análisis. Por otra parte, sabemos que los ciclistas realizaron entrenamientos intensos en edades tempranas, pero no tenemos datos sobre su actividad física en los años siguientes. Los ex atletas son físicamente más activos a medida que envejecen comparados con la población general de su misma edad [389]. Hay una posibilidad de que estos hábitos de ejercicio puedan explicar el aumento del 17% en la longevidad media de los ciclistas, en comparación con la población general.

A pesar de estas limitaciones, concluimos que el ejercicio físico intenso practicado de manera repetitiva y a largo plazo no aumenta la mortalidad o acorta la longevidad. Contrariamente, este tipo de ejercicio aumenta la longevidad.

De hecho, muy recientemente y coincidiendo con nuestros resultados, se ha observado en un estudio llevado a cabo por Marijon y colaboradores una mortalidad significativamente menor en los participantes franceses del TdF en comparación con la población general [390,391]. Estos autores llevaron a cabo este estudio con el objetivo de evaluar y comparar la mortalidad general y las causas específicas de muerte entre los participantes franceses en el TdF desde 1947 hasta 2012 frente a la media comunitaria.

La recomendación más reciente en relación con el ejercicio ha sido: "si un poco es bueno, más puede ser mejor" [154]. A la vista de los resultados, tal vez sería mejor decir: "si un poco es bueno, mucho es mejor si se está bien entrenado".

Sin embargo hay que señalar que este nivel de actividad física no es un objetivo razonable para la mayoría de las personas, especialmente personas mayores o enfermas (diabéticos o pacientes con obesidad).

No pretendemos que la gente se someta a un régimen permanente de ejercicio vigoroso, sino que, contrariamente a las creencias anteriores, el ejercicio competitivo a nivel profesional, si el sujeto está entrenado previamente, sea considerada una práctica saludable que puede prolongar la longevidad.

4.- Estudio del papel del entrenamiento aeróbico en la inducción de la cascada de la biogénesis mitocondrial y sus implicaciones en el envejecimiento músculo-esquelético.

La idea principal de nuestra investigación fue que el envejecimiento provoca la falta de respuesta de PGC-1α a diversos estímulos, siendo el más importante el ejercicio, pero además la exposición al frío o el tratamiento con hormona tiroidea. El papel de las mitocondrias como generadoras clave de EO y también como orgánulo diana de daño asociado a las ROS se postuló por Miquel en la década de los 70 [392]. Un trabajo llevado a cabo en nuestro laboratorio [267] y otro llevado a cabo por el grupo de Bruce Ames [393] utilizando un enfoque metabólico y aproximaciones mediante citometría de flujo proporcionaron tal evidencia.

Un músculo funcional que no ha perdido la capacidad de sintetizar mitocondrias sanas es importante para prevenir la fragilidad, un problema importante en la medicina actual y en particular en geriatría [277,394]. Por lo tanto, la comprensión de los mecanismos moleculares de la mitocondriogénesis en el envejecimiento tiene gran importancia tanto teórica como práctica. La función mitocondrial se adapta en respuesta a la restricción calórica y esta adaptación está involucrada en el incremento de la longevidad [395]. Se ha demostrado que la restricción calórica activa PGC-1α [395-397], pudiendo ser una estrategia eficaz en el retraso de fenotipos celulares en el músculo-esquelético inducido por el envejecimiento [398]. PGC-1α es crítico para la adaptación de la mitocondriogénesis muscular al ejercicio físico ya que activa la expresión de NRF-1 que a su vez activa TFAM, un factor necesario para la duplicación del DNA mitocondrial [337]. Esto nos llevó a pensar que la mitocondriogénesis podría verse afectada en el envejecimiento debido a la alteración de la respuesta de PGC-1α en los animales viejos en comparación con los jóvenes. Para entender el papel de PGC-1α (un factor redox sensible) en la regulación de la mitocondriogénesis muscular utilizamos animales

que KO de PGC-1α. Encontramos una sorprendente similitud en la respuesta molecular al ejercicio en la vía mitocondriogénica entre las ratas viejas y ratones a los que se les ha eliminado el gen que codifica para la proteína PGC-1α.

La vía completa en la que participan PGC-1α→NRF-1 y finalmente citocromo C (un indicador de la masa mitocondrial) respondió positivamente al entrenamiento físico en ratas jóvenes, pero no lo hicieron en las viejas. Éste era precisamente el mismo comportamiento que en los ratones KO de PGC-1α.

Se podría argumentar que la intensidad del entrenamiento no fue suficiente para activar la biogénesis mitocondrial en ratones KO de PGC-1α o en ratas viejas. Sin embargo, la Tabla 10 muestra que la intensidad y la duración del régimen de entrenamiento aplicado fueron suficientes para inducir una mejora significativa en la capacidad de resistencia. PGC-1α protege frente a la atrofia muscular [248] y recientemente se ha demostrado que es necesario para la prevención de la disminución del contenido mitocondrial asociado al envejecimiento mediante el entrenamiento [289].

Por otra parte, PGC-1α se ha descrito que es un elemento clave en la sarcopenia y en enfermedades metabólicas durante el envejecimiento [286]. Animales transgénicos MCK-PGC-1α conservan la función mitocondrial, las uniones neuromusculares y la integridad muscular durante el envejecimiento. Además, el aumento de los niveles de PGC-1α en el músculo-esquelético previene la pérdida de masa muscular mediante la reducción de la apoptosis, la autofagia y la degradación del proteasoma [286].

Como hemos dicho, nuestros estudios basados en las adaptaciones al ejercicio físico en el envejecimiento nos llevaron a la conclusión de que PGC-1α no estaba respondiendo al entrenamiento en los animales viejos. Sospechamos que PGC-1α no puede ser inducido por cualquier tipo de estímulo en los animales viejos. Para probar esta hipótesis, utilizamos dos estímulos de PGC-1α bien conocidos en ratas jóvenes y viejas, es decir, mediante la estimulación con hormona tiroidea (T3) y

aclimatación al frío [243,327]. El tratamiento con T3 o la aclimatación al frío causó una activación muy pronunciada de PGC-1α en animales jóvenes. Sin embargo, hubo una notable falta de activación de PGC-1α por cualquiera de los estímulos utilizados cuando lo analizamos en los animales viejos. Los beneficios para la salud del ejercicio físico crónico pueden ser, al menos parcialmente, debidos a una reducción en la producción de oxidantes mitocondriales [399]. Estos datos ponen en duda la idea muy bien establecida de que el ejercicio genera RLs. Esto fue establecido por primera vez por el grupo de Packer [39] que mostró que las ROS se generan durante la contracción muscular. Los autores postularon que la inducción de la mitocondriogénesis por el ejercicio podría estar mediada por ROS [39]. Nuestro grupo proporcionó la primera evidencia clara de que el ejercicio genera EO sólo cuando es agotador [3,4]. Sin embargo, el ejercicio físico puede ser considerado como un arma de doble filo: cuando se practica de forma intensa causa EO y daño celular, pero cuando se practica con moderación aumenta la expresión de enzimas antioxidantes y por lo tanto debe ser considerado como un antioxidante [199]. Hemos medido el efecto del ejercicio físico sobre el estado de oxidación de las proteínas del músculo-esquelético en ratas jóvenes y viejas, y en ratones WT y KO de PGC-1α. Hemos encontrado que el músculo-esquelético de animales viejos y KO de PGC-1α presentan EO, es decir, un aumento en la carbonilación de proteínas en condiciones de reposo. La oxidación de proteínas no aumentó significativamente después del entrenamiento en cualquier grupo experimental, concordando con nuestra idea de que el entrenamiento no aumenta el EO [199].

Trabajos previos de nuestro laboratorio, así como de otros, han demostrado que interfiriendo con los RLs mediante antioxidantes se puede obstaculizar la activación de la mitocondriogénesis por el ejercicio [40,201,400]. En el caso del envejecimiento, observamos un escenario diferente. Nuestros datos demuestran que existen niveles elevados de ROS de forma crónica en el músculo-esquelético de animales viejos y KO de PGC-1α. Consideramos que, en estas circunstancias, la

respuesta al ejercicio de las vías de señalización celular redox-sensibles pueden verse obstaculizadas. Sin embargo, esta hipótesis debe ser confirmada en futuras investigaciones.

PGC-1α se identifica actualmente como una nueva diana terapéutica para el tratamiento de la disfunción mitocondrial relacionada con la edad en el músculo-esquelético y en la sarcopenia [248]. Por otra parte, recientemente, un interesante trabajo basado en el papel fundamental de PGC-1α conecta los cambios nucleares y mitocondriales en el envejecimiento [401]. Nuestro estudio pone de manifiesto la importancia de mantener una capacidad de respuesta normal de PGC-1α (que se ha perdido en el envejecimiento) para mantener la función muscular normal. Una interpretación esquemática de nuestros resultados se muestra en la Figura 24.

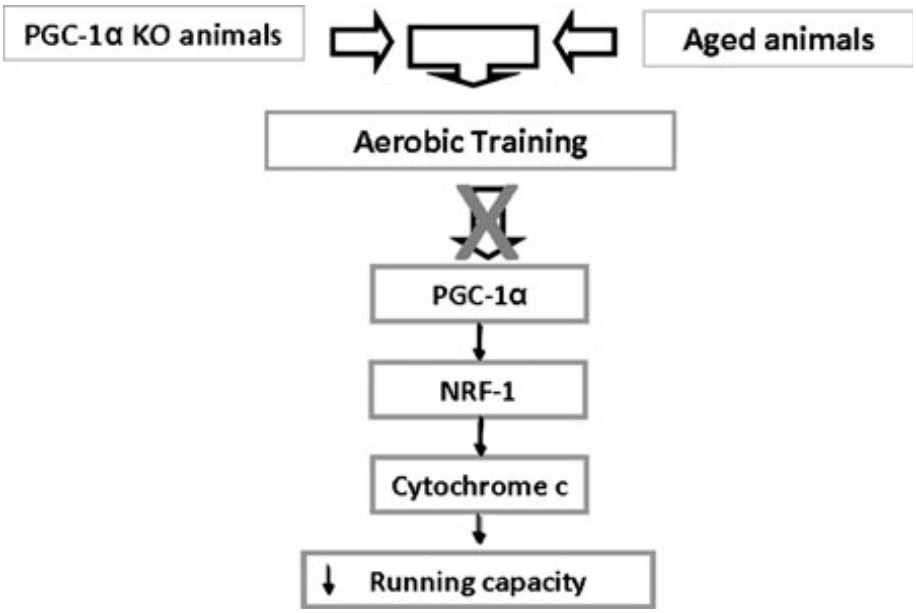


Figura 24. *Interpretación esquemática de nuestros resultados [23].*

RESUMEN: CONCLUSIONES

E. CONCLUSIONES FINALES

A la luz de los resultados obtenidos, podemos extraer las conclusiones que se muestran a continuación:

1.- La xantina oxidasa está implicada en el daño músculo-esquelético y cardiovascular en futbolistas profesionales determinado mediante la cuantificación de biomarcadores clásicos tales como: CK, LDH, AST, mioglobina, CK-MB y Hs-TnT, así como de nuevos biomarcadores tales como MR-proADM y GDF15. Por lo tanto, la administración de alopurinol es una estrategia segura y económica para minimizar no sólo el daño musculo-esquelético, sino también el daño cardiovascular en jugadores de fútbol profesional.

2.- Los niveles de suPAR sérico no están influenciados ni por el ejercicio físico ni por la administración de alopurinol, lo que tiene implicaciones clínicas importantes. En primer lugar, la medición de suPAR –frente a parámetros como la PCR o la IL-6– es fiable independientemente del ejercicio físico realizado por el paciente. El alopurinol se utiliza cada vez más en pacientes con diversos tipos de patología cardiovascular. En dichos pacientes también se utilizan comúnmente la PCR y la IL-6 para su control. Así pues, la evidencia de que el alopurinol no modifica significativamente los niveles séricos de suPAR sería relevante para su uso como un marcador fiable en pacientes que reciben el inhibidor de la XO.

3.- La copeptina y la MR-proADM pueden ser considerados como biomarcadores innovadores con aplicación clínica en medicina del deporte, ya que puede ser utilizado para descartar daño celular en sujetos que hayan sido

sometidos a ejercicio físico de muy larga duración (ultramaratón). Aunque se necesitan más estudios para determinar el papel exacto de estos péptidos, nuestros resultados abren la posibilidad de utilizar estas señales periféricas como posibles biomarcadores de daño inducido por ejercicio físico.

4.- El ejercicio físico de muy larga duración (ultramaratón) aumenta la concentración de NGAL y creatinina. Por lo tanto, su evaluación puede ser aconsejable para controlar la función renal en sujetos que han realizado recientemente un ejercicio físico de larga duración, así como para la identificación de atletas con mayor riesgo de desarrollar daño renal durante este tipo de ejercicio.

5.- El deporte de alto nivel (corredores del TdF) no se asocia con disminuciones en la longevidad de sus practicantes sino con incrementos en la misma. En nuestra opinión, los profesionales sanitarios y la población en general no deben tener la impresión de que el ejercicio físico intenso y/o las competiciones deportivas aeróbicas de alto nivel tienen efectos nocivos para la salud en términos de longevidad. Por el contrario nuestros resultados muestran que el ciclismo de alto nivel, si el sujeto está entrenado previamente, es una práctica saludable que puede prolongar la longevidad.

6.- El envejecimiento provoca una falta de respuesta de PGC-1α a diversos estímulos con la consiguiente afectación de la activación mitocondriogénica en el músculo esquelético. Encontramos una sorprendente similitud en la respuesta molecular al ejercicio en la vía mitocondriogénica en ratones a los que se les ha suprimido PGC-1α frente a las ratas viejas. Nuestros resultados muestran que los animales viejos se comportan como animales deficientes en PGC-1α.

RESUMEN: BIBLIOGRAFÍA

F. BIBLIOGRAFÍA

1. Commoner B, Townsend J, Pake GE (1954) Free radicals in biological materials. Nature 174:689-691
2. Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL, Vina J (2005) Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 567:113-120
3. Gomez-Cabrera MC, Pallardo FV, Sastre J, Vina J, Garcia-del-Moral L (2003) Allopurinol and markers of muscle damage among participants in the Tour de France. Jama 289:2503-2504
4. Sastre J, Asensi M, Gasco E, Pallardo FV, Ferrero JA, Furukawa T, Vina J (1992) Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. Am J Physiol 263:R992-995
5. Viña J, Severa E, Asensi M, Sastre J, Pallardó F, Ferrero JA, García de la Asunción J, Antón V, Marín J (1996) Exercise causes blood glutathione oxidation in chronic obstructive pulmonary disease. Prevention by oxygen therapy. Journal of Applied Physiology 81:2199-2202
6. Heunks LM, Vina J, van Herwaarden CL, Folgering HT, Gimeno A, Dekhuijzen PN (1999) Xanthine oxidase is involved in exercise-induced oxidative stress in chronic obstructive pulmonary disease. Am J Physiol 277:R1697-1704
7. Harris CM, Sanders SA, Massey V (1999) Role of the flavin midpoint potential and NAD binding in determining NAD versus oxygen reactivity of xanthine oxidoreductase. J Biol Chem 274:4561-4569
8. Viña J, Gimeno A, Sastre J, Desco C, Asensi M, Pallardo FV, Cuesta A, Ferrero JA, Terada LS, Repine JE (2000) Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. IUBMB Life 49:539-544
9. Viña J, Gomez-Cabrera MC, Lloret A, Marquez R, Minana JB, Pallardo FV, Sastre J (2000) Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. IUBMB Life 50:271-277
10. Vina J, Gomez-Cabrera MC, Lloret A, Marquez R, Minana JB, Pallardo FV, Sastre J (2000) Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. IUBMB Life 50:271-277
11. Sanchis-Gomar F, Pareja-Galeano H, Gomez-Cabrera MC, Candel J, Lippi G, Salvagno GL, Mann GE, Viña J (2013 (in press)) Allopurinol prevents cardiac and skeletal muscle damage in soccer players. Scand J Med Sci Sports

12. Sanchis-Gomar F, Bonaguri C, Aloe R, Pareja-Galeano H, Martinez-Bello V, Gomez-Cabrera MC, Candel J, Vina J, Lippi G (2013) Effects of acute exercise and xanthine oxidase inhibition on novel cardiovascular biomarkers. Transl Res 162:102-109
13. Sanchis-Gomar F, Bonaguri C, Pareja-Galeano H, Gomez-Cabrera MC, Candel J, Vina J, Lippi G (2013) Effects of acute exercise and allopurinol administration on soluble urokinase plasminogen activator receptor (suPAR). Clin Lab 59:207-210
14. Lippi G, Sanchis-Gomar F, Salvagno GL, Aloe R, Schena F, Guidi GC (2012) Variation of serum and urinary neutrophil gelatinase associated lipocalin (NGAL) after strenuous physical exercise. Clin Chem Lab Med 50:1585-1589
15. Lippi G, Schena F, Sanchis-Gomar F, Guidi G (2013 (in press)) Serum copeptin and mid-region pro-adrenomedullin (MRproADM) after an ultramarathon. J Clin Lab Anal
16. Sanchis-Gomar F (2013) The skeletal muscle-metabolism axis in prostate-cancer therapy. N Engl J Med 367:2257-2258
17. Vina J, Sanchis-Gomar F, Martinez-Bello V, Gomez-Cabrera MC (2012) Exercise acts as a drug; the pharmacological benefits of exercise. Br J Pharmacol 167:1-12
18. Sanchis-Gomar F, Olaso-Gonzalez G, Corella D, Gomez-Cabrera MC, Vina J (2011) Increased average longevity among the "Tour de France" cyclists. Int J Sports Med 32:644-647
19. Vina J, Gomez-Cabrera MC, Borras C, Froio T, Sanchis-Gomar F, Martinez-Bello VE, Pallardo FV (2009) Mitochondrial biogenesis in exercise and in ageing. Adv Drug Deliv Rev 61:1369-1374
20. Sanchis-Gomar F, Gomez-Cabrera MC, Vina J (2011) The loss of muscle mass and sarcopenia: non hormonal intervention. Exp Gerontol 46:967-969
21. Gomez-Cabrera MC, Sanchis-Gomar F, Garcia-Valles R, Pareja-Galeano H, Gambini J, Borras C, Vina J (2012) Mitochondria as sources and targets of damage in cellular aging. Clin Chem Lab Med 50:1287-1295
22. Vina J, Borras C, Sanchis-Gomar F, Martinez-Bello VE, Olaso-Gonzalez G, Gambini J, Ingles M, Gomez-Cabrera MC (2013) Pharmacological Properties of Physical Exercise in The Elderly. Curr Pharm Des
23. Derbre F, Gomez-Cabrera MC, Nascimento AL, Sanchis-Gomar F, Martinez-Bello VE, Tresguerres JA, Fuentes T, Gratas-Delamarche A, Monsalve M, Vina J (2011) Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age (Dordr) 34:669-679
24. Gomez-Cabrera MC, Martinez A, Santangelo G, Pallardo FV, Sastre J, Vina J (2006) Oxidative stress in marathon runners: interest of antioxidant supplementation. Br J Nutr 96 Suppl 1:S31-33

25. Derbre F, Ferrando B, Gomez-Cabrera MC, Sanchis-Gomar F, Martinez-Bello VE, Olaso-Gonzalez G, Diaz A, Gratas-Delamarche A, Cerda M, Vina J (2012) Inhibition of Xanthine Oxidase by Allopurinol Prevents Skeletal Muscle Atrophy: Role of p38 MAPKinase and E3 Ubiquitin Ligases. PLoS One 7:e46668
26. Armstrong RB, Ogilvie RW, Schwane JA (1983) Eccentric exercise-induced injury to rat skeletal muscle. J Appl Physiol 54:80-93
27. Kyparos A, Matziari C, Albani M, Arsos G, Deligiannis A (2001) A decrease in soleus muscle force generation in rats after downhill running. Can J Appl Physiol 26:323-335
28. Lieber RL, Friden J (1999) Mechanisms of muscle injury after eccentric contraction. J Sci Med Sport 2:253-265
29. Ruiz JR, Moran M, Arenas J, Lucia A (2011) Strenuous endurance exercise improves life expectancy: it's in our genes. Br J Sports Med 45:159-161
30. Brancaccio P, Lippi G, Maffulli N (2010) Biochemical markers of muscular damage. Clin Chem Lab Med 48:757-767
31. Lippi G, Schena F, Montagnana M, Salvagno GL, Guidi GC (2008) Influence of acute physical exercise on emerging muscular biomarkers. Clin Chem Lab Med 46:1313-1318
32. Eijsvogels TM, Shave R, van Dijk A, Hopman MT, Thijssen DH (2011) Exercise-induced cardiac troponin release: real-life clinical confusion. Curr Med Chem 18:3457-3461
33. Lippi G, Cervellin G, Banfi G, Plebani M (2011) Cardiac troponins and physical exercise. It's time to make a point. Biochem Med (Zagreb) 21:55-62
34. Lippi G, Schena F, Salvagno GL, Tarperi C, Aloe R, Guidi GC (2011) Comparison of conventional and highly-sensitive troponin I measurement in ultra-marathon runners. J Thromb Thrombolysis 33:338-342
35. Lippi G, Schena F, Dipalo M, Montagnana M, Salvagno GL, Aloe R, Guidi GC (2012) Troponin I measured with a high sensitivity immunoassay is significantly increased after a half marathon run. Scand J Clin Lab Invest
36. Lippi G, Schena F, Montagnana M, Salvagno GL, Banfi G, Guidi GC (2011) Significant variation of traditional markers of liver injury after a half-marathon run. Eur J Intern Med 22:e36-38
37. Yared K, Wood MJ (2009) Is marathon running hazardous to your cardiovascular health? The jury is still out. Radiology 251:3-5
38. Ibrahim B, Stoward PJ (1978) The histochemical localization of xanthine oxidase. Histochem J 10:615-617

39. Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107:1198-1205
40. Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, Sastre J, Vina J (2008) Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 87:142-149
41. Tsutsui H, Kinugawa S, Matsushima S (2011) Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol 301:H2181-2190
42. Moorhouse PC, Grootveld M, Halliwell B, Quinlan JG, Gutteridge JM (1987) Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett 213:23-28
43. Banfi G, Colombini A, Lombardi G, Lubkowska A (2012) Metabolic markers in sports medicine. Adv Clin Chem 56:1-54
44. Schardinger F (1902) Uber das verhalten der kulmilch gegen methylenblau und seine verwendung zur unterscheidung von ungekochter und gekochter milch. Z Untersuch Nahrungs Genussmittel 5 1113-1121
45. Reid MB, Shoji T, Moody MR, Entman ML (1992) Reactive oxygen in skeletal muscle. II. Extracellular release of free radicals. J Appl Physiol 73:1805-1809
46. Lee C, Okabe E (1995) Hydroxyl radical-mediated reduction of Ca(2+)-ATPase activity of masseter muscle sarcoplasmic reticulum. Jpn J Pharmacol 67:21-28
47. Reid MB (2001) Nitric oxide, reactive oxygen species, and skeletal muscle contraction. Med Sci Sports Exerc 33:371-376
48. Linder N, Rapola J, Raivio KO (1999) Cellular expression of xanthine oxidoreductase protein in normal human tissues. Lab Invest 79:967-974
49. Stofan DA, Callahan LA, Di MA, Nethery DE, Supinski GS (2000) Modulation of release of reactive oxygen species by the contracting diaphragm. Am J Respir Crit Care Med 161:891-898
50. Barclay JK, Hansel M (1991) Free radicals may contribute to oxidative skeletal muscle fatigue. Can J Physiol Pharmacol 69:279-284
51. Hellsten Y, Frandsen U, Orthenblad N, Sjodin B, Richter EA (1997) Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. J Physiol 498 (Pt 1):239-248
52. Kadambi A, Skalak TC (2000) Role of leukocytes and tissue-derived oxidants in short-term skeletal muscle ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 278:H435-443
53. Laughlin MH, Hale CC, Novela L, Gute D, Hamilton N, Ianuzzo CD (1991) Biochemical characterization of exercise-trained porcine myocardium. J Appl Physiol 71:229-235

54. Hellsten-Westing Y (1993) Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle. Histochemistry 100:215-222
55. Sahlin K, Ekberg K, Cizinsky S (1991) Changes in plasma hypoxanthine and free radical markers during exercise in man. Acta Physiol Scand 142:275-281
56. Hellsten-Westing Y, Sollevi A, Sjodin B (1991) Plasma accumulation of hypoxanthine, uric acid and creatine kinase following exhausting runs of differing durations in man. Eur J Appl Physiol Occup Physiol 62:380-384
57. Sahlin K, Broberg S, Ren JM (1989) Formation of inosine monophosphate (IMP) in human skeletal muscle during incremental dynamic exercise. Acta Physiol Scand 136:193-198
58. Hellsten Y (1994) Xanthine dehydrogenase and purine metabolism in man. With special reference to exercise. Acta Physiol Scand Suppl 621:1-73
59. Sahlin K (1991) Control of energetic processes in contracting human skeletal muscle. Biochem Soc Trans 19:353-358
60. Arabadjis PG, Tullson PC, Terjung RL (1993) Purine nucleoside formation in rat skeletal muscle fiber types. Am J Physiol 264:C1246-1251
61. Hellsten-Westing Y, Kaijser L, Ekblom B, Sjodin B (1994) Exchange of purines in human liver and skeletal muscle with short-term exhaustive exercise. Am J Physiol 266:R81-86
62. Granger DN, Perry MA, Kvietys PR, Taylor AE (1981) Interstitium-to-blood movement of macromolecules in the absorbing small intestine. Am J Physiol 241:G31-36
63. Elion GB, Kovensky A, Hitchings GH (1966) Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. Biochem Pharmacol 15:863-880
64. Massey V, Komai H, Palmer G, Elion GB (1970) On the mechanism of inactivation of xanthine oxidase by allopurinol and other pyrazolo[3,4-d]pyrimidines. J Biol Chem 245:2837-2844
65. Mountjoy M, Andersen LB, Armstrong N, Biddle S, Boreham C, Bedenbeck HP, Ekelund U, Engebretsen L, Hardman K, Hills AP, et al (2011) International Olympic Committee consensus statement on the health and fitness of young people through physical activity and sport. Br J Sports Med 45:839-848
66. Armstrong N, Tomkinson G, Ekelund U (2011) Aerobic fitness and its relationship to sport, exercise training and habitual physical activity during youth. Br J Sports Med 45:849-858
67. Lippi G, Schena F, Salvagno GL, Tarperi C, Aloe R, Guidi GC (2012) Comparison of conventional and highly-sensitive troponin I measurement in ultra-marathon runners. J Thromb Thrombolysis 33:338-342
68. Poortmans JR (1984) Exercise and renal function. Sports Med 1:125-153

69. Castenfors J (1977) Renal function during prolonged exercise. Ann N Y Acad Sci 301:151-159
70. McAllister RM (1998) Adaptations in control of blood flow with training: splanchnic and renal blood flows. Med Sci Sports Exerc 30:375-381
71. Poortmans JR, Vanderstraeten J (1994) Kidney function during exercise in healthy and diseased humans. An update. Sports Med 18:419-437
72. Delanghe JR, Cobbaert C, Harmoinen A, Jansen R, Laitinen P, Panteghini M (2011) Focusing on the clinical impact of standardization of creatinine measurements: a report by the EFCC Working Group on Creatinine Standardization. Clin Chem Lab Med 49:977-982
73. Soni SS, Pophale R, Ronco C (2011) New biomarkers for acute renal injury. Clin Chem Lab Med 49:1257-1263
74. Waikar SS, Betensky RA, Emerson SC, Bonventre JV (2012) Imperfect gold standards for kidney injury biomarker evaluation. J Am Soc Nephrol 23:13-21
75. Urbschat A, Obermuller N, Haferkamp A (2011) Biomarkers of kidney injury. Biomarkers 16 Suppl 1:S22-30
76. de Bree FM, Burbach JP (1998) Structure-function relationships of the vasopressin prohormone domains. Cell Mol Neurobiol 18:173-191
77. Katan M, Morgenthaler N, Widmer I, Puder JJ, Konig C, Muller B, Christ-Crain M (2008) Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level. Neuro Endocrinol Lett 29:341-346
78. Reichlin T, Hochholzer W, Stelzig C, Laule K, Freidank H, Morgenthaler NG, Bergmann A, Potocki M, Noveanu M, Breidthardt T, et al (2009) Incremental value of copeptin for rapid rule out of acute myocardial infarction. J Am Coll Cardiol 54:60-68
79. Keller T, Tzikas S, Zeller T, Czyz E, Lillpopp L, Ojeda FM, Roth A, Bickel C, Baldus S, Sinning CR, et al (2010) Copeptin improves early diagnosis of acute myocardial infarction. J Am Coll Cardiol 55:2096-2106
80. Giannitsis E, Kehayova T, Vafaie M, Katus HA (2011) Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. Clin Chem 57:1452-1455
81. Charpentier S, Maupas-Schwalm F, Cournot M, Elbaz M, Botella JM, Lauque D (2012) Combination of copeptin and troponin assays to rapidly rule out non-ST elevation myocardial infarction in the emergency department. Acad Emerg Med 19:517-524
82. Lippi G, Aloe R, Dipalo M, Cervellin G (2012) Combination of copeptin and highly sensitive troponin I for diagnosing acute myocardial infarction at emergency department admission. Clin Lab 58:357-358; author reply 359-360

83. Khan SQ, Dhillon OS, O'Brien RJ, Struck J, Quinn PA, Morgenthaler NG, Squire IB, Davies JE, Bergmann A, Ng LL (2007) C-terminal provasopressin (copeptin) as a novel and prognostic marker in acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) study. Circulation 115:2103-2110
84. Neuhold S, Huelsmann M, Strunk G, Stoiser B, Struck J, Morgenthaler NG, Bergmann A, Moertl D, Berger R, Pacher R (2008) Comparison of copeptin, B-type natriuretic peptide, and amino-terminal pro-B-type natriuretic peptide in patients with chronic heart failure: prediction of death at different stages of the disease. J Am Coll Cardiol 52:266-272
85. Balling L, Kistorp C, Schou M, Egstrup M, Gustafsson I, Goetze JP, Hildebrandt P, Gustafsson F (2012) Plasma copeptin levels and prediction of outcome in heart failure outpatients: relation to hyponatremia and loop diuretic doses. J Card Fail 18:351-358
86. Tentzeris I, Jarai R, Farhan S, Perkmann T, Schwarz MA, Jakl G, Wojta J, Huber K (2011) Complementary role of copeptin and high-sensitivity troponin in predicting outcome in patients with stable chronic heart failure. Eur J Heart Fail 13:726-733
87. Luchner A (2012) Copeptin for acute coronary syndrome: diamond or decoy? Clin Chem Lab Med 50:191-192
88. Kehl DW, Iqbal N, Fard A, Kipper BA, De La Parra Landa A, Maisel AS (2012) Biomarkers in acute myocardial injury. Transl Res 159:252-264
89. Enhorning S, Wang TJ, Nilsson PM, Almgren P, Hedblad B, Berglund G, Struck J, Morgenthaler NG, Bergmann A, Lindholm E, et al (2010) Plasma copeptin and the risk of diabetes mellitus. Circulation 121:2102-2108
90. Katan M, Christ-Crain M (2010) The stress hormone copeptin: a new prognostic biomarker in acute illness. Swiss Med Wkly 140:w13101
91. Kolditz M, Halank M, Schulte-Hubbert B, Bergmann S, Albrecht S, Hoffken G (2012) Copeptin predicts clinical deterioration and persistent instability in community-acquired pneumonia. Respir Med
92. Meisel A, Meisel C, Harms H, Hartmann O, Ulm L (2012) Predicting post-stroke infections and outcome with blood-based immune and stress markers. Cerebrovasc Dis 33:580-588
93. Lin Q, Fu F, Chen H, Zhu B (2012) Copeptin in the assessment of acute lung injury and cardiogenic pulmonary edema. Respir Med
94. Hinson JP, Kapas S, Smith DM (2000) Adrenomedullin, a multifunctional regulatory peptide. Endocr Rev 21:138-167
95. Morgenthaler NG, Struck J, Alonso C, Bergmann A (2005) Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. Clin Chem 51:1823-1829
96. Wild PS, Schnabel RB, Lubos E, Zeller T, Sinning CR, Keller T, Tzikas S, Lackner KJ, Peetz D, Rupprecht HJ, et al (2011) Midregional

proadrenomedullin for prediction of cardiovascular events in coronary artery disease: results from the AtheroGene study. Clin Chem 58:226-236

97. Brouwers FP, de Boer RA, van der Harst P, Struck J, de Jong PE, de Zeeuw D, Gans RO, Gansevoort RT, Hillege HL, van Gilst WH, et al (2012) Influence of age on the prognostic value of mid-regional pro-adrenomedullin in the general population. Heart
98. von Haehling S, Filippatos GS, Papassotiriou J, Cicoira M, Jankowska EA, Doehner W, Rozentryt P, Vassanelli C, Struck J, Banasiak W, et al (2010) Mid-regional pro-adrenomedullin as a novel predictor of mortality in patients with chronic heart failure. Eur J Heart Fail 12:484-491
99. Katan M, Fluri F, Schuetz P, Morgenthaler NG, Zweifel C, Bingisser R, Kappos L, Steck A, Engelter ST, Muller B, et al (2010) Midregional pro-atrial natriuretic peptide and outcome in patients with acute ischemic stroke. J Am Coll Cardiol 56:1045-1053
100. Palladini G, Barassi A, Perlini S, Milani P, Foli A, Russo P, Albertini R, Obici L, Lavatelli F, Sarais G, et al (2011) Midregional proadrenomedullin (MR-proADM) is a powerful predictor of early death in AL amyloidosis. Amyloid 18:216-221
101. Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Muller B (2005) Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an observational study. Crit Care 9:R816-824
102. Bello S, Lasierra AB, Minchole E, Fandos S, Ruiz MA, Vera E, de Pablo F, Ferrer M, Menendez R, Torres A (2011) Prognostic power of proadrenomedullin in community-acquired pneumonia is independent of aetiology. Eur Respir J 39:1144-1155
103. Sarda Sanchez M, Calzada Hernandez J, Hernandez Bou S, Claret Teruel G, Velasco Rodriguez J, Luaces Cubells C (2012) Pro-adrenomedullin usefulness in the management of children with community-acquired pneumonia, a preliminary prospective observational study. BMC Res Notes 5:363
104. Maisel A, Mueller C, Nowak RM, Peacock WF, Ponikowski P, Mockel M, Hogan C, Wu AH, Richards M, Clopton P, et al (2011) Midregion prohormone adrenomedullin and prognosis in patients presenting with acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. J Am Coll Cardiol 58:1057-1067
105. Cinar O, Cevik E, Acar A, Kaya C, Ardic S, Comert B, Yokusoglu M, Bilgi C, Meisner M, Madsen T (2012) Evaluation of mid-regional pro-atrial natriuretic peptide, procalcitonin, and mid-regional pro-adrenomedullin for the diagnosis and risk stratification of ED patients with dyspnea. Am J Emerg Med
106. Shah RV, Truong QA, Gaggin HK, Pfannkuche J, Hartmann O, Januzzi JL, Jr. (2012) Mid-regional pro-atrial natriuretic peptide and pro-

adrenomedullin testing for the diagnostic and prognostic evaluation of patients with acute dyspnoea. Eur Heart J

107. Maeder MT, Brutsche MH, Arenja N, Socrates T, Reiter M, Meissner J, Staub D, Morgenthaler NG, Bergmann A, Mueller C (2010) Biomarkers and peak oxygen uptake in patients with chronic lung disease. Respiration 80:543-552
108. Schlittenhardt D, Schober A, Strelau J, Bonaterra GA, Schmiedt W, Unsicker K, Metz J, Kinscherf R (2004) Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages in vitro and in arteriosclerotic lesions. Cell Tissue Res 318:325-333
109. Hsiao EC, Koniaris LG, Zimmers-Koniaris T, Sebald SM, Huynh TV, Lee SJ (2000) Characterization of growth-differentiation factor 15, a transforming growth factor beta superfamily member induced following liver injury. Mol Cell Biol 20:3742-3751
110. Albertoni M, Shaw PH, Nozaki M, Godard S, Tenan M, Hamou MF, Fairlie DW, Breit SN, Paralkar VM, de Tribolet N, et al (2002) Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. Oncogene 21:4212-4219
111. Breen EC, Johnson EC, Wagner H, Tseng HM, Sung LA, Wagner PD (1996) Angiogenic growth factor mRNA responses in muscle to a single bout of exercise. J Appl Physiol 81:355-361
112. Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ (1999) Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. Am J Physiol 276:H679-685
113. Richardson RS, Wagner H, Mudaliar SR, Henry R, Noyszewski EA, Wagner PD (1999) Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. Am J Physiol 277:H2247-2252
114. Autiero M, Luttun A, Tjwa M, Carmeliet P (2003) Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. J Thromb Haemost 1:1356-1370
115. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernolle V, De Mol M, Wu Y, Bono F, Devy L, Beck H, et al (2001) Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med 7:575-583
116. Shibuya M (2001) Structure and dual function of vascular endothelial growth factor receptor-1 (Flt-1). Int J Biochem Cell Biol 33:409-420

117. Li T, Zhu Y, Qin CY, Yang Z, Fang A, Xu S, Ren W (2012) Expression and prognostic significance of vascular endothelial growth factor receptor 1 in hepatocellular carcinoma. J Clin Pathol
118. Dhakal HP, Naume B, Synnestvedt M, Borgen E, Kaaresen R, Schlichting E, Wiedswang G, Bassarova A, Holm R, Giercksky KE, et al (2012) Expression of vascular endothelial growth factor and vascular endothelial growth factor receptors 1 and 2 in invasive breast carcinoma: prognostic significance and relationship with markers for aggressiveness. Histopathology
119. Suryawanshi A, Veiga-Parga T, Reddy PB, Rajasagi NK, Rouse BT (2012) IL-17A differentially regulates corneal vascular endothelial growth factor (VEGF)-A and soluble VEGF receptor 1 expression and promotes corneal angiogenesis after herpes simplex virus infection. J Immunol 188:3434-3446
120. Harris AL, Reusch P, Barleon B, Hang C, Dobbs N, Marme D (2001) Soluble Tie2 and Flt1 extracellular domains in serum of patients with renal cancer and response to antiangiogenic therapy. Clin Cancer Res 7:1992-1997
121. Jelkmann W (2001) Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chem 47:617-623
122. Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, Maiguel D, Karumanchi SA, Yap HK, Saleem M, et al (2011) Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat Med 17:952-960
123. Blasi F, Carmeliet P (2002) uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol 3:932-943
124. Huai Q, Mazar AP, Kuo A, Parry GC, Shaw DE, Callahan J, Li Y, Yuan C, Bian C, Chen L, et al (2006) Structure of human urokinase plasminogen activator in complex with its receptor. Science 311:656-659
125. Sidenius N, Sier CF, Ullum H, Pedersen BK, Lepri AC, Blasi F, Eugen-Olsen J (2000) Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. Blood 96:4091-4095
126. Kofoed K, Eugen-Olsen J, Petersen J, Larsen K, Andersen O (2008) Predicting mortality in patients with systemic inflammatory response syndrome: an evaluation of two prognostic models, two soluble receptors, and a macrophage migration inhibitory factor. Eur J Clin Microbiol Infect Dis 27:375-383
127. Eugen-Olsen J, Andersen O, Linneberg A, Ladelund S, Hansen TW, Langkilde A, Petersen J, Pielak T, Moller LN, Jeppesen J, et al (2010) Circulating soluble urokinase plasminogen activator receptor predicts

cancer, cardiovascular disease, diabetes and mortality in the general population. J Intern Med 268:296-308
128. Backes Y, van der Sluijs KF, Mackie DP, Tacke F, Koch A, Tenhunen JJ, Schultz MJ (2011) Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection: a systematic review. Intensive Care Med
129. Tong Z, Kunnumakkara AB, Wang H, Matsuo Y, Diagaradjane P, Harikumar KB, Ramachandran V, Sung B, Chakraborty A, Bresalier RS, et al (2008) Neutrophil gelatinase-associated lipocalin: a novel suppressor of invasion and angiogenesis in pancreatic cancer. Cancer Res 68:6100-6108
130. Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, Thoren P, Hansson GK (2006) Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler Thromb Vasc Biol 26:136-142
131. Roudkenar MH, Kuwahara Y, Baba T, Roushandeh AM, Ebishima S, Abe S, Ohkubo Y, Fukumoto M (2007) Oxidative stress induced lipocalin 2 gene expression: addressing its expression under the harmful conditions. J Radiat Res 48:39-44
132. O'Donnell MP (1986) Definition of health promotion: Part II: Levels of programs. Am J Health Promot 1:6-9
133. Caspersen CJ, Powell KE, Christenson GM (1985) Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep 100:126-131
134. Lee IM, Skerrett PJ (2001) Physical activity and all-cause mortality: what is the dose-response relation? Med Sci Sports Exerc 33:S459-471; discussion S493-454
135. Lyons AS, Petrucelli RJja. Medicine : an illustrated history. New York: H. N. Abrams; 1978:616 p.
136. Speed C, Jaques R (2010) High-performance sports medicine: an ancient but evolving field. Br J Sports Med 45:81-83
137. Fox SM, 3rd, Haskell WL (1968) Physical activity and the prevention of coronary heart disease. Bull N Y Acad Med 44:950-967
138. Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW (1953) Coronary heart-disease and physical activity of work. Lancet 265:1053-1057; contd
139. Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW (1953) Coronary heart-disease and physical activity of work. Lancet 265:1111-1120; concl
140. Paffenbarger RS, Hale WE (1975) Work activity and coronary heart mortality. N Engl J Med 292:545-550
141. Paffenbarger RS, Jr., Brand RJ, Sholtz RI, Jung DL (1978) Energy expenditure, cigarette smoking, and blood pressure level as related to death from specific diseases. Am J Epidemiol 108:12-18

142. Macera CA, Hootman JM, Sniezek JE (2003) Major public health benefits of physical activity. Arthritis Rheum 49:122-128
143. Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH, Gibbons LW (1989) Physical fitness and all-cause mortality. A prospective study of healthy men and women. Jama 262:2395-2401
144. Myers J, Kaykha A, George S, Abella J, Zaheer N, Lear S, Yamazaki T, Froelicher V (2004) Fitness versus physical activity patterns in predicting mortality in men. Am J Med 117:912-918
145. Hu FB, Willett WC, Li T, Stampfer MJ, Colditz GA, Manson JE (2004) Adiposity as compared with physical activity in predicting mortality among women. N Engl J Med 351:2694-2703
146. Bouchard C, Shephard RJ, Stephens T. Physical activity, fitness, and health : international proceedings and consensus statement : [Second International Consensus Symposium on Physical Activity, Fitness, and Health, held May 5 to May 9, 1992, in Toronto, Canada]. Champaign, Ill. ; Leeds: Human Kinetics Publishers; 1994:xxiv, 1055 p.
147. Warburton DE, Nicol CW, Bredin SS (2006) Health benefits of physical activity: the evidence. Cmaj 174:801-809
148. Pedersen BK, Saltin B (2006) Evidence for prescribing exercise as therapy in chronic disease. Scand J Med Sci Sports 16 Suppl 1:3-63
149. Hansen J, Brandt C, Nielsen AR, Hojman P, Whitham M, Febbraio MA, Pedersen BK, Plomgaard P (2011) Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. Endocrinology 152:164-171
150. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, et al (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150:366-376
151. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, et al (2012) A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481:463-468
152. Dutra DB, Bueno PG, Silva RN, Nakahara NH, Selistre-Araujo HS, Nonaka KO, Leal AM (2012) Expression of myostatin, myostatin receptors and follistatin in diabetic rats submitted to exercise. Clin Exp Pharmacol Physiol 39:417-422
153. Dunstan DW, Daly RM, Owen N, Jolley D, Vulikh E, Shaw J, Zimmet P (2005) Home-based resistance training is not sufficient to maintain improved glycemic control following supervised training in older individuals with type 2 diabetes. Diabetes Care 28:3-9
154. Lee IM (2007) Dose-response relation between physical activity and fitness: even a little is good; more is better. JAMA 297:2137-2139

155. Blair SN, LaMonte MJ, Nichaman MZ (2004) The evolution of physical activity recommendations: how much is enough? Am J Clin Nutr 79:913S-920S
156. (1978) American College of Sports Medicine position statement on the recommended quantity and quality of exercise for developing and maintaining fitness in healthy adults. Med Sci Sports 10:vii-x
157. Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC, et al (1995) Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA 273:402-407
158. (1996) Physical activity and cardiovascular health. NIH Consensus Development Panel on Physical Activity and Cardiovascular Health. JAMA 276:241-246
159. O'Donovan G, Blazevich AJ, Boreham C, Cooper AR, Crank H, Ekelund U, Fox KR, Gately P, Giles-Corti B, Gill JM, et al (2010) The ABC of Physical Activity for Health: a consensus statement from the British Association of Sport and Exercise Sciences. J Sports Sci 28:573-591
160. Warburton DE, Nicol CW, Bredin SS (2006) Prescribing exercise as preventive therapy. Cmaj 174:961-974
161. Church TS, Earnest CP, Skinner JS, Blair SN (2007) Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. JAMA 297:2081-2091
162. Wen CP, Wai JP, Tsai MK, Yang YC, Cheng TY, Lee MC, Chan HT, Tsao CK, Tsai SP, Wu X (2011) Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet
163. Teramoto M, Bungum TJ Mortality and longevity of elite athletes. J Sci Med Sport 13:410-416
164. Ruiz JR, Moran M, Arenas J, Lucia A (2010) Strenuous endurance exercise improves life expectancy: it's in our genes. Br J Sports Med 45:159-161
165. Moorstein B (1968) Life expectancy of Ivy League rowing crews. JAMA 205:106
166. Hartley PH, Llewellyn GF (1939) Longevity of Oarsmen. Br Med J 1:657-662
167. Prout C (1972) Life expectancy of college oarsmen. JAMA 220:1709-1711
168. Polednak AP (1972) Longevity and cause of death among Harvard College athletes and their classmates. Geriatrics 27:53-64

169. Benito B, Gay-Jordi G, Serrano-Mollar A, Guasch E, Shi Y, Tardif JC, Brugada J, Nattel S, Mont L (2011) Cardiac arrhythmogenic remodeling in a rat model of long-term intensive exercise training. Circulation 123:13-22
170. Drezner JA (2008) Contemporary approaches to the identification of athletes at risk for sudden cardiac death. Curr Opin Cardiol 23:494-501
171. Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO (2009) Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980-2006. Circulation 119:1085-1092
172. Teramoto M, Bungum TJ (2010) Mortality and longevity of elite athletes. J Sci Med Sport 13:410-416
173. Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE (1993) Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators. N Engl J Med 329:1677-1683
174. Thompson PD, Franklin BA, Balady GJ, Blair SN, Corrado D, Estes NA, 3rd, Fulton JE, Gordon NF, Haskell WL, Link MS, et al (2007) Exercise and acute cardiovascular events placing the risks into perspective: a scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism and the Council on Clinical Cardiology. Circulation 115:2358-2368
175. Shephard RJ (2001) Absolute versus relative intensity of physical activity in a dose-response context. Med Sci Sports Exerc 33:S400-418; discussion S419-420
176. Wannamethee SG, Shaper AG, Walker M (1998) Changes in physical activity, mortality, and incidence of coronary heart disease in older men. Lancet 351:1603-1608
177. Hakim AA, Petrovitch H, Burchfiel CM, Ross GW, Rodriguez BL, White LR, Yano K, Curb JD, Abbott RD (1998) Effects of walking on mortality among nonsmoking retired men. N Engl J Med 338:94-99
178. Murphy MH, Blair SN, Murtagh EM (2009) Accumulated versus continuous exercise for health benefit: a review of empirical studies. Sports Med 39:29-43
179. Boreham CA, Kennedy RA, Murphy MH, Tully M, Wallace WF, Young I (2005) Training effects of short bouts of stair climbing on cardiorespiratory fitness, blood lipids, and homocysteine in sedentary young women. Br J Sports Med 39:590-593
180. Lee DC, Artero EG, Sui X, Blair SN (2010) Mortality trends in the general population: the importance of cardiorespiratory fitness. J Psychopharmacol 24:27-35
181. Kujala UM (2011) Born to be rich, physically active, fit and healthy? Scand J Med Sci Sports 20:367

182. Matsakas A, Narkar VA (2010) Endurance exercise mimetics in skeletal muscle. Curr Sports Med Rep 9:227-232
183. Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56:831-838
184. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, et al (2003) PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34:267-273
185. Dunn AL, Trivedi MH, O'Neal HA (2001) Physical activity dose-response effects on outcomes of depression and anxiety. Med Sci Sports Exerc 33:S587-597; discussion 609-510
186. Neeper SA, Gomez-Pinilla F, Choi J, Cotman C (1995) Exercise and brain neurotrophins. Nature 373:109
187. Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW (1996) Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Res 726:49-56
188. Mandroukas K, Krotkiewski M, Hedberg M, Wroblewski Z, Bjorntorp P, Grimby G (1984) Physical training in obese women. Effects of muscle morphology, biochemistry and function. Eur J Appl Physiol Occup Physiol 52:355-361
189. Shephard RJ, Futcher R (1997) Physical activity and cancer: how may protection be maximized? Crit Rev Oncog 8:219-272
190. Westerlind KC (2003) Physical activity and cancer prevention--mechanisms. Med Sci Sports Exerc 35:1834-1840
191. Thompson PD, Crouse SF, Goodpaster B, Kelley D, Moyna N, Pescatello L (2001) The acute versus the chronic response to exercise. Med Sci Sports Exerc 33:S438-445; discussion S452-433
192. Sakamoto K, Goodyear LJ (2002) Invited review: intracellular signaling in contracting skeletal muscle. J Appl Physiol 93:369-383
193. Ji LL, Gomez-Cabrera MC, Steinhafel N, Vina J (2004) Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. Faseb J 18:1499-1506
194. Goodyear LJ, Chang PY, Sherwood DJ, Dufresne SD, Moller DE (1996) Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. Am J Physiol 271:E403-408
195. Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL, Vina J (2005) Decreasing Xanthine Oxidase Mediated Oxidative Stress Prevents Useful Cellular Adaptations to Exercise in Rats. J Physiol
196. Berchtold MW, Brinkmeier H, Muntener M (2000) Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 80:1215-1265

197. Reid MB (2001) Invited Review: redox modulation of skeletal muscle contraction: what we know and what we don't. J Appl Physiol 90:724-731
198. Salminen A, Vihko V (1983) Endurance training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation in vitro. Acta Physiol Scand 117:109-113
199. Gomez-Cabrera MC, Domenech E, Vina J (2008) Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med 44:126-131
200. Salminen A, Vihko V (1983) Lipid peroxidation in exercise myopathy. Exp Mol Pathol 38:380-388
201. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Bluher M (2009) Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A 106:8665-8670
202. Borras C, Gomez-Cabrera MC, Vina J (2011) The dual role of p53: DNA protection and antioxidant. Free Radic Res 45:643-652
203. Huang LE, Arany Z, Livingston DM, Bunn HF (1996) Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 271:32253-32259
204. Palomero J, Broome CS, Rasmussen P, Mohr M, Nielsen B, Nybo L, McArdle A, Drust B (2008) Heat shock factor activation in human muscles following a demanding intermittent exercise protocol is attenuated with hyperthermia. Acta Physiol (Oxf) 193:79-88
205. Gomez-Cabrera MC, Vina J, Ji LL (2009) Interplay of oxidants and antioxidants during exercise: implications for muscle health. Phys Sportsmed 37:116-123
206. Bortz WM, 2nd, Angwin P, Mefford IN, Boarder MR, Noyce N, Barchas JD (1981) Catecholamines, dopamine, and endorphin levels during extreme exercise. N Engl J Med 305:466-467
207. Carr DB, Bullen BA, Skrinar GS, Arnold MA, Rosenblatt M, Beitins IZ, Martin JB, McArthur JW (1981) Physical conditioning facilitates the exercise-induced secretion of beta-endorphin and beta-lipotropin in women. N Engl J Med 305:560-563
208. Harber VJ, Sutton JR (1984) Endorphins and exercise. Sports Med 1:154-171
209. Vaynman S, Gomez-Pinilla F (2006) Revenge of the "sit": how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. J Neurosci Res 84:699-715
210. Dishman RK, Berthoud HR, Booth FW, Cotman CW, Edgerton VR, Fleshner MR, Gandevia SC, Gomez-Pinilla F, Greenwood BN, Hillman

CH, et al (2006) Neurobiology of exercise. Obesity (Silver Spring) 14:345-356
211. van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 96:13427-13431
212. Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K (2001) Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 58:498-504
213. Grealy MA, Johnson DA, Rushton SK (1999) Improving cognitive function after brain injury: the use of exercise and virtual reality. Arch Phys Med Rehabil 80:661-667
214. Siuciak JA, Boylan C, Fritsche M, Altar CA, Lindsay RM (1996) BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. Brain Res 710:11-20
215. Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22:3251-3261
216. Vissing J, Andersen M, Diemer NH (1996) Exercise-induced changes in local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 16:729-736
217. Czurko A, Hirase H, Csicsvari J, Buzsaki G (1999) Sustained activation of hippocampal pyramidal cells by 'space clamping' in a running wheel. Eur J Neurosci 11:344-352
218. McCloskey DP, Adamo DS, Anderson BJ (2001) Exercise increases metabolic capacity in the motor cortex and striatum, but not in the hippocampus. Brain Res 891:168-175
219. Lejoyeux M, Avril M, Richoux C, Embouazza H, Nivoli F (2008) Prevalence of exercise dependence and other behavioral addictions among clients of a Parisian fitness room. Compr Psychiatry 49:353-358
220. Freimuth M, Moniz S, Kim SR (2011) Clarifying exercise addiction: differential diagnosis, co-occurring disorders, and phases of addiction. Int J Environ Res Public Health 8:4069-4081
221. Sussman S, Sussman AN (2011) Considering the definition of addiction. Int J Environ Res Public Health 8:4025-4038
222. Black A, Black MM, Gensini G (1975) Exertion and acute coronary artery injury. Angiology 26:759-783
223. Siscovick DS, Weiss NS, Fletcher RH, Lasky T (1984) The incidence of primary cardiac arrest during vigorous exercise. N Engl J Med 311:874-877

224. Albert CM, Mittleman MA, Chae CU, Lee IM, Hennekens CH, Manson JE (2000) Triggering of sudden death from cardiac causes by vigorous exertion. N Engl J Med 343:1355-1361
225. Baracos VE (1989) Exercise inhibits progressive growth of the Morris hepatoma 7777 in male and female rats. Can J Physiol Pharmacol 67:864-870
226. Thompson HJ, Ronan AM, Ritacco KA, Tagliaferro AR (1989) Effect of type and amount of dietary fat on the enhancement of rat mammary tumorigenesis by exercise. Cancer Res 49:1904-1908
227. Jones LW, Eves ND, Courneya KS, Chiu BK, Baracos VE, Hanson J, Johnson L, Mackey JR (2005) Effects of exercise training on antitumor efficacy of doxorubicin in MDA-MB-231 breast cancer xenografts. Clin Cancer Res 11:6695-6698
228. Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA, Ray CA (2004) American College of Sports Medicine position stand. Exercise and hypertension. Med Sci Sports Exerc 36:533-553
229. Tipton CM (1999) Exercise training for the treatment of hypertension: a review. Clin J Sport Med 9:104
230. Howatson G, van Someren KA (2008) The prevention and treatment of exercise-induced muscle damage. Sports Med 38:483-503
231. Vina J, Sanchis-Gomar F, Martinez-Bello V, Gomez-Cabrera M (2012) Exercise acts as a drug; the pharmacological benefits of exercise. Br J Pharmacol 167:1-12
232. Vigen R, Maddox TM, Allen LA (2012) Aging of the United States Population: Impact on Heart Failure. Curr Heart Fail Rep
233. Abbatecola AM, Fumagalli A, Bonardi D, Guffanti EE (2011) Practical management problems of chronic obstructive pulmonary disease in the elderly: acute exacerbations. Curr Opin Pulm Med 17 Suppl 1:S49-54
234. Demontiero O, Vidal C, Duque G (2012) Aging and bone loss: new insights for the clinician. Ther Adv Musculoskelet Dis 4:61-76
235. Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA, Macera CA, Heath GW, Thompson PD, Bauman A (2007) Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 39:1423-1434
236. Goodyear LJ (2008) The exercise pill--too good to be true? N Engl J Med 359:1842-1844
237. Narkar VA, Fan W, Downes M, Yu RT, Jonker JW, Alaynick WA, Banayo E, Karunasiri MS, Lorca S, Evans RM (2011) Exercise and PGC-1alpha-independent synchronization of type I muscle metabolism and vasculature by ERRgamma. Cell Metab 13:283-293

238. Wojtaszewski JF, Nielsen P, Hansen BF, Richter EA, Kiens B (2000) Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle. J Physiol 528 Pt 1:221-226
239. Winder WW, Taylor EB, Thomson DM (2006) Role of AMP-activated protein kinase in the molecular adaptation to endurance exercise. Med Sci Sports Exerc 38:1945-1949
240. Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, Mihaylova MM, Nelson MC, Zou Y, Juguilon H, et al (2008) AMPK and PPARdelta agonists are exercise mimetics. Cell 134:405-415
241. Jorgensen SB, Treebak JT, Viollet B, Schjerling P, Vaulont S, Wojtaszewski JF, Richter EA (2007) Role of AMPKalpha2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. Am J Physiol Endocrinol Metab 292:E331-339
242. Jager S, Handschin C, St-Pierre J, Spiegelman BM (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 104:12017-12022
243. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92:829-839
244. Koves TR, Li P, An J, Akimoto T, Slentz D, Ilkayeva O, Dohm GL, Yan Z, Newgard CB, Muoio DM (2005) Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. J Biol Chem 280:33588-33598
245. Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, et al (2002) Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature 418:797-801
246. Lin J, Handschin C, Spiegelman BM (2005) Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1:361-370
247. Schiaffino S, Sandri M, Murgia M (2007) Activity-dependent signaling pathways controlling muscle diversity and plasticity. Physiology (Bethesda) 22:269-278
248. Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL, Spiegelman BM (2006) PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc Natl Acad Sci U S A 103:16260-16265
249. Schuler M, Ali F, Chambon C, Duteil D, Bornert JM, Tardivel A, Desvergne B, Wahli W, Chambon P, Metzger D (2006) PGC1alpha

expression is controlled in skeletal muscles by PPARbeta, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. Cell Metab 4:407-414

250. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, et al (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127:1109-1122
251. Ungvari Z, Sonntag WE, de Cabo R, Baur JA, Csiszar A (2011) Mitochondrial protection by resveratrol. Exerc Sport Sci Rev 39:128-132
252. Momken I, Stevens L, Bergouignan A, Desplanches D, Rudwill F, Chery I, Zahariev A, Zahn S, Stein TP, Sebedio JL, et al (2011) Resveratrol prevents the wasting disorders of mechanical unloading by acting as a physical exercise mimetic in the rat. FASEB J 25:3646-3660
253. Ringholm S, Olesen J, Pedersen JT, Brandt CT, Halling JF, Hellsten Y, Prats C, Pilegaard H (2013) Effect of lifelong resveratrol supplementation and exercise training on skeletal muscle oxidative capacity in aging mice; impact of PGC-1alpha. Exp Gerontol 48:1311-1318
254. Gliemann L, Schmidt JF, Olesen J, Bienso RS, Peronard SL, Grandjean SU, Mortensen SP, Nyberg M, Bangsbo J, Pilegaard H, et al (2013) Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. J Physiol 591:5047-5059
255. Carey AL, Kingwell BA (2009) Novel pharmacological approaches to combat obesity and insulin resistance: targeting skeletal muscle with 'exercise mimetics'. Diabetologia 52:2015-2026
256. Kuida K, Haydar TF, Kuan CY, Gu Y, Taya C, Karasuyama H, Su MS, Rakic P, Flavell RA (1998) Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. Cell 94:325-337
257. Schroeter H, Boyd CS, Ahmed R, Spencer JP, Duncan RF, Rice-Evans C, Cadenas E (2003) c-Jun N-terminal kinase (JNK)-mediated modulation of brain mitochondria function: new target proteins for JNK signalling in mitochondrion-dependent apoptosis. Biochem J 372:359-369
258. Zhou Q, Lam PY, Han D, Cadenas E (2009) Activation of c-Jun-N-terminal kinase and decline of mitochondrial pyruvate dehydrogenase activity during brain aging. FEBS Lett 583:1132-1140
259. Ahmed S, Passos JF, Birket MJ, Beckmann T, Brings S, Peters H, Birch-Machin MA, von Zglinicki T, Saretzki G (2008) Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. J Cell Sci 121:1046-1053
260. Holloszy JO (2004) Adaptations of skeletal muscle mitochondria to endurance exercise: a personal perspective. Exerc Sport Sci Rev 32:41-43
261. Holloszy JO, Booth FW (1976) Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38:273-291

262. Harman D (1956) Aging: a theory based on free radical and ratiation chemistry. J Gerontol 11:298-300
263. Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. Physiol Rev 59:527-605
264. Miquel J, Economos AC, Fleming J, Johnson JE, Jr. (1980) Mitochondrial role in cell aging. Exp Gerontol 15:575-591
265. Rockstein M, Chesky J, Philpott DE, Takahashi A, Johnson JE, Jr., Miquel J (1975) An electron microscopic investigation of age-dependent changes in the flight muscle of Musca domestica L. Gerontologia 21:216-223
266. de la Asuncion JG, Millan A, Pla R, Bruseghini L, Esteras A, Pallardo FV, Sastre J, Vina J (1996) Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. Faseb J 10:333-338
267. Sastre J, Pallardo FV, Pla R, Pellin A, Juan G, O'Connor JE, Estrela JM, Miquel J, Vina J (1996) Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. Hepatology 24:1199-1205
268. Shigenaga MK, Hagen TM, Ames BN (1994) Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci U S A 91:10771-10778
269. Sastre J, Millan A, Garcia de la Asuncion J, Pla R, Juan G, Pallardo, O'Connor E, Martin JA, Droy-Lefaix MT, Vina J (1998) A Ginkgo biloba extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress. Free Radic Biol Med 24:298-304.
270. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jager S, Handschin C, Zheng K, Lin J, Yang W, et al (2006) Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. Cell 127:397-408
271. Gadaleta MN, Cormio A, Pesce V, Lezza AM, Cantatore P (1998) Aging and mitochondria. Biochimie 80:863-870
272. Wolfe RR (2006) The underappreciated role of muscle in health and disease. Am J Clin Nutr 84:475-482
273. Vanitallie TB (2003) Frailty in the elderly: contributions of sarcopenia and visceral protein depletion. Metabolism 52:22-26
274. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, et al (2001) Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56:M146-156
275. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, et al (2010) Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing 39:412-423
276. Rosenberg IH (1997) Sarcopenia: origins and clinical relevance. J Nutr 127:990S-991S

277. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, Evans WJ (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 330:1769-1775
278. Gill TM, Gahbauer EA, Han L, Allore HG (2010) Trajectories of disability in the last year of life. N Engl J Med 362:1173-1180
279. Drew B, Phaneuf S, Dirks A, Selman C, Gredilla R, Lezza A, Barja G, Leeuwenburgh C (2003) Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. Am J Physiol Regul Integr Comp Physiol 284:R474-480
280. McKiernan SH, Colman RJ, Lopez M, Beasley TM, Aiken JM, Anderson RM, Weindruch R (2011) Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle. Exp Gerontol 46:23-29
281. Cassano P, Sciancalepore AG, Lezza AM, Leeuwenburgh C, Cantatore P, Gadaleta MN (2006) Tissue-specific effect of age and caloric restriction diet on mitochondrial DNA content. Rejuvenation Res 9:211-214
282. Navarro A, Boveris A (2007) The mitochondrial energy transduction system and the aging process. Am J Physiol Cell Physiol 292:C670-686
283. Marzetti E, Wohlgemuth SE, Lees HA, Chung HY, Giovannini S, Leeuwenburgh C (2008) Age-related activation of mitochondrial caspase-independent apoptotic signaling in rat gastrocnemius muscle. Mech Ageing Dev 129:542-549
284. Chowanadisai W, Bauerly KA, Tchaparian E, Wong A, Cortopassi GA, Rucker RB Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression. J Biol Chem 285:142-152
285. Hood DA, Irrcher I, Ljubicic V, Joseph AM (2006) Coordination of metabolic plasticity in skeletal muscle. J Exp Biol 209:2265-2275
286. Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT (2009) Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. Proc Natl Acad Sci U S A 106:20405-20410
287. Saleem A, Carter HN, Iqbal S, Hood DA Role of p53 within the regulatory network controlling muscle mitochondrial biogenesis. Exerc Sport Sci Rev 39:199-205
288. Mammucari C, Rizzuto R Signaling pathways in mitochondrial dysfunction and aging. Mech Ageing Dev 131:536-543
289. Leick L, Lyngby SS, Wojtasewski JF, Pilegaard H (2010) PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. Exp Gerontol 45:336-342

290. Yen TC, Chen YS, King KL, Yeh SH, Wei YH (1989) Liver mitochondrial respiratory functions decline with age. Biochem Biophys Res Commun 165:944-1003
291. Tummino PJ, Gafni A (1991) A comparative study of succinate-supported respiration and ATP/ADP translocation in liver mitochondria from adult and old rats. Mech Ageing Dev 59:177-188
292. Cadenas E, Davies KJ (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29:222-230
293. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford, UK: Oxford Univ. Press; 2007
294. Powers SK, Jackson MJ (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 88:1243-1276
295. Sies H (1985) Oxidative stress. London: Academic
296. Sies H, Cadenas E (1985) Oxidative stress: damage to intact cells and organs. Philos Trans R Soc Lond B Biol Sci 311:617-631
297. Jones DP (2006) Redefining oxidative stress. Antioxid Redox Signal 8:1865-1879
298. Strehler BL (1985) Aging: a challenge to science, society, and the individual. Clin Geriatr Med 1:5-13
299. Vina J, Borras C, Abdelaziz KM, Garcia-Valles R, Gomez-Cabrera MC (2013) The free radical theory of aging revisited: the cell signaling disruption theory of aging. Antioxid Redox Signal 19:779-787
300. Vina J, Borras C, Miquel J (2007) Theories of ageing. IUBMB Life 59:249-254
301. Miquel J, de Juan E, Sevila I (1992) Oxygen-induced mitochondrial damage and aging. EXS 62:47-57
302. Yang W, Hekimi S A mitochondrial superoxide signal triggers increased longevity in Caenorhabditis elegans. PLoS Biol 8:e1000556
303. Sohal RS, Orr WC (1992) Relationship between antioxidants, prooxidants, and the aging process. Ann N Y Acad Sci 663:74-84.
304. García de la Asunción J, Millan A, Pla R, Bruseghini L, Esteras A, Pallardo FV, Sastre J, Viña J (1996) Mitochondiral glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. FASEB J 10:333-338
305. Sohal RS, Sohal BH (1991) Hydrogen peroxide release by mitochondria increases during aging. Mech Ageing Dev 57:187-202.
306. Barja G, Cadenas S, Rojas C, Perez-Campo R, Lopez-Torres M (1994) Low mitochondrial free radical production per unit O2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds. Free Radic Res 21:317-327.

307. Richter C, Park JW, Ames BN (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci USA 85:6465-6467
308. Corbisier P, Remacle J (1990) Involvement of mitochondria in cell degeneration. European J Cell Biol 51:173-182
309. Ali SS, Xiong C, Lucero J, Behrens MM, Dugan LL, Quick KL (2006) Gender differences in free radical homeostasis during aging: shorter-lived female C57BL6 mice have increased oxidative stress. Aging Cell 5:565-574
310. Borras C, Sastre J, Garcia-Sala D, Lloret A, Pallardo FV, Vina J (2003) Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. Free Radic Biol Med 34:546-552
311. Cutler RG (1991) Antioxidants and aging. Am J Clin Nutr 53:373S-379S
312. Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25:585-621
313. Lambert AJ, Brand MD (2007) Research on mitochondria and aging, 2006-2007. Aging Cell 6:417-420
314. Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11:298-300
315. Lloret A, Badia MC, Mora NJ, Pallardo FV, Alonso MD, Vina J (2009) Vitamin E paradox in Alzheimer's disease: it does not prevent loss of cognition and may even be detrimental. J Alzheimers Dis 17:143-149
316. Perez VI, Bokov A, Van Remmen H, Mele J, Ran Q, Ikeno Y, Richardson A (2009) Is the oxidative stress theory of aging dead? Biochim Biophys Acta 1790:1005-1014
317. Olmos Y, Valle I, Borniquel S, Tierrez A, Soria E, Lamas S, Monsalve M (2009) Mutual Dependence of Foxo3a and PGC-1{alpha} in the Induction of Oxidative Stress Genes. J Biol Chem 284:14476-14484
318. Hathcock JN, Azzi A, Blumberg J, Bray T, Dickinson A, Frei B, Jialal I, Johnston CS, Kelly FJ, Kraemer K, et al (2005) Vitamins E and C are safe across a broad range of intakes. Am J Clin Nutr 81:736-745
319. Ji LL, Gomez-Cabrera MC, Vina J (2006) Exercise and hormesis: activation of cellular antioxidant signaling pathway. Ann N Y Acad Sci 1067:425-435
320. Vina J, Borras C, Gomez-Cabrera MC, Orr WC (2006) Part of the series: from dietary antioxidants to regulators in cellular signalling and gene expression. Role of reactive oxygen species and (phyto)oestrogens in the modulation of adaptive response to stress. Free Radic Res 40:111-119
321. CLSI. Procedures for the handling and processing of blood specimens for common laboratory tests. NCCLS H18-A4. In. 4º ed; 2010

322. Lippi G, Schena F, Salvagno GL, Sanchis-Gomar F, Guidi GC (2013) Serum copeptin and mid-region pro-adrenomedullin (MR-proADM) after an ultramarathon. J Clin Lab Anal
323. Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jager S, Vianna CR, Reznick RM, et al (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell 119:121-135
324. Powers SK, Criswell D, Lawler J, Ji LL, Martin D, Herb RA, Dudley G (1994) Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. Am J Physiol 266:R375-380
325. Lawler JM, Powers SK, Visser T, Van Dijk H, Kordus MJ, Ji LL (1993) Acute exercise and skeletal muscle antioxidant and metabolic enzymes: effects of fiber type and age. Am J Physiol 265:R1344-1350
326. Davies KJ, Packer L, Brooks GA (1981) Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. Arch Biochem Biophys 209:539-554
327. Irrcher I, Adhihetty PJ, Sheehan T, Joseph AM, Hood DA (2003) PPARgamma coactivator-1alpha expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. Am J Physiol Cell Physiol 284:C1669-1677
328. Romagnoli M, Gomez-Cabrera MC, Perrelli MG, Biasi F, Pallardo FV, Sastre J, Poli G, Vina J (2010) Xanthine oxidase-induced oxidative stress causes activation of NF-kappaB and inflammation in the liver of type I diabetic rats. Free Radic Biol Med 49:171-177
329. Banfi G, Del Fabbro M, Lippi G (2009) Serum creatinine concentration and creatinine-based estimation of glomerular filtration rate in athletes. Sports Med 39:331-337
330. Tian Y, Tong TK, Lippi G, Huang C, Shi Q, Nie J (2011) Renal function parameters during early and late recovery periods following an all-out 21-km run in trained adolescent runners. Clin Chem Lab Med 49:993-997
331. Banfi G, Del Fabbro M, Lippi G (2008) Creatinine values during a competitive season in elite athletes involved in different sport disciplines. J Sports Med Phys Fitness 48:479-482
332. Lippi G, Banfi G, Salvagno GL, Franchini M, Guidi GC (2008) Glomerular filtration rate in endurance athletes. Clin J Sport Med 18:286-288
333. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A (2007) Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 11:R31
334. Lucia A, Earnest C, Arribas C (2003) The Tour de France: a physiological review. Scand J Med Sci Sports 13:275-283

335. Padilla S, Mujika I, Santisteban J, Impellizzeri FM, Goiriena JJ (2008) Exercise intensity and load during uphill cycling in professional 3-week races. Eur J Appl Physiol 102:431-438.
336. Leick L, Wojtaszewski JF, Johansen ST, Kiilerich K, Comes G, Hellsten Y, Hidalgo J, Pilegaard H (2008) PGC-1alpha is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle. Am J Physiol Endocrinol Metab 294:E463-474
337. Hood DA (2001) Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. J Appl Physiol 90:1137-1157
338. Terjung RL (1979) The turnover of cytochrome c in different skeletal-muscle fibre types of the rat. Biochem J 178:569-574
339. Proske U, Morgan DL (2001) Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol 537:333-345
340. Giza E, Micheli LJ (2005) Soccer injuries. Med Sport Sci 49:140-169
341. Borras C, Gambini J, Gomez-Cabrera MC, Sastre J, Pallardo FV, Mann GE, Vina J (2006) Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NFkappaB. Faseb J 20:2136-2138
342. Ji LL, Gomez-Cabrera MC, Vina J (2007) Role of nuclear factor kappaB and mitogen-activated protein kinase signaling in exercise-induced antioxidant enzyme adaptation. Appl Physiol Nutr Metab 32:930-935
343. Kang C, O'Moore KM, Dickman JR, Ji LL (2009) Exercise activation of muscle peroxisome proliferator-activated receptor-gamma coactivator-1alpha signaling is redox sensitive. Free Radic Biol Med 47:1394-1400
344. Ryan MJ, Jackson JR, Hao Y, Leonard SS, Alway SE (2011) Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice. Free Radic Biol Med
345. McCord JM (1985) Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 312:159-163
346. Brancaccio P, Maffulli N, Limongelli FM (2007) Creatine kinase monitoring in sport medicine. Br Med Bull 81-82:209-230
347. Noman A, Ang DS, Ogston S, Lang CC, Struthers AD (2010) Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. Lancet 375:2161-2167
348. Lippi G, Schena F, Guidi GC (2006) Health benefits of physical activity. CMAJ 175:776; author reply 777
349. Aizer A, Gaziano JM, Cook NR, Manson JE, Buring JE, Albert CM (2009) Relation of vigorous exercise to risk of atrial fibrillation. Am J Cardiol 103:1572-1577

350. Neilan TG, Januzzi JL, Lee-Lewandrowski E, Ton-Nu TT, Yoerger DM, Jassal DS, Lewandrowski KB, Siegel AJ, Marshall JE, Douglas PS, et al (2006) Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston marathon. Circulation 114:2325-2333
351. Kim JH, Malhotra R, Chiampas G, d'Hemecourt P, Troyanos C, Cianca J, Smith RN, Wang TJ, Roberts WO, Thompson PD, et al (2012) Cardiac arrest during long-distance running races. N Engl J Med 366:130-140
352. Lippi G, Maffulli N (2009) Biological influence of physical exercise on hemostasis. Semin Thromb Hemost 35:269-276
353. Mellin V, Isabelle M, Oudot A, Vergely-Vandriesse C, Monteil C, Di Meglio B, Henry JP, Dautreaux B, Rochette L, Thuillez C, et al (2005) Transient reduction in myocardial free oxygen radical levels is involved in the improved cardiac function and structure after long-term allopurinol treatment initiated in established chronic heart failure. Eur Heart J 26:1544-1550
354. Papezikova I, Pekarova M, Kolarova H, Klinke A, Lau D, Baldus S, Lojek A, Kubala L (2012) Uric acid modulates vascular endothelial function through the down regulation of nitric oxide production. Free Radic Res
355. Lippi G, Montagnana M, Franchini M, Favaloro EJ, Targher G (2008) The paradoxical relationship between serum uric acid and cardiovascular disease. Clin Chim Acta 392:1-7
356. Anker SD, Doehner W, Rauchhaus M, Sharma R, Francis D, Knosalla C, Davos CH, Cicoira M, Shamim W, Kemp M, et al (2003) Uric acid and survival in chronic heart failure: validation and application in metabolic, functional, and hemodynamic staging. Circulation 107:1991-1997
357. Hew-Butler T, Hoffman MD, Stuempfle KJ, Rogers IR, Morgenthaler NG, Verbalis JG (2011) Changes in copeptin and bioactive vasopressin in runners with and without hyponatremia. Clin J Sport Med 21:211-217
358. Burge J, Knechtle B, Knechtle P, Gnadinger M, Rust CA, Rosemann T (2011) Maintained serum sodium in male ultra-marathoners--the role of fluid intake, vasopressin, and aldosterone in fluid and electrolyte regulation. Horm Metab Res 43:646-652
359. L'Abate P, Wiegert S, Struck J, Wellmann S, Cannizzaro V (2013) Determinants of plasma copeptin: A systematic investigation in a pediatric mechanical ventilation model. Respir Physiol Neurobiol 185:222-227
360. Montoro-Garcia S, Hernandez-Romero D, Jover E, Garcia-Honrubia A, Vilchez JA, Casas T, Martinez P, Climent V, Caballero L, Valdes M, et al (2012) Growth differentiation factor-15, a novel biomarker related with disease severity in patients with hypertrophic cardiomyopathy. Eur J Intern Med 23:169-174

361. Munk PS, Valborgland T, Butt N, Larsen AI (2011) Response of growth differentiation factor-15 to percutaneous coronary intervention and regular exercise training. Scand Cardiovasc J 45:27-32
362. Stahrenberg R, Edelmann F, Mende M, Kockskamper A, Dungen HD, Luers C, Binder L, Herrmann-Lingen C, Gelbrich G, Hasenfuss G, et al (2010) The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. Eur J Heart Fail 12:1309-1316
363. Tchou I, Margeli A, Tsironi M, Skenderi K, Barnet M, Kanaka-Gantenbein C, Papassotiriou I, Beris P (2009) Growth-differentiation factor-15, endoglin and N-terminal pro-brain natriuretic peptide induction in athletes participating in an ultramarathon foot race. Biomarkers 14:418-422
364. Bailey AP, Shparago M, Gu JW (2006) Exercise increases soluble vascular endothelial growth factor receptor-1 (sFlt-1) in circulation of healthy volunteers. Med Sci Monit 12:CR45-50
365. Kivela R, Silvennoinen M, Lehti M, Jalava S, Vihko V, Kainulainen H (2008) Exercise-induced expression of angiogenic growth factors in skeletal muscle and in capillaries of healthy and diabetic mice. Cardiovasc Diabetol 7:13
366. Lippi G, Schena F, Montagnana M, Salvagno GL, Guidi GC (2008) Acute influence of aerobic physical exercise on procalcitonin. Eur J Clin Invest 38:784-785
367. Scherr J, Braun S, Schuster T, Hartmann C, Moehlenkamp S, Wolfarth B, Pressler A, Halle M (2011) 72-h kinetics of high-sensitive troponin T and inflammatory markers after marathon. Med Sci Sports Exerc 43:1819-1827
368. Pacher P, Nivorozhkin A, Szabo C (2006) Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol Rev 58:87-114
369. Sahib AS, Al-Jawad FH, Alkaisy AA (2010) Effect of antioxidants on the incidence of wound infection in burn patients. Ann Burns Fire Disasters 23:199-205
370. Gobbi P, Lo Presti MS, Fernández AR, Enders JE, Fretes R, Gea S, Paglini-Oliva PA, Rivarola HW (2007) Allopurinol is effective to modify the evolution of Trypanosoma cruzi infection in mice. Parasitol Res 101:1459-1462
371. Maeder MT, Staub D, Brutsche MH, Arenja N, Socrates T, Reiter M, Meissner J, Morgenthaler NG, Bergmann A, Struck J, et al (2010) Copeptin response to clinical maximal exercise tests. Clin Chem 56:674-676
372. Bracher A, Knechtle B, Gnadinger M, Burge J, Rust CA, Knechtle P, Rosemann T (2012) Fluid intake and changes in limb volumes in male ultra-marathoners: does fluid overload lead to peripheral oedema? Eur J Appl Physiol 112:991-1003

373. Clarkson PM (2007) Exertional rhabdomyolysis and acute renal failure in marathon runners. Sports Med 37:361-363
374. Sanchez LD, Corwell B, Berkoff D (2006) Medical problems of marathon runners. Am J Emerg Med 24:608-615
375. Bonventre JV (1988) Mediators of ischemic renal injury. Annu Rev Med 39:531-544
376. McCullough PA, Chinnaiyan KM, Gallagher MJ, Colar JM, Geddes T, Gold JM, Trivax JE (2011) Changes in renal markers and acute kidney injury after marathon running. Nephrology (Carlton) 16:194-199
377. Spiropoulos A, Goussetis E, Margeli A, Premetis E, Skenderi K, Graphakos S, Baltopoulos P, Tsironi M, Papassotiriou I (2010) Effect of inflammation induced by prolonged exercise on circulating erythroid progenitors and markers of erythropoiesis. Clin Chem Lab Med 48:199-203
378. Cowland JB, Borregaard N (1997) Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics 45:17-23
379. Decavele AS, Dhondt L, De Buyzere ML, Delanghe JR (2011) Increased urinary neutrophil gelatinase associated lipocalin in urinary tract infections and leukocyturia. Clin Chem Lab Med 49:999-1003
380. Bloomer RJ, Goldfarb AH, Wideman L, McKenzie MJ, Consitt LA (2005) Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. J Strength Cond Res 19:276-285
381. Le Meur Y, Paraf F, Szelag JC, Aldigier JC, Leroux-Robert C (1998) Acute renal failure in a marathon runner: role of glomerular bleeding in tubular injury. Am J Med 105:251-252
382. Pollard T, Weiss I (1970) Acute renal necrosis in patient with march hemoglobinuria. N Engl J Med 283:15-16
383. Woodcock J, Franco OH, Orsini N, Roberts I (2010) Non-vigorous physical activity and all-cause mortality: systematic review and meta-analysis of cohort studies. Int J Epidemiol doi: 10.1093/ije/dyq104
384. Lucia A, Hoyos J, Chicharro JL (2001) Physiology of professional road cycling. Sports Med 31:325-337
385. Chakravarty EF, Hubert HB, Lingala VB, Fries JF (2008) Reduced disability and mortality among aging runners: a 21-year longitudinal study. Arch Intern Med 168:1638-1646
386. Kujala UM, Tikkanen HO, Sarna S, Pukkala E, Kaprio J, Koskenvuo M (2001) Disease-specific mortality among elite athletes. Jama 285:44-45
387. Ruiz JR, Moran M, Arenas J, Lucia A (2010) Strenuous endurance exercise improves life expectancy: it's in our genes. Br J Sports Med doi:10.1136/bjsm.2010.075085

388. Fogelholm M, Kaprio J, Sarna S (1994) Healthy lifestyles of former Finnish world class athletes. Med Sci Sports Exerc 26:224-229
389. Sarna S, Sahi T, Koskenvuo M, Kaprio J (1993) Increased life expectancy of world class male athletes. Med Sci Sports Exerc 25:237-244
390. Zaidi A, Sharma S (2013) Reduced mortality in former Tour de France participants: the benefits from intensive exercise or a select genetic tour de force? Eur Heart J
391. Marijon E, Tafflet M, Antero-Jacquemin J, El Helou N, Berthelot G, Celermajer DS, Bougouin W, Combes N, Hermine O, Empana JP, et al (2013) Mortality of French participants in the Tour de France (1947-2012). Eur Heart J
392. Johnson JE, Mehler WR, Miquel J (1975) A fine structural study of degenerative changes in the dorsal column nuclei of aging mice. Lack of protection by vitamin E. J Gerontol 30:395-411
393. Hagen TM, Yowe DL, Bartholomew JC, Wehr CM, Do KL, Park JY, Ames BN (1997) Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. Proc Natl Acad Sci U S A 94:3064-3069
394. Gill TM, Baker DI, Gottschalk M, Peduzzi PN, Allore H, Byers A (2002) A program to prevent functional decline in physically frail, elderly persons who live at home. N Engl J Med 347:1068-1074
395. Anderson RM, Barger JL, Edwards MG, Braun KH, O'Connor CE, Prolla TA, Weindruch R (2008) Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. Aging Cell 7:101-111
396. Anderson RM, Weindruch R (2009) Metabolic reprogramming, caloric restriction and aging. Trends Endocrinol Metab 21:134-141
397. Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, et al (2005) Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science 310:314-317
398. McKiernan SH, Colman RJ, Lopez M, Beasley TM, Aiken JM, Anderson RM, Weindruch R (2010) Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle. Exp Gerontol 46:23-29
399. Judge S, Jang YM, Smith A, Selman C, Phillips T, Speakman JR, Hagen T, Leeuwenburgh C (2005) Exercise by lifelong voluntary wheel running reduces subsarcolemmal and interfibrillar mitochondrial hydrogen peroxide production in the heart. Am J Physiol Regul Integr Comp Physiol 289:R1564-1572
400. Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS, Wadley GD (2010) Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. Med Sci Sports Exerc 43:1017-1024

401. Kelly DP (2011) Cell biology: Ageing theories unified. Nature 470:342-343

RESUMEN: APÉNDICES

4. APÉNDICES

APÉNDICE 1: Factor de impacto y área temática de las revistas

Revista	Factor de Impacto	Área temática	Quartil
N Engl J Med	53.3	Medicine, general & internal	Q1
Adv Drug Deliv Rev	13.57	Pharmacology & pharmacy	Q1
Br J Pharmacol	5.06	Pharmacology & pharmacy	Q1
Age (Dordr)	3.95	Geriatrics & gerontology	Q1
Exp Gerontol	3.74	Geriatrics & gerontology	Q1
Transl Res	3.49	Medicine, general & internal	Q1
Curr Pharm Des	3.31	Pharmacology & pharmacy	Q1
Scand J Med Sci Sports	3.21	Sports sciences	Q1
Int J Sports Med	2.43	Sports sciences	Q1
Clin Chem Lab Med	2.15	Medical laboratory technology	Q2
J Clin Lab Anal	1.4	Medical laboratory technology	Q3
Clin Lab	1.1	Medical laboratory technology	Q3

Media de factor de impacto: 8.06

APÉNDICE 2: Copia de los trabajos publicados

SCANDINAVIAN JOURNAL OF MEDICINE & SCIENCE IN SPORTS

Allopurinol prevents cardiac and skeletal muscle damage in soccer players

Journal:	*Scandinavian Journal of Medicine and Science in Sports*
Manuscript ID:	SJMSS-O-411-13
Manuscript Type:	Original Article
Date Submitted by the Author:	31-Jul-2013
Complete List of Authors:	Sanchis-Gomar, Fabian; University of Valencia, Physiology Pareja-Galeano, Helios; University of Valencia, Physiology Gomez-Cabrera, Carmen; University of Valencia , Physiology Candel, Jorge; 2Medical Services Valencia CF., Lippi, Giuseppe; Clinical Chemistry Laboratory, Academic Hospital of Parma, Salvagno, Gian Luca; Clinical Chemistry Laboratory, Academic Hospital of Parma, Mann, Giovanni; King's College London, Cardiovascular Division Vina, Jose; University of Valencia, Physiology;
Keywords:	xanthine oxidase, free radicals, injury, performance

Scandinavian Journal of Medicine & Science in Sports

Title: Allopurinol prevents cardiac and skeletal muscle damage in soccer players

Authors: F Sanchis-Gomar[1]*, H Pareja-Galeano[1]*, MC Gomez-Cabrera[1], J Candel[2], G Lippi[3], GL Salvagno[3], GE Mann[4], and J Viña[1]

Affiliations: [1]Department of Physiology. University of Valencia. Fundacion Investigacion Hospital Clinico Universitario/INCLIVA. Spain. [2]Medical Services Valencia CF. [3]Clinical Chemistry Laboratory, Academic Hospital of Parma, Parma, Italy. [4]Cardiovascular Division, BHF Centre of Research Excellence, School of Medicine, King's College London, UK.

* These two authors have contributed equally to this work

Address correspondence to:

Jose Vina

Department of Physiology. Faculty of Medicine. University of Valencia.

Av. Blasco Ibañez, 15, Valencia. 46010 Spain

Phone: (34) 96 386 46 50; Fax: (34) 96 386 46 42

Email: jose.vina@uv.es

Conflict of Interest: The authors declare that no conflict of interest exists.

Running Title: Allopurinol and muscle damage

ABSTRACT

High intensity exercise is associated with an increased risk of skeletal and cardiac muscle injuries. Xanthine oxidase (XO), a free radical generating enzyme, is involved in tissue damage produced during exhaustive exercise. Our aim was to test whether allopurinol, a powerful inhibitor of XO widely used in clinical practice, may be effective in preventing exercise-induced muscle, cardiac and liver injury in soccer players. Twelve soccer players from a top-level professional team were randomized into two experimental groups. One received 300 mg of allopurinol before a match of the premier Spanish Football League and the other placebo (cellulose). Serum samples were obtained before and after the match for assessment of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), myoglobin, creatine kinase-MB isoenzyme (CK-MB), highly-sensitive troponin T (Hs-TnT), gamma glutamyltransferase (GGT), alanine aminotransferase (ALT), uric acid, and lipid hydroperoxides. A soccer match induces an increase in serum markers of muscle or cardiac damage that can be prevented by allopurinol. No differences in the physical activity profile during the match were observed between groups. Uric acid and lipid peroxidation were lower in allopurinol-treated individuals than in placebo. Allopurinol represents an effective and inexpensive pharmacological agent to prevent tissue damage in soccer players.

Key words: Xanthine oxidase, free radicals, injury, performance.

INTRODUCTION

Soccer is the most popular organized sport worldwide (Giza & Micheli 2005). Its practice may be associated with micro-injury due to eccentric contractions that, as a result of the demands placed on the muscle by the competitive event, progress to major damage (Proske & Morgan 2001). Investigations in adult male soccer players identified an incidence of 10–35 injuries per 1,000 game hours, and almost every player incurs one performance-limiting injury per year (Dvorak & Junge 2000; Hawkins & Fuller 1999). The Fédération Internationale de Football Association (FIFA) estimates that the average worldwide medical cost of a soccer injury is USD 150, leading to an estimated annual cost of USD 30 billion (Dvorak & Junge 2000). In professional soccer, the average cost due to injury approximates USD 70 million per season (Dvorak & Junge 2000). The rise in youth soccer participation, and the subsequent cost associated with injuries, places an enormous economic pressure on healthcare systems.

The diagnosis of muscular lesions in soccer players is usually made by clinical criteria combined with imaging of the lesion and blood tests to detect the presence of specific muscle markers (Guerrero et al. 2008). CK, LDH, and AST activities, and myoglobin levels are among the most widely used serum markers of muscle injury (Banfi et al. 2012). We have previously found that XO-derived free radicals are, at least in part, responsible of the muscle damage in top professional cycling atheletes (Gomez-Cabrera et al. 2003). However, the role of XO in soccer, which includes eccentric muscular contractions, has to our knowledge not been examined. Xanthine oxidase also contributes to exercise-induced myocardial ischaemia (Noman et al. 2010), and therefore inhibition of XO may lead to several beneficial effects on cardiac and vascular function. Allopurinol improves endothelial function (George et al. 2006) and myocardial efficiency by decreasing the energy cost and improving hemodynamics of

the left ventricle (Cappola et al. 2001; Mellin et al. 2005). It also decreases myocardial oxygen demand per unit of cardiac output and prolongs exercise in stable angina pectoris (Noman, Ang 2010). Thus, the aim of this study was to establish whether allopurinol prevents skeletal muscle, heart and liver damage in top level soccer players.

MATERIALS AND METHODS

Ethics Statement

The study complies with the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. The experimental protocol was approved by the Ethics Research Committee of the Faculty of Medicine, University of Valencia, Spain.

Study design

Twelve professional soccer players (age 25±2 years; weight 75.0±8.2 Kg; height 1.8±0.1 m; body muscle mass 36.2±3.0 Kg; body fat mass 8.1±1.0 Kg) were randomized into two experimental groups. An oral dose of 300 mg of allopurinol was administered to six participants four hours before a match of the premier Spanish Football League. The remaining six participants received placebo (cellulose). The match was played at 10 p.m. and during the second half of the match two players were changed. These players were also included in the study, whereas the goalkeeper was excluded. The players were offered soft drinks (with no antioxidant vitamins added) *ad libitum* during the match to prevent dehydration. Venous blood samples were obtained before the match (Baseline) and twelve hours later (Post-Match). All blood samples were collected in vacuum tubes with no additives after overnight fasting, at the same hour and under the same conditions (temperature and humidity), by venipuncture of a superficial vein of the antecubital fossa after the subjects had remained seated for 10 min. All sample tubes were left in upright position for 45 min at room temperature (20º C) to allow complete blood clotting before centrifugation (CLSI 2010). Serum was then separated by centrifugation at 1500xg for 15 min at room temperature, stored in aliquots and kept frozen at -80º C until measurement. No specimen was discarded due to unsatisfactory phlebotomy, difficulty in locating venous access, missing vein, visible

hemolysis or lipemia. Written informed consent was obtained prior to participation. Allopurinol is not among the list of drugs prohibited by World Anti-Doping Agency (WADA) or FIFA.

Laboratory measurements

All serum aliquots were thawed at the same time. The concentrations of serum uric acid, AST, ALT, GGT, LDH and CK were tested in duplicate immediately after thawing with an enzymatic colorimetric method using a Roche Cobas® 6000 apparatus (Roche Diagnostics GmbH), according to the manufacturer's specifications. Serum CK-MB, myoglobin, and highly-sensitive troponin T were assayed using Roche Elecsys 2010 (Roche Diagnostics GmbH, Penzberg, Germany). Serum lipid hydroperoxides were determined as malondialdehyde (MDA) by high performance liquid chromatography following the protocol previously described by Wong et al (Wong et al. 1987).

Statistical analysis

A two-way analysis of variance with repeated measures was used to determine the difference between the two parameters during the experimental intervention (Baseline versus Post-Match). When a difference was found, multiple comparisons using the Tukey post hoc test were performed. The statistical analysis was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK).

RESULTS

Effect of allopurinol on markers of skeletal muscle damage in soccer players

Players who received placebo showed a significant increase in serum activities of CK, LDH and AST, and in serum levels of myoglobin 12 hours after the match. Allopurinol prevented the exercise-induced increases of CK, AST and myoglobin and partially, those of LDH (See Table 1).

Effect of allopurinol on markers of cardiac damage in soccer players

Serum levels of CK-MB and Hs-TnT were assessed as specific biomarkers of myocardial injury. CK-MB and Hs-TnT increased significantly in the placebo group but not in the allopurinol treated group. Significant differences were observed between placebo and allopurinol groups after the match (See Table 1).

Lack of effect of allopurinol on markers of hepatic damage in soccer players

ALT serum activity was measured as biomarker of liver injury. ALT activity increased significantly after the match in both placebo and allopurinol groups, highlighting a lack of effect of allopurinol on ALT activity in the liver (See Table 1).

Effect of allopurinol on serum uric acid and plasma lipid peroxidation in soccer players

In proof-of-principle experiments to establish a role of XO-derived free radicals in exercise-induced tissue damage, we determined serum uric acid levels in the soccer players. Uric acid is an end-product of XO activity. Soccer players supplemented with allopurinol, but not those who received placebo, exhibited a significant decrease in serum uric acid levels 12 hours after the match. XO-derived free radicals have been involved in lipid peroxidation in top level professional athletes (Gomez-Cabrera, Pallardo 2003). Significant differences were also observed in serum lipid peroxidation, determined as malondialdehyde (MDA), between placebo and allopurinol groups after

the match (see Table 1). Therefore, allopurinol prevents exercise-induced lipid peroxidation as reflected by MDA measurements.

To exclude the possibility that changes in biomarkers were due to hemoconcentration, we measured serum sodium, calcium and GGT levels. As there were no significant differences in both placebo and allopurinol treated groups (results not shown), we conclude that the observed changes in biomarkers are not a consequence of hemoconcentration.

Anthropometric variables and physical activity profiles in soccer players

The following activity profiles were measured during the match: total distance run, distance covered at high intensity, and distance covered in shorter sprints. We failed to observe any significant difference between placebo and allopurinol treated groups (See Table S1 in which the anthropometric variables and physical activity profiles during a competitive match, in soccer players, are shown), and also found no differences in the following anthropometric parameters: weight, height, fat mass, and muscle mass.

DISCUSSION

Eccentric exercise may trigger muscle damage, weakness and soreness (Proske & Morgan 2001). In some sports such as soccer, the micro-damage from eccentric exercise may progress to more tears, potentially leading to major injuries as a result of the demands placed on the muscle by the competitive event (Proske & Morgan 2001). Soccer injuries challenge sports medicine physicians and trainers to develop effective treatment and prevention programmes (Giza & Micheli 2005). A reliable approach to prevent soccer muscle injuries may counteract muscle damage caused by the repeated contractions performed both in training and in competition, without negatively affecting the soccer player's performance. We have previously shown that XO, a free radical generating enzyme involved in the ischemia-reperfusion injury (McCord 1985), causes muscle damage associated with exhaustive exercise (Gomez-Cabrera et al. 2006; Gomez-Cabrera, Pallardo 2003; Vina et al. 2000). The new finding is that allopurinol prevents tissue damage specifically caused by top level professional soccer.

The professional soccer match induced a significant increase in serum markers of skeletal muscle damage (CK, LDH, AST and myoglobin) that was effectively prevented by administration of allopurinol. Even though the serum levels of these markers depend on age, gender, race, muscle mass, and physical activity (Brancaccio et al. 2007), no differences were found in these parameters between the allopurinol and placebo groups before exercise.

We measured oxidative damage to lipids by determining lipid hydroperoxides (estimated by the formation of MDA). We have determined the MDA because it is a reliable indicator of lipid peroxidation in human samples (Breusing et al. 2010). As previously reported with professional cyclists, allopurinol prevents exercise-induced lipid peroxidation in soccer players (Gomez-Cabrera, Pallardo 2003).

No differences in performance during the match were found between groups. Indeed, we observed no differences between placebo and allopurinol-treated players in their activity profile based on the total distance run/covered, distance covered at high intensity, and distance covered in short sprints, (see Supplementary Material, Table S1, for the anthropometric variables and physical activity profiles in our soccer players). These findings are consistent with our previous report on the benefits of allopurinol for cyclists in the Tour de France (Gomez-Cabrera, Pallardo 2003).

It has been recently reported that allopurinol prolongs total exercise time during an exercise tolerance test, in patients with stable chronic angina pectoris (Noman, Ang 2010). We did not observe any ergogenic effect of allopurinol, probably because this study was not designed to identify such an effect but rather the beneficial effects of allopurinol for muscle integrity.

There is unquestionable evidence showing the beneficial effects of exercise to prevent and to treat several human disorders such as cardiovascular disease, osteoporosis and cancer. We have recently introduced the concept that exercise behaves as a drug (Lippi et al. 2006; Vina et al. 2012). However, long-term intense endurance training promotes heart chamber-specific remodeling and susceptibility to ventricular arrhythmia (Aizer et al. 2009; Benito et al. 2011). Strenuous exercise can generate transitory ischemia, myocardial stress, and diastolic left ventricular dysfunction often inducing an increase in biochemical parameters measured in the diagnosis of heart diseases (Kim et al. 2012; Lippi & Maffulli 2009; Neilan et al. 2006). We found a significant increase in serum markers of cardiac damage, CK-MB and Hs-TnT, after the soccer match, which could be prevented by administration of allopurinol.

The exercise-related increase in cardiac biomarkers has been extensively described, but a definitive pathophysiological explanation has not been found (Banfi, Colombini

2012). It seems plausible that these increments do not reflect clinically threatening myocardial damage, but rather the onset of reversible injury to cardiomyocytes (Banfi, Colombini 2012; Lippi et al. 2011). The beneficial effects of allopurinol treatment in different cardiovascular pathologies have been proven. In experimental chronic heart failure, long-term allopurinol treatment improves left ventricular hemodynamic and prevents left ventricular remodeling. These long-term effects are, at least partially, caused by a transient reduction of myocardial free radicals by allopurinol (Mellin, Isabelle 2005). Allopurinol is also a useful and safe anti-ischemic drug for patients with chronic stable angina (Noman, Ang 2010). There is a causal association between hyperuricemia and cardiovascular risk (Papezikova et al. 2012). Elevated uric acid levels have been associated with an increased risk of cardiovascular events, coronary disease, and stroke (Lippi et al. 2008). It has also been shown that uric acid has a predictive value for mortality in chronic heart failure (Anker et al. 2003). Thus, allopurinol administration is a safe, non-expensive way to minimize not only muscle damage but also cardiac damage in elite soccer players.

PERSPECTIVE

Soccer lesions, at both professional and amateur levels, cause an enormous burden to society in terms of suffering of the participants and also in economic terms (a point we detail in the paper). Because allopurinol is such a safe drug, the possibility could be considered that players could be given allopurinol to prevent cardiac and skeletal muscle damage. Thus, this is not a paper dealing with a very specific sports physiology problem, but rather a general one which may be of interest to the wide readership of your Journal. The fact that professional players in the last decade have started playing three games a week (as opposed to earlier when they only played one every weekend) gives more strength to the preventive action of allopurinol for these individuals.

FINANCIAL DISCLOSURE

This work was supported by grants: SAF2010-19498, FPU-AP2009-4755, ISCIII2006-RED13-027, PROMETEO2010/074, EU Funded COSTB35 and CM1001, and FEDER funds from the EU. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript

REFERENCES

Aizer A, Gaziano JM, Cook NR, Manson JE, Buring JE, Albert CM. Relation of vigorous exercise to risk of atrial fibrillation. Am J Cardiol. 2009: 103: 1572-1577.

Anker SD, Doehner W, Rauchhaus M, Sharma R, Francis D, Knosalla C, Davos CH, Cicoira M, Shamim W, Kemp M, Segal R, Osterziel KJ, Leyva F, Hetzer R, Ponikowski P, Coats AJ. Uric acid and survival in chronic heart failure: validation and application in metabolic, functional, and hemodynamic staging. Circulation. 2003: 107: 1991-1997.

Banfi G, Colombini A, Lombardi G, Lubkowska A. Metabolic markers in sports medicine. Adv Clin Chem. 2012: 56: 1-54.

Benito B, Gay-Jordi G, Serrano-Mollar A, Guasch E, Shi Y, Tardif JC, Brugada J, Nattel S, Mont L. Cardiac arrhythmogenic remodeling in a rat model of long-term intensive exercise training. Circulation. 2011: 123: 13-22.

Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull. 2007: 81-82: 209-230.

Breusing N, Grune T, Andrisic L, Atalay M, Bartosz G, Biasi F, Borovic S, Bravo L, Casals I, Casillas R, Dinischiotu A, Drzewinska J, Faber H, Fauzi NM, Gajewska A, Gambini J, Gradinaru D, Kokkola T, Lojek A, Luczaj W, Margina D, Mascia C, Mateos R, Meinitzer A, Mitjavila MT, Mrakovcic L, Munteanu MC, Podborska M, Poli G, Sicinska P, Skrzydlewska E, Vina J, Wiswedel I, Zarkovic N, Zelzer S, Spickett CM. An inter-laboratory validation of methods of lipid peroxidation measurement in UVA-treated human plasma samples. Free Radic Res. 2010: 44: 1203-1215.

Cappola TP, Kass DA, Nelson GS, Berger RD, Rosas GO, Kobeissi ZA, Marban E, Hare JM. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation. 2001: 104: 2407-2411.

CLSI. Procedures for the handling and processing of blood specimens for common laboratory tests. NCCLS H18-A4. 4º ed, 2010.

Dvorak J, Junge A. Football injuries and physical symptoms. A review of the literature. Am J Sports Med. 2000: 28: S3-9.

George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. Circulation. 2006: 114: 2508-2516.

Giza E, Micheli LJ. Soccer injuries. Med Sport Sci. 2005: 49: 140-169.

Gomez-Cabrera MC, Martinez A, Santangelo G, Pallardo FV, Sastre J, Vina J. Oxidative stress in marathon runners: interest of antioxidant supplementation. Br J Nutr. 2006: 96 Suppl 1: S31-33.

Gomez-Cabrera MC, Pallardo FV, Sastre J, Vina J, Garcia-del-Moral L. Allopurinol and markers of muscle damage among participants in the Tour de France. Jama. 2003: 289: 2503-2504.

Guerrero M, Guiu-Comadevall M, Cadefau JA, Parra J, Balius R, Estruch A, Rodas G, Bedini JL, Cusso R. Fast and slow myosins as markers of muscle injury. Br J Sports Med. 2008: 42: 581-584; discussion 584.

Hawkins RD, Fuller CW. A prospective epidemiological study of injuries in four English professional football clubs. Br J Sports Med. 1999: 33: 196-203.

Kim JH, Malhotra R, Chiampas G, d'Hemecourt P, Troyanos C, Cianca J, Smith RN, Wang TJ, Roberts WO, Thompson PD, Baggish AL. Cardiac arrest during long-distance running races. N Engl J Med. 2012: 366: 130-140.

Lippi G, Cervellin G, Banfi G, Plebani M. Cardiac troponins and physical exercise. It's time to make a point. Biochem Med (Zagreb). 2011: 21: 55-62.

Lippi G, Maffulli N. Biological influence of physical exercise on hemostasis. Semin Thromb Hemost. 2009: 35: 269-276.

Lippi G, Montagnana M, Franchini M, Favaloro EJ, Targher G. The paradoxical relationship between serum uric acid and cardiovascular disease. Clin Chim Acta. 2008: 392: 1-7.

Lippi G, Schena F, Guidi GC. Health benefits of physical activity. CMAJ. 2006: 175: 776; author reply 777.

McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985: 312: 159-163.

Mellin V, Isabelle M, Oudot A, Vergely-Vandriesse C, Monteil C, Di Meglio B, Henry JP, Dautreaux B, Rochette L, Thuillez C, Mulder P. Transient reduction in myocardial free oxygen radical levels is involved in the improved cardiac function and structure after long-term allopurinol treatment initiated in established chronic heart failure. Eur Heart J. 2005: 26: 1544-1550.

Neilan TG, Januzzi JL, Lee-Lewandrowski E, Ton-Nu TT, Yoerger DM, Jassal DS, Lewandrowski KB, Siegel AJ, Marshall JE, Douglas PS, Lawlor D, Picard MH, Wood MJ. Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston marathon. Circulation. 2006: 114: 2325-2333.

Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: a randomised, placebo controlled crossover trial. Lancet. 2010: 375: 2161-2167.

Papezikova I, Pekarova M, Kolarova H, Klinke A, Lau D, Baldus S, Lojek A, Kubala L. Uric acid modulates vascular endothelial function through the down regulation of nitric oxide production. Free Radic Res. 2012.

Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol. 2001: 537: 333-345.

Vina J, Gomez-Cabrera MC, Lloret A, Marquez R, Minana JB, Pallardo FV, Sastre J. Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. IUBMB Life. 2000: 50: 271-277.

Vina J, Sanchis-Gomar F, Martinez-Bello V, Gomez-Cabrera M. Exercise acts as a drug; the pharmacological benefits of exercise. Br J Pharmacol. 2012: 167: 1-12.

Wong SH, Knight JA, Hopfer SM, Zaharia O, Leach CN, Jr., Sunderman FW, Jr. Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clin Chem. 1987: 33: 214-220.

Table 1. Biochemical parameters of muscular, cardiac, liver, and oxidative damage in soccer players. Effect of allopurinol administration.

	Placebo			Allopurinol			Ratio of effect
	Basal	Post-match	Δ	Basal	Post-match	Δ	
Skeletal muscle							
CK (U/L)	229 ± 123	1287 ± 1092$^{\#\#}$	1103	199 ± 218	592 ± 536*	379	2.9
LDH (U/L)	199 ± 36	449 ± 69$^{\#\#\#}$	180	200 ± 42	353 ± 58$^{\$\$\$,**}$	151	1.2
AST (U/L)	22 ± 3	55 ± 36$^{\#\#}$	21	22 ± 6	30 ± 9*	6	3.5
Myoglobin (ng/mL)	33.3 ± 5.3	121.8 ± 98.5$^{\#\#}$	88.0	36.2 ± 31.2	63.6 ± 18.4*	15.8	5.6
Cardiac muscle							
CK-MB (µg/L)	2.4 ± 1.6	9.3 ± 9.8$^{\#}$	6.5	1.9 ± 1.9	4.3 ± 3.7	2.2	2.9
Hs-TnT (ng/L)	5.5 ± 2.5	14.1 ± 3.4$^{\#}$	8.6	4.8 ± 3.8	9.5 ± 5.8*	6.6	1.3
Liver							
ALT (U/L)	7 ± 3	19 ± 4$^{\#\#\#}$	11	8 ± 2	22 ± 4$^{\$\$\$}$	13	0.8
Oxidative damage							
MDA (nmol/mL)	0.6 ± 0.4	0.9 ± 0.3	0.3	0.6 ± 0.3	0.6 ± 0.3*	0.0	-854

ALT (alanine aminotransferase), AST (aspartate aminotransferase), CK (creatine kinase), CK-MB (creatine kinase myocardicisoenzyme), Hs-TnT (high sensitive troponin T), LDH (lactate dehydrogenase), MDA (malondialdehyde). Values as means ± SD (n=6 placebo; n=6 allopurinol). Δ is defined as the difference between post-match and basal. Ratio of effect is calculated as the Δ (placebo) / Δ (allopurinol).

*p<0.05 and **p<0.01 between placebo and allopurinol groups in post-match;

$^{\$}$p<0.05 and $^{\$\$\$}$p<0.001 in allopurinol group between basal and post-match;

$^{\#}$p<0.05, $^{\#\#}$p<0.01 and $^{\#\#\#}$p<0.001 in placebo group between basal and post-match

SUPLEMENTARY APPENDIX

Table S1. Anthropometric variables and physical activity profiles during a competitive match, in soccer players.

SUPPLEMENTARY APPENDIX

Table S1. Anthropometric variables and physical activity profiles during a competitive match, in soccer players.

		Placebo	Allopurinol
Anthropometric variables			
Age (years)		26.3 ± 3.4	25.1 ± 2.2
Weight (Kilograms)		78.9 ± 8.9	71.8 ± 6.3
Height (meters)		1.8 ± 0.1	1.8± 0.1
Muscle mass (Kilograms)		37.5 ± 3.3	35.0 ± 2.6
Fat mass (Kilograms)		8.8± 1.5	7.6 ± 1.1
Physical activity profiles			
Total distance (m)	1st half	5508.3 ± 524.2	5321.6 ± 444.2
	2nd half	5235.8 ± 1195.4	5258.6 ± 523.0
Distance covered at HI (21Km/h-24Km/h)	1st half	349.3 ± 153.0	194.4 ± 81.6
	2nd half	359.0 ± 91.4	298.0 ± 126.1
Distance covered in sprint (>24Km/h) (m)	1st half	197.0 ± 98.9	69.0 ± 43.8
	2nd half	160.8 ± 37.3	169.2 ± 110.2

HI: High Intensity; m: meters. Values as means ± SD (n=6 placebo; n=6 allopurinol).

Effects of acute exercise and xanthine oxidase inhibition on novel cardiovascular biomarkers

FABIAN SANCHIS-GOMAR, CHIARA BONAGURI, ROSALIA ALOE, HELIOS PAREJA-GALEANO, VLADIMIR MARTINEZ-BELLO, MARI CARMEN GOMEZ-CABRERA, JORGE CANDEL, JOSE VIÑA, and GIUSEPPE LIPPI

VALENCIA, SPAIN; AND PARMA, ITALY

Several sports have been associated with a postexercise increase of cardiac, liver, and skeletal muscle biomarkers of injury. Exhaustive or acute physical exercise causes an increased generation of reactive oxygen species, resulting in cellular injury. Thus, exercise and training may trigger pathophysiological changes in serum concentrations of a variety of biomarkers. In this study, we aimed to evaluate the variation of novel biomarkers of stress and cardiovascular disease such as copeptin, midregional part of proadrenomedullin (MR-proADM), growth differentiation factor 15 (GDF15), soluble vascular endothelial growth factor receptor, and placental growth factor along with uric acid before and after acute high-intensity exercise and allopurinol administration. We also assessed whether allopurinol administration may affect the circulating levels of these biomarkers by inhibition of XO activity. This is a double-blind, placebo-controlled study in which 12 professional football players were divided into 2 experimental groups. An oral dose of 300 mg of allopurinol was administered to one group of six participants 4 hours before a match of the Spanish Football League, whereas the other 6 participants received placebo (cellulose). Venous blood samples were obtained before the match (baseline) and twelve hours afterwards (post-match). Serum MR-proADM levels increased significantly in the placebo group, whereas serum GDF15 levels increased significantly in both the placebo and allopurinol group after the match. No differences in the other parameters tested were found after the match in any experimental group. The trend toward postexercise increase of serum MR-proADM and GDF15 levels shows that the metabolism of these proteins is clearly imbalanced after exercise, which thereby represents a potential source of biological variability in their clinical assessment. (Translational Research 2013;162:102–109)

Abbreviations: ADM = adrenomedullin; GDF15 = growth differentiation factor 15; MR-proADM = midregional part of proadrenomedullin; PlGF = placental growth factor; sVEGFR-1/sFLT-1 = vascular endothelial growth factor receptor-1; UA = uric acid; VEGF = vascular endothelial growth factor; XO = xanthine oxidase

From the Department of Physiology, Faculty of Medicine, University of Valencia, Fundación Investigación Hospital Clínico Universitario/Fundación para la Investigación del Hospital Clínico de la Comunidad Valenciana, Valencia, Spain; Azienda Ospedaliero-Universitaria di Parma, U.O. di Diagnostica Ematochimica, Dipartimento di Patologia e Medicina di Laboratorio, Parma, Italy; Valencia C.F Medical Services, Valencia, Spain; Department of Teaching of Musical, Visual and Corporal Expression. Faculty of Teaching, University of Valencia, Spain.

This work was supported by grants SAF2010-19498, ISCIII2006-RED13-027, PROMETEO2010/074, EU Funded COSTB3,5 and CM1001, and FEDER funds from the EU.

Conflict of interest: All authors have read the journal's policy on disclosure of potential conflicts of interest. The authors report no conflict of interest.

Submitted for publication November 27, 2012; revision submitted January 31, 2013; accepted for publication February 21, 2013.

Reprint requests: Fabian Sanchis-Gomar, Department of Physiology, Faculty of Medicine, University of Valencia, Av. Blasco Ibañez, 15, Valencia, 46010 Spain; e-mail: fabian.sanchis@uv.es.

1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2013.02.006

AT A GLANCE COMMENTARY

Sanchis Goman F, et al.

Background

High intensity exercise is associated with cardiac muscle damage, thus, increasing the risk of heart disorders in high-level sportsmen. Xanthine oxidase (XO), a free radical generating enzyme, is involved in the tissue damage produced during exhaustive exercise. Thus, its inhibition may exert several beneficial cardiac and vascular effects. Allopurinol improves endothelial function and myocardial efficiency, decreases myocardial oxygen demand per unit of cardiac output, and prolongs exercise in stable angina pectoris.

Translational Significance

Midregional part of proadrenomedullin (MR-proADM) and growth differentiation factor 15 (GDF15) are clearly influenced by exercise, which thereby represent a potential source of biological variability in their clinical assessment.

Several sports such as cycling and long-distance running (eg, marathon and ultra-marathon), have been associated with a postexercise increase of cardiac, liver, and skeletal muscle biomarkers of injury, including cardiac troponins, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, creatine kinase, and lactate dehydrogenase among others,[1-7] as well as with radiological findings suggestive of myocardial damage and fibrosis.[8] Several cellular sources of reactive oxygen species production exist in skeletal muscle, including xanthine oxidase (XO). Xanthine oxidoreductase is an intracellular enzyme involved in purine catabolism, which catalyzes the reduction of hypoxanthine and xanthine to uric acid (UA).[9] Exhaustive or acute physical exercise causes an increased generation of reactive oxygen species, and therefore, an oxidative stress in muscle and in other organs, which finally result in cellular injury.[10-12] Oxidative stress is clearly involved in the pathophysiology of cardiac remodeling and heart failure, as well as in skeletal muscle damage induced by exhaustive exercise.[13] Allopurinol is a well-known inhibitor of xanthine oxidoreductase widely used in clinical practice[14] and is also a promising drug to prevent muscle oxidative damage during exhaustive physical exercise, as previously reported.[11,15]

It has been proven that exercise and training may trigger pathophysiological changes in urinary and serum concentrations of a variety of laboratory parameters, and its assessment enables to monitor the damage at a specific tissue level (ie, liver, kidney, muscle, heart). This aspect has inherently increased the use of traditional biomarkers of tissue injury by clinical chemists and sport physicians during the past decades.[16] New initiatives aim to accelerate the search for new innovative and promising biomarkers, which should allow a better monitoring of sport performance, diagnosis of sport-related injuries, prediction of overtraining, and even the identification of the most suitable period for return to play after injury.

Copeptin is the C-terminal portion of provasopressin, a 39-amino acid glycopeptide, which is produced during cleavage of arginine vasopressin or antidiuretic hormone.[17] Importantly, copeptin correlates with the individual stress level[18] and the combination of copeptin with cardiac troponins, in patients with chest pain, may improve the early diagnosis of acute coronary syndrome.[19-23] Additionally, copeptin provides reliable prognostic information in patients after an acute myocardial infarction or chronic heart failure.[24-29] Increased levels of this biomarker may also predict an increased risk for diabetes independently of conventional risk factors.[30] The prognostic value of copeptin has been well established in other severe diseases, including sepsis, pneumonia, lower respiratory tract infections, and stroke.[31-34]

Adrenomedullin (ADM) is a 52-amino acid vasoactive peptide that plays an important role in microcirculation and endothelial dysfunction. The precursor preproadrenomedullin (preproADM), which is transcriptionally induced by insulin, hypoxia, and several inflammatory stimuli, is further activated to ADM after cleavage of another biologically active peptide known as proadrenomedullin N-terminal 20 peptide (PAMP) and 2 peptides flanking ADM (ie, the midregional part of proADM [MR-proADM 45–92] and the COOH terminus of the molecule [proADM 153–185].[35] The mid-regional portion of pro-adrenomedullin (MR-proADM) is more stable than ADM and, hence, better suited for use in clinical practice and assessment in stored samples.[36] Recent evidence shows that MR-proADM is an independent predictor of events in patients with symptomatic coronary artery disease,[37] cardiovascular events,[38] chronic heart failure,[39] stroke,[40] and other severe disorders such as amyloidosis,[41] sepsis,[42] community-acquired peumonia,[43,44] and acute dyspnea.[45-47] In addition, Maeder et al observed that MR-proADM were associated with peak oxygen consumption independent of lung function in patients with chronic lung disease.[48] However, the physiological function of MR-proADM is still under investigation. Although both copeptin and MR-proADM

are emerging as putative cardiovascular biomarkers, little is known about the influence of physical exercise on their concentration.

Growth differentiation factor 15 (GDF15) is a member of the transforming growth factor β superfamily. Several studies have shown that inflammation or injury upregulate GDF15 expression in the heart.[49,50] Moreover, this novel biomarker has been found to be associated with vascular disorders such as atherosclerosis, hypertension, ischemia, and heart failure,[51] suggesting that it may act as a stress signal for the cardiomyocytes.

In healthy skeletal muscle several animal and human studies have shown increased vascular endothelial growth factor (VEGF) expression after acute exercise.[52-54] Placental growth factor (PlGF) is a member of the VEGF family of growth factors that is expressed in several inflammatory cell types and acts as a crucial mediator of hematopoietic stem cell recruitment and later stages of angiogenesis.[55] PlGF mainly mediates arteriogenesis in response to ischemia or tissue damage.[56] The vascular endothelial growth factor receptor-1 (sVEGFR-1/sFLT-1) is a receptor tyrosine kinase specific for the angiogenic factors VEGF (VEGF-A), PlGF, and VEGF-B.[57] The level of sVEGFR1/sFLT-1 changes in several pathologies[58-60] and have prognostic value.[61] It has been also suggested that sVEGFR1/sFLT-1 levels should be presented together with VEGF levels in experimental and pathological conditions.[62] Nevertheless, very little is also known about the regulation of both sVEGFR-1/sFLT-1 and PlGF expression by physical exercise.

Therefore, in this study we aimed to evaluate the variation of these novel biochemical markers of stress and cardiovascular disease after acute high-level physical exercise. We also sought to compare whether allopurinol administration may affect the circulating levels of these biomarkers attributable to the inhibition XO activity. For this purpose, we measured copeptin, MR-proADM, GDF15, sVEGFR-1/sFLT-1, and PlGF along with UA before and after acute high-intensity exercise and allopurinol administration.

METHODS

Study design. This is a double-blind, placebo-controlled study in which 12 professional soccer players (age 25 $\pm$ 2 years; weight 75.0 $\pm$ 8.2 kg; height 1.8 $\pm$ 0.1 m; body fat free mass 36.2 $\pm$ 3.0 kg; body fat 8.1 $\pm$ 1.0 kg) were randomized into 2 experimental groups. An oral dose of 300 mg of allopurinol was administered to 1 group of 6 participants 4 hours before a match of the Spanish Football League; the other 6 participants received placebo (cellulose). The match was played at 10 PM. During the second half of the match 2 players were changed. These players were also included in the study, whereas the goalkeeper was excluded. The players were offered beverages *ad libitum* during the match to prevent excessive dehydration. Venous blood samples were obtained before the match (baseline) and 12 hours afterwards (post-match). All blood samples were collected in vacuum tubes with no additives after an overnight fasting, at the same hour and in the same conditions (temperature and humidity), from venipuncture of a superficial vein of the antecubital fossa after the subjects had remained seated for 10 minutes. Serum was immediately separated (by centrifugation at 1500 $\times$ g, 15 minutes, room temperature) and stored at -20°C until measurement.

Written informed consent was obtained prior to participation, and the study complies with the World Medical Association Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects. The experimental protocol was approved by the Committee on Ethics in Research of the Faculty of Medicine, University of Valencia, Spain. Allopurinol is not among the list of drugs prohibited by World Anti-Doping Agency or "Fédération Internationale de Football Association."

Laboratory measurements. Serum copeptin and MR-proADM were assayed by Time Resolved Amplified Kryptat Emission on Kryptor (Thermo Scientific BRAHMS, Henningsdorf, Germany). According to the manufacturer's specification, the intra- and interassay imprecision of copeptin immunoassay at concentrations of 20 and 50 pmol/L are 6%-12% and 8%-13%, respectively. The analytical detection limit, the 97.5% percentile of the male reference range as well as the cut-off of copeptin in the diagnostics of cardiovascular disorders, and AMI have been set at 4.8, 19.1 pmol/L and 14 pmol/L, respectively.[19] According to the manufacturer's specification, the intra- and interassay imprecision of MR-proADM immunoassay at concentrations ranging from 0.5 to 6 nmol/L is comprised between 4% and 11%. The analytical detection limit and 95% percentile of the reference range are 0.05 and 0.52 nmol/L, respectively. Serum VEGFR1/Flt-1, PlGF, and GDF15 were assayed with the Quantikine Human Soluble VEGF R1/Flt-1 Immunoassay, Quantikine ELISA Human PlGF Immunoassay, and Quantikine Human GDF15 Immunoassay (R&D Systems, Inc, Minneapolis, Minn). According to manufacturer's quote, the intra- and interassay imprecision are 3.6%-7.0% and 10.9%-11% for the PlGF immunoassay, and 1.8%-2.8% and 4.7%-6.0% for the GDF15 immunoassay, respectively. The

concentration of serum uric acid was tested with an enzymatic colorimetric method on Roche Cobas (Roche Diagnostics, Indianapolis, Ind). According to manufacturer's specifications, the total imprecision of this test is comprised between 0.30% and 0.50%.

Statistical analysis. For statistical analysis of results, the mean was taken as the measurement of the main tendency and the standard deviation was taken as the dispersion measurement. The normal distribution of each variable was established with the Shapiro-Wilk normality test. A 2-way analysis of variance with repeated measures was used to determine the difference on the 2 parameters during the experimental intervention (baseline and post-match). When an interaction effect was found, multiple comparisons using the Tukey post hoc test were performed. The statistical analysis was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK), and the alpha level for statistical significance was set at $P < 0.05$.

RESULTS

No significant differences in body mass were found between groups, and the body weight remained unchanged until the end of the study in all the experimental groups (The results are presented in Table 1).

The athletes supplemented with allopurinol, but not those who received placebo, exhibited a significant decrease in serum concentration of UA after the match (5.1 ± 1.2 vs 3.8 ± 0.7 mg/dL; $P = 0.03$).

Serum copeptin concentration exhibited a nonsignificant decrease in the placebo group (from 9.8 ± 6.2 to 6.9 ± 2.3 pmol/L; $P = 0.18$) and a nonsignificant increase in the allopurinol group (from 9.4 ± 3.6 to 12.2 ± 4.2 pmol/L; $P = 0.10$) after the match. No significant differences were observed between placebo and allopurinol groups at baseline (9.8 ± 6.2 vs 9.4 ± 3.6 pmol/L; $P = 0.40$), whereas the difference between placebo and allopurinol groups after the game was statistically significant (6.9 ± 2.3 vs 12.2 ± 4.2 pmol/L; $P = 0.02$).

Serum MR-proADM increased significantly from 0.26 ± 0.1 nmol/L to 0.36 ± 0.06 nmol/L in the placebo group after the match ($P = 0.02$). Similarly, it also increased from 0.29 ± 0.06 nmol/L to 0.35 ± 0.05 nmol/L in the allopurinol group, but this variation was not statistically significant ($P = 0.08$). No significant differences were observed between placebo and allopurinol groups at either baseline (0.26 ± 0.1 vs 0.29 ± 0.06 nmol/L; $P = 0.45$) or after the game (0.36 ± 0.06 vs 0.35 ± 0.05 nmol/L; $P = 0.22$) extractions.

Serum GDF15 levels increased significantly from 227.0 ± 56.6 pg/dL to 299.2 ± 70.6 pg/dL in the placebo group ($P = 0.04$) and from 239.7 ± 73.4 pg/dL to 313.1 ± 83.7 pg/dL in the allopurinol group ($P = 0.04$) after the match extractions. No significant differences were observed between placebo and allopurinol groups at either baseline (227.0 ± 56.6 vs 239.7 ± 73.4 pg/dL; $P = 0.37$) or after the game (299.2 ± 70.6 vs 313.1 ± 83.7 pg/dL; $P = 0.38$) extractions.

For serum PlGF concentration, no statistical significant changes were found before or after the match in either placebo (2.62 ± 0.2 vs 2.51 ± 0.3 pg/dL; $P = 0.29$) or allopurinol (2.81 ± 0.3 vs 2.79 ± 0.3 pg/dL; $P = 0.37$) groups. No significant differences were observed between placebo and allopurinol groups at either baseline (2.62 ± 0.2 vs 2.81 ± 0.3 pg/dL; $P = 0.12$) or after the game (2.51 ± 0.3 vs 2.79 ± 0.3 pg/dL; $P = 0.10$) extractions.

Serum VEGFR1/Flt-1 concentration increased from 55.5 ± 9.3 to 62.12 ± 13.0 pg/dL in the placebo group ($P = 0.18$) and from 56.4 ± 11.9 to 65.8 ± 8.8 pg/dL in the allopurinol group ($P = 0.06$) after the match, but both changes were not statistically significant. No significant differences were observed between placebo and allopurinol groups at either baseline (55.5 ± 9.3 vs 56.4 ± 11.9 pg/dL; $P = 0.44$) or after the game (62.12 ± 13.0 vs 65.8 ± 8.8 pg/dL; $P = 0.30$) extractions.

DISCUSSION

The role of allopurinol as an antioxidant in exercise has been extensively studied by our group[2–5] and other research teams.[6,7] All of these studies aimed to determine the involvement of XO as a source of free radicals in skeletal muscle. However, in this study we evaluated the variation of novel biomarkers of stress and cardiovascular disease before and after acute high-intensity exercise and the effect of allopurinol administration.

A large number of conventional and innovative cardiovascular biomarkers are regarded as promising indicators of the level of exercise-induced damage. Biomarkers are critical for evaluating the impact of different exercise intensities and regimes in sport medicine, cardiology, and clinical chemistry. In this context, the identification of novel and sensitive biomarkers is essential. The aim of this work was to assess copeptin, MR-proADM, GDF15, sVEGFR-1/sFLT-1, and PlGF as biomarkers of exercise-induced damage using serum samples of professional football players. Furthermore, we also investigated whether allopurinol administration may affect the circulating levels of these biomarkers by inhibiting XO activity.

Two recent studies have evaluated copeptin levels in relation to hydration status in ultramarathoners. Hew-

Table 1. The table shows the trends of the serum biomarkers in football players who received placebo or allopurinol before and after the match

	Placebo		Allopurinol	
	Basal	Post-match	Basal	Post-match
UA (mg/dL)	5.2 ± 0.6	5.3 ± 0.8	5.1 ± 1.2	3.8 ± 0.7†
Copeptin (pmol/L)	9.8 ± 6.2	6.9 ± 2.3	9.4 ± 3.6	12.2 ± 4.2*
MR-proADM (nmol/L)	0.26 ± 0.10	0.36 ± 0.06‡	0.29 ± 0.06	0.35 ± 0.05
GDF15 (pg/dL)	227.0 ± 56.6	299.2 ± 70.6‡	239.8 ± 73.4	313.1 ± 83.7†
PlGF (pg/dL)	2.62 ± 0.20	2.51 ± 0.30	2.81 ± 0.30	2.79 ± 0.30
sVEGFR1/sFLT-1 (pg/dL)	55.5 ± 9.3	62.12 ± 13.0	56.4 ± 11.9	65.8 ± 8.8

Abbreviations: GDF15, growth differentiation factor 15; *MR-proADM,* midregional part of proadrenomedullin; *PlGF,* placental growth factor; *sVEGFR1/sFLT-1,* soluble vascular endothelial growth factor receptor; *UA,* uric acid.
Values as means ± SD (n = 6 placebo; n = 6 allopurinol).
*$P < 0.05$ between placebo and allopurinol groups in post-match.
†$P < 0.05$ in allopurinol group between basal and post-match.
‡$P < 0.05$ in placebo group between basal and post-match.

Butler et al reported that copeptin levels significantly increased during, as well as at the end, of extreme distance runs. The authors found significant correlations between copeptin and the percentage of plasma volume change.[63] Likewise, Bürge et al observed that plasma copeptin concentration extraordinarily increased by nearly 12 times in 50 male undergoing a 100-km ultramarathon and that a correlation existed between the changes in copeptin and that in serum sodium.[64] In our study, we failed to find any significant variation in copeptin concentration, although this finding can be explained by the different experimental protocol that we followed, regarding intensity and duration of exercise (ie, football match vs strenuous long-duration running). In addition, we collected the samples 12 hours after the match, whereas in the previous references studies, the samples were collected immediately after the exercise. It has been recently shown that copeptin has a short plasma half-life of approximately 23-47 minutes.[65] Intriguingly, allopurinol administration increased the serum copeptin concentration after the game.

To the best of our knowledge, there are no studies that have investigated the variation of MR-proADM after exercise. Adrenomedullin typically reflects an overflow from local sites of production and action, frequently caused by endothelial damage.[35] In certain conditions, the increased concentration of adrenomedullin has a hormonal function, causing a general decrease in vascular resistance and a fall in blood pressure.[35] In our study, a significant increase in serum MR-proADM was recorded after the match in the placebo group, although we also showed that allopurinol administration, and thereby XO inhibition, was effective in preventing the exercise-induced serum MR-proADM increments. Regardless of the underlying causes supporting the increase of MR-proADM, the finding that XO activity is involved in the variation of this marker is interesting and may also have some clinical implications and applications (eg, monitoring of patients with hyperuricemia). We noted that the concentration of MR-proADM was remarkably increased after acute high-intensity exercise. In fact, since the hypothesis that an exercise-induced increase of MR-proADM has occurred should be ruled out before using this biomarker for assessment of cardiovascular risk, myocardial injury, and cellular damage. It is also worthwhile mentioning that its increase can play a pathophysiological role, promoting favorable or unfavorable biological effects that may be elucidated once the definitive role of this hormone has been established.

Higher levels of GDF15 are associated with conditions of severe disease in hypertrophic cardiomyopathy.[66] However, moderate exercise training (ie, 3 times a week, for 1 hour, over 6 months) did not affect circulating levels of GDF15 in patients with stable coronary artery disease.[67] GDF15 is elevated in heart failure with normal ejection fraction and in systolic heart failure.[68] Additionally, it is independently associated with impairment in exercise capacity and in physical components of quality of life. Moreover, it has been reported that diagnostic performance of GDF15 is at least as good as that of N-terminal prohormone of brain natriuretic peptide, and the combination of these markers may so improve the diagnostic accuracy of natriuretic peptides in patients with heart failure.[68] Tchou et al reported that circulating GDF15 serum concentrations increased after an ultramarathon foot race.[69] Accordingly we observed a significant increase in serum GDF15 after the match in both the placebo or allopurinol groups. Allopurinol administration did not affect the serum GDF15 concentration, which would

lead us to conclude that serum GDF15 metabolism is independent from allopurinol administration, and thereby, from XO activity.

Bailey et al observed that exercise in healthy volunteers increased circulating sVEGFR-1/sFLT-1, an endogenous VEGF inhibitor, which may functionally decrease plasma levels of free VEGF. A significant positive correlation between percentage increase in plasma levels of sVEGFR-1/sFLT-1 and total peak oxygen consumption during exercise was also described.[70] Kivelä et al, however, failed to observe changes in skeletal muscle VEGFR-1 expression in either healthy or diabetic mice after exercise.[71] In our study, we failed to observe significant increases in the concentration of both sVEGFR-1/sFLT-1 and PlGF after the match in both groups. This would lead us to suggest that these biomarkers are instead virtually insensitive to physical exercise, at least in our experimental conditions of exercise intensity and duration. We have also showed that allopurinol administration did not modify the serum sVEGFR-1/sFLT-1 and PlGF levels, thus, suggesting that the metabolism of these 2 biomarkers is not influenced by XO activity.

This is the first study, to the best of our knowledge, reporting the effects of acute high-intensity exercise and allopurinol administration on several innovative cardiovascular biomarkers in professional sportsmen. Notwithstanding the limited number of subjects studied, the trend toward postexercise increase of serum MR-proADM and GDF15 levels attests that the metabolism of these proteins is clearly influenced after exercise, which thereby represents a potential source of biological variability in their clinical assessment. On the other hand, their responsiveness to the stress following acute exercise opens new scenario on their clinical use, (eg, for monitoring biological responses after cardiopulmonary exercise testing). More research is needed to confirm the surprising effect of allopurinol administration on serum copeptin values in healthy individuals.

REFERENCES

1. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. Clin Chem Lab Med 2010;48:757–67.
2. Lippi G, Schena F, Montagnana M, Salvagno GL, Guidi GC. Influence of acute physical exercise on emerging muscular biomarkers. Clin Chem Lab Med 2008;46:1313–8.
3. Eijsvogels TM, Shave R, van Dijk A, Hopman MT, Thijssen DH. Exercise-induced cardiac troponin release: real-life clinical confusion. Curr Med Chem 2011;18:3457–61.
4. Lippi G, Cervellin G, Banfi G, Plebani M. Cardiac troponins and physical exercise. It's time to make a point. Biochem Med (Zagreb) 2011;21:55–62.
5. Lippi G, Schena F, Salvagno GL, Tarperi C, Aloe R, Guidi GC. Comparison of conventional and highly-sensitive troponin I measurement in ultra-marathon runners. J Thromb Thrombolysis 2011;33:338–42.
6. Lippi G, Schena F, Dipalo M, et al. Troponin I measured with a high sensitivity immunoassay is significantly increased after a half marathon run. Scand J Clin Lab Invest 2012;72:467–70.
7. Lippi G, Schena F, Montagnana M, Salvagno GL, Banfi G, Guidi GC. Significant variation of traditional markers of liver injury after a half-marathon run. Eur J Intern Med 2011;22:e36–8.
8. Yared K, Wood MJ. Is marathon running hazardous to your cardiovascular health? The jury is still out. Radiology 2009;251:3–5.
9. Ibrahim B, Stoward PJ. The histochemical localization of xanthine oxidase. Histochem J 1978;10:615–7.
10. Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982;107:1198–205.
11. Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL, Vina J. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 2005;567:113–20.
12. Gomez-Cabrera MC, Domenech E, Romagnoli M, et al. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 2008;87:142–9.
13. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol 2011;301:H2181–90.
14. Moorhouse PC, Grootveld M, Halliwell B, Quinlan JG, Gutteridge JM. Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett 1987;213:23–8.
15. Gomez-Cabrera MC, Martinez A, Santangelo G, Pallardo FV, Sastre J, Vina J. Oxidative stress in marathon runners: interest of antioxidant supplementation. Br J Nutr 2006;96(Suppl 1): S31–3.
16. Banfi G, Colombini A, Lombardi G, Lubkowska A. Metabolic markers in sports medicine. Adv Clin Chem 2012;56:1–54.
17. de Bree FM, Burbach JP. Structure-function relationships of the vasopressin prohormone domains. Cell Mol Neurobiol 1998;18: 173–91.
18. Katan M, Morgenthaler N, Widmer I, et al. Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level. Neuro Endocrinol Lett 2008;29:341–6.
19. Reichlin T, Hochholzer W, Stelzig C, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. J Am Coll Cardiol 2009;54:60–8.
20. Keller T, Tzikas S, Zeller T, et al. Copeptin improves early diagnosis of acute myocardial infarction. J Am Coll Cardiol 2010;55: 2096–106.
21. Giannitsis E, Kehayova T, Vafaie M, Katus HA. Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. Clin Chem 2011;57:1452–5.
22. Charpentier S, Maupas-Schwalm F, Cournot M, Elbaz M, Botella JM, Lauque D. Combination of copeptin and troponin assays to rapidly rule out non-ST elevation myocardial infarction in the emergency department. Acad Emerg Med 2012;19:517–24.
23. Lippi G, Aloe R, Dipalo M, Cervellin G. Combination of copeptin and highly sensitive troponin I for diagnosing acute myocardial infarction at emergency department admission. Clin Lab 2012; 58:357–8. author reply 9–60.
24. Khan SQ, Dhillon OS, O'Brien RJ, et al. C-terminal provasopressin (copeptin) as a novel and prognostic marker in acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) study. Circulation 2007;115:2103–10.
25. Neuhold S, Huelsmann M, Strunk G, et al. Comparison of copeptin, B-type natriuretic peptide, and amino-terminal pro-B-type

natriuretic peptide in patients with chronic heart failure: prediction of death at different stages of the disease. J Am Coll Cardiol 2008;52:266–72.
26. Balling L, Kistorp C, Schou M, et al. Plasma copeptin levels and prediction of outcome in heart failure outpatients: relation to hyponatremia and loop diuretic doses. J Card Fail 2012;18:351–8.
27. Tentzeris I, Jarai R, Farhan S, et al. Complementary role of copeptin and high-sensitivity troponin in predicting outcome in patients with stable chronic heart failure. Eur J Heart Fail 2011;13:726–33.
28. Luchner A. Copeptin for acute coronary syndrome: diamond or decoy? Clin Chem Lab Med 2012;50:191–2.
29. Kehl DW, Iqbal N, Fard A, Kipper BA, De La Parra Landa A, Maisel AS. Biomarkers in acute myocardial injury. Transl Res 2012;159:252–64.
30. Enhorning S, Wang TJ, Nilsson PM, et al. Plasma copeptin and the risk of diabetes mellitus. Circulation 2010;121:2102–8.
31. Katan M, Christ-Crain M. The stress hormone copeptin: a new prognostic biomarker in acute illness. Swiss Med Wkly 2010; 140:w13101.
32. Kolditz M, Halank M, Schulte-Hubbert B, Bergmann S, Albrecht S, Hoffken G. Copeptin predicts clinical deterioration and persistent instability in community-acquired pneumonia. Respir Med 2012;106:1320–8.
33. Meisel A, Meisel C, Harms H, Hartmann O, Ulm L. Predicting post-stroke infections and outcome with blood-based immune and stress markers. Cerebrovasc Dis 2012;33:580–8.
34. Lin Q, Fu F, Chen H, Zhu B. Copeptin in the assessment of acute lung injury and cardiogenic pulmonary edema. Respir Med 2012; 106:1268–77.
35. Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. Endocr Rev 2000;21:138–67.
36. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. Clin Chem 2005;51:1823–9.
37. Wild PS, Schnabel RB, Lubos E, et al. Midregional proadrenomedullin for prediction of cardiovascular events in coronary artery disease: results from the AtheroGene study. Clin Chem 2011; 58:226–36.
38. Brouwers FP, de Boer RA, van der Harst P, et al. Influence of age on the prognostic value of mid-regional pro-adrenomedullin in the general population. Heart 2012;98:1348–53.
39. von Haehling S, Filippatos GS, Papassotiriou J, et al. Mid-regional pro-adrenomedullin as a novel predictor of mortality in patients with chronic heart failure. Eur J Heart Fail 2010;12:484–91.
40. Katan M, Fluri F, Schuetz P, et al. Midregional pro-atrial natriuretic peptide and outcome in patients with acute ischemic stroke. J Am Coll Cardiol 2010;56:1045–53.
41. Palladini G, Barassi A, Perlini S, et al. Midregional proadrenomedullin (MR-proADM) is a powerful predictor of early death in AL amyloidosis. Amyloid 2011;18:216–21.
42. Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Muller B. Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an observational study. Crit Care 2005;9:R816–24.
43. Bello S, Lasierra AB, Minchole E, et al. Prognostic power of proadrenomedullin in community-acquired pneumonia is independent of aetiology. Eur Respir J 2011;39:1144–55.
44. Sarda Sanchez M, Calzada Hernandez J, Hernandez Bou S, Claret Teruel G, Velasco Rodriguez J, Luaces Cubells C. Pro-adrenomedullin usefulness in the management of children with community-acquired pneumonia, a preliminary prospective observational study. BMC Res Notes 2012;5:363.
45. Maisel A, Mueller C, Nowak RM, et al. Midregion prohormone adrenomedullin and prognosis in patients presenting with acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. J Am Coll Cardiol 2011;58:1057–67.
46. Cinar O, Cevik E, Acar A, et al. Evaluation of mid-regional pro-atrial natriuretic peptide, procalcitonin, and mid-regional pro-adrenomedullin for the diagnosis and risk stratification of ED patients with dyspnea. Am J Emerg Med 2012;30: 1915–20.
47. Shah RV, Truong QA, Gaggin HK, Pfannkuche J, Hartmann O, Januzzi JL Jr. Mid-regional pro-atrial natriuretic peptide and pro-adrenomedullin testing for the diagnostic and prognostic evaluation of patients with acute dyspnoea. Eur Heart J 2012;33: 2197–205.
48. Maeder MT, Brutsche MH, Arenja N, et al. Biomarkers and peak oxygen uptake in patients with chronic lung disease. Respiration 2010;80:543–52.
49. Schlittenhardt D, Schober A, Strelau J, et al. Involvement of growth differentiation factor-15/macrophage inhibitory cytokine-1 (GDF-15/MIC-1) in oxLDL-induced apoptosis of human macrophages in vitro and in arteriosclerotic lesions. Cell Tissue Res 2004;318:325–33.
50. Hsiao EC, Koniaris LG, Zimmers-Koniaris T, Sebald SM, Huynh TV, Lee SJ. Characterization of growth-differentiation factor 15, a transforming growth factor beta superfamily member induced following liver injury. Mol Cell Biol 2000;20: 3742–51.
51. Albertoni M, Shaw PH, Nozaki M, et al. Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. Oncogene 2002;21: 4212–9.
52. Breen EC, Johnson EC, Wagner H, Tseng HM, Sung LA, Wagner PD. Angiogenic growth factor mRNA responses in muscle to a single bout of exercise. J Appl Physiol 1996;81: 355–61.
53. Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ. Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. Am J Physiol 1999;276:H679–85.
54. Richardson RS, Wagner H, Mudaliar SR, Henry R, Noyszewski EA, Wagner PD. Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. Am J Physiol 1999;277:H2247–52.
55. Autiero M, Luttun A, Tjwa M, Carmeliet P. Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. J Thromb Haemost 2003;1:1356–70.
56. Carmeliet P, Moons L, Luttun A, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. Nat Med 2001;7:575–83.
57. Shibuya M. Structure and dual function of vascular endothelial growth factor receptor-1 (Flt-1). Int J Biochem Cell Biol 2001; 33:409–20.
58. Li T, Zhu Y, Qin CY, et al. Expression and prognostic significance of vascular endothelial growth factor receptor 1 in hepatocellular carcinoma. J Clin Pathol 2012;65:808–14.
59. Dhakal HP, Naume B, Synnestvedt M, et al. Expression of vascular endothelial growth factor and vascular endothelial growth factor receptors 1 and 2 in invasive breast carcinoma: prognostic significance and relationship with markers for aggressiveness. Histopathology 2012;61:350–64.
60. Suryawanshi A, Veiga-Parga T, Reddy PB, Rajasagi NK, Rouse BT. IL-17A differentially regulates corneal vascular endothelial growth factor (VEGF)-A and soluble VEGF receptor 1

expression and promotes corneal angiogenesis after herpes simplex virus infection. J Immunol 2012;188:3434–46.
61. Harris AL, Reusch P, Barleon B, Hang C, Dobbs N, Marme D. Soluble Tie2 and Flt1 extracellular domains in serum of patients with renal cancer and response to antiangiogenic therapy. Clin Cancer Res 2001;7:1992–7.
62. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chem 2001;47:617–23.
63. Hew-Butler T, Hoffman MD, Stuempfle KJ, Rogers IR, Morgenthaler NG, Verbalis JG. Changes in copeptin and bioactive vasopressin in runners with and without hyponatremia. Clin J Sport Med 2011;21:211–7.
64. Burge J, Knechtle B, Knechtle P, Gnadinger M, Rust CA, Rosemann T. Maintained serum sodium in male ultra-marathoners–the role of fluid intake, vasopressin, and aldosterone in fluid and electrolyte regulation. Horm Metab Res 2011;43: 646–52.
65. L'Abate P, Wiegert S, Struck J, Wellmann S, Cannizzaro V. Determinants of plasma copeptin: A systematic investigation in a pediatric mechanical ventilation model. Respir Physiol Neurobiol 2013;185:222–7.
66. Montoro-Garcia S, Hernandez-Romero D, Jover E, et al. Growth differentiation factor-15, a novel biomarker related with disease severity in patients with hypertrophic cardiomyopathy. Eur J Intern Med 2012;23:169–74.
67. Munk PS, Valborgland T, Butt N, Larsen AI. Response of growth differentiation factor-15 to percutaneous coronary intervention and regular exercise training. Scand Cardiovasc J 2011;45:27–32.
68. Stahrenberg R, Edelmann F, Mende M, et al. The novel biomarker growth differentiation factor 15 in heart failure with normal ejection fraction. Eur J Heart Fail 2010;12:1309–16.
69. Tchou I, Margeli A, Tsironi M, et al. Growth-differentiation factor-15, endoglin and N-terminal pro-brain natriuretic peptide induction in athletes participating in an ultramarathon foot race. Biomarkers 2009;14:418–22.
70. Bailey AP, Shparago M, Gu JW. Exercise increases soluble vascular endothelial growth factor receptor-1 (sFlt-1) in circulation of healthy volunteers. Med Sci Monit 2006;12:CR45–50.
71. Kivela R, Silvennoinen M, Lehti M, Jalava S, Vihko V, Kainulainen H. Exercise-induced expression of angiogenic growth factors in skeletal muscle and in capillaries of healthy and diabetic mice. Cardiovasc Diabetol 2008;7:13.

Clin. Lab. 2013;59:XXX-XXX

SHORT COMMUNICATION

Effects of Acute Exercise and Allopurinol Administration on Soluble Urokinase Plasminogen Activator Receptor (suPAR)

FABIAN SANCHIS-GOMAR [1], CHIARA BONAGURI [2], HELIOS PAREJA-GALEANO [1], MARI CARMEN GOMEZ-CABRERA [1], JORGE CANDEL [3], JOSE VIÑA [1], GIUSEPPE LIPPI [2]

[1] *Department of Physiology, Faculty of Medicine, University of Valencia, Fundación Investigación Hospital Clínico Universitario/INCLIVA, Valencia, Spain*

[2] *Azienda Ospedaliero-Universitaria di Parma, U.O. di Diagnostica Ematochimica, Dipartimento di Patologia e Medicina di Laboratorio, Parma, Italy*

[3] *Valencia C.F Medical Services, Valencia, Spain*

SUMMARY

Background: **Although physical exercise acutely increases the most widely used inflammatory biomarkers, there is no information on its effect on soluble urokinase plasminogen activating receptor (suPAR), a circulating biomarker increasingly used for the assessment of systemic inflammation.**

Methods: **suPAR was assessed with the quantitative suPARnostic® Standard ELISA Assay (Virogates, Birkerød, Denmark) in 12 professional football players before and after a football match. The athletes were divided into two experimental groups. An oral dose of 300 mg of allopurinol was administered to one group of six participants four hours before a match; the other six participants received placebo.**

Results: **Serum suPAR concentration did not vary significantly after the match in both the placebo and allopurinol group. No significant differences were observed between placebo and allopurinol groups at baseline and after the game.**

Conclusions: **At variance with other consolidated inflammatory biomarkers, suPAR is not influenced by either physical exercise or administration of xanthine oxidase inhibitors.**

(Clin. Lab. 2013;59:xx-xx. DOI: 10.7754/Clin.Lab.2013.120728)

KEY WORDS

physical exercise, soluble urokinase plasminogen activator receptor, suPAR, allopurinol

INTRODUCTION

Physical exercise is widely advocated to prevent chronic disorders such as cardiovascular disease, cancer, osteoporosis, and type 2 diabetes [1]. It has also been demonstrated that endurance exercise improves life expectancy [2,3]. However, strenuous physical exercise can generate free radicals that can cause muscle damage [4]. Xanthine oxidase (XO), a free radical-generating enzyme, may cause oxidative stress (OS) damage associated with exhaustive exercise [5]. Exercise also exerts mechanical and metabolic stress to the body, which contributes to a commonly experienced sub-clinical pathological response involving OS and subsequent inflammation [6]. To counteract these potential oxidative injuries, allopurinol administration may be effective in preventing muscle damage and lipid peroxidation as shown in Tour de France participants, the most important cycling event worldwide, and in marathon runners [7,8].

The soluble urokinase plasminogen activating receptor (suPAR) is a circulating protein, with a molecular weight of between 20 to 50 kDa depending on the degree of glycosylation and proteolytic cleavage [9,10]. suPAR is present at very low concentrations in human blood under physiological conditions, whereas increased plasma levels reliably mirror immune activation and can thereby be considered a valuable marker for several infectious diseases [9,10]. This protein is expressed by several immunoregulatory cells, including

Short Communication accepted August 18, 2012

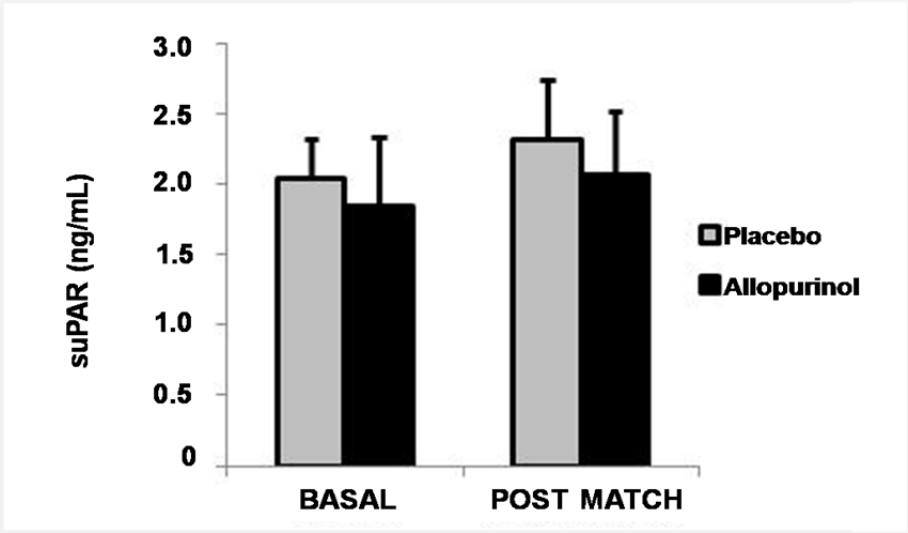


Figure 1. Kinetics of soluble urokinase plasminogen activating receptor (suPAR) in twelve professional football players before and after a football match. The athletes were also divided to receive an oral dose of 300 mg allopurinol (n = 6) or placebo (n = 6).

neutronphils, activated T-cells, and macrophages [11], so that high levels are always observed in association with inflammation, disease progression, and fatal outcome [12,13]. It has been shown that circulating suPAR predicts cancer, cardiovascular disease, diabetes, and mortality in the general population [14], while systemic levels of suPAR correlate positively with markers of organ dysfunction and severity-of-disease classification system scores [15].

The principal aim of this study was to investigate whether acute exhaustive exercise affects circulating suPAR levels among participants in a strenuous sporting event. We then also assessed whether allopurinol administration may affect the circulating levels of this novel biomarker by inhibition of XO.

MATERIALS AND METHODS

Study design

Twelve professional football players (age 25 ± 2 years; weight 75 ± 8 Kg) were divided into two experimental groups. An oral dose of 300 mg of allopurinol was administered to one group of six participants four hours before a match of the Spanish Football League; the other six participants received a placebo. The match was played at 10 p.m. During the second half of the match two players were changed. These players were also included in the study, whereas the goalkeeper was excluded. The players were offered beverages *ad libitum* during the match to prevent excessive dehydration. Venous blood samples were obtained before the match (baseline) and twelve hours afterwards (post-match). All blood samples were collected in vacuum tubes with no additives after an overnight fasting, at the same hour, and under the same conditions (temperature and humidity), from venipuncture of a superficial vein of the antecubital fossa while the subjects had remained seated for not less than 10 minutes. Serum was immediately separated (1500x*g*, 15 minutes, RT) and stored at -20ºC until testing. Written informed consent was obtained prior to participation. The study complies with the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. The experimental protocol was approved by the Committee on Ethics in Research of the Faculty of Medicine, University of Valencia, Spain. Allopurinol is not among the list of drugs prohibited by World Anti-Doping Agency (WADA) or "Fédération Internationale de Football Association" (FIFA).

Measurement of suPAR

The concentration of suPAR was assessed with the quantitative suPARnostic® Standard ELISA Assay (Virogates, Birkerød, Denmark). The test is a simplified double monoclonal antibody sandwich assay, in which samples and peroxidase-conjugated anti-suPAR are mixed in microwells prior to incubation in anti-suPAR

precoated optically clear microwells. The standard is calibrated against an internal gold standard and all values are calculated back to this to ensure samples from different labs and/or different assay lots can be directly compared when the suPARnostic® kit is used. The linearity of the assay is between 2.0 to 15.6 ng/mL, the total imprecision - expressed as total coefficient of variation (CV%) - between 2.3 and 6.0%.

Statistical analysis

For statistical analysis of results, the mean was taken as the measurement of the main tendency and the standard deviation was taken as the dispersion measurement. A two-way analysis of variance with repeated measures was used to determine the difference between the two parameters during the experimental intervention (Baseline and Post-Match). When an interaction effect was found, multiple comparisons using the Turkey post hoc test were performed. The statistical analysis was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK), and the alpha level for statistical significance was set at $p < 0.05$.

RESULTS

No significant differences in body mass were found between groups, and the body weight remained unchanged until the end of the study in all the experimental groups (data not shown). Figure 1 shows the serum suPAR levels before and after the match in both groups (placebo and allopurinol). Serum suPAR concentration increased from 2.0 ± 0.3 to 2.3 ± 0.4 ng/mL in the placebo group ($p = 0.14$) and from 1.8 ± 0.5 to 2.0 ± 0.4 ng/mL in the allopurinol group ($p = 0.20$). No statistically significant changes were found before or after the match in either the placebo or allopurinol groups. Moreover, no significant differences were observed between placebo and allopurinol groups at either baseline (2.0 ± 0.3 versus 1.8 ± 0.5; $p = 0.20$) or after the match (2.3 ± 0.4 versus 2.0 ± 0.4; $p = 0.18$).

DISCUSSION

The potential influence of biological variability is one of the most critical issues in the complex pipeline that encompasses discovery, clinical validation, and routine assessment of novel diagnostic biomarkers. Physical exercise, in particular, has a strong and acute influence on the most widely used inflammatory biomarkers, including procalcitonin (PCT), C Reactive Protein (CRP), and Interleukin-6 (IL-6). We have recently shown that the serum levels of PCT may increase up to 4-fold after a modest bulk of physical exercise [16]. Analogously, the concentrations of IL-6 and CRP were reported to be remarkably increased after running, up to 16- and 28-fold, respectively [17]. Physical exercise should hence be considered a potential confounding factor, which may substantially impair the diagnostic performance of PCT, CRP, and IL-6. suPAR is a unique risk marker that behaves as a "master alarm" for a patient risk status. An increased serum level of suPAR reflects an ongoing critical condition characterized by increased risk of mortality, whereas decreasing levels of suPAR are a valuable index of patient improvement and effectiveness of therapeutic intervention [11-14]. The results of the present study, clearly showing that the serum levels of suPAR are not significantly influenced by either physical exercise or allopurinol administration, have relevant clinical implications. First, the measurement of suPAR - at variance with that of PCT, CRP and IL-6 - is still strongly reliable regardless of the physical exercise performed by the patient. It is also noteworthy that allopurinol, the archetypal inhibitor of XO which has been the cornerstone of the therapeutic management of hyperuricemia for decades, is increasingly used in patients with various forms of tissue and vascular injuries, such as acute coronary syndrome, chronic heart failure, inflammatory diseases, as well as septic shock [18], burns, wounds [19], and other localized forms of infection [20], wherein PCT, CRP, and IL-6 are commonly monitored. The evidence that allopurinol does not significantly modify the serum levels of suPAR would, hence, enable its use also in patients receiving XO inhibitors.

Acknowledgement:

This work was supported by grants SAF2009-08334 from the Spanish Ministry of Education and Science (MEC); ISCIII2006-RED13-027 from the "Red Temática de investigación cooperativa en envejecimiento y fragilidad (RETICEF), PROMETEO2010/074 and EU Funded COSTB35. This study has been co-financed by FEDER funds from the European Union. Sanchis-Gomar F was a recipient of a research fellowship from the Conselleria d' Educació, Generalitat Valenciana, "VALi+d program". Pareja-Galeano H was a recipient of a FPU fellowship from the Spanish Ministry of Education and Science (AP2009-4755).

Declaration of Interest:

None of the authors had any conflicts of interest with the funding agencies or professional relationships with companies or manufacturers who may benefit from the results of the present study.

References:

1. Vina J, Sanchis-Gomar F, Martinez-Bello V, Gomez Cabrera M. Exercise acts as a drug. Pharmacological benefits of exercise. Br J Pharmacol 2012;167:1-12.

2. Sanchis-Gomar F, Olaso-Gonzalez G, Corella D, Gomez-Cabrera MC, Vina J, Increased Average Longevity among the "Tour de France" Cyclistas Int J Sports Med 2011;32:644-7.

3. Ruiz Jr, Moran M, Arenas J, Lucia A. Strenuous endurance exercise improves life expectancy: it's in our genes. Br J Sport Med 2011;45:159-61.

4. Sastre J, Asensi M, Gasco E, et al. Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. Am J Physiol 1992;263:R992-5.

5. Vina J, Gomez-Cabrera MC, Lloret A, et al. Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants. IUBMB Life 2000;50:271-7.

6. Pyne DB. Exercise-induced muscle damage and inflammation: a review. Aust J Sci Med Sport 1994;26:49-58.

7. Gomez-Cabrera MC, Pallardo FV, Sastre J, Vina J, Garcia-del-Moral L. Allopurinol and markers of muscle damage among participants in the Tour de France. JAMA 2003;289:2503-4.

8. Gomez-Cabrera MC, Martinez A, Santangelo G, et al. Oxidative stress in marathon runners interest of antioxidant supplementation. Br J Nutr 2006;96 Suppl 1:S31-3.

9. Wei C, El Hindi S, Li J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. Nat Med 2011;17: 952-60.

10. Blasi F, Carmeliet P, uPAR: a versatile signalling orchestrator. Nat Rev Mol Cell Biol 2002;3:932-43.

11. Huai Q, Mazar AP, Kuo A, et al. Structure of human urokinase plasminogen activator in complex with its receptor. Science 2006; 311:656-9.

12. Sidenius N, Sier CF, Ullum H, et al. Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. Blood 2000;96:4091-5.

13. Kofoed K, Eugen-Olsen J, Petersen J, Larsen K, Andersen O. Predicting mortality in patients with systemic inflammatory response syndrome: an evaluation of two prognostic models, two soluble receptors and a macrophage migration inhibitory factor. Eur J Clin Microbiol Infect Dis 2008;27:375-83.

14. Eugen-Olsen J, Andersen O, Linneberg A, et al. Circulating soluble urokinase plasminogen activator receptor predicts cancer, 14. Cardiovascular disease, diabetes and mortality in the general population. J Intern Med 2010;268:296-308.

15. Backes Y, van der Sluijs KF, Mackie DP, et al. Usefulness of suPAR as a biological marker in patients with systemic inflammation or infection a systematic review. Intensive Care Med 2011;37 (11):1897-8.

16. Lippi G, Schena F, Montagnana M, Salvagno GL, Guidi GC. Acute influence of aerobic physical exercise on procalcitonin. Eur J Clin Invest 2008;38:784-5.

17. Scherr J, Braun S, Schuster T, et al. 72-h kinetics of high-sensitive troponin T and inflammatory markers after marathon. Med Sci Sports Exerc 2011,43:1819-27.

18. Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol Rev 2006;58:87-114.

19. Sahib AS, Al-Jawad FH, Alkaisy AA. Effect of antioxidants on the incidence of wound infection in burn patients. Ann Burns Fire Disasters 2010;23:199-205.

20. Gobbi P, Lo Presti MS, Fernandez AR, et al. Allopurinol is effecttive to modify the evolution of Trypansoma cruzi infection in mice. Parasitol Res 2007;101:1459-62.

Correspondence:
Fabian Sanchis Gomar
Department of Physiology. Faculty of Medicine.
University of Valencia.
Av. Blasco Ibañez, 15, Valencia
46010 Spain
Phone: +34 96 386 46 46
Fax: +34 96 386 46 42
Email: fabian.sanchis@uv.es

Journal of Clinical Laboratory Analysis 0: 1–6 (2013)

Serum Copeptin and Midregion Proadrenomedullin (MR-proADM) After an Ultramarathon

Giuseppe Lippi,[1]* Federico Schena,[2] Gian Luca Salvagno,[3] Fabian Sanchis-Gomar,[4] and Gian Cesare Guidi[3]

[1]U.O. di Diagnostica Ematochimica, Dipartimento di Patologia e Medicina di Laboratorio, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy

[2]Sezione di Scienze Neurologiche Neuropsicologiche, Morfologiche e Motorie, Università di Verona, Verona, Italy

[3]Sezione di Chimica Clinica, Dipartimento di Scienze della Vita e della Riproduzione, Università di Verona, Verona, Italy

[4]Department of Physiology, Faculty of Medicine, University of Valencia, Valencia, Spain

Background: Although it is widely acknowledged that physical activity confers several health benefits, it remains uncertain whether strenuous and physically demanding exercise might determine biological effects that might turn to be ultimately unfavorable for health. Copeptin and midregion proadrenomedullin (MR-proADM) are emerging cardiovascular and stress biomarkers, but little is known about the influence of strenuous physical exercise on their concentrations. *Methods:* The present study was performed to investigate the variation of copeptin and MR-proADM, along with that of serum creatinine and estimated glomerular filtration rate before and after a 60 km ultramarathon in 16 healthy Caucasian males. *Results:* The serum concentrations of both copeptin and MR-proADM remarkably increased after the 60 km run, by 6.4 times (interquartile range (IQR), 2.710.4) and 2.3 times (IQR, 1.8–2.6), respectively. A highly significant correlation was observed between the increase of creatinine and MR-proADM, but not between serum creatinine and copeptin. The percentage of subjects exhibiting values above the upper limit of the reference range in male was 0% for both copeptin and MR-proADM before the ultramarathon, but increased to respectively 81 and 63% postexercise. *Conclusion:* The evidence that an ultramarathon causes a substantial increase of copeptin and MR-proADM raises doubts as to whether exhaustive exercise might be considered globally beneficial or even safe, especially in unfit or/and untrained population. J. Clin. Lab. Anal. 0:1–6, 2013.

Key words: sport; physical exercise; marathon; copeptin; midregion proadrenomedullin

INTRODUCTION

It is widely acknowledged that physical activity and exercise confer several benefits for healthier lifestyle as well as in the growing battle against obesity, cardiovascular disease, and other chronic disorders such as diabetes, osteoporosis, decline of cognitive function, and cancer (1). The International Olympic Committee has recently endorsed recommendations advocating that all governments should encourage physical exercise and health promotion on global health and regional agency agendas (1). Although it is hence clear that sedentary behavior or an inadequate high-level physical exercise in unfit or/and unhealthy population might be deleterious, it remains uncertain whether strenuous and physically demanding exercise might determine short- and long-term biological effects that might turn to be ultimately unfavorable for health

*Correspondence to: Giuseppe Lippi, U.O. Diagnostica Ematochimica, Dipartimento di Patologia e Medicina di Laboratorio, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci, 14, 43126 Parma, Italy. E-mail: glippi@ao.pr.it; ulippi@tin.it

Received 18 January 2013; Accepted 31 October 2013
DOI 10.1002/jcla.21720
Published online in Wiley Online Library (wileyonlinelibrary.com).

(2). It has been recently observed that exhaustive exercise in physically fit and trained people is a healthy practice, which may even increase life expectancy (3). On the other hand, however, several sports modalities such as cycling and long-distance running (e.g., marathon and ultramarathon) have been associated with a remarkable increase in biomarkers of cardiac injury (4–7), as well as with radiological findings suggestive of myocardial necrosis and fibrosis (8). Oxidative stress is clearly involved in the pathophysiology of cardiac remodeling and heart failure as well as in skeletal muscle damage induced by exhaustive exercise (9). Exhaustive or acute physical exercise causes an increase in the generation of reactive oxygen species, and therefore raises the oxidative stress in muscle and in other organs, resulting in cell damage (10, 11).

Copeptin is the C-terminal portion of provasopressin, a 39 amino acid glycopeptide of unknown function, which is produced during cleavage of arginine vasopressin (AVP), also known as antidiuretic hormone (12). AVP is mainly released from the neurohypophysis to maintain water balance by the kidney, and thereby contributes to osmotic and cardiovascular homeostasis. Besides the clinical significance as a surrogate marker of AVP secretion, there is increasing evidence that the combined determination of copeptin with cardiac troponins in patients with chest pain might accelerate the diagnosis of acute coronary syndrome or acute myocardial infarction (AMI) (13–15), as well as mirror disease severity and help predict unfavorable outcomes in patients with chronic stable heart failure (16). Additional studies have also suggested that copeptin assessment may provide reliable prognostic information in patients after an AMI (17), as well as in those with chronic heart failure (18). Increased levels of copeptin might also predict an increased risk for diabetes independently of conventional risk factors (19). The prognostic value of copeptin has been established in other severe diseases such as sepsis, pneumonia, lower respiratory tract infections, and stroke (20). Most importantly, it was also recently demonstrated that copeptin concentration correlates with the individual stress level (21).

Adrenomedullin (ADM) is a 52 amino acid vasoactive peptide that plays an important role in microcirculation and endothelial dysfunction. It is mainly expressed from the adrenal medulla and adipose tissue, as well as from endothelial and vascular smooth muscle cells, immune cells, and several endocrine glands (22). The precursor pre-proADM, which is transcriptionally induced by insulin, hypoxia, and several inflammatory stimuli, is further activated to ADM after cleavage of another biologically active peptide known as proADM N-terminal 20 peptide (PAMP) and two peptides flanking ADM, that is, the midregional part of proADM (MR-proADM 45–92) and the carboxy-terminus of the molecule (proADM 153–185). Recent evidence attests that MR-proADM is an independent predictor for events in patients with symptomatic coronary artery disease (23), chronic heart failure (24), stroke (25), and other severe disorders such as amyloidosis (26), sepsis (27), community-acquired pneumonia (28), and acute dyspnea (29), even if the causal contribution of MR-proADM to the pathogenesis of these pathologies remains unclear.

Although both copeptin and MR-proADM can hence be considered emerging cardiovascular biomarkers, little is known about the influence of strenuous physical exercise on their concentrations, especially on that of the latter peptide. The present study was performed to investigate the value of copeptin and MR-proADM as biochemical markers of cellular damage after high-level physical exercise. For this purpose, we measured copeptin, MR-proADM, serum creatinine, and estimated glomerular filtration rate (EGFR) before and after a 60 km ultramarathon run.

MATERIALS AND METHODS

Study Design

The population study consisted of 16 healthy Caucasian males (mean age: 41 years, range: 34–50 years), who had been engaged in specific endurance training for at least 4 years (mean training regimen: 248 ± 32 min/week; maximal oxygen uptake, VO_2: 65 ± 2 ml/kg/min). The athletes concluded a 60 km ultramarathon equipped with a heart rate (HR) monitor at $82 \pm 4\%$ VO_{2max}. The percentage was calculated according to VO_2/HR relationship assessed with incremental test. None of the subjects suffered from acute or chronic diseases, reported regular or recent medication intake, including antioxidants and nicotine. Strenuous exercise was also precluded for at least 36 h before this study. The ultramarathon began at 08:00 A.M. and was run on a hilly and demanding course, on a cloudy and partially rainy day, with temperatures ranging from 6 to 8°C and humidity from 54 to 87%. Blood samples were collected at fast, 20 min before the participants warmed up (pre), and 10 min after the end of the ultramarathon (post). All subjects gave an informed consent for being tested, and the study was carried out in agreement with the Declaration of Helsinki and under the terms of all relevant local legislation. Blood was collected in vacuum tubes containing no additives (Becton-Dickinson, Oxford, UK), separated by centrifugation at $3{,}000 \times g$ within 30 min from venipuncture and stored at −70°C.

Laboratory Measurements

Serum creatinine was measured by Jaffe, rate-blanked, and compensated assay on a Beckman Coulter AU5800,

employing proprietary reagents (Beckman Coulter Inc., Brea, CA). The kidney function was also defined by EGFR, according to the formula developed and validated in the Modification of Diet in Renal Disease (MDRD) study, and further modified by Levey et al. for methods traceable to the serum creatinine reference system (30). The resulting equation is as follows: EGFR = 175 × (serum creatinine$^{-1.154}$) × (age$^{-0.203}$) × 1.212 (if black) × 0.742 (if female), and it does not require a body weight variable because it normalizes GFR for a standard body surface area of 1.73 m^2. The lower limit of the reference range for this formula is conventionally fixed at <60 ml/min/1.73 m^2.

Serum copeptin and ADM were assayed by Time-Resolved Amplified Kryptat Emssion (TRACE) on Kryptor (Thermo Scientific B·R·A·H·M·S, Germany). According to the manufacturer's specification, the intra- and interassay imprecision of copeptin immunoassay at concentrations of 20 and 50 pmol/l are 6–12% and 8–13%, respectively. The analytical detection limit, the 97.5% percentile of the male reference range as well as the cut-off of copeptin in the diagnostics of cardiovascular disorders and AMI have been set at 4.8, 19.1 pmol/l and 14 pmol/l, respectively (13). According to the manufacturer's specification, the intra- and interassay imprecision of MR-proADM immunoassay at concentrations ranging from 0.5 to 6 nmol/l are comprised between 4 and 11%. The analytical detection limit and 95% percentile of the reference range are 0.05 and 0.52 nmol/l, respectively.

Statistical Analysis

The Wilcoxon signed-rank test and the $\chi 2$ test (for categorical variables) were used to evaluate the significance of exercise-induced variations. Data with a nonnormal distribution were normalized using a logarithmic transformation prior to analysis. Statistical analyses were carried out with Analyse-it for Excel software (Analyse-it Software, Leeds, UK) and the level of statistical significance was set at $P < 0.05$. Data are presented as the median and interquartile range (IQR). The postmarathon results have also been adjusted for plasma volume change, according to the formula of Dill and Costill, as described elsewhere (31).

RESULTS

All the athletes completed successfully the ultramarathon, with no clinically meaningful symptoms. The main results of this study are shown in Table 1. The serum concentration of creatine significantly increased by 31% (IQR, 13–58%) after the run, whereas the EGFR decreased by the same extent. The serum concentrations of both copeptin and MR-proADM remarkably increased after the 60 km run, by 6.4 times (IQR, 2.7–10.4) and 2.3 times (IQR, 1.8–2.6; Fig. 1), respectively. A highly significant correlation was observed between the delta increase (i.e., the ratio between the post- and prerun value) of serum creatinine and MR-proADM ($r = 0.680$; $P = 0.004$), but not between serum creatinine and copeptin ($r = -0.064$; $P = 0.81$; Fig. 2). Finally, the percentage of subjects exhibiting values above the upper limit of the reference range in male was 0% for both copeptin and MR-proADM before the ultramarathon, but increased to respectively 13 (81%) and 10 (63%) postexercise.

TABLE 1. Variation of Serum Creatinine, EGFR, Copeptin, and MR-proADM After a 60 km Ultramarathon Run in 16 Male Athletes

	Prerun	Postrun	*P*
Creatinine (μmol/l)	68 (60–76)	92 (72–114)	<0.001
EGFR (ml/min/1.73 m^2)	111 (98–127)	80 (61–106)	<0.001
Copeptin (pmol/l)	5.9 (5.0–10.3)	40.6 (20.3–100.2)	<0.001
MR-proADM (nmol/l)	0.29 (0.27–0.34)	0.68 (0.51–0.80)	<0.001

DISCUSSION

There is only one study that has assessed the variation of copeptin after short-term exercise, two after long-distance endurance exercise, whereas none has investigated the variation of MR-proADM after exercise to the best of our knowledge. Maeder et al. originally studied 161 patients undergoing cardiopulmonary exercise testing (CPET) with upright symptom-limited cycle ergometer tests (mean exercise time of 6.3 min and increments of 10–25 W/min) (32), and observed a significant increase (i.e., median increase of 59%) of copeptin concentration from rest to peak at exercise. Interestingly, the percent change in copeptin was independently associated with maximal work rate as well as with the previous use of several cardiac medications by virtue of their surrogate status for the presence of coronary artery disease or previous AMI. Hew-Butler et al. reported that copeptin levels significantly increased during and at the end of extreme distance runs despite decreases in plasma Na^{2+}, thus suggesting the existence of a nonosmotic vasopressin stimulation during this strenuous exercise activity (33). Bürge et al. also investigated 50 male ultramarathoners undergoing a 100 km ultramarathon and found that serum creatinine increased by nearly 30% after the exercise (34), whereas plasma copeptin concentration remarkably increased by nearly 12 times, and hence to a greater extent as that observed in our study (i.e., 6.4 times) (35). Interestingly, the change in copeptin was not associated with the number of urinations, urine excretion, or fluid intake during the race. This is consistent with our findings, showing

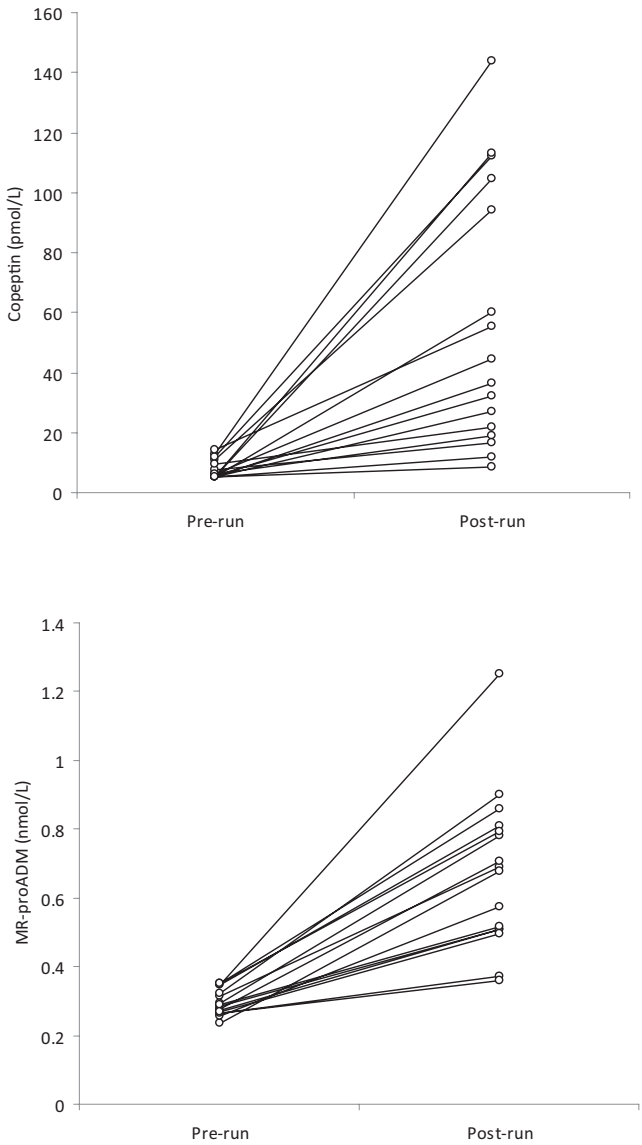


Fig. 1. Variation of copetin and MR-proADM after a 60 km ultramarathon run in 16 male athletes.

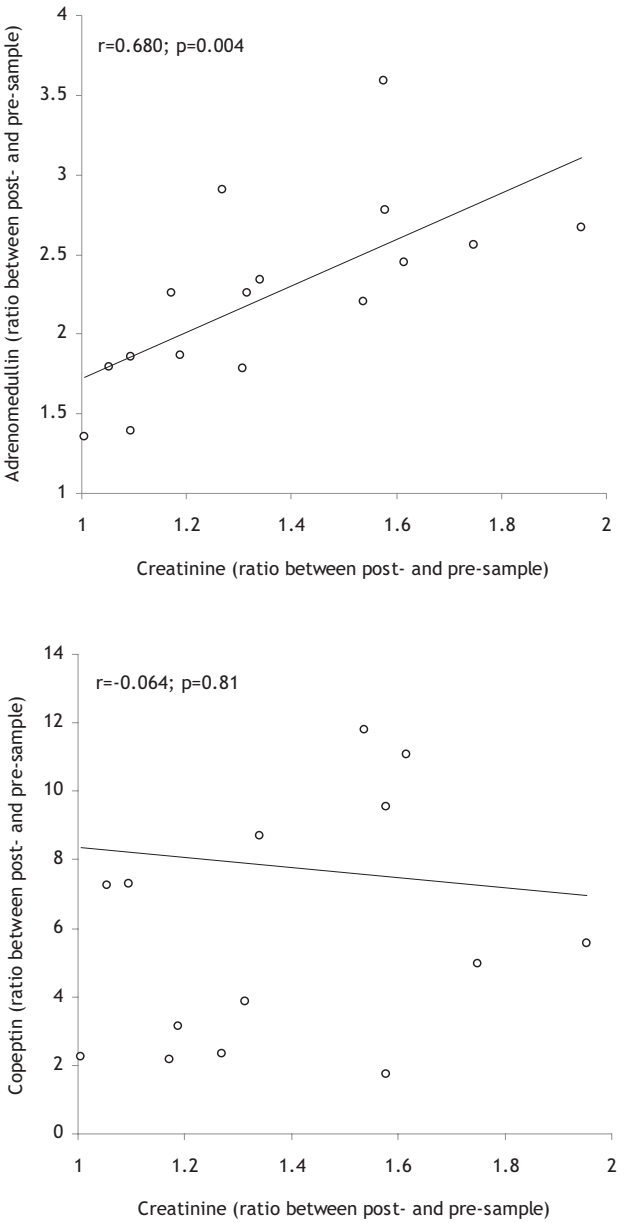


Fig. 2. Linear regression analysis and Spearman's correlation coefficient (r) between the delta increase (i.e., the ratio between the post- and prerun value) of creatinine and copetin, and creatinine and MR-proADM.

that the postexercise increase of serum copeptin was not related to that of serum creatinine, thereby suggesting that the variation of this marker is not related to renal function and/or fluid homeostasis, but might be attributable to other factors such as stressor events, which activate the hypothalamic–pituitary–adrenal axis (20, 21). AVP is synthesized in the hypothalamus essentially in response to high osmolality and arterial hypovolaemia but is also substantially released in the context of the stress response, which might thereby include strenuous exercise as well (20).

The significant association between variation of serum creatinine and MR-proADM suggests that renal function and hypovolaemia might have partially contributed to its variation. A systemic increase in ADM typically reflects an overflow from local sites of production and action, mostly caused by endothelial damage (e.g., in chronic heart failure and septic shock) rather than from physiological secretion by the adrenal gland (22). In certain conditions increased plasma ADM has, in fact, a hormonal function, causing a general decrease in vascular resistance and a fall in blood pressure (22), whereas the hormonal effects of vasopressin include vasoconstriction,

stimulation of release of adrenocorticotropic hormone, and prolonged activation of vasopressin receptors, which mediate antidiuretic effects and myocardium hypertrophy (17). In the same group of athletes, we have previously measured troponin I with both a highly sensitive and a contemporary-sensitive troponin I immunoassays, observing that the values of these biomarkers significantly increased after the trial. Nevertheless, the diagnostic cutoff was exceeded in a minority of cases (i.e., 20%) and the overall increase was limited, thus compatible with transitory and reversible myocardial ischemia rather than attributable to myocardiocyte necrosis (7). As such, the variation of both copeptin and MR-proADM is likely due to the cardiac stress evoked by the strenuous aerobic exercise, rather than due to necrotic effects, including myocardial damage.

Regardless of the underlying causes supporting the increase of both copeptin and MR-proADM, the results of this study have clinical implications. In agreement with previous studies, the concentration of copeptin, as well as that of MR-proADM, is remarkably increased after long-distance running. Therefore, the hypothesis of an exercise-induced increase of both copeptin and MR-proADM should be considered before using these biomarkers for assessment of cardiovascular risk, myocardial injury, and cellular damage. Then, the evidence that a 60 km run causes a clinically meaningful increase of cardiac troponins (7), but also of copeptin and MR-proADM, raises several doubts as to whether exhaustive exercise might be considered globally beneficial or even safe, especially in unfit or/and untrained population. It is also worthwhile mentioning that the increase of both peptides can play a pathophysiological role, promoting unfavorable health effects.

All these factors support the evidence of a strong association of both biomarkers with adverse health outcomes throughout a wide spectrum of clinical conditions. Copeptin and MR-proADM should be considered as innovative biomarkers suitable for application in clinical and sports medicine, since it can be safely used to rule out cellular damage in patients who had undergone recent exhaustive exercise. Although further studies are needed to determine the exact role of these peptides, our results open the possibility to use these peripheral signals as possible biomarkers of exercise-induced damage and stress.

REFERENCES

1. Mountjoy M, Andersen LB, Armstrong N, et al. International Olympic Committee consensus statement on the health and fitness of young people through physical activity and sport. Br J Sports Med 2011;45:839–848.
2. Armstrong N, Tomkinson G, Ekelund U. Aerobic fitness and its relationship to sport, exercise training and habitual physical activity during youth. Br J Sports Med 2011;45:849–858
3. Sanchis-Gomar F, Olaso-Gonzalez G, Corella D, Gomez-Cabrera MC, Viña J. Increased average longevity among the "tour de france" cyclists. Int J Sports Med 2011;32:644–647.
4. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. Clin Chem Lab Med 2010;48:757–767.
5. Lippi G, Schena F, Montagnana M, Salvagno GL, Guidi GC. Influence of acute physical exercise on emerging muscular biomarkers. Clin Chem Lab Med 2008;46:1313–1318.
6. Lippi G, Cervellin G, Banfi G, Plebani M. Cardiac troponins and physical exercise. It's time to make a point. Biochem Med (Zagreb) 2011;21:55–62.
7. Lippi G, Schena F, Salvagno GL, Tarperi C, Aloe R, Guidi GC. Comparison of conventional and highly-sensitive troponin I measurement in ultra-marathon runners. J Thromb Thrombolysis 2012;33:338–342.
8. Yared K, Wood MJ. Is marathon running hazardous to your cardiovascular health? The jury is still out. Radiology 2009;251:3–5.
9. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol 2011;301:H2181–H2190.
10. Gomez-Cabrera MC, Borrás C, Pallardó FV, Sastre J, Ji LL, Viña J. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 2005;567(Pt 1):113–120.
11. Gomez-Cabrera MC, Domenech E, Romagnoli M, et al. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 2008;87:142–149.
12. de Bree FM, Burbach JP. Structure-function relationships of the vasopressin prohormone domains. Cell Mol Neurobiol 1998;18:173–191.
13. Reichlin T, Hochholzer W, Stelzig C, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. J Am Coll Cardiol 2009;54:60–68.
14. Keller T, Tzikas S, Zeller T, et al. Copeptin improves early diagnosis of acute myocardial infarction. J Am Coll Cardiol 2010;55:2096–2106.
15. Giannitsis E, Kehayova T, Vafaie M, Katus HA. Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. Clin Chem 2011;57:1452–1455.
16. Tentzeris I, Jarai R, Farhan S, et al. Complementary role of copeptin and high-sensitivity troponin in predicting outcome in patients with stable chronic heart failure. Eur J Heart Fail 2011;13:726–733.
17. Khan SQ, Dhillon OS, O'Brien RJ, et al. C-terminal provasopressin (copeptin) as a novel and prognostic marker in acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) study. Circulation 2007;115:2103–2110.
18. Neuhold S, Huelsmann M, Strunk G, et al. Comparison of copeptin, B-type natriuretic peptide, and amino-terminal pro-B-type natriuretic peptide in patients with chronic heart failure: Prediction of death at different stages of the disease. J Am Coll Cardiol 2008;52:266–272.
19. Enhörning S, Wang TJ, Nilsson PM, et al. Plasma copeptin and the risk of diabetes mellitus. Circulation 2010;121:2102–2108.
20. Katan M, Christ-Crain M. The stress hormone copeptin: A new prognostic biomarker in acute illness. Swiss Med Wkly 2010;140:w13101.
21. Katan M, Morgenthaler N, Widmer I, et al. Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level. Neuro Endocrinol Lett 2008;29:341–346.
22. Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. Endocr Rev 2000;21:138–167.
23. Wild PS, Schnabel RB, Lubos E, et al. Midregional proadrenomedullin for prediction of cardiovascular events in coronary

artery disease: Results from the AtheroGene study. Clin Chem 2012;58:226–236.
24. von Haehling S, Filippatos GS, Papassotiriou J, et al. Mid-regional pro-adrenomedullin as a novel predictor of mortality in patients with chronic heart failure. Eur J Heart Fail 2010;12:484–491.
25. Katan M, Fluri F, Schuetz P, et al. Midregional pro-atrial natriuretic peptide and outcome in patients with acute ischemic stroke. J Am Coll Cardiol 2010;56:1045–1053.
26. Palladini G, Barassi A, Perlini S, et al. Midregional proadrenomedullin (MR-proADM) is a powerful predictor of early death in AL amyloidosis. Amyloid 2011;18:216–221.
27. Christ-Crain M, Morgenthaler NG, Struck J, Harbarth S, Bergmann A, Müller B. Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: An observational study. Crit Care 2005;9:R816–824.
28. Bello S, Lasierra AB, Mincholé E, et al. Prognostic power of proadrenomedullin in community-acquired peumonia is independent on aetiology. Eur Respir J 2012;39:1144–1155.
29. Maisel A, Mueller C, Nowak RM, et al. Midregion prohormone adrenomedullin and prognosis in patients presenting with acute dyspnea: Results from the BACH (Biomarkers in Acute Heart Failure) trial. J Am Coll Cardiol 2011;58:1057–1067.
30. Levey AS, Coresh J, Greene T, et al. Expressing the Modification of Diet in Renal Disease study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53:766–772.
31. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 1974;37: 247–248.
32. Maeder MT, Staub D, Brutsche MH, et al. Copeptin response to clinical maximal exercise tests. Clin Chem 2010;56:674–676.
33. Hew-Butler T, Hoffman MD, Stuempfle KJ, Rogers IR, Morgenthaler NG, Verbalis JG. Changes in copeptin and bioactive vasopressin in runners with and without hyponatremia. Clin J Sport Med 2011;21:211–217.
34. Bürge J, Knechtle B, Knechtle P, Gnädinger M, Rüst AC, Rosemann T. Maintained serum sodium in male ultra-marathoners—The role of fluid intake, vasopressin, and aldosterone in fluid and electrolyte regulation. Horm Metab Res 2011;43: 646–652.
35. Bracher A, Knechtle B, Gnädinger M, et al. Fluid intake and changes in limb volumes in male ultra-marathoners: Does fluid overload lead to peripheral oedema? Eur J Appl Physiol 2012;112:991–1003.

Clin Chem Lab Med 2012;50(9):1585–1589 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/cclm-2011-0954

Variation of serum and urinary neutrophil gelatinase associated lipocalin (NGAL) after strenuous physical exercise

Giuseppe Lippi[1,*], Fabian Sanchis-Gomar[2], Gian Luca Salvagno[3], Rosalia Aloe[1], Federico Schena[4] and Gian Cesare Guidi[3]

[1] U.O. di Diagnostica Ematochimica, Dipartimento di Patologia e Medicina di Laboratorio, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy
[2] Department of Physiology, Faculty of Medicine, University of Valencia, Valencia, Spain
[3] Sezione di Chimica Clinica, Dipartimento di Scienze della Vita e della Riproduzione, Università di Verona, Verona, Italy
[4] Sezione di Scienze Neurologiche Neuropsicologiche, Morfologiche e Motorie, Università di Verona, Verona, Italy

Abstract

Background: Strenuous exercise may trigger acute complications, such as exertional rhabdomyolysis and gastrointestinal complaint. As less is known about the potential renal impairment after long distance running, we assessed creatinine and neutrophil gelatinase associated lipocalin (NGAL) in serum (sNGAL) and urine (uNGAL) before and after an ultramarathon.
Methods: The study population consisted of 16 trained male athletes who ran a 60 km ultramarathon. Blood and spot urine samples were collected 20 min before and immediately after the run. Creatinine was assessed by Jaffe assay on Beckman Coulter AU5800 and renal function was expressed as estimated glomerular filtration rate (eGFR) by MDRD formula. NGAL was measured by fully-automated immunoassay NGAL Test™ on AU 5800.
Results: Serum and urinary creatinine increased significantly by 38% and 78%, respectively. The eGFR contextually decreased by 31%. sNGAL, uNGAL and uNGAL/creatinine ratio increased by 1.6-fold, 7.7-fold and 2.9-fold. In six of 16 athletes (38%), the acute post-exercise increase of serum creatinine met the criteria of acute kidney injury. No significant relationship was found between pre-exercise, post-exercise values and post-exercise variation of sNGAL, uNGAL and uNGAL/creatinine ratio. A significant correlation was found between pre- and post-exercise changes of serum creatinine and sNGAL, but not with either uNGAL or uNGAL/creatinine ratio.
Conclusions: The acute variations of serum creatinine and uNGAL attest that renal impairment is likely to develop after long distance running. The uNGAL seems more independent from creatinine variation and extra-renal sources, and thereby more reliable for monitoring the renal involvement in these types of kidney impairment.

Keywords: creatinine; neutrophil gelatinase associated lipocalin (NGAL); physical exercise; sports.

Introduction

It is now well established that the renal blood flow is about 1.2 L/min at rest, that is approximately 20% of the cardiac output (1). Physical activity induces profound changes in hemodynamics. The increased blood flow through the muscles during exercise causes, in fact, a dramatic decrease of circulation in the splanchnic and renal districts, which seems to be directly proportional to extent and duration of the physical effort (e.g., in heavy exercise the renal blood flow may fall to 25% of the resting value) (1, 2). Several mechanisms have been advocated to support the perturbation of renal hemodynamics in sportsmen, including an increased sympathetic nervous system outflow as well as a heightened activity of two important vasoconstrictors, such as angiotensin II and vasopressin (3). These changes are counterbalanced by a local mechanism of autoregulation, which is aimed to preserve the glomerular filtration rate (GFR). The resulting increased filtration fraction, which can be twice as high as in the resting condition, partially limits the transfer of metabolites or substances through the glomeruli and reduces the extent of exercise proteinuria (4).

A post-exercise, transient proteinuria (i.e., with half-time of approx. 1 h) is commonplace in athletes, and appears to be directly related to the intensity of the exercise (4). It is mainly due to a combined mechanism of increased clearance of plasma proteins due to increased glomerular permeability, and a partial inhibition of tubular reabsorption of macromolecules (3). Whether a prolonged and heavy reduction of the blood flow in the kidney may be associated with a parenchymal injury is uncertain and the clarification of this issue has been somehow limited by the lack of a reliable biomarker for renal damage. Historically, the diagnostic criteria for acute kidney injury (AKI) have been essentially based on acute variations of serum creatinine or urine output. Although the former parameter is still the gold standard for diagnosing impaired renal function (5), its assessment in the setting of

*Corresponding author: Prof. Giuseppe Lippi, U.O. Diagnostica Ematochimica, Azienda Ospedaliero-Universitaria di Parma, Via Gramsci, 14, 43126 – Parma, Italy
Phone: +39-0521-703050/703197,
E-mail: glippi@ao.pr.it; ulippi@tin.it
Received December 25, 2011; accepted January 16, 2012; previously published online February 14, 2012

AKI carries several drawbacks, such as dependence from age, gender, nutritional status, drugs, muscle mass, as well as a suboptimal positive predictive value in patients with prerenal azotemia. The negative predictive value of serum creatinine is also limited by the large "renal reserve", as a significant variation of serum creatinine becomes evident when over half of the GFR is lost (6, 7), so that the diagnosis of AKI may be substantially delayed by its slow kinetics. The assessment of urine output also carries some caveats, as the negative predictive value is low (i.e., oliguria is not commonplace in all patients with AKI) and the positive predictive value is also limited by the fact that oliguric patients do not always develop AKI (6).

Several urinary and serum proteins have been recently proposed and studied as potential biomarkers of AKI, including neutrophil gelatinase associated lipocalin (NGAL), cystatin-C (Cys-C), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), monocyte chemotactic peptide (MCP-1), netrin-1, liver-type fatty acid binding proteins (L-FABP). Among these, the best clinical evidence has been accumulated so far for NGAL (6, 8). NGAL is a 25-kDa acute phase protein largely produced by the kidney tubule, which is also up-regulated in other pathological conditions, such as pancreatic and rectal cancer, diabetes, atherosclerosis and myocardial infarction (6, 9, 10). The members of the lipocalin family can also be overexpressed by reactive oxygen species (ROS) (11), which can be in turn generated by exhaustive or acute physical exercise (12).

Thus, as renal ischemia due to strenuous aerobic exercise may cause a serious renal tissue damage and, therefore, an AKI, the aim of this study was to assess acute variations in NGAL and in creatinine after performing strenuous physical exercise. For this purpose, we measured urinary NGAL (uNGAL), serum NGAL (sNGAL), urinary and serum creatinine, the urinary NGAL/creatinine ratio and finally, the estimated glomerular filtration rate (eGFR) in 16 athletes before and after a 60-km ultramarathon run.

Materials and methods

The study population consisted in 16 trained Caucasian male athletes (mean age: 42 years, range: 34–52 years), who had been engaged in specific endurance training for 3 to 10 years (mean training regimen: 240±32 min/week; maximal oxygen uptake, VO_2: 65±2 mL/kg/min). All athletes run a 60-km ultramarathon equipped with a heart rate (HR) monitor at 80±4% VO_{2max} (the% was calculated according to the VO_2/HR relationship assessed with incremental test). None of the athletes had acute or chronic diseases, or reported intake of medications, including antioxidants or nicotine. Strenuous exercise was averted 36–48 h before the trial. The trial began at 08:00 a.m. and was carried out on a hilly and demanding course, on a cloudy and partially rainy day, with temperature from 6°C to 8°C, and humidity from 54% to 87%. The athletes were offered beverages *ad libitum* during the run to prevent excessive dehydration. Blood and spot urine samples were collected after a fasting period of 8 h, 20 min before the participants warmed up ("pre-exercise"), and within 10 min after completion of the run ("post-exercise"). All subjects gave informed consent for being tested, and the study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation. Blood was collected in vacuum tubes containing no additives (Becton-Dickinson, Oxford, UK). Creatinine and albumin were assessed by Jaffe, rate blanked and compensated assay and bromocresol green, respectively, on a Beckman Coulter AU5800 (Beckman Coulter Inc., Brea, CA, USA), with proprietary reagents. The renal function was also reported as eGFR, with the equation developed and validated in the Modification of Diet in Renal Disease (MDRD) study and further modified by Levey et al. for methods traceable to the serum creatinine reference system (13), as follows: $eGFR=175\times(\text{serum creatinine}^{-1.154})\times(\text{age}^{-0.203})$. The lower limit of the reference range for this formula is conventionally fixed at <60 mL/min/1.73 m^2. sNGAL and uNGAL were measured with the fully-automated immunoassay NGAL Test™ (BioPorto Diagnostics A/S, Gentofte, Denmark, distributed in Italy by Sentinel Diagnostics, Milan, Italy), on a Beckman Coulter AU 5800. The analytical characteristics of this assay have been previously described. Briefly, the intra- and inter-assay imprecision is comprised between 1.0% and 2.3%, the method shows optimal linearity between 18 and 790 ng/mL and the calculated diagnostic cut-off is 200 ng/mL for both serum and urine (14). The Wilcoxon matched pairs and the χ^2 (for categorical variables) tests were used to evaluate the significance of exercise-induced variations. Data with a non-normal distribution were normalized using a logarithmic transformation prior to analysis. Statistical analysis was performed using with ANALYSE-IT for Excel software (Analyse-It Software, Leeds, UK) and the level of statistical significance was set at $p<0.05$. Data are shown as medians and interquartile range (IQR).

Results

The main findings of this study are shown in Table 1 and Figure 1. The plasma volume changes (i.e., hemoconcentration) after the runs, as calculated from pre- (4.51 g/L, IQR 4.31–4.70 g/L) and post-exercise (4.80 g/L; IQR 4.63–4.96 g/L; $p<0.01$) variation of albumin concentration, was 5.6%

Table 1 Variation of creatinine, estimated glomerular filtration rate (eGFR) and neutrophil gelatinase associated lipocalin (NGAL) after an ultramarathon run.

	Pre-exercise	Post-exercise	p-Value
Serum creatinine, µmol/L	68 (58–76)	98 (76–118)	<0.001
Urinary creatinine, µmol/L	106 (76–142)	168 (138–257)	<0.001
eGFR, mL/min/1.73 m^2	112 (97–133)	75 (57–99)	<0.001
Serum NGAL, ng/mL	105 (86–158)	196 (139–290)	<0.001
Urinary NGAL, ng/mL	4.4 (0.5–33.9)	35.6 (12.5–86.3)	<0.001
Urinary NGAL to creatinine ratio	0.05 (0.01–0.26)	0.17 (0.06–0.60)	<0.001

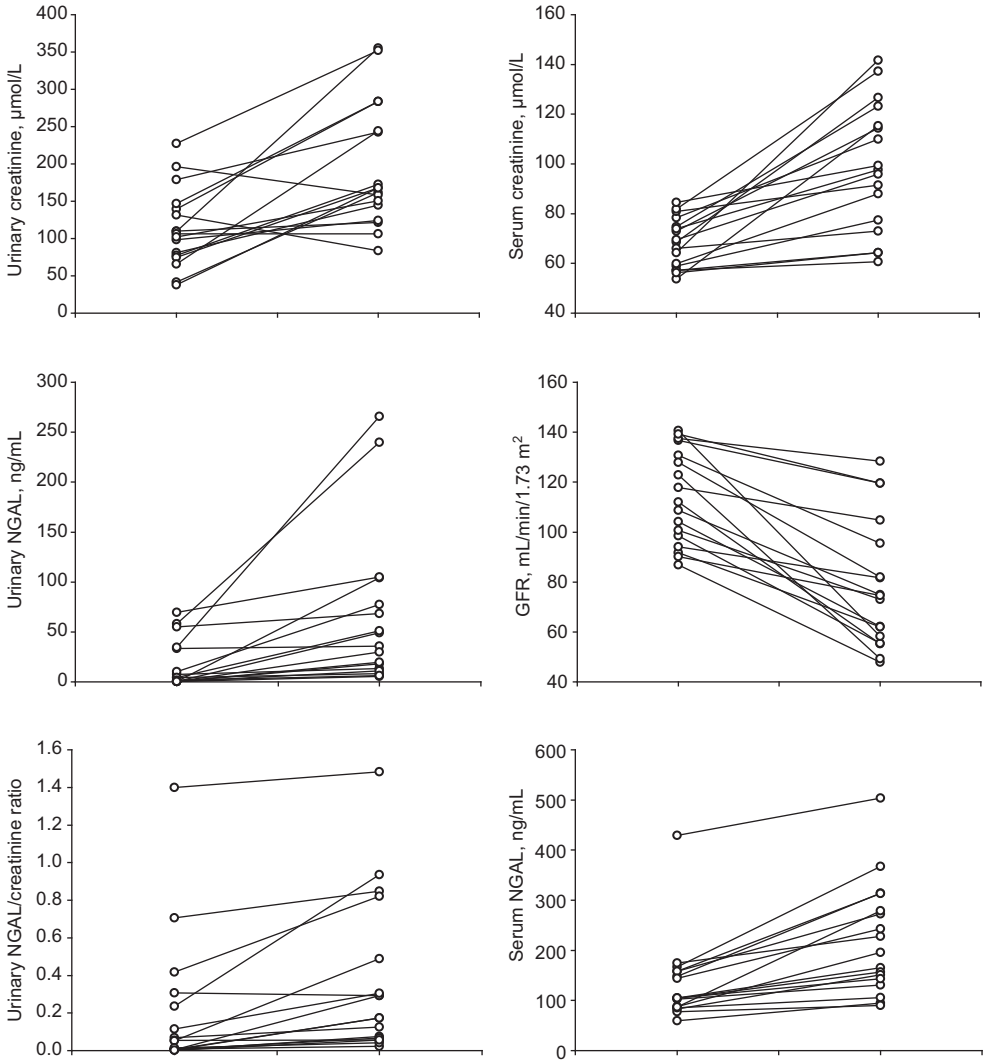


Figure 1 Variation of creatinine, estimated glomerular filtration rate (eGFR) and neutrophil gelatinase associated lipocalin (NGAL) after an ultramarathon run.

(IQR 2.2%–7.0%). Basically, all parameters tested exhibited a substantial variation post-exercise. In agreement with previous findings (15–18), serum and urinary creatinine increased by 38% (IQR 14%–66%) and 78% (IQR 21%–158%), respectively. The eGFR contextually decreased by 31% (IQR 14%–44%). sNGAL, uNGAL and uNGAL/creatinine ratio increased by 1.6-fold (IQR 1.3–2.0 fold), 7.7-fold (IQR 1.9–37.3 fold) and 2.9-fold (IQR 1.6–25.8 fold) respectively (Figure 1). Abnormal values of eGFR (i.e., <60 mL/min/1.73 m^2) were observed in none of the athletes pre-exercise and in five athletes (29%; p=0.015) post-exercise. In six of 16 athletes (i.e., 38%), the acute increase of serum creatinine met the Acute Kidney Injury Network (AKIN) criteria for AKI (Stage 1), which are defined as 50% rise in serum creatinine (19). As regards NGAL, values above the calculated cut-off of the test (i.e., 200 ng/mL) were observed in one athlete for sNGAL and in none for uNGAL pre-exercise, as well as in one for sNGAL (i.e., 6% vs. 6%; p=ns) and two for uNGAL (i.e., 0% vs. 12%; p=0.144) post-exercise. Interestingly, we failed to observe any significant relationship between pre-exercise sNGAL and uNGAL concentrations (r=–0.147, p=0.574), as well as in pre-exercise sNGAL and uNGAL/creatinine ratio (r=–0.110; p=0.673). Likewise, no significant relationship was found between post-exercise sNGAL and uNGAL values (r=0.085; p=0.746) and in post-exercise sNGAL and uNGAL/creatinine ratio (r=0.192; p=0.460). No significant correlation was found in the post-exercise variation of NGAL expressed as a ratio between post- and pre-exercise values (sNGAL vs. uNGAL r=0.025; p=0.925, sNGAL vs. uNGAL/creatinine ratio r=–0.016; p=0.950). Finally, a highly significant correlation was found between pre- and post-exercise changes of serum creatinine and sNGAL, (r=0.813; p<0.001), but not between pre- and post-exercise changes of serum creatinine and either uNGAL (r=0.248; p=0.338) or uNGAL/creatinine ratio (r=0.254; p=0.325).

Discussion

It is widely acknowledged that strenuous exercise, especially marathon running, may be associated with several acute complications, including a significant damage to the skeletal muscle, the process typically known as "exertional rhabdomyolysis" (20, 21), gastrointestinal complaints due to decreased mesenteric blood flow (22), as well as rare cardiac and thrombotic events (23). Although the identical pathological mechanisms (e.g., hemodynamic imbalance, inflammation or oxidative stress) that can trigger gastrointestinal and heart damage may also affect the kidney, less is known about the potential acute renal impairment developing after strenuous endurance exercise. Acute renal failure may be a serious consequence of renal ischemia due to a dramatic reduction of renal blood flow (24), but there are only two studies that have investigated the changes in renal markers after long distance running to the best of our knowledge. McCullough et al. assessed the concentration of serum creatinine, serum cystatin C, uNGAL and urinary KIM-1 in 25 athletes (13 women and 12 men) after a marathon running (25). At variance with our study, baseline values were, however, collected four weeks before the marathon, thereby rather far from the trial. Peak values of all biomarkers were also measured immediately after the race, although only uNGAL and not sNGAL was measured. In analogy with our study, nearly 40% of marathon runners (vs. 38% in our investigation) experienced a transient rise in serum creatinine that met the criteria of AKI. A parallel elevation of Cys-C and supportive elevations of uNGAL and urinary KIM-1 were reported. In particular, the concentration of uNGAL increased from 8.2±4.0 to 47.0±28.6 ng/mL, which is globally comparable to the rise observed in our study (from 4.4 to 35.6 ng/mL). Spiropoulos et al. investigated the variation of lipocalin-2 (i.e., a secreted member of the lipocalin protein family that seems effective to inhibit the production of erythrocytes) in healthy athletes after an ultradistance foot race. In agreement with our findings, a remarkable increase of lipocalin-2 concentrations was reached immediately after the race, which lead the authors to suggest that exercise-induced inflammation might be an important modulator of lipocalins production (26).

The results of our study are thereby in agreement with those of McCullough et al., but put forward several other interesting aspects. First, we have shown for the first time that, along with uNGAL, also the serum concentration of this renal biomarker dramatically increases after long-distance running. Interestingly, the serum increase appears also more homogenous among the athletes (e.g., the IQR range of the serum variation is comprised between 1.3 and 2.0 fold, whereas that in urine, ranges from 1.9 to 37.3 fold). The lack of correlation between sNGAL and uNGAL, either considering uNGAL alone or the ratio with urinary creatinine in all conditions assessed (i.e., pre-, post-exercise values and post-exercise variation) attests instead that sNGAL and uNGAL follow a rather different kinetics and a potential differential metabolism, in exertional renal impairment. The highly significant correlation observed between acute variation of serum creatinine and sNGAL, as well as the synthesis and release of this protein by sources other than the kidney (e.g., neutrophils, bone marrow, uterus, prostate, salivary gland, stomach, colon, trachea, lung and liver) (27) seems to attest that the serum increase in our study might be largely independent from the renal impairment, but rather attributable to exercise-induced inflammation (e.g., leukocyte production or decrease glomerular filtration) (26, 28). Conversely, the different pattern of increase observed in uNGAL would make the urinary assessment of this biomarker more independent from creatinine variation and extra-renal sources, and thereby more reliable to monitor the renal involvement during this and similar types of kidney impairment.

Interestingly, one athlete exhibited a very high sNGAL (but not uNGAL) basal concentration (i.e., 429 ng/mL), which further increased post-exercise, up to 503 ng/mL. Although the source of this anomalous data can not be definitely established, this subject also exhibited leukocytosis pre-exercise (i.e., 9.2×10^3/μL, 70% neutrophils), whereas the leukocytes count was normal in all the other athletes (i.e., $<9.0\times10^3$/μL). Therefore, it is highly conceivable that this increased sNGAL value at baseline might have been supported by extra-renal causes, most likely by increased release by neutrophils.

Regardless of the mechanisms underlying our findings, it is well known that when the blood flow to the kidneys decays and becomes inadequate to supply metabolic demands, a number of pathophysiological changes occur, which can ultimately result in inflammation, cell death and tissue dysfunction (24). Exercise induces thoughtful changes in renal hemodynamics and protein excretion. Renal plasma flow drops during exercise in relation to the intensity of exercise and renal blood flow may fall to 25% of the resting value when strenuous work is performed (1). Moreover, acute physical exertion has been shown to induce an augmented generation of reactive oxygen and nitrogen species (RONS) that both can trigger an oxidative stress (29). This would contribute to support an increase of sNGAL, as a ROS-mediated overexpression of lipocalin family members has already been clearly demonstrated (11). Intraluminal obstruction due to hematuria of glomerular origin caused by the prolonged and intense effort has occasionally been reported in marathon runners and may be an alternative cause (30, 31). Nevertheless, gross hematuria was absent in our athlete population, and it is thereby unlikely that this mechanism has contributed substantially in our setting. The development of extreme hemoconcentration cannot be considered a reliable explanation for our findings as the athletes were offered beverages *ad libitum* during the run to prevent excessive dehydration. Moreover, the plasma volume change (i.e., hemoconcentration), calculated from post-exercise variation of albumin concentration (i.e., 4.80 vs. 4.51 g/L; median increase +5.6%), would only partly explain the serum (+1.6-fold) and urinary (+7.7-fold) increase of NGAL.

Therefore, all these factors support the evidence that strenuous physical exercise increases NGAL and creatinine concentration as a consequence of induced adaptation mechanisms, such as the reduction in renal blood flow, increases in oxidative cellular damage, rises in protein excretion and inflammation. Consequently, it is likely that strenuous aerobic exercise might cause some sort of renal tissue impairment.

As uNGAL and serum creatinine can be easily and rapidly assessed in clinical laboratories, and probably reflect two different pathways of kidney impairment (i.e., tubular and glomerular, respectively), their assessment may be advisable to monitor the renal function in patients who have recently performed strenuous physical exercise, as well as for identifying those athletes more at risk of developing renal injury during strenuous aerobic exercise.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Poortmans JR. Exercise and renal function. Sports Med 1984;1:125–53.
2. Castenfors J. Renal function during prolonged exercise. Ann N Y Acad Sci 1977;301:151–9.
3. McAllister RM. Adaptations in control of blood flow with training: splanchnic and renal blood flows. Med Sci Sports Exerc 1998;30:375–81.
4. Poortmans JR, Vanderstraeten J. Kidney function during exercise in healthy and diseased humans. An update. Sports Med 1994;18:419–37.
5. Delanghe JR, Cobbaert C, Harmoinen A, Jansen R, Laitinen P, Panteghini M. Focusing on the clinical impact of standardization of creatinine measurements: a report by the EFCC Working Group on Creatinine Standardization. Clin Chem Lab Med 2011;49:977–82.
6. Soni SS, Pophale R, Ronco C. New biomarkers for acute renal injury. Clin Chem Lab Med 2011;49:1257–63.
7. Waikar SS, Betensky RA, Emerson SC, Bonventre JV. Imperfect Gold Standards for Kidney Injury Biomarker Evaluation. J Am Soc Nephrol 2012;23:13–21.
8. Urbschat A, Obermüller N, Haferkamp A. Biomarkers of kidney injury. Biomarkers 2011;16 Suppl 1:S22–30.
9. Tong Z, Kunnumakkara AB, Wang H, Matsuo Y, Diagaradjane P, Harikumar KB, et al. Neutrophil gelatinase-associated lipocalin: a novel suppressor of invasion and angiogenesis in pancreatic cancer. Cancer Res 2008;68:6100–8.
10. Hemdahl AL, Gabrielsen A, Zhu C, Eriksson P, Hedin U, Kastrup J, et al. Expression of neutrophil gelatinase-associated lipocalin in atherosclerosis and myocardial infarction. Arterioscler Thromb Vasc Biol 2006;26:136–42.
11. Roudkenar MH, Kuwahara Y, Baba T, Roushandeh AM, Ebishima S, Abe S, et al. Oxidative stress induced lipocalin 2 gene expression: addressing its expression under the harmful conditions. J Radiat Res 2007;48:39–44.
12. Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, et al. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 2008;87:142–9.
13. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F; Chronic Kidney Disease Epidemiology Collaboration. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. Clin Chem 2007;53:766–72.
14. Lippi G, Aloe R, Storelli A, Cervellin G, Trenti T. Evaluation of NGAL Test™, a fully-automated neutrophil gelatinase-associated lipocalin (NGAL) immunoassay on Beckman Coulter AU 5822. Clin Chem Lab Med 2012;50:1581–4.
15. Banfi G, Del Fabbro M, Lippi G. Serum creatinine concentration and creatinine-based estimation of glomerular filtration rate in athletes. Sports Med 2009;39:331–7.
16. Tian Y, Tong TK, Lippi G, Huang C, Shi Q, Nie J. Renal function parameters during early and late recovery periods following an all-out 21-km run in trained adolescent runners. Clin Chem Lab Med 2011;49:993–7.
17. Banfi G, Del Fabbro M, Lippi G. Creatinine values during a competitive season in elite athletes involved in different sport disciplines. J Sports Med Phys Fitness 2008;48:479–82.
18. Lippi G, Banfi G, Salvagno GL, Franchini M, Guidi GC. Glomerular filtration rate in endurance athletes. Clin J Sport Med 2008;18:286–8.
19. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. Acute Kidney Injury Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007;11:R31.
20. Clarkson PM. Exertional rhabdomyolysis and acute renal failure in marathon runners. Sports Med 2007;37:361–3.
21. Brancaccio P, Lippi G, Maffulli N. Biochemical markers of muscular damage. Clin Chem Lab Med 2010;48:757–67.
22. Sanchez LD, Corwell B, Berkoff D. Medical problems of marathon runners. Am J Emerg Med 2006;24:608–15.
23. Lippi G, Maffulli N. Biological influence of physical exercise on hemostasis. Semin Thromb Hemost 2009;35:269–76.
24. Bonventre JV. Mediators of ischemic renal injury. Annu Rev Med 1988;39:531–44.
25. McCullough PA, Chinnaiyan KM, Gallagher MJ, Colar JM, Geddes T, Gold JM, et al. Changes in renal markers and acute kidney injury after marathon running. Nephrology (Carlton). 2011;16:194–9.
26. Spiropoulos A, Goussetis E, Margeli A, Premetis E, Skenderi K, Graphakos S, et al. Effect of inflammation induced by prolonged exercise on circulating erythroid progenitors and markers of erythropoiesis. Clin Chem Lab Med 2010;48:199–203.
27. Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics 1997;45:17–23.
28. Decavele AS, Dhondt L, De Buyzere ML, Delanghe JR. Increased urinary neutrophil gelatinase associated lipocalin in urinary tract infections and leukocyturia. Clin Chem Lab Med 2011;49:999–1003.
29. Bloomer RJ, Goldfarb AH, Wideman L, McKenzie MJ, Consitt LA. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. J Strength Cond Res 2005;19:276–85.
30. Le Meur Y, Paraf F, Szelag JC, Aldigier JC, Leroux-Robert C. Acute renal failure in a marathon runner: role of glomerular bleeding in tubular injury. Am J Med 1998;105:251–2.
31. Pollard T, Weiss I. Acute renal necrosis in patient with march hemoglobinuria. N Engl J Med 1970;283:15–6.

Increased Average Longevity among the "Tour de France" Cyclists

Authors

F. Sanchis-Gomar[1], G. Olaso-Gonzalez[1], D. Corella[2], M. C. Gomez-Cabrera[1], J. Vina[1]

Affiliations

[1] Department of Physiology, Faculty of Medicine, University of Valencia, Spain
[2] Department of Preventive Medicine and Public Health and CIBER Fisiopatología de la Obesidad y Nutrición, Faculty of Medicine, University of Valencia, Spain

Key words
- life expectancy
- mortality
- physical activity
- endurance
- lifestyle

Abstract

It is widely held among the general population and even among health professionals that moderate exercise is a healthy practice but long term high intensity exercise is not. The specific amount of physical activity necessary for good health remains unclear. To date, longevity studies of elite athletes have been relatively sparse and the results are somewhat conflicting. The Tour de France is among the most gruelling sport events in the world, during which highly trained professional cyclists undertake high intensity exercise for a full 3 weeks. Consequently we set out to determine the longevity of the participants in the Tour de France, compared with that of the general population. We studied the longevity of 834 cyclists from France (n = 465), Italy (n = 196) and Belgium (n = 173) who rode the Tour de France between the years 1930 and 1964. Dates of birth and death of the cyclists were obtained on December 31st 2007. We calculated the percentage of survivors for each age and compared them with the values for the pooled general population of France, Italy and Belgium for the appropriate age cohorts. We found a very significant increase in average longevity (17%) of the cyclists when compared with the general population. The age at which 50% of the general population died was 73.5 vs. 81.5 years in Tour de France participants. Our major finding is that repeated very intense exercise prolongs life span in well trained practitioners. Our findings underpin the importance of exercising without the fear that becoming exhausted might be bad for one's health.

accepted after revision
January 11, 2011

Bibliography
DOI http://dx.doi.org/10.1055/s-0031-1271711
Published online: 2011
Int J Sports Med

ISSN 0172-4622

Correspondence
Dr. Mari Carmen Gomez-Cabrera, PhD
Department of Physiology
Faculty of Medicine
University of Valencia
Avda. Blasco Ibañez 15
46013 Valencia
Spain
Tel.: + 34/963/864 646
Fax: + 34/963/864 642
carmen.gomez@uv.es

Introduction

A consensus is growing on the importance of the relationship between physical activity and health and wellness, but the specific amount of physical activity necessary for good health remains unclear [8]. Continued debate as to how much, what type, how often, what intensity, and how long the physical activity should be performed, has led to the promulgation of numerous different public health and clinical recommendations [1]. Public health recommendations for physical activity are 30 min of moderate-intensity activity per day, which provides substantial benefits for sedentary adults. However this amount of exercise may be insufficient to prevent unhealthy weight gain. Blair et al. recommend, for persons exercising 30 min per day, that they try to build up to 60 min per day. It is considered that this may provide additional health benefits [1]. It is widely held among the general population and even among health professionals that although moderate exercise is a healthy practice, vigorous competitive exercise is not [17].

It is now clear that persons (healthy or unhealthy) who undergo regular exercise show a reduction in their risk of mortality [15,21], but the effects of competitive sports on health are uncertain [6,22]. In fact it has been hypothesized that the increased risk of cardiovascular disease observed in very highly trained athletes might be related to the frequent exposure to oxidative stress associated with strenuous exercise [9]. In a previous work we showed that Tour de France participants display mild muscle damage as evidenced by an increase in plasma activity of cytosolic enzymes such as creatine kinase or aspartate aminotransferase [5].

To date, longevity studies of elite athletes have been relatively sparse and the results are somewhat conflicting [19,20]. In one report coming out of a single country (Finland), athletes participating in multiple sports were tracked for longevity, and it was reported that long-distance

runners and cross-country skiers live significantly longer than the general population [10,11]. In contrast, a second study showed that individuals whose energy expenditure is in the range of 3500 kcal per week exhibit a mortality rate higher than that of the sedentary population [16].

Professional road cycling is an extreme endurance sport. Approximately 30000–35000 km are ridden each year in training and competition. Some races, such as the Tour de France (TdF) last 21 days (~100 h of competition) during which professional cyclists cover >3500 km (see **Table 1**). In some phases of such a demanding sport, exercise intensity is surprisingly high, since professional cyclists must complete prolonged periods of exercise at high percentages (~90%) of their maximal oxygen uptake (VO_{2max}) [14]. Therefore, the TdF is among the most gruelling sport events in the world, during which highly trained professional cyclists undertake long-term high intensity exercise for a full 3 weeks period.

Based on these observations, we decided to test the hypothesis that the exercise regimen to which elite cyclists are subjected does indeed have a life shortening effect relative to the general population. Consequently we set out to determine the longevity of the participants of the TdF and compared it with that of the general population born between 1892 and 1942 (i.e., the years in which the cyclists studied were born).

Methods

Study population

Of the 1318 participants, who have cycled the TdF between the years 1930 and 1964, only 1229 riders are considered for which there is proof of their date of birth and death. Cyclists who did not complete all stages of the TdF were excluded. Other exclusion criteria for this study were: cycling before 1930 or after 1964 and being born in a country that has contributed less than 100 participants during this period. Of these 1229 cyclists, all men, 834 came from France (n=465), Italy (n=196) and Belgium (n=173), representing the 68% of participants in the TdF in the years studied. The remainder came from 21 different countries (see **Fig. 1**), each represented by only a small number of cyclists. To simplify matters, we focused on the 3 countries with the largest contingent of the TdF participants and compared the longevity of their cyclists with that of the average population in their respective countries. Furthermore, these countries have a demographic record of the population since the nineteenth century, allowing comparison of the survival rates of the riders of the TdF with the general population. Only very scattered data can be obtained in the literature on cyclists who rode the TdF before 1930. Of course, of those who rode after 1964, many are still alive and the rate of survival cannot be calculated. This leaves us with a broad sample of 834 cyclists (of a possible total of 1318), which constitute a representative population of the TdF participants. Survival rates for riders of the TdF between 1930 and 1964 correspond to years of birth between 1892 and 1942. This was compared with that of the general population, i.e., men born between 1892 and 1942 (the years in which the cyclists studied were born). Our data come from 3 official electronic websites (www.letour.fr, www.cyclingarchives.com, www.memoire-du-cyclisme.net) which contain detailed information about every cyclist who has ever taken part in the TdF, including dates of birth and death. The average age of death of the male general population in the countries of origin was calculated from data obtained from the human mortality database (www.mortality.org).

Dates of birth and death and the percentage of survivors for each age, on December 31st 2007, were recorded to calculate the curve both for general population and for cyclists. The percentage of survival of cyclists born in each year (from 1930 to 1964) was plotted and compared with the calculated values for the pooled general population of France, Italy and Belgium for the appropriate age cohorts.

The variable named "percentage of survivors" was defined as follows: number of persons born in a given year who were alive on December 31st 2007 divided by number of persons born in that given year. The variable "age" was calculated as: 2007 – year of birth. The same applies for the TdF participants. This study meets the required ethical standards of this journal [7].

Statistical analysis

This was a case-control study. Statistical analysis was performed with SPSS (Chicago, IL, USA) software for Windows (version 17.0). Polynomial regression curves for each population were adjusted and areas under each curve were measured. Statistical significance of the difference in areas was calculated by the z- statistic. The non parametric Mann-Whitney U test was applied for the comparison of the mean of the percent survival for each population. The alpha level for statistical significance was set at $p<0.05$.

Table 1 Some features of the Tour de France concluded between 1930 and 1964.

	TOURS 1930–1964
average of total kilometres performed per tour	4537.7 ± 238.5 (km)
average total time employed per tour	138.0 ± 16.2 (h)
average of completed tours per cyclist	2.4 ± 2.0 (times)
average age of cyclists who competed in the tours	27.3 ± 3.6 (years)
average speed (all tours)	33.1 ± 2.7 (km/h)

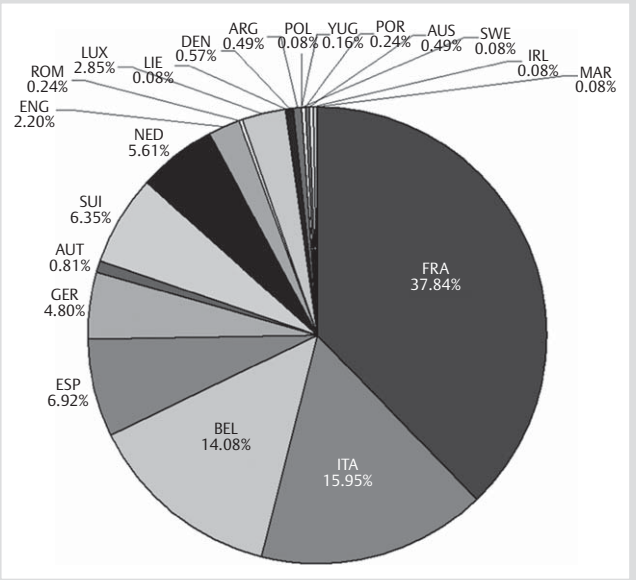


Fig. 1 Distribution by countries of the cyclists who participated in the TdF between 1930 and 1964. ARG: Argentina; AUS: Australia; AUT: Austria; BEL: Belgium; DEN: Denmark; ENG: England; ESP: Spain; FRA: France; GER: Germany; ITA: Italy; IRL: Ireland; LIE: Liechtenstein; LUX: Luxembourg; MAR: Morocco; NED: Netherlands; POL: Poland; POR: Portugal; ROM: Romania; SWE: Sweden; SUI: Switzerland; YUG: Yugoslavia. Algeria is not represented because it has only one rider representative.

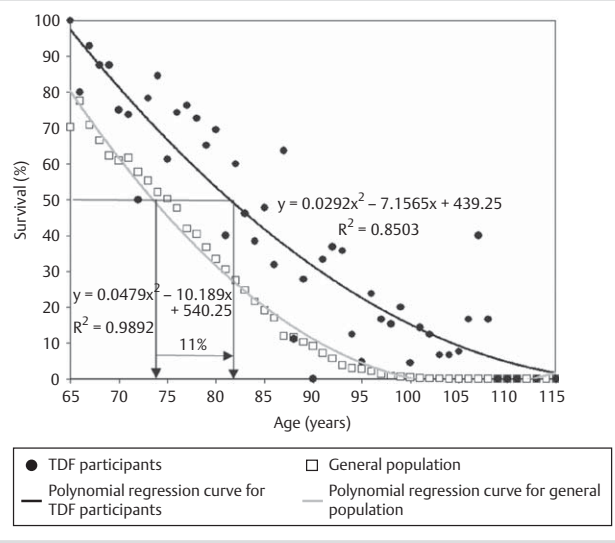


Fig. 2 Percentage of survival related to age in TdF participants and in the general population. Persons born between 1892 and 1942 have been studied. Average life span of TdF participants is higher ($p = 0.004$; 17.5%) than the general population of the same country in which the cyclists were born. The age at which 50% of the general population died was 73.5 vs. 81.5 years in TdF participants, i.e., 11% increase.

Results

Fig. 2 shows that longevity of the TdF participants is significantly higher than that of the general population (when comparing the area under the curve corresponding to the TdF participants with that of the general population $p < 0.05$). The average of survival between 65 and 115 years was 39.1% in the participants of the Tour, while for the general population it was 21.5%. The age at which 50% of the general population died was 73.5 vs. 81.5 years in the TdF participants, i.e., 11% increase (see **Fig. 2**). Note that the values in ordinates are percentage of participants alive. For instance, of the 5 persons who were born in 1900 and who rode the TdF between 1930 and 1964, 2 were still alive on December 31st 2007, i.e., 40%. Considering all the ages studied, the average percentage of survival of the TdF participants (area under the curve) was 17% higher than that of the general population.

Discussion

Low levels of physical activity (2.5 h/week of moderate intensity) reduce mortality by 19% [23]. Increasing this to a 1 h session 7 days a week (7 h/week) could increase the benefit to 24% [23]. Professional cyclists' levels of activity are ~30 h/week of moderate-high intensity activity [14]. The effect of this level of exercise on mortality has been studied very scarcely. In our study we show that professional cyclists' exhibit increased life span.

In our opinion, physicians, health professionals and general population should not hold the impression that strenuous exercise and/or high-level aerobic competitive sports have deleterious effects, are bad for one's health, and shorten life. Recently, it has been shown that non-vigorous physical activity reduces the risk of all-cause mortality [23]. In another study, Chakravarty et al. have concluded that vigorous exercise at middle and older ages is associated with reduced disability in later life and a notable survival advantage [2]. This study demonstrates that even higher levels of activity also increase longevity. We have to keep in mind that our data are limited to 1964 which is perhaps before the time some of today's most dangerous drugs were used, e.g. anabolic steroids, blood doping, etc. Moreover, endurance cyclists are a select group and may be a selected population because people in poor health are less likely to become cyclists and thus they are likely to have healthier habits than the general population [11]. However, while this work was in process, a study published by Ruiz et al. showed that the association between strenuous aerobic exercise, undertaken by elite athletes, and increased life expectancy is not biased by a genetic selection [17]. Their results indicated that top level athletes have similar disease-trait-related genotype scores to those observed in non-athletes [17]. However other lifestyle factors could also contribute to the increased average longevity among the Tour de France participants. Former athletes seem to smoke less, consume less alcohol and have a healthier diet than the general population [4]. Our results are in accordance with previous studies in which it has been demonstrated that an improvement in cardiorespiratory fitness are associated with a lower risk of mortality from all-causes [12]. In our opinion, the critical beneficial factor is also being physically fit. Many patients and clinicians are confused about what amount of exercise is needed for health [3].

We are aware of some unavoidable limitations of our study. For instance we do not have information on co-morbidities or causes of death of the population studied. Likewise, the more conventional longevity analysis to estimate a population survival curve from a sample, the Kaplan-Meier curve, could not be used because the control population is not closed, for instance human migrations cannot be excluded from our analysis. Moreover, we know that the cyclists undertook strenuous bouts of exercise in their early life, but we do not have data about their physical activity in subsequent years. Former athletes are physically more active as they age than the age-matched general population [18]. There is a possibility that these exercise habits are likely to explain the 17% increase in average longevity in the cyclists when compared with the general population.

In spite of these limitations, we conclude that long-term repetitive strenuous exercise does not increase mortality or shorten life span. On the contrary, this type of exercise lengthens life span (see **Fig. 2**). The general recommendation should be to train and perform exercise frequently. The most recent recommendation regarding exercise was: "Even a little is good; more may be better!" [13]. In view of our results, perhaps it would be better to say: "Even a little is good; a lot is better if you are well trained".

However it should be noted that this level of physical activity is not a plausible and reasonable goal for most people, especially for unfit, middle-aged and older people, or unhealthy persons (diabetics or obese patients). We do not claim that one should subject himself to a lifelong regimen of strenuous exercise but rather that, contrary to previous beliefs, professional competitive exercise, if the subject is previously trained, is a healthy practice that may prolong life span.

Acknowledgements

We thank Mrs. Marilyn Noyes and Dr. William Orr, for their kind help in reviewing the manuscript. The results of the present

study do not constitute endorsement by ACSM. Funding received This work was supported by grants: BFU2007-65 803/BFI, DPS2008-06968, ISCIII2006-RED13-027 from the "Red Temática de investigación cooperativa en envejecimiento y fragilidad (RETICEF) and COST B35 Action.

Conflict of Interest: None of the authors has a conflict of interest.

References

1 *Blair SN*, *LaMonte MJ*, *Nichaman MZ*. The evolution of physical activity recommendations: how much is enough? Am J Clin Nutr 2004; 79: 913S–920S
2 *Chakravarty EF*, *Hubert HB*, *Lingala VB*, *Fries JF*. Reduced disability and mortality among aging runners: a 21-year longitudinal study. Arch Intern Med 2008; 168: 1638–1646
3 *Church TS*, *Earnest CP*, *Skinner JS*, *Blair SN*. Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. JAMA 2007; 297: 2081–2091
4 *Fogelholm M*, *Kaprio J*, *Sarna S*. Healthy lifestyles of former Finnish world class athletes. Med Sci Sports Exerc 1994; 26: 224–229
5 *Gomez-Cabrera MC*, *Pallardo FV*, *Sastre J*, *Vina J*, *Garcia-del-Moral L*. Allopurinol and markers of muscle damage among participants in the Tour de France. JAMA 2003; 289: 2503–2504
6 *Harris KM*, *Henry JT*, *Rohman E*, *Haas TS*, *Maron BJ*. Sudden death during the triathlon. JAMA 2010; 303: 1255–1257
7 *Harriss DJ*, *Atkinson G*. International Journal of Sports Medicine – Ethical Standards in Sport and Exercise Science Research. Int J Sports Med 2009; 30: 701–702
8 *Kesaniemi YK*, *Danforth E Jr*, *Jensen MD*, *Kopelman PG*, *Lefebvre P*, *Reeder BA*. Dose-response issues concerning physical activity and health: an evidence-based symposium. Med Sci Sports Exerc 2001; 33: S351–S358
9 *Knez WL*, *Coombes JS*, *Jenkins DG*. Ultra-endurance exercise and oxidative damage: implications for cardiovascular health. Sports Med 2006; 36: 429–441
10 *Kujala UM*. Essay: Does training adversely affect long-term health? Lancet 2005; 366 (Suppl 1): S55–S56
11 *Kujala UM*, *Tikkanen HO*, *Sarna S*, *Pukkala E*, *Kaprio J*, *Koskenvuo M*. Disease-specific mortality among elite athletes. JAMA 2001; 285: 44–45
12 *Lee DC*, *Artero EG*, *Sui X*, *Blair SN*. Mortality trends in the general population: the importance of cardiorespiratory fitness. J Psychopharmacol 2010; 24: 27–35
13 *Lee IM*. Dose-response relation between physical activity and fitness: even a little is good; more is better. JAMA 2007; 297: 2137–2139
14 *Lucia A*, *Hoyos J*, *Chicharro JL*. Physiology of professional road cycling. Sports Med 2001; 31: 325–337
15 *Myers J*, *Prakash M*, *Froelicher V*, *Do D*, *Partington S*, *Atwood JE*. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med 2002; 346: 793–801
16 *Paffenbarger RS Jr*, *Hyde RT*, *Wing AL*, *Hsieh CC*. Physical activity, all-cause mortality, and longevity of college alumni. N Engl J Med 1986; 314: 605–613
17 *Ruiz JR*, *Moran M*, *Arenas J*, *Lucia A*. Strenuous endurance exercise improves life expectancy: it's in our genes. Br J Sports Med 2010, doi:10.1136/bjsm.2010.075085
18 *Sarna S*, *Sahi T*, *Koskenvuo M*, *Kaprio J*. Increased life expectancy of world class male athletes. Med Sci Sports Exerc 1993; 25: 237–244
19 *Schnohr P*. Athletic activity and longevity. Lancet 1972; 2: 605
20 *Schnohr P*. Longevity and causes of death in male athletic champions. Lancet 1971; 2: 1364–1366
21 *Sun Q*, *Townsend MK*, *Okereke OI*, *Franco OH*, *Hu FB*, *Grodstein F*. Physical activity at midlife in relation to successful survival in women at age 70 years or older. Arch Intern Med 2010; 170: 194–201
22 *Teramoto M*, *Bungum TJ*. Mortality and longevity of elite athletes. J Sci Med Sport 2010; 13: 410–416
23 *Woodcock J*, *Franco OH*, *Orsini N*, *Roberts I*. Non-vigorous physical activity and all-cause mortality: systematic review and meta-analysis of cohort studies. Int J Epidemiol 2010, doi: 10.1093/ije/dyq104

Send Orders for Reprints to reprints@benthamscience.net

Current Pharmaceutical Design, **2014,** *20,* **000-000**

Pharmacological Properties of Physical Exercise in the Elderly

Jose Viña*, Consuelo Borras, Fabian Sanchis-Gomar, Vladimir E Martinez-Bello, Gloria Olaso-Gonzalez, Juan Gambini, Marta Ingles[1] and Mari Carmen Gomez-Cabrera

Department of Physiology. Fundacion Investigacion Hospital Clinico Universitario/INCLIVA. [1]Department of Physiotherapy. Faculty of Physiotherapy. University of Valencia. Spain

Abstract: Scientific evidence links physical activity to several benefits. Recently, we proposed the idea that exercise can be regarded as a drug. As with many drugs, dosage is of great importance. However, to issue a public recommendation of physical activity in aging is not an easy task. Exercise in the elderly needs to be carefully tailored and individualized with the specific objectives of the person or group in mind.

The beneficial effects of exercise in two of the main age-related diseases, sarcopenia and Alzheimer's Disease, are dealt with at the beginning of this report. Subsequently, dosage of exercise and the molecular signaling pathways involved in its adaptations are discussed. Exercise and aging are associated with oxidative stress so the paradox arises, and is discussed, as to whether exercise would be advisable for the aged population from an oxidative stress point of view. Two of the main redox-sensitive signaling pathways altered in old skeletal muscle during exercise, NF-κB and PGC-1α, are also reviewed.

The last section of the manuscript is devoted to the age-associated diseases in which exercise is contraindicated. Finally, we address the option of applying exercise mimetics as an alternative for disabled old people.

The overall denouement is that exercise is so beneficial that it should be deemed a drug both for young and old populations. If old adults adopted a more active lifestyle, there would be a significant delay in frailty and dependency with clear benefits to individual well-being and to the public's health.

Keywords: Health, adaptation, inactivity, training, aging, NF-κB, PGC-1α.

INTRODUCTION

The definition of health provided by the WHO is "Physical, mental, and social well-being, not merely the absence of disease and infirmity". Being physically fit is defined as the physiological condition of well-being that enables one to fulfil one's obligations of daily life (physical fitness related to health) and perform sport (physical fitness related to performance) or both of these concepts.

When an organism lives in new surroundings particular functions are enhanced and compensate for difficulties presented by the new situation [1]. The difficulties themselves activate the specific changes [2]. During exercise, several homeostatic systems are challenged and the ability of these systems to maintain or return to homeostasis in response to these challenges is improved. Thus, adaptation is the purpose of exercise training. Aging is associated with a loss of efficiency of virtually all physiological functions. In general terms, aging causes a loss in the capacity to maintain the internal milieu of the individual when faced with changes in the external atmosphere [3]. Thus, old individuals (men or women $\geq$ 65 years) lose the capacity to maintain homeostasis and their response to exercise differs from young subjects. However, regular physical activity is considered of the utmost importance for a healthy lifestyle at all ages, but especially in the old population. The benefits of an active lifestyle clearly outweigh the potential risks of exercise, particularly in older people. Disability levels in a vigorously exercising population remain below that of non-exercisers, and age-related increases in disability are delayed by approximately fifteen years [4]. Following a regular physical activity program increases the age of onset of chronic illness and shortens the time between the onset of morbidity and death. This represents a significant improvement in the quality of life of the elderly and results in major reductions in the cost of treating their medical conditions [4, 5].

*Address correspondence to this author at the Department of Physiology. Faculty of Medicine. University of Valencia., Av. Blasco Ibañez, 15, Valencia, E46010 Spain; Tel: (34) 96 386 46 50; Fax: (34) 96 386 46 42;
E-mail: jose.vina@uv.es

Physical exercise has become one of the key issues for the prevention of functional impairment and some chronic, degenerative diseases among the elderly, whose number increases continuously in Western societies [6, 7]. In developed countries, people over 65 will represent 20- 25% of the population by 2025 and nearly 35% by 2050 [8]. I. L. Nascher, the founder of the clinical field of geriatrics in the US, described the concept of healthspan (without using the term) as a goal of being productive and happy for an individual's entire lifespan, rather than seeking longevity despite severely hindering impairments of body and mind [8, 9]. Exercise can postpone detrimental aspects of aging and contribute to healthspan. Maximizing healthspan and preventing dysfunction are at least as important as extending lifespan [10]. We have recently observed that life-long spontaneous exercise does not prolong lifespan but prevents age-associated frailty in animals [11]. A concern of health providers is whether increasing longevity will increase disability and health costs [12]. Elderly individuals often say they would rather keep on feeling healthy than merely live longer [10].

Physical exercise is an extremely favorable activity for modulating health and lifespan. Here we review the advantages to health it provides the aged population with. Physical activity ("any movement of the body") and exercise ("physical activity that is typified by specific and purposeful training") are different concepts [13]. However, for the sake of clarity we shall refer to both these concepts interchangeably in this review as some of the reports we refer to use these expressions as synonyms [1].

The amount of time spent being engaged in exercising declines with age [14]. Although after the retirement age (65 years) some people show increased participation in activities of light-moderate intensity, overall, exercise declines progressively as we grow old [14]. In 1991, 38% of the adults over 55 years reported no leisure time-physical activity during the previous month, in a telephone survey performed in Columbia [14]. While only 24% of adults between 18-34 years of age reported no leisure-time physical activity. Regrettably, physical inactivity and a sedentary lifestyle prevail in modern society and they affect especially the older population.

1381-6128/14 $58.00+.00

PHYSICAL ACTIVITY AND HEALTH, TWO INTERRELATED CONCEPTS AT OLD AGE

The idea that physical activity promotes health dates from ancient China (2500 BC) [1, 15]. In Greco-Roman times, Hippocrates and Galen emphasized the need to recommend exercise for wellbeing [16]. In this regard Plato said: "Lack of activity destroys the good condition of every human being while movement and methodical physical exercise saves and preserves it" [17].

Morris and co-workers [18], comparing subjects performing dissimilar work, concluded that exercise is linked to health. They reported that London bus conductors presented a significantly lower frequency of Coronary Heart Disease (CHD) than the drivers of these vehicles who were more inactive. Subsequently, they compared postmen with more inactive postal office workers and found a significant inverse correlation between CHD and physical activity [19]. In the 70's, Paffenbarger and collaborators evaluated the rise in the relative risk of mortality due to any reason and to particular diseases related to sendentariness [20, 21]. Karvonen *et al.* reported that Finnish elite skiers lived 2.8 – 4.3 years longer than most men in Finland [22].

Epidemiological research has shown the advantages of practicing exercise in the prevention and treatment of numerous diseases. All subjects who increase their intensity of exercise and fitness reduce their relative risk of death (by 20 – 35%) [23, 24]. Moreover, different studies have shown that the lack of physical activity and low levels of physical fitness are associated with increased all-cause mortality rates [14, 25].

Therefore, regular physical exercise clearly brings about health benefits and lowers the risk of premature death due to all causes, especially due to heart disease, particularly in all asymptomatic subjects.

A small increment in ~1000 kcal per week in energy expenditure from physical activity or a 1-MET (Metabolic Equivalent) increase in fitness is linked with a 20% decrease in mortality [26]. One MET represents the approximate rate of oxygen consumption of an adult at rest which is about 3.5 $mL \cdot min^{-1} \cdot kg^{-1}$. In $kilocalories \cdot min^{-1}$ is about 1.2 for a 70 Kg person [14].

The advantages of physical exercise are obvious for both healthy subjects and patients. Aging is associated with an increased susceptibility to many forms of stress, as well as trauma and infections [3]. The functioning of the immune system is impaired and this gives rise to both an increase in auto-immune disease as well as to an increased susceptibility to infections [3]. The incidence of cancer increases exponentially with age, and the same happens with neurodegenerative diseases [3]. The prevalence of all these devastating conditions decreases with exercise as it promotes physiological capacities in older adults [14].

Indeed, randomized and observational tests have proved that regular exercise helps in the treatment of various chronic diseases [27, 28]. Proof exists for prescription of physical activity in primary and secondary prevention of heart and lung disease, metabolic disorders, bone, muscle and joint diseases, cancer, and depression [6, 28]. Although physical exercise works as a medication for these maladies, as with all drugs the following parameters should be taken into account to obtain optimal clinical results: the dose (volume and intensity of the exercise), administration frequency (hours of exercise a week), nature of the exercise (resistance or aerobic), systemic and psychoactive effects, as well as secondary effects and contraindications [29].

THE DOSING OF EXERCISE AT OLD AGE

Dosage is vital in clinical medicine and all drugs on the market must present details on their safety and efficacy. One must take a minimum of physical exercise to achieve health benefits, and while such benefits increase if one intensifies the amount of exercise taken, the benefits can turn into disadvantages if a certain amount of exercise is exceeded [30]. However, the dose response, minimum dose, and the maximum safe dose of exercise has not been defined, especially at old age [30]. In 1991, Beers and co-workers published a set of criteria for inadequate drug prescription in the elderly. They reviewed the prescription medicines that should be avoided, excessive dosage, and excessive duration of treatment [31]. Pharmacotherapy in the elderly requires an understanding of the age-dependent changes in function and composition of the body [32]. The most important pharmacokinetic change in old age is a decrease in the excretory capacity of the kidney. But, as mentioned before, aging is characterized by a progressive loss of functional capacities of most, if not all, organs.

Thus, if we consider that exercise can act as a drug, the intensity of aerobic training must be carefully titrated at old age. There is an ongoing debate on how much, how often, what intensity, what type, and how lengthy physical activity in the elderly should be. This is important for issuing public health recommendations [33].

Exercise intensity can be measured in different ways [30]. It can be expressed relative to a 10-point scale, oxygen consumption (VO_2), or heart rate [34]. Moderate-intensity activities are those in which heart rate and breathing are raised, but it is still possible to speak comfortably. This occurs at around 4–6 METs. Brisk walking at 3.0 mph (80.4 $m \cdot min^{-1}$) is one of such activities. Vigorous-intensity activities are those in which the heart rate is higher, breathing is heavier, and conversation is harder (about 6–8 METs) [34], for instance jogging.

For healthy adults, it has been shown that exercising even at 50% of the recommendations (seventy-two minutes of moderate exercise a week) appears sufficient to provide some improvement in fitness. However, at this low exercise dosage cardiovascular risk factors (blood pressure, lipid profile, and weight) do not improve [35]. In fact, for many individuals, up to sixty minutes of daily physical activity are more appropriate if weight control is the primary goal [30]. Thus, the dose-response ratio between physical activity and health outcomes are different. The evaluation of the minimum amount of physical activity (lower dose) necessary to achieve beneficial effects has been the subject of intense research. Wen and co-workers have recently found that fifteen minutes a day or ninety minutes a week of moderate-intensity exercise is of benefit in terms of life expectancy, even for subjects with cardiovascular risks [36] (See Table **1**).

The guidelines discussed above are generally appropriate for young to middle-aged adults.

To promote and maintain health, older adults need moderate-intensity aerobic physical activity for a minimum of thirty minutes five days a week, or vigorous intensity aerobic activity for a minimum of twenty minutes three days a week. Also, combinations of moderate and vigorous-intensity activity can be performed to meet this recommendation. Moderate-intensity aerobic activity involves a moderate level of effort relative to an individual's aerobic fitness. On a 10-point scale, where sitting is 0 and all-out effort is 10, moderate-intensity activity is a 5 or 6 and produces noticeable increases in heart rate and breathing. On the same scale, vigorous-intensity activity is a 7 or 8 and produces large increases in heart rate and breathing. An important characteristic of exercise in aging is the heterogeneity of fitness in the old population. Thus, for some older adults a moderate-intensity walk is a slow walk, and for others it is a brisk walk. This recommended amount of aerobic activity is in addition to routine activities of daily living of light-intensity (e.g., self care, cooking, casual walking, or shopping) or moderate-intensity activities lasting less than ten minutes in duration (e.g., walking around the home or office, walking from the parking lot) [37].

Recently we have shown that training status is a very relevant factor in the prescription of the exercise "dose" [38]. Unfit people can obtain significant improvement in physical fitness with a low

Table 1. Historical evolution in physical activity recommendations and guidelines.

	Physical Activity Recommendations			
	Intensity	**Minutes**	**Frequency**	**Reference**
Older adults (ages >65)	Moderate exercise (5 to 6 on a 10-point scale)	30 minutes/day in bouts of at least 10 min each	A minimum of 5 days/week	[165]
	Vigorous intensity (7 to 8 on a 10-point scale)	At least 20 minutes/days	A minimum of 3 days/week	[165]
Healthy adults (ages 18-45)	Moderate exercise	Thirty minutes/day (150 per week)	Most days of the week (5 days/week)	[180]
	Vigorous exercise	75 minutes/week		[180]

training intensity while people with a higher fitness level need a greater exercise intensity to achieve further improvement in fitness [39]. In the last decades, the participation of elderly trained people in endurance events, such as marathon running, has significantly increased [40]. Thus, it is important that the type and intensity of physical activity is decided by taking into consideration the training status not only in young [29], but also in old individuals.

Special precaution should be taken when prescribing exercise to older adults, who commonly have chronic medical conditions, low fitness levels, and/or functional limitations [37]. For instance, it has been shown that vigorous activities are not essential for the reduction of cardiovascular risk in men over sixty. Regular physical activity is enough to achieve a significant decrease in mortality in this population. Thus, the greatest benefit to health is gained from sustained moderate exercise, above which there appears to be no further benefit to health in older men [41, 42].

The preceding recommendations address the role of aerobic exercise to promote and maintain health in old age. However, other fitness components can also be trained. It has been shown that people who maintain or improve their strength, flexibility, and balance are able to perform daily activities better and avoid frailty and disability, especially as they advance into older age [37]. Thus, it is recommended that older adults perform strengthening activities at least twice a week, activities that improve balance at least three times a week, and minimize the time they spend in sedentary postures [43]. Muscle strengthening activities, as well as balance exercise and flexibility activity reduce the risk of falls and promote physical independence [37]. This is highly important to prevent sarcopenia (see next section) which is one of the main causes of the frailty syndrome. Frailty in older adults has been characterized as a syndrome of weakness, declines in activity, weight loss, and vulnerability to adverse health outcomes [44]. It has a tremendous impact on the older individual, their family, and society as a whole. The interest in this syndrome has been growing over the last decade because frailty is the main risk factor for disability in older people and also predicts other adverse outcomes like falls, hospitalization, and death [44, 45]. Recently we have found that life-long spontaneous exercise does not prolong longevity (average or maximal) but prevents frailty in a controlled mouse population [11].

SYSTEMIC ADAPTATIONS TO EXERCISE DURING AGING

Virtually all physiological functions lose efficiency with aging. It causes loss in vision, hearing capacity, memory, motor coordination, and other neural functions of physiological importance. The force and elasticity of the skeletal muscular system deteriorate; there is a lower pulmonary ventilation, lower glomeral filtration in the kidneys, and a lower maximal blood flow through the heart. Age-associated glucose intolerance has also been reported [3]. Vital organs suffer a phenomenon associated with atrophy or degeneration. Among the most notable ones are organs that are composed of post mitotic cells such as neurons, myocardial, and skeletal muscle cells. Aging is also associated with an increased susceptibility to trauma, infections and many other forms of stress [3]. The functioning of the immune system is impaired and this gives rise to both an increase in auto-immune disease as well as an increased susceptibility to infections. In this scenario, the exercise-induced adaptations are especially important in old age. These adaptations are particularly evident in the cardio respiratory, musculoskeletal system, body composition, and metabolism [28, 46]. Moreover, the documented health benefits of exercise also include diminished symptoms of depression and anxiety, among others [47].

Skeletal muscle is the main target of exercise training. Modifications in skeletal muscle are crucial for enhancing endurance and metabolic efficiency [48]. Aging has been associated with a decrease in mitochondrial biogenesis, induced by different stimuli, in skeletal muscle [49]. A functional muscle that has not lost the capacity to synthesize healthy mitochondria is an important contributor to the prevention of frailty [50, 51]. Endurance exercise induces an increase in mitochondriogenesis, and an increase in fatty acid oxidation that ultimately leads to an increase in aerobic capacity and retards diseases such as obesity, type 2 diabetes, and cardiovascular diseases [52, 53].

Exercise-induced improved psychological well-being has also been extensively reported (e.g., through reduced stress, anxiety, and depression) [54]. The beneficial effects of exercise on cognitive function are well known in both young and aged animals [55]. There is no doubt that exercise training is a good strategy to prevent Alzheimer's Disease (AD) [56]. Exercise is associated with an increased expression of neurotrophic factors in several brain areas which are related to better memory and improved cognitive function.

The Brain-derived neurotrophic factor (BDNF) can enhance the survival and differentiation of neurons and voluntary exercise has been shown to increase it [57]. Aged brains are responsive to exercise-induced BDNF expression [58]. Neurotransmitter systems are also affected by exercise. Treadmill running decreases dopamine depletion in the striatum of Parkinsonian rats, suggesting that exercise may be a potential intervention to reduce the incidence of Parkinson's disease [59]. The exercise-induced increase in serotonin and acetylcholine levels also seems to play a role in exercise-induced benefits on cognition [60].

Exercise is particularly important for the prevention and management of many chronic diseases such as diabetes, osteoporosis, hypertension, obesity, cancer, and depression [28]. Physical activity results in specific adaptations that affect individual states in all of these diseases. For instance, adaptations that affect glucose homeo-

stasis, in type 2 diabetes, are of great importance. Several changes occur as a result of regular physical activity, including increased glycogen synthase and hexokinase activities, increased GLUT-4 mRNA and protein expression, and improved muscle capillary density (resulting in improved glucose delivery to the muscle) [61].

Exercise causes a significant reduction in the incidence of cancer (specifically colon and breast cancer) [6, 62]. Possible explanations include reductions in fat stores, increased energy expenditure offsetting a high fat diet, activity-related changes in sex hormone levels, immune function, insulin and insulin-like growth factors, free radical generation**,** and direct effects on tumor cell biology [63]. This is especially relevant in the elderly because the incidence of cancer increases exponentially with age [3].

EXERCISE, AS A PSYCHOACTIVE DRUG. ROLE IN ALZHEIMER'S DISEASE

Exercise training can favorably influence cognitive function [64, 65]. It improves learning, memory, quality of sleep [66], and counteracts the mental decline that comes with age [67]. Regular exercise facilitates functional recovery from brain injury [68], and depression [69, 70]. This may be explained because is a very powerful stimulus to the induction of neurogenesis in the adult dentate gyrus [66] that can contribute to remodeling hippocampal synaptic circuits and to enhancing cognitive function. Exercise training can also mitigate the consequences of acute exposure to different types of psychological stress [65]. The exercise-induced alterations in serotonergic and in norephinephrinergic systems can explain these responses [65]. Most of the positive effects of exercise, as mentioned previously, have been related to the induction, in different brain areas, of neurotrophic proteins, including the BDNF, glyal-derived neurotrophic factor (GDNF), and insulin growth factor (IGF). Exercise also induces transient increases in local cerebral glucose uptake and in cerebral blood flow in the different brain areas, in both rats and humans [71]. This is very important because memory disturbances in the normal elderly are intimately related to hypoxia, a reduction in blood supply, and glucose hypometabolism in the hippocampus and a number of key brain areas [72].

The incidence of neurodegenerative diseases increases exponentially with age. Alzheimer's disease is the most frequent cause of dementia in Western societies. The prevalence worldwide is estimated to be as high as 24 million. The incidence of Alzheimer's is expected to double every 20 years until 2040 [73]. Exercise has clear beneficial effects on AD patients. Recently, a specific walking program has been shown to stabilize the progressive cognitive dysfunction in patients in the latter stages of AD, improving not only their activities of daily living, but also their cognitive state [74]. Results supporting that idea have been found previously. Blankevoort's systematic review about interventions in patients with dementia indicates that physical exercise produces an improvement in their physical function at every stage of the illness [75]. Furthermore, AD patients recruited by the National Health Service Memory Clinic in the UK who followed an intervention consisting in fifteen exercises focused on fine motor involvement, balance and hand-eye coordination besides the Brain Gym Programme, experienced significant improvement in attention and visual memory [76]. These are just some examples of an abundant literature on the use of physical exercise as treatment in AD. Physical exercise can play an important role not only in the treatment of AD but also in its prevention. Data from animal models of AD has shown the protective effect of voluntary exercise on the disease by reducing amyloid deposition and enhancing amyloid beta clearance, the major constituent of plaques in AD. Treadmill exercise has also been shown to ameliorate the accumulation of phosphorylated tau, an essential component of neurofibrillary tangles in AD. An improvement in learning and memory has also been found. These findings are consistent with studies in normal and aged animals, showing that exercise improves learning and memory through increased hippocampal plasticity [77].

The studies performed in humans support the findings in animals. A recent example is a prospective study carried out on a cohort of 716 old people without dementia who were participating in the Rush Memory and Aging Project. Total daily exercise and physical activity were measured by actigraphy. After four years of follow-up, 71 people had developed AD. They conclude that a higher level of physical activity was associated with a lower risk of AD [78]. The opposite situation has also been described. Sedentary lifestyle (with less than 5 hours of activity per week) has been significantly associated with more than double risk for dementia [79]. Although there is an enormous variability in assessment and outcome measures, the positive association between physical exercise with AD risk is clear.

PREVENTION OF SARCOPENIA BY PHYSICAL EXERCISE

Irwin Rosenberg in 1989 proposed the use of the Greek term sarcopenia (meaning 'sarx' for flesh and 'penia' for loss) to describe the loss of muscle mass among older people, a loss that can be dramatic [80]. This age-associated loss of muscle mass is linked to a loss of muscle quality and strength [81]. Various cellular and molecular factors are involved in the development of sarcopenia, such as changes in muscle fibre phenotype, protein muscle synthesis and degradation, mitochondrial function, and cytokines [10]. Muscle fibre loss occurs as early as age 25, but the situation becomes dramatic at age 80 when a loss of 40% of muscle mass occurs [82]. Functionally debilitating sarcopenia affects approximately 7% of adults over 70, and up to 20% of those over 80. The estimated cost of sarcopenia-related health issues to the US health care system is more than 18 billion dollars annually.

Interventions intended to prevent or delay sarcopenia are clearly desired. Although the number of causative factors is high, physical inactivity has been linked to a higher risk of sarcopenia [81]. There is a plethora of experimental data on the potential therapeutic benefits of physical activity and exercise [83-85]. Physical activity elicits a wide range of beneficial metabolic and functional adaptations in the aged skeletal muscle, improving physical capacity [86], contractile protein synthesis [87], mitochondrial function [88], and insulin action [89]. The exercise-induced stimulation of muscular protein anabolism in older persons is achieved through the activation of the mammalian target of rapamycin (mTOR) [90]. Moreover, the down-regulation of myocyte apoptosis through exercise training is linked to the preservation of muscle integrity and improved physical performance in later life [91].

Both aerobic and resistance exercise are beneficial to the elderly, but resistance exercise is doubtless the most recommended strategy to prevent sarcopenia due to its direct effects on skeletal muscle [92, 93]. Aerobic exercise increases VO_{2max}, cardiac stroke volume, reduces heart rate and blood pressure, and reduces body fat percentage in the old population [94]. However, this type of exercise does not significantly prevent the age-related decrease in muscle mass and strength [95]. Resistance exercise is the performance of dynamic or static contractions against external resistance of varying intensities [96]. This type of training can be performed at old age by using hand weights, light free weights, or stretching bands [97]. Muscle protein synthesis decreases during an exercise session, but it increases during recovery from resistance exercise for up to 48 hours [98]. During the immediate post-resistance exercise recovery period in which mTOR and several up- and downstream kinases involved in muscle synthesis are activated [99]; the stimulation of muscle protein synthesis with contractile activity promotes adaptive muscle hypertrophy [100]. Resistance training counteracts sarcopenia by increasing muscle mass and strength in older adults and the frail elderly [83]. Moreover, it has also been associated with improvements in aerobic fitness, function, and performance of ac-

tivities of daily living [101, 102]. Thus, physical exercise is currently considered the primary countermeasure to prevent sarcopenia [103]. Furthermore, resistance exercise helps even very old, frail persons to retain the ability of improving muscle mass, strength, and physical performance [50]. The lack of motivation of most persons is a handicap of physical exercise in counteracting age-related muscle loss. In addition, many older adults may not be able to engage in physical exercise due to comorbidity and disabling conditions. Therefore, the clinical status of old people should be taken into consideration and individualize exercise programs [104]. Overall, the old population is encouraged to maintain high levels of physical exercise to prevent muscle mass loss and, therefore, sarcopenia.

THE OXIDATIVE STRESS PARADOX. DOES EXERCISE ALLEVIATE OR INCREASE FREE RADICAL PRODUCTION AT OLD AGE?

Both exercise and aging increase reactive oxygen species (ROS), which can result in damage to cells [105] and aging also causes an increase in mitochondrial free radical production [3]. Exercise increases free radical production mainly by increasing the activity of oxidases, such as NAD(P)H and xanthine oxidases [106]. ROS can cause oxidative stress if they overwhelm the cellular antioxidant defenses [105], therefore it is not clear whether aged individuals are more susceptible to some of the harmful effects of rigorous exercise as a result of increased exposure to ROS [107].

Skeletal muscle generates ROS during contractile activity. Research in this area started in 1954, when the first data showing that free radicals are present in muscle were published [108]. However, the first suggestion that exercise was associated with an increase in lipid peroxidation did not appear until the late 1970s [109], and it was not until the 1980s that the first link between muscle function and free radical biology was identified. Koren and co-workers showed that free radical content is elevated in isolated frog limb muscles stimulated to contract repetitively [110]. Shortly afterward, a ground-breaking report was published showing a 2- to 3-fold increase in free radical content in skeletal muscle from rats run to exhaustion [106]. These findings were associated with three aspects of fatigue that are now well recognized: increased lipid peroxidation, decreased control of mitochondrial respiration, and decreased integrity of the sarcoplasmic reticulum. The same study showed that Vitamin E deficiency magnified these three changes, indicating that exercise-induced changes were sensitive to both free radical production and antioxidant buffering [106]. Since then, research in the area has grown rapidly. It is now clear that intense muscular contractile activity can result in oxidative stress not only in animals but also in humans. For instance, we found that during the Tour de France, cyclists showed significant increases in plasma malondialdehyde levels [111]. We also reported similar results in athletes after marathon running [112].

There are several potential tissue sources in which ROS may be produced during exercise: heart, lungs, white blood cells, and skeletal muscle have been the most studied [113, 114]. At the subcellular level, several sources of free radicals have been studied in skeletal muscle during exercise [115]. In the past it was assumed that an increase in oxygen consumption by mitochondria would lead to an increase in $O_2^{\bullet-}$ formation. However, recent research suggests that mitochondria may not be the dominant source of ROS during exercise [115, 116]. The role of xanthine oxidase (XO) in oxidant generation during high-intensity intermittent exercise has long been recognized [112, 117, 118]. Depletion of ATP during demanding muscle contraction results in an accumulation of hypoxanthine and xanthine and conversion of xanthine dehydrogenase to XO. These conditions set the stage for generating $O_2^{\bullet-}$ when oxygen is replenished to relatively hypoxic muscle [119]. Allopurinol, an inhibitor of XO, widely used in the clinical practice to treat gout, decreases muscle oxidative stress after exhaustive exercise both in humans and in rats [111, 120, 121].

There are many theories of aging [3]. One of the most prominent is the free radical theory of aging which was initially proposed by Harman in the 1950s [122]. It proposes that free radicals derived from oxygen are responsible for damage associated with aging. The antioxidant systems are unable to counterbalance all the free radicals continuously generated during the life of the cell. This results in oxidative damage in the cell and thus in tissues. There is a great deal of experimental proof in support of this theory. The finding in the laboratory of Britton Chance, that ~2% of oxygen consumed by mitochondria in state 4 is converted to hydrogen peroxide, underpinned the role of mitochondria in ROS production [123]. These experiments led Jaime Miquel to refine the free radical theory of aging and in the '70s he formulated the mitochondrial free radical theory of aging. The main contributions of Miquel were emphasizing the importance of mitochondrial DNA as a target of oxidants produced during aging, and pointing out that mitochondriogenesis might be impaired in aging [124].

The mitochondrial theory of aging, although recently questioned [125], has been tested in various laboratories and there are many reports in its support [126, 127]. The continuous free radical generation by mitochondria during the whole life span causes a chronic oxidative stress that plays a critical role in aging [122, 128]. Antioxidant enzyme activities in skeletal muscle are increased at old age [129, 130]. However, protein and mRNA levels of antioxidant enzymes are either decreased or unaltered [131, 132]. Some biological factors (reviewed in the next section) prevent aged skeletal muscle from achieving the higher levels of adaptation normally seen in young muscles, thus leading to loss of mass and function. Aging also increases the incidence of muscle injury, and the inflammatory response can lead old muscle to further oxidative stress [107]. Moreover, muscle repair and regeneration capacity is reduced at old age [107]. In this scenario, the question of whether exercise is harmful or beneficial for the old skeletal muscle, in terms of oxidative stress, seems appropriate. Elderly persons who are physically active benefit from exercise-induced adaptation in cellular antioxidant defense systems [107]. Bejma and Ji found high levels of ROS generation when old muscles were subjected to an acute bout of exercise [133]. However, the same authors found that exercise training (10 weeks) resulted in lower levels of skeletal muscle and myocardium lipid peroxidation [134, 135]. It has also been shown that exercise training improves muscle mitochondrial respiratory control in old trained rats when challenged with external oxidants [107]. Moreover, improved muscle mechanics, strength, and endurance make old muscles less vulnerable to acute injury and chronic inflammation. These findings show that the adaptability of muscle to training found in the young animals does not completely disappear at old age [107]. However, due to the increased susceptibility of aged muscle to oxidative stress and even to damage, there are some aspects that should be taken into consideration with regard to the aged population participating in regular exercise training. Old individuals should carefully select a progressive exercise protocol to minimize oxidative stress [107].

SIGNALING PATHWAYS REGULATED BY EXERCISE IN SKELETAL MUSCLE, ALTERED DURING AGING

Exercise regulates cellular functions by different stimuli: alterations in metabolite concentrations, a shift in the ATP/ADP ratio, changes in Ca^{+2} and intracellular pH, and activation of redox-sensitive signaling pathways [136, 137] (See Fig. **1**). Physical exercise can activate the mitogen-activated protein kinase signaling pathways (MAPKs), including the extracellular signal-regulated kinases 1 and 2 (ERK1/2) [138], the p38 kinase [139], and the c-Jun NH2-terminal kinase (JNK) [138]. It can also increase the activity of the AMP-activated protein kinase (AMPK), Akt, SIRT1, and

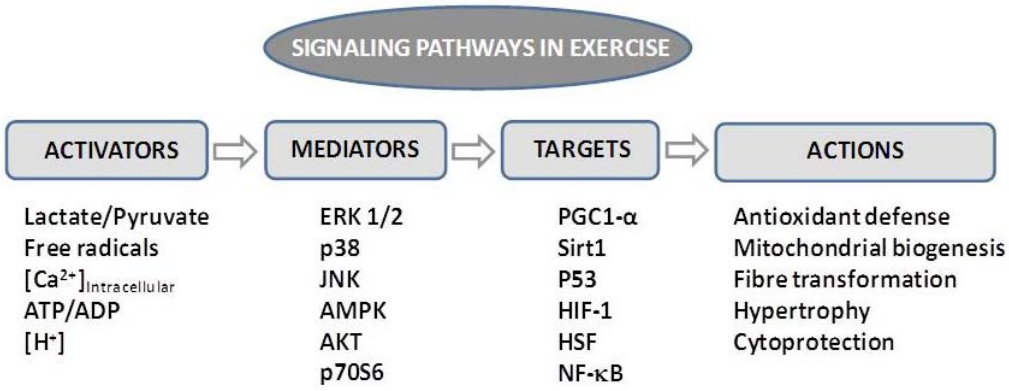


Fig. (1). Cell signaling pathways involved in exercise-induced adaptations in skeletal muscle.

p70S6 kinase [136, 140]. The Ca^{+2}-regulated signaling has also been extensively shown. In addition to triggering muscle contraction through the troponin system, Ca^{+2} is involved in the regulation of relevant intracellular proteins, such as protein kinase C, calcineurin, and calmodulin kinase, which mediate cellular signal transduction [141]. More recently, it has been shown that ROS play multiple regulatory roles in cells, such as the control of gene expression, regulation of cell signaling pathways, and modulation of skeletal muscle force production [142]. Thus, a moderate amount of free radicals may be seen as beneficial as they act as signals to enhance defenses, rather than deleterious as they are when cells are exposed to high levels of these radicals [143]. A major conclusion that can be drawn from these results is that exercise itself is an antioxidant, because training increases the expression of antioxidant enzymes [116].

The following are some of the most relevant redox-sensitive signaling pathways modulated by exercise: the peroxisome proliferator-activated receptor-γ coactivator-1α and β (PGC-1α and PGC-1β) [144, 145], p53 [146], the hypoxia-inducible factor 1 (HIF-1) [147], the heat shock factor (HSF) [148], the nuclear Factor-κB (NF-κB), and the MAPK signaling pathways [137, 139]. Several important adaptations in skeletal muscle, such as mitochondrial biogenesis, antioxidant defense, hypertrophy, cytoprotection, and fiber transformation are regulated primarily by these pathways; thus, its regulation is tightly controlled [114]. Aging impairs most of the cell signaling transduction pathways modulated by exercise in skeletal muscle [49, 149, 150]. For instance, Williamson *et al.* [151] reported higher resting activities of ERK 1/2, $p90^{RSK}$, p38, and JNK/SAPK in the leg muscle of old men compared to young ones. However, aged muscles decreased MAPK enzyme activities after an acute bout of resistance contraction, whereas young ones increased these enzyme activities.

Nuclear Factor-κB is one of the main redox sensitive transcription factors and has a key role in regulating the immune response to infection [152]. NF-κB is also constitutively activated at old age, which leads to the higher basal expression of pro-inflammatory cytokines, chemokines, adhesion molecules, and ROS-generating enzymes such as cyclooxigenase. Chronic activation of NF-κB has been identified as a main reason for aged-related muscle wasting and sarcopenia [153]. Yu and Chung [154] demonstrated that 4-hydroxynonenal, a lipid peroxidation product often found in aged muscle, could activate the following pathway: p38→ NIK→IKK→ NF-κB. Since NF-κB activation often leads to increased pro-inflammatory cytokine expression, this vicious cycle was hypothesized as the basis for the inflammation theory of aging. The chronic activation of NF-κB in skeletal muscle at old age seems to be part of a general cellular adaptive response aimed at providing protection against subsequent, damaging insults [150]. However, there is a failure to activate NF-κB fully in the skeletal muscle of old animals following contractile activity [150] although the mechanisms responsible for this fall are unclear.

Another relevant co-activator affected by aging is PGC-1α which acts as a master regulator of energy metabolism and mitochondrial biogenesis by coordinating the activity of multiple transcription factors [155]. Aging has been associated, in skeletal muscle, with reductions in mitochondrial oxidative phosphorylation activity, mitochondrial DNA mutations, reductions in mitochondrial DNA content, decreased activities of the mitochondrial electron transport chain, and altered apoptotic signaling [156]. Thus, the promotion of mitochondriogenesis is critical to prevent aging in skeletal muscle. We have recently shown that muscle from old rats presents a marked loss in mitochondriogenesis and that this may be due to a lack of induction of PGC-1α [157]. We found a striking similarity between the response to exercise training in PGC-1α KO mice and in old rats. In young rats, PGC-1α was activated in skeletal muscle not only by training but also by exposure to the cold or triiodothyronine. However, in old animals, we found an age-associated lack of expression of PGC-1α in response to exercise training or to any of the other stimuli tested in rat skeletal muscle. Our study highlighted the importance of maintaining a normal PGC-1α responsiveness to maintain normal muscle function [157].

The mechanisms responsible for the failure of the activation of relevant cell signaling pathways in the old skeletal muscle by exercise are unclear but they seem to be involved in the process and intervene in age-related development of muscle deterioration [150]. We have recently hypothesized that lifelong exercise training may prevent the failure of old muscles to respond to exercise [11].

CONTRAINDICATIONS OF EXERCISE AT OLD AGE

Under some circumstances, physical exercise is not useful to increase quality of life. Older individuals are living with a significant burden of chronic disease, geriatric impairments in cognition, vision, hearing, and reduced physiological reserve. They have an increased susceptibility to age-related diseases, such as cancer, cardiovascular and neurodegenerative diseases, and insulin-independent diabetes. Although both the heart and the lung significantly benefit from physical activity, there are some contraindications when exercise is performed by subjects suffering from heart or pulmonary diseases [1].

For instance and regarding coronary heart disease, exercise is contraindicated until the condition has been stable for at least five days; dyspnea at rest, aortic stenosis, pericarditis, myocarditis, endocarditis, fever, and severe hypertension are all contraindications of exercise [6]. The incidence of heart failure increases with age, largely due to the development of heart failure risk factors such as

hypertension and coronary artery disease. Thus, exercise should be prescribed with caution to these individuals [158].

There are no absolute contraindications to very moderate exercise in chronic obstructive pulmonary disease patients [6]. However, acute exacerbations of the disease are associated with increased mortality and hospitalization, especially in older patients [159]. As in asthma patients, a pause in training is recommended when an acute exacerbation occurs. In cases of infection, a pause in training is recommended until the patient has been asymptomatic for a day, whereafter training can be slowly resumed [6].

Exercise is contraindicated in cases of acute joint inflammation if pain worsens after training [6]. With increasing age, there is a significant reduction in bone formation. Age-related bone loss is also seen in men [160]. The training of patients with osteoporosis should include activities with a low risk of falling [6].

Cancer in older persons is an increasingly common problem, due to the progressive prolongation of the life-expectancy of the Western population [161]. Exercise is contraindicated in cancer patients being treated with chemotherapy or radiotherapy when a leukocyte concentration falls below $0.5x10^9$/L, hemoglobin below 10 g/dL, thrombocyte concentration below $20x10^9$/L, and temperature above 38ºC. Patients with bone metastases should not perform strength conditioning at high load [6].

Diabetes affects 10.9 million US adults aged 65 years and older [162]. In diabetic patients (both type I and II) exercise should be postponed if blood glucose is >2.5 g/L together with ketonuria and >3.0 g/L even without ketonuria, in both cases before it is corrected.

In patients with hypertension and active proliferative retinopathy, high-intensity training or training involving Valsalva-like maneuvers should be avoided. Patients with neuropathy and incipient foot ulcers should refrain from activities entailing the bearing of the patient's own body weight. Hypertension is highly prevalent among older adults, and aging of the population will substantially increase the prevalence of this condition. Age-related endothelial dysfunction and increased arterial stiffness contribute to the increased prevalence of hypertension, particularly systolic hypertension, among the elderly [163]. Hypertensive patients with a blood pressure higher than 180/105 should begin pharmacotherapy before regular physical activity is initiated [164].

EXERCISE MIMETICS. AN ALTERNATIVE FOR DISABLED OLD PEOPLE?

Physical inactivity is a pressing public health issue [165]. But exercise may be impractical because of physical limitations or side effects. This stimulates the search for exercise mimetics (or "exercise pills") that mimic exercise [166] (See Fig. **2**).

As mentioned in a previous section, exercise improves performance by activating several pathways particularly in skeletal muscle. AMPK is activated by exercise and is fundamental for an exercise-mediated switch to aerobic myofibers in the muscle [167]. AMPK suppresses anabolic and stimulates catabolic pathways to restore cellular ATP levels [48, 168]. Recently, it has been reported that 5'-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) can mimic the exercise effect by increasing the GLUT-4 protein, hexokinase activity, resting glycogen content, and muscle mitochondria [169]. AICAR is an acute activator of AMPK, which is taken up by cells and mimics the effects of AMP on AMPK [170]. One of the main characteristics of the aging muscle is a decline in mitochondrial function [156]. Thus, the activation of AMPK is a potential target to restore this deficiency. Conflicting results have been published on the effect of AICAR treatments in the skeletal muscle of old animals. Reznick and co-workers reported that AMPK activation was abolished in old Fischer 344 rats in response to AICAR [170]. On the other hand, similar AICAR-stimulated activation of AMPK in old compared to young rat muscles have been reported by other research groups [171]. More research is needed to elucidate the potential beneficial effects of AICAR in old individuals.

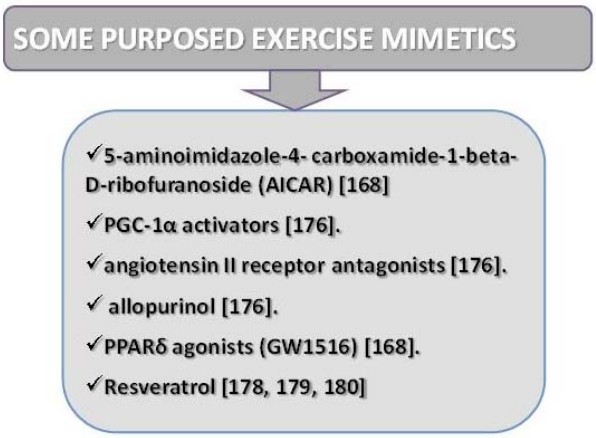


Fig. (2). Exercise mimetics. An alternative for disabled old people.

We have recently proposed several pharmacological, non-hormonal interventions to prevent the age-associated loss of muscle mass and sarcopenia, such as angiotensin II receptor antagonists, allopurinol, and PGC-1α activators [172]. Targeting PGC-1α activity may be a fruitful approach to delay the aging process. One promising candidate is resveratrol [173], a plant polyphenol commonly found in red wine that has been considered as an exercise mimetic. High doses of resveratrol improve endurance [174] and may act as a caloric restriction mimetic [175, 176]. Sirt1 has been identified as its putative primary target. It is known that Sirt1 is a positive regulator of PGC-1α [174] and as such has the potential to influence pathways involved in mitochondrial biogenesis and oxidative metabolism. As Sirt1 is also activated with exercise it has been suggested that resveratrol promotes mitochondrial biogenesis and decreases mitochondrial oxidative stress in a Sirt1-dependent fashion [174, 177], thus potentiating the possibility of replacing damaged mitochondria in aged animals. However, there are discrepancies on the role of resveratrol as an exercise mimetic in aging. It improves parameters of aging at low doses in mice although its impact on aging in primates is not known [178]. Further work is needed to determine if resveratrol has the potential to be an effective therapeutic agent to treat muscle functional impairment in aging [179].

Finally, activation or over-expression of the transcription factor PPARδ in muscle also results in an increase in mitochondrial biogenesis and in the proportion of oxidative muscle fibers [169]. This results in increased running endurance and protection against diet-induced obesity and type 2 diabetes. Thus, several potent and selective PPARδ agonists, such as GW1516 have also been proposed as endurance exercise mimetic drugs [169]. To our knowledge, GW1516 has not been yet tested in old animals.

In our opinion, the applicability of these compounds currently is limited in both the young and old populations.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

We thank Mrs Marilyn Noyes for her kind help in reviewing the manuscript.This work was supported by grants SAF2010-19498, from the Spanish Ministry of Education and Science (MEC); IS-

CIII2006-RED13-027 and ISCIII2012-RED-43-029 from the "Red Tematica de investigacion cooperativa en envejecimiento y fragilidad" (RETICEF), PROMETEO2010/074 from "Conselleria de Sanitat de la Generalitat Valenciana", 35NEURO GentxGent from "Fundacio Gent Per Gent de la Comunitat Valenciana" and EU Funded CM1001 and FRAILOMIC-HEALTH.2012.2.1.1-2. This study has been co-financed by FEDER funds from the European Union.

ABBREVIATIONS

AD	=	Alzheimer's Disease
AICAR	=	5'-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside
AMPK	=	AMP-activated protein kinase
BDNF	=	Brain-derived neurotrophic factor
CHD	=	Coronary Heart Disease
ERK1/2	=	Extracellular signal-regulated kinase 1 and 2
GDNF	=	Glyal-derived neurotrophic factor
IGF	=	Insulin Growth Factor
JNK	=	c-Jun NH2-terminal kinase
MAPK	=	Mitogen activated protein kinase
MET	=	Metabolic Equivalent (Estimated oxygen cost of 3.5 $mL \cdot min^{-1} \cdot Kg^{-1}$)
MnSOD	=	Mitochondrial superoxide dismutase
mTOR	=	Mammalian target of rapamycin
PGC-1α	=	Peroxisome proliferator-activated receptor-γ coactivator-1α
PPARδ	=	Peroxisome proliferator-activated receptor δ
ROS	=	Reactive oxygen species
SCD	=	Sudden cardiac death
VO_2	=	Oxygen consumption
XO	=	Xanthine oxidase

REFERENCES

[1] Vina J, Sanchis-Gomar F, Martinez-Bello V, *et al*. Exercise acts as a drug; the pharmacological benefits of exercise. Br J Pharmacol 2012; 167: 1-12.

[2] Adolph EF. Physiological adaptations: hypertrophies and superfunctions. Am Sci 1972; 60: 608-17.

[3] Vina J, Borras C, Miquel J. Theories of ageing. IUBMB Life 2007; 59: 249-54.

[4] Fries JF. Physical activity, the compression of morbidity, and the health of the elderly. J R Soc Med 1996; 89: 64-8.

[5] Ji LL, Leeuwenburgh C, Leichtweis S, *et al*. Oxidative stress and aging. Role of exercise and its influences on antioxidant systems. Ann N Y Acad Sci 1998; 854: 102-17.

[6] Pedersen BK, Saltin B. Evidence for prescribing exercise as therapy in chronic disease. Scand J Med Sci Sports 2006; 16 Suppl 1: 3-63.

[7] Manini TM, Newman AB, Fielding R, *et al*. Effects of exercise on mobility in obese and nonobese older adults. Obesity (Silver Spring) 2010; 18: 1168-75.

[8] Goetzl EJ. Is aging a drug target? FASEB J 2011; 25: 2509-11.

[9] Nascher IL, Jacobi A. Geriatrics: the diseases of old age and their treatment including physiological old age, home and institutional care, and medico-legal relations. K. Paul, French, Trubner: [S.l.] 1919.

[10] Kirkland JL, Peterson C. Healthspan, translation, and new outcomes for animal studies of aging. J Gerontol A Biol Sci Med Sci 2009; 64: 209-12.

[11] Garcia-Valles R, Ibañez-Sania S, Sanchis-Gomar F, *et al*. Long Life Spontaneous Exercise Does Not Prolong Lifespan But Prevents Frailty In C57BL/6J. Healthspan Vs Lifespan. 7th Congress of the EUGMS. Revista Española de Geriatría y Gerontología (Espec Cong) 2011; 46: 224.

[12] Fries JF. Aging, natural death, and the compression of morbidity. N Engl J Med 1980; 303: 130-5.

[13] Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep 1985; 100: 126-31.

[14] Pate RR, Pratt M, Blair SN, *et al*. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA 1995; 273: 402-7.

[15] Lyons AS, Petrucelli RJja. Medicine : an illustrated history. H. N. Abrams: New York 1978.

[16] Speed C, Jaques R. High-performance sports medicine: an ancient but evolving field. Br J Sports Med 2010; 45: 81-3.

[17] Fox SM, 3rd, Haskell WL. Physical activity and the prevention of coronary heart disease. Bull N Y Acad Med 1968; 44: 950-67.

[18] Morris JN, Heady JA, Raffle PA, *et al*. Coronary heart-disease and physical activity of work. Lancet 1953; 265: 1053-7; contd.

[19] Morris JN, Heady JA, Raffle PA, *et al*. Coronary heart-disease and physical activity of work. Lancet 1953; 265: 1111-20; concl.

[20] Paffenbarger RS, Hale WE. Work activity and coronary heart mortality. N Engl J Med 1975; 292: 545-50.

[21] Paffenbarger RS, Jr., Brand RJ, Sholtz RI, *et al*. Energy expenditure, cigarette smoking, and blood pressure level as related to death from specific diseases. Am J Epidemiol 1978; 108: 12-8.

[22] Karvonen MJ, Klemola H, Virkajarvi J, *et al*. Longevity of endurance skiers. Med Sci Sports 1974; 6: 49-51.

[23] Macera CA, Hootman JM, Sniezek JE. Major public health benefits of physical activity. Arthritis Rheum 2003; 49: 122-8.

[24] Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., *et al*. Physical fitness and all-cause mortality. A prospective study of healthy men and women. Jama 1989; 262: 2395-401.

[25] Paffenbarger RS, Jr., Hyde RT, Wing AL, *et al*. Physical activity, all-cause mortality, and longevity of college alumni. N Engl J Med 1986; 314: 605-13.

[26] Myers J, Kaykha A, George S, *et al*. Fitness versus physical activity patterns in predicting mortality in men. Am J Med 2004; 117: 912-8.

[27] Bouchard C, Shephard RJ, Stephens T. Physical activity, fitness, and health : international proceedings and consensus statement : [Second International Consensus Symposium on Physical Activity, Fitness, and Health, held May 5 to May 9, 1992, in Toronto, Canada]. Human Kinetics Publishers: Champaign, Ill. ; Leeds 1994.

[28] Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. Cmaj 2006; 174: 801-9.

[29] Vina J, Sanchis-Gomar F, Martinez-Bello V, *et al*. Exercise acts as a drug; the pharmacological benefits of exercise. Br J Pharmacol 2012; 167: 1-12.

[30] Lee IM. Dose-response relation between physical activity and fitness: even a little is good; more is better. JAMA 2007; 297: 2137-9.

[31] Beers MH, Fink A, Beck JC. Screening recommendations for the elderly. Am J Public Health 1991; 81: 1131-40.

[32] Turnheim K. Drug dosage in the elderly. Is it rational? Drugs Aging 1998; 13: 357-79.

[33] Blair SN, LaMonte MJ, Nichaman MZ. The evolution of physical activity recommendations: how much is enough? Am J Clin Nutr 2004; 79: 913S-920S.

[34] Warburton DE, Nicol CW, Bredin SS. Prescribing exercise as preventive therapy. Cmaj 2006; 174: 961-74.

[35] Church TS, Earnest CP, Skinner JS, *et al*. Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. Jama 2007; 297: 2081-91.

[36] Wen CP, Wai JP, Tsai MK, *et al*. Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet 2011.

[37] Nelson ME, Rejeski WJ, Blair SN, *et al*. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 2007; 39: 1435-45.

[38] Sanchis-Gomar F, Olaso-Gonzalez G, Corella D, *et al*. Increased average longevity among the "Tour de France" cyclists. Int J Sports Med 2011; 32: 644-7.

[39] Shephard RJ. Absolute versus relative intensity of physical activity in a dose-response context. Med Sci Sports Exerc 2001; 33: S400-18; discussion S419-20.

[40] Lepers R, Cattagni T. Do older athletes reach limits in their performance during marathon running? Age (Dordr) 2012; 34: 773-81.
[41] Wannamethee SG, Shaper AG, Walker M. Changes in physical activity, mortality, and incidence of coronary heart disease in older men. Lancet 1998; 351: 1603-8.
[42] Hakim AA, Petrovitch H, Burchfiel CM, *et al*. Effects of walking on mortality among nonsmoking retired men. N Engl J Med 1998; 338: 94-9.
[43] WHO Global Recommendations on Physical Activity for Health 2010.
[44] Fried LP, Tangen CM, Walston J, *et al*. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001; 56: M146-56.
[45] Ensrud KE, Ewing SK, Cawthon PM, *et al*. A comparison of frailty indexes for the prediction of falls, disability, fractures, and mortality in older men. J Am Geriatr Soc 2009; 57: 492-8.
[46] Lee DC, Artero EG, Sui X, *et al*. Mortality trends in the general population: the importance of cardiorespiratory fitness. J Psychopharmacol 2010; 24: 27-35.
[47] Kujala UM. Born to be rich, physically active, fit and healthy? Scand J Med Sci Sports 2011; 20: 367.
[48] Matsakas A, Narkar VA. Endurance exercise mimetics in skeletal muscle. Curr Sports Med Rep 2010; 9: 227-32.
[49] Derbre F, Gomez-Cabrera MC, Nascimento AL, *et al*. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age (Dordr) 2012; 34: 669-79.
[50] Fiatarone MA, O'Neill EF, Ryan ND, *et al*. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 1994; 330: 1769-75.
[51] Gill TM, Gahbauer EA, Han L, *et al*. Trajectories of disability in the last year of life. N Engl J Med 2010; 362: 1173-80.
[52] Holloszy JO, Coyle EF. Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 1984; 56: 831-8.
[53] Mootha VK, Lindgren CM, Eriksson KF, *et al*. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003; 34: 267-73.
[54] Dunn AL, Trivedi MH, O'Neal HA. Physical activity dose-response effects on outcomes of depression and anxiety. Med Sci Sports Exerc 2001; 33: S587-97; discussion 609-10.
[55] Neeper SA, Gomez-Pinilla F, Choi J, *et al*. Exercise and brain neurotrophins. Nature 1995; 373: 109.
[56] Pareja-Galeano H, Brioche T, Sanchis-Gomar F, *et al*. [Effects of physical exercise on cognitive alterations and oxidative stress in an APP/PSN1 transgenic model of Alzheimer's disease]. Rev Esp Geriatr Gerontol 2012; 47: 198-204.
[57] Neeper SA, Gomez-Pinilla F, Choi J, *et al*. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Res 1996; 726: 49-56.
[58] Adlard PA, Perreau VM, Cotman CW. The exercise-induced expression of BDNF within the hippocampus varies across life-span. Neurobiol Aging 2005; 26: 511-20.
[59] Poulton NP, Muir GD. Treadmill training ameliorates dopamine loss but not behavioral deficits in hemi-parkinsonian rats. Exp Neurol 2005; 193: 181-97.
[60] Kramer AF, Erickson KI, Colcombe SJ. Exercise, cognition, and the aging brain. J Appl Physiol 2006; 101: 1237-42.
[61] Mandroukas K, Krotkiewski M, Hedberg M, *et al*. Physical training in obese women. Effects of muscle morphology, biochemistry and function. Eur J Appl Physiol Occup Physiol 1984; 52: 355-61.
[62] Shephard RJ, Futcher R. Physical activity and cancer: how may protection be maximized? Crit Rev Oncog 1997; 8: 219-72.
[63] Westerlind KC. Physical activity and cancer prevention--mechanisms. Med Sci Sports Exerc 2003; 35: 1834-40.
[64] Vaynman S, Gomez-Pinilla F. Revenge of the "sit": how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. J Neurosci Res 2006; 84: 699-715.
[65] Dishman RK, Berthoud HR, Booth FW, *et al*. Neurobiology of exercise. Obesity (Silver Spring) 2006; 14: 345-56.
[66] van Praag H, Christie BR, Sejnowski TJ, *et al*. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 1999; 96: 13427-31.
[67] Laurin D, Verreault R, Lindsay J, *et al*. Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 2001; 58: 498-504.
[68] Grealy MA, Johnson DA, Rushton SK. Improving cognitive function after brain injury: the use of exercise and virtual reality. Arch Phys Med Rehabil 1999; 80: 661-7.
[69] Siuciak JA, Boylan C, Fritsche M, *et al*. BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. Brain Res 1996; 710: 11-20.
[70] Shirayama Y, Chen AC, Nakagawa S, *et al*. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002; 22: 3251-61.
[71] Vissing J, Andersen M, Diemer NH. Exercise-induced changes in local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 1996; 16: 729-36.
[72] Daulatzai MA. Early stages of pathogenesis in memory impairment during normal senescence and Alzheimer's disease. J Alzheimers Dis 2010; 20: 355-67.
[73] Mayeux R, Stern Y. Epidemiology of Alzheimer disease. Cold Spring Harb Perspect Med; 2.
[74] Venturelli M, Scarsini R, Schena F. Six-month walking program changes cognitive and ADL performance in patients with Alzheimer. Am J Alzheimers Dis Other Demen 2011; 26: 381-8.
[75] Blankevoort CG, van Heuvelen MJ, Boersma F, *et al*. Review of effects of physical activity on strength, balance, mobility and ADL performance in elderly subjects with dementia. Dement Geriatr Cogn Disord 2010; 30: 392-402.
[76] Yaguez L, Shaw KN, Morris R, *et al*. The effects on cognitive functions of a movement-based intervention in patients with Alzheimer's type dementia: a pilot study. Int J Geriatr Psychiatry; 26: 173-81.
[77] O'Callaghan RM, Griffin EW, Kelly AM. Long-term treadmill exposure protects against age-related neurodegenerative change in the rat hippocampus. Hippocampus 2009; 19: 1019-29.
[78] Buchman AS, Bennett DA. Amyloid pathology in persons with "normal" cognition. Neurology 2012; 78: 228-9.
[79] Norton MC, Dew J, Smith H, *et al*. Lifestyle behavior pattern is associated with different levels of risk for incident dementia and Alzheimer's disease: the Cache County study. J Am Geriatr Soc 2012
60: 405-12.
[80] Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr 1997; 127: 990S-991S.
[81] Cruz-Jentoft AJ, Landi F, Topinkova E, *et al*. Understanding sarcopenia as a geriatric syndrome. Curr Opin Clin Nutr Metab Care 2010; 13: 1-7.
[82] Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J Neurol Sci 1988; 84: 275-94.
[83] Fiatarone MA, Marks EC, Ryan ND, *et al*. High-intensity strength training in nonagenarians. Effects on skeletal muscle. JAMA 1990; 263: 3029-34.
[84] Peterson MD, Rhea MR, Sen A, *et al*. Resistance exercise for muscular strength in older adults: a meta-analysis. Ageing Res Rev 2010; 9: 226-37.
[85] Steib S, Schoene D, Pfeifer K. Dose-response relationship of resistance training in older adults: a meta-analysis. Med Sci Sports Exerc 2010; 42: 902-14.
[86] Coggan AR, Spina RJ, King DS, *et al*. Skeletal muscle adaptations to endurance training in 60- to 70-yr-old men and women. J Appl Physiol 1992; 72: 1780-6.
[87] Balagopal P, Schimke JC, Ades P, *et al*. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am J Physiol Endocrinol Metab 2001; 280: E203-8.
[88] Menshikova EV, Ritov VB, Fairfull L, *et al*. Effects of exercise on mitochondrial content and function in aging human skeletal muscle. J Gerontol A Biol Sci Med Sci 2006; 61: 534-40.
[89] Kahn SE, Larson VG, Beard JC, *et al*. Effect of exercise on insulin action, glucose tolerance, and insulin secretion in aging. Am J Physiol 1990; 258: E937-43.
[90] Fujita S, Rasmussen BB, Cadenas JG, *et al*. Aerobic exercise overcomes the age-related insulin resistance of muscle protein metabolism by improving endothelial function and Akt/mammalian target of rapamycin signaling. Diabetes 2007; 56: 1615-22.

[91] Marzetti E, Calvani R, Bernabei R, *et al*. Apoptosis in skeletal myocytes: a potential target for interventions against sarcopenia and physical frailty - a mini-review. Gerontology 2012; 58: 99-106.
[92] Rolland Y, Czerwinski S, Abellan Van Kan G, *et al*. Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives. J Nutr Health Aging 2008; 12: 433-50.
[93] Abellan van Kan G, Andre E, Bischoff Ferrari HA, *et al*. Carla Task Force on Sarcopenia: propositions for clinical trials. J Nutr Health Aging 2009; 13: 700-7.
[94] Lemura LM, von Duvillard SP, Mookerjee S. The effects of physical training of functional capacity in adults. Ages 46 to 90: a meta-analysis. J Sports Med Phys Fitness 2000; 40: 1-10.
[95] Hawkins S, Wiswell R. Rate and mechanism of maximal oxygen consumption decline with aging: implications for exercise training. Sports Med 2003; 33: 877-88.
[96] Phillips SM. Resistance exercise: good for more than just Grandma and Grandpa's muscles. Appl Physiol Nutr Metab 2007; 32: 1198-205.
[97] Visvanathan R, Chapman I. Preventing sarcopaenia in older people. Maturitas 2010; 66: 383-8.
[98] Phillips SM, Tipton KD, Aarsland A, *et al*. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol 1997; 273: E99-107.
[99] Drummond MJ, Dreyer HC, Pennings B, *et al*. Skeletal muscle protein anabolic response to resistance exercise and essential amino acids is delayed with aging. J Appl Physiol 2008; 104: 1452-61.
[100] Phillips SM. Protein requirements and supplementation in strength sports. Nutrition 2004; 20: 689-95.
[101] Martin Ginis KA, Latimer AE, Brawley LR, *et al*. Weight training to activities of daily living: helping older adults make a connection. Med Sci Sports Exerc 2006; 38: 116-21.
[102] Vincent KR, Braith RW, Feldman RA, *et al*. Improved cardiorespiratory endurance following 6 months of resistance exercise in elderly men and women. Arch Intern Med 2002; 162: 673-8.
[103] Frankel JE, Bean JF, Frontera WR. Exercise in the elderly: research and clinical practice. Clin Geriatr Med 2006; 22: 239-56; vii.
[104] Jones TE, Stephenson KW, King JG, *et al*. Sarcopenia--mechanisms and treatments. J Geriatr Phys Ther 2009; 32: 83-9.
[105] Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. Clarendon Press: Oxford 1985.
[106] Davies KJ, Quintanilha AT, Brooks GA, *et al*. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982; 107: 1198-205.
[107] Ji LL. Exercise at old age: does it increase or alleviate oxidative stress? Ann N Y Acad Sci 2001; 928: 236-47.
[108] Commoner B, Townsend J, Pake GE. Free radicals in biological materials. Nature 1954; 174: 689-91.
[109] Dillard CJ, Litov RE, Tappel AL. Effects of dietary vitamin E, selenium, and polyunsaturated fats on *in vivo* lipid peroxidation in the rat as measured by pentane production. Lipids 1978; 13: 396-402.
[110] Koren A, Schara M, Sentjurc M. EPR measurements of free radicals during tetanic contractions of frog skeletal muscle. . Period Biol. 1980; 82: 399-401
[111] Gomez-Cabrera MC, Pallardo FV, Sastre J, *et al*. Allopurinol and markers of muscle damage among participants in the Tour de France. Jama 2003; 289: 2503-4.
[112] Gomez-Cabrera MC, Martinez A, Santangelo G, *et al*. Oxidative stress in marathon runners: interest of antioxidant supplementation. Br J Nutr 2006; 96 Suppl 1: S31-3.
[113] Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 2008; 88: 1243-76.
[114] Gomez-Cabrera MC, Vina J, Ji LL. Interplay of oxidants and antioxidants during exercise: implications for muscle health. Phys Sportsmed 2009; 37: 116-23.
[115] Jackson MJ, Pye D, Palomero J. The production of reactive oxygen and nitrogen species by skeletal muscle. J Appl Physiol 2007; 102: 1664-70.
[116] Gomez-Cabrera MC, Domenech E, Vina J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med 2008; 44: 126-31.
[117] Hellsten Y, Ahlborg G, Jensen-Urstad M, *et al*. Indication of *in vivo* xanthine oxidase activity in human skeletal muscle during exercise. Acta Physiol Scand 1988; 134: 159-60.
[118] Stofan DA, Callahan LA, Di MA, *et al*. Modulation of release of reactive oxygen species by the contracting diaphragm. Am J Respir Crit Care Med 2000; 161: 891-8.
[119] Harris CM, Sanders SA, Massey V. Role of the flavin midpoint potential and NAD binding in determining NAD versus oxygen reactivity of xanthine oxidoreductase. J Biol Chem 1999; 274: 4561-9.
[120] Gomez-Cabrera MC, Borras C, Pallardo FV, *et al*. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 2005; 567: 113-20.
[121] Gomez-Cabrera MC, Close GL, Kayani A, *et al*. Effect of xanthine oxidase-generated extracellular superoxide on skeletal muscle force generation. American Journal of Physiology:Regulatory, Integrative and Comparative Physiology 2009; Under review.
[122] Harman D. Aging: a theory based on free radical and ratiation chemistry. J. Gerontol 1956; 11: 298-300.
[123] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979; 59: 527-605.
[124] Miquel J, Economos AC, Fleming J, *et al*. Mitochondrial role in cell aging. Exp Gerontol 1980; 15: 575-91.
[125] Yang W, Hekimi S. A mitochondrial superoxide signal triggers increased longevity in Caenorhabditis elegans. PLoS Biol; 8: e1000556.
[126] Sohal RS, Orr WC. Relationship between antioxidants, prooxidants, and the aging process. Ann N Y Acad Sci 1992; 663: 74-84.
[127] García de la Asunción J, Millan A, Pla R, *et al*. Mitochondiral glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. FASEB J. 1996; 10: 333-338.
[128] Fulle S, Protasi F, Di Tano G, *et al*. The contribution of reactive oxygen species to sarcopenia and muscle ageing. Exp Gerontol 2004; 39: 17-24.
[129] Ji LL, Dillon D, Wu E. Alteration of antioxidant enzymes with aging in rat skeletal muscle and liver. Am J Physiol 1990; 258: R918-23.
[130] Luhtala TA, Roecker EB, Pugh T, *et al*. Dietary restriction attenuates age-related increases in rat skeletal muscle antioxidant enzyme activities. J Gerontol 1994; 49: B231-8.
[131] Hollander J, Bejma J, Ookawara T, *et al*. Superoxide dismutase gene expression in skeletal muscle: fiber-specific effect of age. Mech Ageing Dev 2000; 116: 33-45.
[132] Oh-ishi S, Toshinai K, Kizaki T, *et al*. Effects of aging and/or training on antioxidant enzyme system in diaphragm of mice. Respir Physiol 1996; 105: 195-202.
[133] Bejma J, Ji L. Aging and acute exercise inhance free radical generation in rat skeletal muscle. J. Appl. Physiol. 1999; 87: 465-470.
[134] Leeuwenburgh C, Fiebig R, Chandwaney R, *et al*. Aging and exercise training in skeletal muscle: responses of glutathione and antioxidant enzyme systems. Am. J. Physiol. 1994; 267: R439-45.
[135] Fiebig R, Leeuwenburgh C, Gore M, *et al*. The interactive effects of aging and training on myocardial antioxidant enzymes and oxidative stress. Age 1996; 19: 83-89.
[136] Sakamoto K, Goodyear LJ. Invited review: intracellular signaling in contracting skeletal muscle. J Appl Physiol 2002; 93: 369-83.
[137] Ji LL, Gomez-Cabrera MC, Steinhafel N, *et al*. Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. Faseb J 2004; 18: 1499-506.
[138] Goodyear LJ, Chang PY, Sherwood DJ, *et al*. Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. Am J Physiol 1996; 271: E403-8.
[139] Gomez-Cabrera MC, Borras C, Pallardo FV, *et al*. Decreasing Xanthine Oxidase Mediated Oxidative Stress Prevents Useful Cellular Adaptations to Exercise in Rats. J Physiol 2005.
[140] Gambini J, Gomez-Cabrera MC, Borras C, *et al*. Free [NADH]/[NAD(+)] regulates sirtuin expression. Arch Biochem Biophys 2011; 512: 24-9.
[141] Berchtold MW, Brinkmeier H, Muntener M. Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 2000; 80: 1215-65.
[142] Reid MB. Invited Review: redox modulation of skeletal muscle contraction: what we know and what we don't. J Appl Physiol 2001; 90: 724-31.
[143] Salminen A, Vihko V. Endurance training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation *in vitro*. Acta Physiol Scand 1983; 117: 109-13.
[144] Gomez-Cabrera MC, Domenech E, Romagnoli M, *et al*. Oral administration of vitamin C decreases muscle mitochondrial biogene-

sis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 2008; 87: 142-9.

[145] Ristow M, Zarse K, Oberbach A, *et al.* Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A 2009; 106: 8665-70.

[146] Borras C, Gomez-Cabrera MC, Vina J. The dual role of p53: DNA protection and antioxidant. Free Radic Res 2011; 45: 643-52.

[147] Huang LE, Arany Z, Livingston DM, *et al*. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 1996; 271: 32253-9.

[148] Palomero J, Broome CS, Rasmussen P, *et al.* Heat shock factor activation in human muscles following a demanding intermittent exercise protocol is attenuated with hyperthermia. Acta Physiol (Oxf) 2008; 193: 79-88.

[149] Ji LL. Exercise-induced modulation of antioxidant defense. Ann N Y Acad Sci 2002; 959: 82-92.

[150] Vasilaki A, McArdle F, Iwanejko LM, *et al*. Adaptive responses of mouse skeletal muscle to contractile activity: The effect of age. Mech Ageing Dev 2006; 127: 830-9.

[151] Williamson D, Gallagher P, Harber M, *et al.* Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle. J Physiol 2003; 547: 977-87.

[152] Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. Cell 1986; 47: 921-8.

[153] Cai D, Frantz JD, Tawa NE, Jr., *et al.* IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 2004; 119: 285-98.

[154] Yu BP, Chung HY. Adaptive mechanisms to oxidative stress during aging. Mech Ageing Dev 2006; 127: 436-43.

[155] Puigserver P, Wu Z, Park CW, *et al.* A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998; 92: 829-39.

[156] Gomez-Cabrera MC, Sanchis-Gomar F, Garcia-Valles R, *et al.* Mitochondria as sources and targets of damage in cellular aging. Clin Chem Lab Med 2012; 50: 1287-95.

[157] Derbre F, Gomez-Cabrera MC, Nascimento AL, *et al.* Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age (Dordr) 2011; 34: 669-79.

[158] Vigen R, Maddox TM, Allen LA. Aging of the United States Population: Impact on Heart Failure. Curr Heart Fail Rep 2012.

[159] Abbatecola AM, Fumagalli A, Bonardi D, *et al.* Practical management problems of chronic obstructive pulmonary disease in the elderly: acute exacerbations. Curr Opin Pulm Med 2011; 17 Suppl 1: S49-54.

[160] Demontiero O, Vidal C, Duque G. Aging and bone loss: new insights for the clinician. Ther Adv Musculoskelet Dis 2012; 4: 61-76.

[161] Carreca I, Balducci L, Extermann M. Cancer in the older person. Cancer Treat Rev 2005; 31: 380-402.

[162] Caspersen CJ, Thomas GD, Boseman LA, *et al.* Aging, diabetes, and the public health system in the United States. Am J Public Health 2012; 102: 1482-97.

Received: June 13, 2013 **Accepted: September 24, 2013**

[163] Pimenta E, Oparil S. Management of hypertension in the elderly. Nat Rev Cardiol 2012; 9: 286-96.

[164] Pescatello LS, Franklin BA, Fagard R, *et al.* American College of Sports Medicine position stand. Exercise and hypertension. Med Sci Sports Exerc 2004; 36: 533-53.

[165] Haskell WL, Lee IM, Pate RR, *et al.* Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 2007; 39: 1423-34.

[166] Goodyear LJ. The exercise pill--too good to be true? N Engl J Med 2008; 359: 1842-4.

[167] Wojtaszewski JF, Nielsen P, Hansen BF, *et al.* Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle. J Physiol 2000; 528 Pt 1: 221-6.

[168] Winder WW, Taylor EB, Thomson DM. Role of AMP-activated protein kinase in the molecular adaptation to endurance exercise. Med Sci Sports Exerc 2006; 38: 1945-9.

[169] Narkar VA, Downes M, Yu RT, *et al.* AMPK and PPARdelta agonists are exercise mimetics. Cell 2008; 134: 405-15.

[170] Reznick RM, Zong H, Li J, *et al.* Aging-associated reductions in AMP-activated protein kinase activity and mitochondrial biogenesis. Cell Metab 2007; 5: 151-6.

[171] Thomson DM, Brown JD, Fillmore N, *et al.* AMP-activated protein kinase response to contractions and treatment with the AMPK activator AICAR in young adult and old skeletal muscle. J Physiol 2009; 587: 2077-86.

[172] Sanchis-Gomar F, Gomez-Cabrera MC, Vina J. The loss of muscle mass and sarcopenia: Non hormonal intervention. Exp Gerontol 2011.

[173] Baur JA. Resveratrol, sirtuins, and the promise of a DR mimetic. Mech Ageing Dev; 131: 261-9.

[174] Lagouge M, Argmann C, Gerhart-Hines Z, *et al.* Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006; 127: 1109-22.

[175] Pearson KJ, Baur JA, Lewis KN, *et al.* Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 2008; 8: 157-68.

[176] Barger JL, Kayo T, Vann JM, *et al.* A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice. PLoS One 2008; 3: e2264.

[177] Ungvari Z, Labinskyy N, Mukhopadhyay P, *et al.* Resveratrol attenuates mitochondrial oxidative stress in coronary arterial endothelial cells. Am J Physiol Heart Circ Physiol 2009; 297: H1876-81.

[178] Anderson R, Prolla T. PGC-1alpha in aging and anti-aging interventions. Biochim Biophys Acta 2009; 1790: 1059-66.

[179] Jackson JR, Ryan MJ, Alway SE. Long-term supplementation with resveratrol alleviates oxidative stress but does not attenuate sarcopenia in aged mice. J Gerontol A Biol Sci Med Sci; 66: 751-64.

[180] O'Donovan G, Blazevich AJ, Boreham C, *et al.* The ABC of Physical Activity for Health: a consensus statement from the British Association of Sport and Exercise Sciences. J Sports Sci 2010; 28: 573-91.

and 5 of 11 patients with IgM MGUS (45%), as defined by consensus criteria,[1] were positive for MYD88 L265P expression by either conventional or quantitative AS-PCR assays (Fig. 1).

These studies would suggest that IgM MGUS itself is heterogeneous and that MYD88 L265P is probably a driver mutation. This mutation could give a competitive growth advantage to an early, possibly Waldenström's macroglobulinemia–bound clone and even a predisposition to other mutations that facilitate progression to symptomatic Waldenström's macroglobulinemia. Additional common somatic mutations in *CXCR4*, *ARID1A*, *MUC16*, *TRAF2*, and *TRRAP* have been revealed by whole-genome sequencing in patients with Waldenström's macroglobulinemia, which could serve as triggers for progression from IgM MGUS.[3] Thus, longitudinal genomic studies are needed to delineate such a "multihit" hypothesis and to clarify the role of MYD88 L265P as a precursor to Waldenström's macroglobulinemia, as compared with other IgM-secreting B-cell cancers.

Steven P. Treon, M.D., Ph.D.
Lian Xu, M.S.
Zachary R. Hunter, M.A.

Dana–Farber Cancer Institute
Boston, MA
steven_treon@dfci.harvard.edu

Since publication of their article, the authors report no further potential conflict of interest.

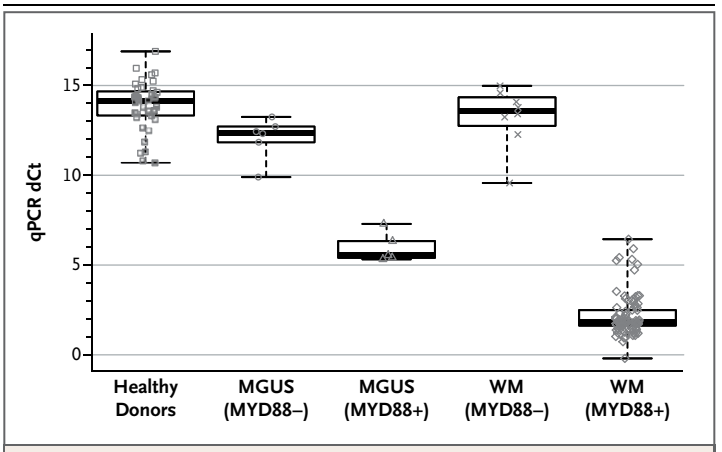


Figure 1. MYD88 L265P Expression in Healthy Donors and in Patients with IgM MGUS and Waldenström's Macroglobulinemia.

Shown are allele-specific values for CD19+ selected cells from 40 healthy donors (10 samples from bone marrow aspirates and 30 from peripheral blood) and from 11 patients with IgM monoclonal gammopathy of undetermined significance (MGUS) and 96 patients with Waldenström's macroglobulinemia (WM) (all samples from bone marrow aspirates) in whom the diagnosis was determined by consensus criteria.[1] The values were calculated by means of quantitative polymerase-chain-reaction (PCR) assay expressed as the difference in cycle threshold (dCt) between the gene of interest and the endogenous control. The disease groups are stratified according to the presence or absence of the MYD88 variant, with smaller qPCR dCT values indicating greater expression. Median values are shown by thick horizontal lines and interquartile and total ranges by box and whisker plots, respectively, for each group with an overlay of individual data points. Pairwise comparisons of MYD88 L265P expression versus wild-type expression were highly significant ($P<0.001$ by Tukey's HSD [honestly significant difference] test).

1. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. Semin Oncol 2003;30:110-5.
2. Xu L, Hunter ZR, Yang G, et al. Detection of the MYD88 L265P mutation in Waldenström's macroglobulinemia using a highly sensitive allele-specific PCR assay. J Clin Oncol 2012;30:Suppl:8042. abstract.
3. Hunter ZR, Xu L, Yang G, et al. Use of whole genome sequencing to identify highly recurrent somatic mutations in Waldenström's macroglobulinemia. J Clin Oncol (in press).
DOI: 10.1056/NEJMc1211959

The Skeletal Muscle–Metabolism Axis in Prostate-Cancer Therapy

TO THE EDITOR: Basaria and Bhasin (Sept. 6 issue)[1] report that men with prostate cancer who are receiving androgen-deprivation therapy have multiple adverse events. The authors included follistatin and irisin between the main targets of investigation to prevent and treat the metabolic side effects associated with androgen-deprivation therapy. It has been shown that exercise induces a marked increase in plasma and muscle follistatin and irisin.[2-4] Follistatin expression changes in both the muscle and fat of diabetic rats and can be modulated by exercise.[5] Concentrations of irisin increase significantly after exercise in both mice and humans, and irisin levels in the blood are correlated with irisin messenger RNA levels in muscle tissue.[4] All these facts underscore the importance of exercise with regard to the overexpression of both myokines and, therefore, with regard to the prevention and treatment of sarcopenia, frailty, and the cardiometabolic complications of androgen-deprivation therapy. Although it might not be feasible for

some patients, physicians should strongly recommend exercise or simple physical activity as an adjuvant therapy for patients who have prostate cancer and are receiving androgen-deprivation therapy.

Fabian Sanchis-Gomar
University of Valencia
Valencia, Spain
fabian.sanchis@uv.es

No potential conflict of interest relevant to this letter was reported.

1. Basaria S, Bhasin S. Targeting the skeletal muscle–metabolism axis in prostate-cancer therapy. N Engl J Med 2012;367:965-7.
2. Hansen J, Brandt C, Nielsen AR, et al. Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. Endocrinology 2011;152:164-71.
3. Wu J, Boström P, Sparks LM, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366-76.
4. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463-8.
5. Dutra DB, Bueno PG, Silva RN, et al. Expression of myostatin, myostatin receptors and follistatin in diabetic rats submitted to exercise. Clin Exp Pharmacol Physiol 2012;39:417-22.

DOI: 10.1056/NEJMc1211955

THE AUTHORS REPLY: We agree with Sanchis-Gomar that men undergoing androgen-deprivation therapy should be encouraged to engage in physical activity.[1] Indeed, recent short-term studies have shown that both resistance and aerobic exercises improve the level of fatigue, quality of life, and muscle strength in men undergoing androgen-deprivation therapy.[2] Physical activity is also likely to be beneficial in improving metabolic factors, even though data from controlled studies are lacking in this patient population. However, owing to a variety of coexisting conditions, some men undergoing androgen-deprivation therapy may not be able to engage in any form of exercise.[3,4] Therefore, research is needed to evaluate the role of novel ligands that can potentially have an effect on the prevention and treatment of frailty and cardiometabolic complications of androgen-deprivation therapy.

Shalender Bhasin, M.D.
Shehzad Basaria, M.D.
Boston University School of Medicine
Boston, MA
shehzad.basaria@bmc.org

Since publication of their article, the authors report no further potential conflict of interest.

1. Basaria S. Androgen deprivation therapy, insulin resistance, and cardiovascular mortality: an inconvenient truth. J Androl 2008;29:534-9.
2. Segal RJ, Reid RD, Courneya KS, et al. Randomized controlled trial of resistance or aerobic exercise in men receiving radiation therapy for prostate cancer. J Clin Oncol 2009;27:344-51.
3. Basaria S, Lieb J II, Tang AM, et al. Long-term effects of androgen deprivation therapy in prostate cancer patients. Clin Endocrinol (Oxf) 2002;56:779-86.
4. Bylow K, Dale W, Mustian K, et al. Falls and physical performance deficits in older patients with prostate cancer undergoing androgen deprivation therapy. Urology 2008;72:422-7.

DOI: 10.1056/NEJMc1211955

There Is More to Life Than Death

TO THE EDITOR: The Perspective article by Hartzband and Groopman (Sept. 13 issue)[1] contains factual errors about the U.S. Preventive Services Task Force (USPSTF) recommendations. The assertion that the USPSTF "concluded that the absolute benefit from routine mammograms in women 40 to 49 years was insufficient to offset the harm" is incorrect. The USPSTF recommendation states that "for biennial screening mammography in women aged 40 to 49 years, there is moderate certainty that the net benefit is small." Thus, the decision "should be an individual one and take patient context into account, including the patient's values regarding specific benefits and harms."[2]

The USPSTF did not base its recommendation against prostate-specific antigen (PSA) screening "largely on the U.S. Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial." As stated in the recommendation, "two major trials of PSA screening were considered by the USPSTF: the U.S. PLCO study and the European Randomized Study of Screening for Prostate Cancer (ERSPC)."[3] The PLCO study showed no reduction in mortality, whereas the ERSPC showed a very small reduction. In making the assessment that the benefit of screening does not outweigh the harms, the USPSTF weighed the very small potential mortality benefit against the known morbidity and mortality from screening, biopsies, and especially overtreatment of screen-detected cancers.

Although the decline in prostate-cancer mortality in the United States over the past 20 years

Experimental Gerontology 46 (2011) 967–969

Contents lists available at SciVerse ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Commentary

The loss of muscle mass and sarcopenia: Non hormonal intervention

Fabian Sanchis-Gomar *, Mari Carmen Gómez-Cabrera, Jose Viña

Department of Physiology, Faculty of Medicine, University of Valencia, Fundacion Investigacion Hospital Clinico Universitario/INCLIVA, Spain

ARTICLE INFO

Article history:
Received 23 July 2011
Received in revised form 26 August 2011
Accepted 28 August 2011
Available online 3 September 2011

Section Editor: Christiaan Leeuwenburgh

Keywords:
Aging
Life span
Muscle atrophy
Immobilization
Hormonal treatments

ABSTRACT

Muscle aging is a key component of the increase in frailty in human populations. The generation of critical levels of power is a prerequisite to perform simple tasks of daily living, such as rising from a chair or climbing stairs. There is great scientific and social interest to determine which behaviors can lead to the maintenance of the muscle mass in young immobilized subjects and in the elderly. Several hormonal treatments have been proposed for the treatment of sarcopenia. However, the side effects associated to these treatments emphasize the need of finding non-toxic and non-hormonal treatments that help increase muscle strength, improve muscle function, and decrease the degree of dependency in the old population. Recently, several studies have shed new light on this topic. Any medical efforts to develop treatments to prevent muscle dysfunction leading to sarcopenia, and eventually frailty, will be a major breakthrough in the public health in advances countries. Moreover, any significant improvement in the loss of muscle function will be a major breakthrough in the health and welfare of the population.

Muscle atrophy occurs as a consequence of denervation, injury, joint immobilization, bed rest, glucocorticoid treatment, sepsis, cancer and aging (Jagoe and Goldberg, 2001). Recent advances in medical care as well as in basic gerontology have led to a significant increase in life span. However, this remarkable increase has also led to enhance frailty and dependency (Vanitallie, 2003). Muscle aging is a key component of the increase in frailty in human and animal populations. There is great scientific and social interest to determine which behaviors can lead to the maintenance of the muscle mass in young immobilized subjects and in the elderly. Recently, several studies have shed new light on this topic.

Burks et al. (Burks et al., 2011) were interested in the beneficial impact on the muscle remodeling process of sarcopenic mice by losartan, an angiotensin II receptor antagonist (ARA) commonly used to treat high blood pressure. In their study, they showed that immobilized mice treated with losartan were protected against loss of muscle mass and that this protective mechanism was mediated by an increased activation of the insulin-like growth factor 1 (IGF-1)/Akt/mammalian target of rapamycin (mTOR) pathway. Thus, blockade of the AT1 (angiotensin II type I) receptor improved muscle remodeling and protected against disuse atrophy.

Moreover, another ARA, telmisartan, has been suggested to improve skeletal muscle function and to prevent adipogenesis and weight gain through activation of PPAR-delta-dependent pathways (Feng et al., 2010; He et al., 2010). Consequently, telmisartan may be used as a pharmacological intervention to treat sarcopenia.

The generation of critical levels of power is a prerequisite to perform simple tasks of daily living, such as rising from a chair or climbing stairs. For a young healthy person these activities can be performed easily. But after a prolonged period of forced inactivity (such as during the recovery of a sport injury or prolonged immobilization) when loss of muscle mass occurs, simple tasks become increasingly difficult. In these situations the loss of muscle mass and function has serious implications for independent living, quality of life and undoubtedly for the recovery for the daily life activities or sport practice. Approximately 25% of free living persons 65 years old or more, suffer from frailty. This is a geriatric syndrome closely related to an increased risk of falling, morbidity and mortality (Seguin and Nelson, 2003). Senile sarcopenia defined as the loss of muscle bulk and force associated to aging is the main component of frailty (Rosenberg, 1997). Several hormonal treatments have been proposed for the treatment of sarcopenia: growth hormone, IGF-1, testosterone, selective androgen receptor modulators (SARMs), hGH secretagogues, stanozolol, estrogens and tibolone (Onder et al., 2009). However, the side effects associated to these treatments emphasize the need of finding non-toxic and non-hormonal treatments that help increase muscle strength, improve muscle function, and decrease the degree of dependency in the old population.

Otherwise, another important tool in the prevention of sarcopenia is exercise. Multitude of studies shows that exercise, including resistance or power training, have positive effects on sarcopenia (Fiatarone et al.,

* Corresponding author at: Department of Physiology, Faculty of Medicine, University of Valencia, Av. Blasco Ibañez, 15, Valencia 46010, Spain. Tel.: +34 96 386 46 50; fax: +34 96 386 46 42.
E-mail address: fabian.sanchis@uv.es (F. Sanchis-Gomar).

0531-5565/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.exger.2011.08.012

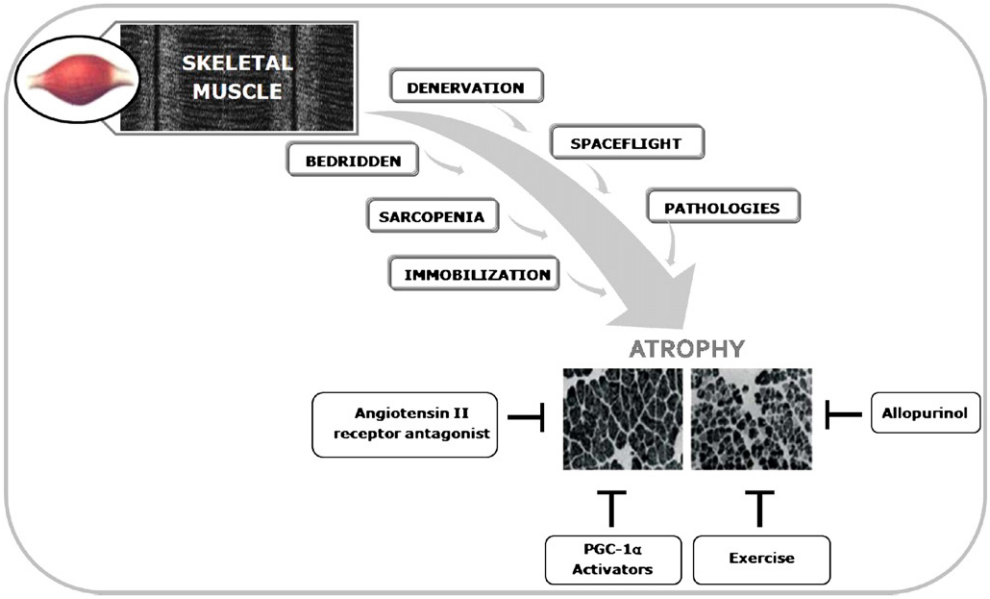


Fig. 1. Possible non hormonal medical interventions to prevent the loss of muscle mass and sarcopenia.

1990; Freiberger et al., 2011; Liu and Latham, 2009; Nicola and Catherine, 2010; Peterson et al., 2010; Steib et al., 2010). Thus, old population is encouraged to maintain high levels of exercise by means of resistance, power, or functional training.

Causes of aging are very complex. Prominent factors involved in the age-related loss of muscle mass are: programmed cell death, oxidative stress, lowering in the rate of protein synthesis, inflammation, lowering levels of anabolic hormones, and finally, mitochondrial dysfunction (Balagopal et al., 2001; Doherty, 2003; Janssen et al., 1993). Many authors have related sarcopenia with alterations in mitochondrial DNA, in both animals and humans (Melov et al., 2007). As postulated by Miquel in the 70s (Miquel et al., 1980; Rockstein et al., 1975), and later confirmed by our group (Sastre et al., 1996), mitochondrial damage is associated with aging, and in particular with aging of the muscle cells.

Michael Reid and coworkers reported in 2004 that allopurinol (an inhibitor of xanthine oxidase commonly used in clinical practice to treat hyperuricemia) causes a decrease in the contractile dysfunction caused by hind limb unloading (Matuszczak et al., 2004). Moreover suppression of xanthine oxidase activity by allopurinol can increase maximal isometric force in the plantar flexor muscles of aged mice after repetitive electrically evoked contractions (Ryan et al., 2011).

Researchers have identified the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) as the master regulator of mitochondriogenesis in mammalian tissues (Puigserver and Spiegelman, 2003; Puigserver et al., 1998; Wu et al., 1999). Modulation of PGC-1α levels in skeletal muscle present an avenue for the prevention and treatment of age-related disorders (Sandri et al., 2006). Recently, we have found an age-associated lack of expression of PGC-1α in response to exercise training or to other stimuli such as cold induction or thyroid hormone treatment (Derbre et al., 2011). We observed that aged rodents behave as PGC-1α KO ones. Our results highlight the role of PGC-1α in the loss of mitochondriogenesis associated with aging and point to this important transcriptional coactivator as a target for pharmacological interventions to prevent age-associated sarcopenia (Derbre et al., 2011).

Promoting the quality of life of elderly persons (health span) by preventing the age-associated decrease in muscle mass is one of the most important challenges in current clinical medicine. On the other hand, preventing the muscle mass loss due for instance to immobilization is one of the most important aims in today's medical care. The translation of discoveries in basic science into clinical care rarely comes easy but drugs such as losartan, telmisartan or allopurinol, all of them approved by the Food and Drug Administration for the treatment other diseases, may be useful to prevent muscle loss in the fields of Geriatrics, Sport Medicine, Traumatology, and Rehabilitation.

To conclude, any medical efforts to develop treatments, such as those reported in the Fig. 1, to prevent muscle dysfunction leading to sarcopenia, and eventually frailty, will be a major breakthrough in the public health in advanced countries. The economic and social consequences of this problem cannot be overestimated. In the US, the total expense associated with frailty and muscle dysfunction in 2009 was 18 billion dollars (Janssen et al., 2004). The amount of resources that we will have to allocate to deal with the vast amount of population who will be dependent is enormous. Any significant improvement in the loss of muscle function will be a major breakthrough in the health and welfare of the population.

References

Balagopal, P., Schimke, J.C., Ades, P., Adey, D., Nair, K.S., 2001. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am. J. Physiol. Endocrinol. Metab. 280, E203–E208.
Burks, T.N., Andres-Mateos, E., Marx, R., Mejias, R., Van Erp, C., Simmers, J.L., Walston, J.D., Ward, C.W., Cohn, R.D., 2011. Losartan restores skeletal muscle remodeling and protects against disuse atrophy in sarcopenia. Sci. Transl. Med. 3, 82ra37.
Derbre, F., Gomez-Cabrera, M.C., Nascimento, A.L., Sanchis-Gomar, F., Martinez-Bello, V.E., Tresguerres, J.A., Fuentes, T., Gratas-Delamarche, A., Monsalve, M., Vina, J., 2011. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age (Dordr). doi:10.1007/s11357-011-9264-y.
Doherty, T.J., 2003. Invited review: aging and sarcopenia. J. Appl. Physiol. 95, 1717–1727.
Feng, X., Luo, Z., Ma, L., Ma, S., Yang, D., Zhao, Z., Yan, Z., He, H., Cao, T., Liu, D., Zhu, Z., 2010. Angiotensin II receptor blocker telmisartan enhances running endurance of skeletal muscle through activation of the PPAR-δ/AMPK pathway. J. Cell. Mol. Med. 15 (7), 1572–1581.
Fiatarone, M.A., Marks, E.C., Ryan, N.D., Meredith, C.N., Lipsitz, L.A., Evans, W.J., 1990. High-intensity strength training in nonagenarians. Effects on skeletal muscle. JAMA 263, 3029–3034.
Freiberger, E., Sieber, C., Pfeifer, K., 2011. Physical activity, exercise, and sarcopenia — future challenges. Wien. Med. Wochenschr. doi:10.1007/s10354-011-0001-z.
He, H., Yang, D., Ma, L., Luo, Z., Ma, S., Feng, X., Cao, T., Yan, Z., Liu, D., Tepel, M., Zhu, Z., 2010. Telmisartan prevents weight gain and obesity through activation of peroxisome proliferator-activated receptor-delta-dependent pathways. Hypertension 55, 869–879.
Jagoe, R.T., Goldberg, A.L., 2001. What do we really know about the ubiquitin–proteasome pathway in muscle atrophy? Curr. Opin. Clin. Nutr. Metab. Care 4, 183–190.
Janssen, Y.M.W., Van Houten, B., Borm, P.A.J., Mossman, B.T., 1993. Biology of disease: cell and tissue responses to oxidative damage. Lab. Invest. 69, 261–274.
Janssen, I., Shepard, D.S., Katzmarzyk, P.T., Roubenoff, R., 2004. The healthcare costs of sarcopenia in the United States. J. Am. Geriatr. Soc. 52, 80–85.
Liu, C.J., Latham, N.K., 2009. Progressive resistance strength training for improving physical function in older adults. Cochrane Database Syst. Rev. CD002759.

Matuszczak, Y., Arbogast, S., Reid, M.B., 2004. Allopurinol mitigates muscle contractile dysfunction caused by hindlimb unloading in mice. Aviat. Space Environ. Med. 75, 581–588.
Melov, S., Tarnopolsky, M.A., Beckman, K., Felkey, K., Hubbard, A., 2007. Resistance exercise reverses aging in human skeletal muscle. PLoS One 2, e465.
Miquel, J., Economos, A.C., Fleming, J., Johnson Jr., J.E., 1980. Mitochondrial role in cell aging. Exp. Gerontol. 15, 575–591.
Nicola, F., Catherine, S., 2010. Dose–response relationship of resistance training in older adults: a meta-analysis. Br. J. Sports Med. 45, 233–234.
Onder, G., Della Vedova, C., Landi, F., 2009. Validated treatments and therapeutics prospectives regarding pharmacological products for sarcopenia. J. Nutr. Health Aging 13, 746–756.
Peterson, M.D., Rhea, M.R., Sen, A., Gordon, P.M., 2010. Resistance exercise for muscular strength in older adults: a meta-analysis. Ageing Res. Rev. 9, 226–237.
Puigserver, P., Spiegelman, B.M., 2003. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr. Rev. 24, 78–90.
Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M., Spiegelman, B.M., 1998. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92, 829–839.
Rockstein, M., Chesky, J., Philpott, D.E., Takahashi, A., Johnson Jr., J.E., Miquel, J., 1975. An electron microscopic investigation of age-dependent changes in the flight muscle of Musca domestica L. Gerontologia 21, 216–223.
Rosenberg, I.H., 1997. Sarcopenia: origins and clinical relevance. J. Nutr. 127, 990S–991S.
Ryan, M.J., Jackson, J.R., Hao, Y., Leonard, S.S., Alway, S.E., 2011. Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice. Free Radic. Biol. Med. 51 (1), 38–52.
Sandri, M., Lin, J., Handschin, C., Yang, W., Arany, Z.P., Lecker, S.H., Goldberg, A.L., Spiegelman, B.M., 2006. PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc. Natl. Acad. Sci. U. S. A. 103, 16260–16265.
Sastre, J., Pallardo, F.V., Pla, R., Pellin, A., Juan, G., O'Connor, J.E., Estrela, J.M., Miquel, J., Vina, J., 1996. Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. Hepatology 24, 1199–1205.
Seguin, R., Nelson, M.E., 2003. The benefits of strength training for older adults. Am. J. Prev. Med. 25, 141–149.
Steib, S., Schoene, D., Pfeifer, K., 2010. Dose–response relationship of resistance training in older adults: a meta-analysis. Med. Sci. Sports Exerc. 42, 902–914.
Vanitallie, T.B., 2003. Frailty in the elderly: contributions of sarcopenia and visceral protein depletion. Metabolism 52, 22–26.
Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R.C., Spiegelman, B.M., 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98, 115–124.

British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2012.01970.x
www.brjpharmacol.org

REVIEW

Exercise acts as a drug; the pharmacological benefits of exercise

J Vina, F Sanchis-Gomar, V Martinez-Bello and MC Gomez-Cabrera

Department of Physiology, University of Valencia, Fundacion Investigacion Hospital Clinico Universitario/INCLIVA, Valencia, Spain

Correspondence
Professor Jose Viña, Department of Physiology, Faculty of Medicine, University of Valencia, Av. Blasco Ibañez, 15, Valencia, 46010 Spain. E-mail: jose.vina@uv.es

Keywords
health; dosing of exercise; contraindications of exercise; sport; training

Received
25 January 2012
Revised
11 March 2012
Accepted
16 March 2012

The beneficial effects of regular exercise for the promotion of health and cure of diseases have been clearly shown. In this review, we would like to postulate the idea that exercise can be considered as a drug. Exercise causes a myriad of beneficial effects for health, including the promotion of health and lifespan, and these are reviewed in the first section of this paper. Then we deal with the dosing of exercise. As with many drugs, dosing is extremely important to get the beneficial effects of exercise. To this end, the organism adapts to exercise. We review the molecular signalling pathways involved in these adaptations because understanding them is of great importance to be able to prescribe exercise in an appropriate manner. Special attention must be paid to the psychological effects of exercise. These are so powerful that we would like to propose that exercise may be considered as a psychoactive drug. In moderate doses, it causes very pronounced relaxing effects on the majority of the population, but some persons may even become addicted to exercise. Finally, there may be some contraindications to exercise that arise when people are severely ill, and these are described in the final section of the review. Our general conclusion is that exercise is so effective that it should be considered as a drug, but that more attention should be paid to the dosing and to individual variations between patients.

Abbreviations

ACSM, American College of Sports Medicine; ACTH, adrenocorticotropic hormone; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; BDNF, brain-derived neurotrophic factor; CHD, coronary heart disease; GDNF, glial cell-derived neurotrophic factor; HIF-1, hypoxia-inducible factor 1; HSF, heat shock factor; HSP, heat shock protein; IGF, insulin growth factor; MET, metabolic equivalent (estimated oxygen cost of 3.5 $mL \cdot min^{-1} \cdot kg^{-1}$); MnSOD, mitochondrial superoxide dismutase; PGC-1α, PPAR-γ coactivator-1α; ROS, reactive oxygen species; SCD, sudden cardiac death; VO_2, oxygen consumption

Exercise, movement and health: definitions

Health promotion is the science and art of helping people change their lifestyle to move towards a state of optimal health (O'Donnell, 1986). The World Health Organization defines health as 'Physical, mental, and social well-being, not merely the absence of disease and infirmity'. Physical fitness is defined as the physiologic state of well-being that allows one to meet the demands of daily living (health-related physical fitness) or that provides the basis for sport performance (performance-related physical fitness), or both. Although we are aware that there is a clear difference between the terms physical activity ('any bodily movement') and exercise ('a subset of physical activity that is characterized by a planned and purposeful training') (Caspersen *et al.*, 1985), in this review, we are going to use these two concepts as synonymous because some of the studies to which we will refer use the terms interchangeably.

Historical background

The hypothesis that physical activity promotes health and longevity is not new. As far back as 2500 BC, in ancient China, records of organized exercise for health promotion

have been found (Lyons and RJja, 1978; Lee and Skerrett, 2001). In Greco-Roman times, 2500 years ago, Hippocrates (460–370 BC) and later Galen (AD 129–210) recognized the need to promote and prescribe exercise for health-related benefits and the need to provide general medical care for the athletic individual (Speed and Jaques, 2010). In this regard, the philosopher Plato (427–347 BC) said: 'Lack of activity destroys the good condition of every human being while movement and methodical physical exercise saves and preserves it' (Fox and Haskell, 1968).

Simple comparisons of men in different occupations provided the first empirical evidence that physical activity was associated with health. The first studies demonstrating a significant inverse relationship between physical activity and coronary heart disease (CHD) were those conducted by Morris *et al*. (1953b) in London early in the 1950s. These authors found that London bus conductors had only 73% the frequency of CHD that was found in the less active bus drivers. Their later comparison of London postmen and less active postal clerks produced much the same findings (Morris *et al*., 1953a). These seminal studies were followed by those of Paffenbarger and collaborators in the 1970s, assessing the increase in the relative risk of death from any cause and from specific diseases associated with physical inactivity (Paffenbarger and Hale, 1975; Paffenbarger *et al*., 1978).

Exercise is beneficial for your health

Exercise is one of the most frequently prescribed therapies both in health and disease. There is irrefutable evidence showing the beneficial effects of exercise both to prevent and to treat several diseases. Researchers have shown that both men and women who report increased levels of physical activity and fitness have reductions in relative risk of death (by about 20%–35%) (Blair *et al*., 1989; Macera *et al*., 2003).

Recent research suggests that modest increments in energy expenditure due to physical activity (~1000 kcal per week) or an increase in physical fitness of 1 MET (metabolic equivalent) is associated with lowering mortality by about 20% (Myers *et al*., 2004). Physically inactive middle-aged women (engaging in less than 1 h of exercise per week) experience a 52% increase in all-cause mortality, a doubling of cardiovascular-related mortality, and a 29% increase in cancer-related mortality when compared with physically active ones (Hu *et al*., 2004). Thus, there is clear evidence that regular physical activity produces significant health effects and reduces the risk of premature death from any cause and from cardiovascular disease in particular amongst asymptomatic men and women.

The benefits of physical activity are evident, not only in healthy persons but also in patients. Observational and randomized trials have shown that regular physical activity contributes to the treatment of several chronic diseases (Bouchard *et al*., 1994; Warburton *et al*., 2006a). There is evidence for prescribing exercise in the primary and secondary prevention of pulmonary and cardiovascular diseases (CHD, chronic obstructive pulmonary disease, hypertension, intermittent claudication); metabolic disorders (type 2 diabetes, dyslipaemia, obesity, insulin resistance); muscle, bone and joint diseases (rheumatoid arthritis, fibromyalgia, chronic fatigue syndrome, osteoporosis); cancer; and depression (Pedersen and Saltin, 2006; Warburton *et al*., 2006a). Even if exercise is an effective therapeutic agent for all of these diseases, as with any other medicine, the dosage (volume and intensity of the exercise), frequency of administration (sessions per week), type (aerobic vs. resistance exercise), systemic and psychoactive effects and contraindications and side effects of the exercise must be taken into account to achieve the best clinical outcome. For instance, both resistance and aerobic training have been shown to be of benefit for the control of diabetes; however, resistance training may have greater benefits for glycaemic control than aerobic training (Dunstan *et al*., 2005).

The dosage of exercise

Dosage is important in clinical medicine and all marketed drugs require data on their efficacy and safety (Lee, 2007). It is known that there is a minimum amount of physical activity for health benefits. These benefits increase with increasing the amount of exercise, but beyond a certain level, adverse effects outweigh benefits (Lee, 2007). Unlike chemical drugs, however, the minimum dose, dose response and maximum safe dose of physical activity are not well understood (Lee, 2007). There is a continuous debate on how much, what type, how often, what intensity and how lengthy physical activity should be. This is important for issuing public health recommendations (Blair *et al*., 2004). Summarizing available information across studies is difficult because investigators have measured exercise intensity in different ways and classified physical activity according to different dose schemes that are often difficult to compare (Lee, 2007). Over the years, various expert groups, based on the best evidence available, have postulated different physical activity recommendations and guidelines (see Table 1).

Intensity levels of physical activity can be expressed relative to oxygen consumption (VO_2) or to heart rate (Warburton *et al*., 2006b). Moderate-intensity activities are those in which heart rate and breathing are raised; but, still, it is possible to speak comfortably. This occurs around 4–6 METs and brisk walking at 3.0 mph (80.4 $m \cdot min^{-1}$) is one such activity. Vigorous-intensity activities are that in which heart rate is higher, breathing is heavier and conversation is harder (about 6–8 METs) (Warburton *et al*., 2006b), for instance jogging. It has been shown that exercising at even 50% of the recommended levels (72 min of moderate exercise a week) appears sufficient to provide some improvement in fitness. However, at this low exercise dosage, cardiovascular risk factors (blood pressure, lipid profile and weight) do not improve (Church *et al*., 2007). In fact, for many individuals, up to 60 min of daily physical activity are more appropriate if weight control is the primary goal (Lee, 2007). Thus, dose–response relations between physical activity and different health outcomes are different. The evaluation of the minimum amount of physical activity (lower dose) necessary to achieve its beneficial effects has been the object of intense research. Wen *et al*. (2011) have recently found that 15 min a day or 90 min a week of moderate-intensity exercise is of

Table 1
Historical evolution in physical activity recommendations and guidelines

	Physical activity recommendations			
	Intensity	Minutes	Frequency	Reference
1970s–1980s	Vigorous exercise (e.g. running)	20 min·day^{-1}	3 times·week^{-1}	(American College of Sports Medicine, 1978)
1990s	Moderate exercise (e.g. brisk walking)	30 min·day^{-1}	Most days of the week	(Pate *et al.*, 1995; Physical activity and cardiovascular health, 1996)
2000s	Moderate exercise	60 min·day^{-1}	3 times·week^{-1}	(Lee, 2007)
2010 (healthy adults ages 18–45)	Moderate exercise	30 min·day^{-1} (150 min week^{-1})	Most days of the week (5 days·week^{-1})	(O'Donovan *et al.*, 2010)
	Vigorous exercise	75 min·week^{-1}		(O'Donovan *et al.*, 2010)

benefit in terms of life expectancy, even for subjects with cardiovascular risks.

Exhaustive exercise and longevity

Although the health benefits of leisure-time physical activity are well documented, the association between vigorous exercise training and mortality or longevity of elite athletes is not fully understood (Teramoto and Bungum, 2010). For centuries, the general belief has been that exhaustive, competitive exercise is harmful and decreases life expectancy (Ruiz *et al.*, 2010). For instance, Moorstein (1968) stated that all members of the 1948 Harvard rowing crew had died early from cardiac diseases. In contrast, it has been shown that participation in endurance competitive sports increases life expectancy. It was found that the life expectancy of oarsmen was higher than that of their non-athletic controls (Hartley and Llewellyn, 1939; Prout, 1972). Karvonen and co-workers found that Finnish champion skiers (born between 1845 and 1910) lived 2.8–4.3 years longer than general male population in Finland. In contrast with most studies of that time, Polednak (1972) reported evidence against the beneficial effects of strenuous exercise. He found differences in longevity and cardiovascular mortality related to the extent of participation in college athletics. Moreover, in a recent animal study, it has been found that long-term vigorous endurance exercise training may in some cases promote adverse cardiac remodelling and produce a substrate for cardiac arrhythmias (Benito *et al.*, 2011). The incidence of sudden cardiac death (SCD) amongst young athletes (estimated to be 1–3 per 100 000 person-years) is higher than in non-athletes and may possibly still be underestimated (Drezner, 2008). However, it has been shown that the most common cause of SCD in young athletes is underlying inherited cardiac disease, such as cardiomyopathies, congenital coronary anomalies and ion channelopathies (Maron *et al.*, 2009). To clarify this apparent contradiction, we determined the longevity of the participants of the Tour de France and compared it with that of the general population born between 1892 and 1942. The Tour de France is amongst the most gruelling sport events in the world. We found an 11% increase in average longevity in Tour de France participants when compared with the general population (Sanchis-Gomar *et al.*, 2011b) (see Figure 1). Thus, the majority of data in human studies support the notion that prescription of regular, vigorous aerobic exercise might be a useful tool, with a dose–effect response to improve the overall health status and longevity of the general population (Ruiz *et al.*, 2010; Teramoto and Bungum, 2010). In our opinion, physicians, health professionals and general population should not be under the impression that strenuous exercise and/or high-level aerobic competitive sports are bad for one's health and shorten one's life. Thus, a dose–response relation appears to exist, such that people who have the highest levels of physical activity and fitness are at lowest risk of premature death (Warburton *et al.*, 2006a).

Training status is a very relevant factor in the prescription of the exercise 'dose'. Increasing the doses of exercise has positive consequences for health in trained individuals (Ruiz *et al.*, 2010; Sanchis-Gomar *et al.*, 2011b), whereas heavy physical exertion can trigger the onset of acute myocardial infarction, particularly in people who are habitually sedentary (Mittleman *et al.* 1993). Results from the same group showed that less active men participating in vigorous activity were more likely to have a myocardial infarction during exercise than the most active men (Thompson *et al.*, 2007).

In the pharmacological treatment of many conditions, physicians typically start with a dose of a drug believed to be the minimum effective dose. If the patient does not respond, this initial dose may then be titrated upwards to a maximum dose, beyond which the adverse effects of the drug are unacceptable for treatment (Lee, 2007). Thus, the intensity of aerobic training may be also titrated in healthy people (Warburton *et al.*, 2006b). Unfit people can get significant improvements in physical fitness with a low training intensity, while those with a higher fitness level need a greater level of exercise intensity to achieve further improvements in fitness (Shephard, 2001). Thus, these fit individuals who have met the physical activity levels recommended for all healthy adults for at least 6 months may obtain additional health benefits by engaging in 300 min or more of moderate-intensity aerobic activity per week, or 150 min or more of vigorous-intensity aerobic activity each week, or equivalent combinations of moderate- and vigorous-intensity aerobic activities (Lee and Skerrett, 2001; O'Donovan *et al.*, 2010). These relatively low doses are, obviously, not applicable to

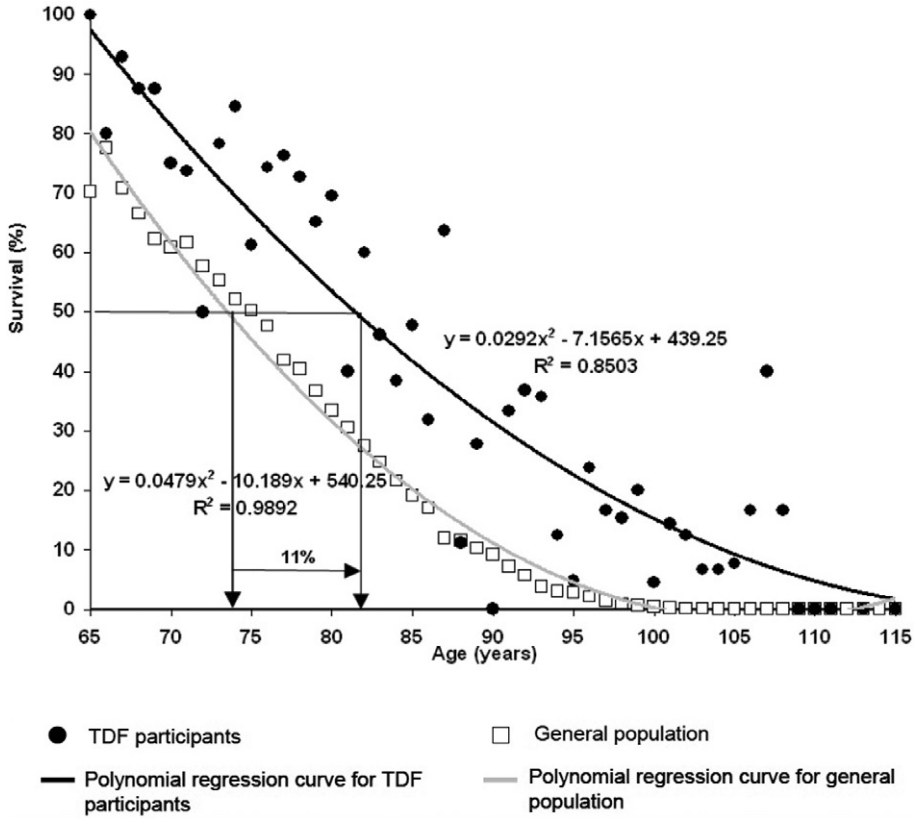


Figure 1

Percentage of survival related to age in Tour de France (TdF) participants and in the general population. Persons born between 1892 and 1942 have been studied. Average life span of Tour de France participants is higher ($P = 0.004$; 17.5%) than the general population of the same country in which the cyclists were born. The age at which 50% of the general population died was 73.5 years, compared with 81.5 years in Tour de France participants (i.e. 11% increase).

high level professional athletes who perform exercise at much higher doses.

The guidelines discussed above are generally appropriate for young to middle-aged adults. But, as with medicines, special considerations should be taken when prescribing exercise for people with special needs such as elderly, children, pregnant women, overweight or obese patients and patients with chronic diseases (Warburton *et al.*, 2006b). For instance, it has been shown that vigorous activities are not essential for the reduction of cardiovascular risk in men over 60. Regular physical activity is enough to achieve a significant decrease in mortality in this population. Thus, the greatest benefit to health is gained from sustained moderate exercise, above which there appears to be no further benefit to health in older men (Hakim *et al.*, 1998; Wannamethee *et al.*, 1998).

Regarding the 'dosage' of exercise, whether it should be performed in either one continuous or two or more accumulated bouts, the available evidence suggest that at least for fitness, accumulated and continuous patterns of exercise training of the same total duration confer similar benefits (Murphy *et al.*, 2009). For instance, it has been shown that five to eight 2 min bouts of stair climbing accumulated over the course of a day confer health benefits, including increases in cardiovascular fitness, compared with non-exercising controls (Boreham *et al.*, 2005).

Although physical activity is beneficial to health with or without weight loss, adults who find it difficult to maintain a normal weight and adults with increased risk of cardiovascular disease or type 2 diabetes, in particular, may benefit from going beyond the levels of activity recommended for all healthy adults and gradually progressing towards meeting the recommendations for conditioned individuals (O'Donovan *et al.*, 2010).

Systemic adaptations to exercise

The exercise-induced adaptations are especially evident in the cardiorespiratory and musculoskeletal systems, and body composition and metabolism (Warburton *et al.*, 2006a; Lee *et al.*, 2010). But the documented health benefits of exercise also include diminished symptoms of depression and anxiety (Kujala, 2011).

Skeletal muscle is the main target of exercise training. Modifications in skeletal muscle are crucial for enhancing endurance and metabolic efficiency (Matsakas and Narkar, 2010). Muscle fibres are commonly classified as type I slow-twitch or oxidative fibres, with a high mitochondrial content, and type II fast-twitch or glycolytic fibres, which have fewer mitochondria. Endurance exercise induces an increase in

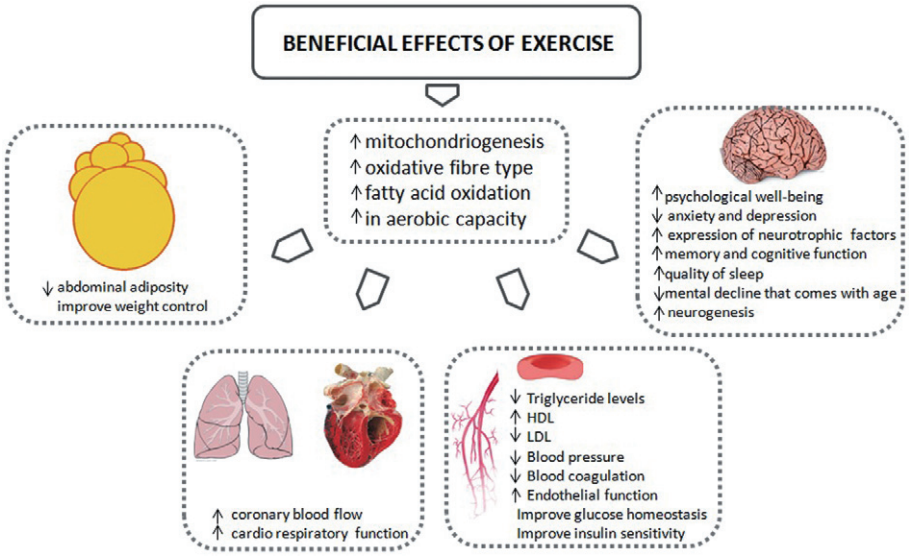


Figure 2
Health benefits of exercise in tissues and organs.

mitochondriogenesis, a shift in fibre distribution from glycolytic to oxidative and an increase in fatty acid oxidation that ultimately leads to an increase in aerobic capacity and retards diseases such as obesity, type 2 diabetes and cardiovascular diseases (Holloszy and Coyle, 1984; Mootha *et al.*, 2003).

It has been shown that regular exercise can reduce abdominal adiposity and improve weight control (Warburton *et al.*, 2006a), enhance lipoprotein profiles (e.g. reduce triglyceride levels, increase high density lipoprotein and decrease low-density lipoprotein levels), improve glucose homeostasis and insulin sensitivity, reduce blood pressure, improve autonomic tone, reduce systemic inflammation; decrease blood coagulation, improve coronary blood flow, augment cardiac function and enhance endothelial function (Warburton *et al.*, 2006a).

Regular physical activity is also associated with improved psychological well-being (e.g. through reduced stress, anxiety and depression) (Dunn *et al.*, 2001). The beneficial effects of exercise on cognitive function are well known (Neeper *et al.*, 1995). The mechanism behind this is not fully understood, but it seems to be associated with an increased expression of neurotrophic factors in some brain areas. Increased expression of these factors is related to better memory and improved cognitive function. Brain-derived neurotrophic factor (BDNF) can enhance the survival and differentiation of neurons, and voluntary exercise has been shown to increase it (Neeper *et al.*, 1996). Psychological well-being is particularly important for the prevention and management of cardiovascular disease, but it also has important implications for the prevention and management of other chronic diseases such as diabetes, osteoporosis, hypertension, obesity, cancer and depression (Warburton *et al.*, 2006a). It has been shown that physical activity results in specific adaptations that affect individual states in all of these diseases. For instance, adaptations that affect glucose homeostasis, in type 2 diabetes, are of great importance. Several changes occur as a result of regular physical activity, including increased glycogen synthase and hexokinase activities, increased mRNA and protein expression of the glucose transporter GLUT-4 and improved muscle capillary density (resulting improved glucose delivery to the muscle) (Mandroukas *et al.*, 1984).

Exercise causes a significant reduction in cancer rates (specifically colon and breast cancer) (Shephard and Futcher, 1997; Pedersen and Saltin, 2006). Possible explanations include reductions in fat stores, increased energy expenditure offsetting a high fat diet, activity-related changes in sex hormone levels, immune function, insulin and insulin-like growth factors, free radical generation and direct effects on the tumour cell biology (Westerlind, 2003).

The majority of the proposed mechanisms have been discussed in the context of chronic adaptations by regular physical activity. However, it has been shown that isolated exercise sessions (separate doses of exercise) also elicit transient, but still beneficial, changes in risk factors for chronic diseases (Thompson *et al.*, 2001). Many of the training adaptations derive from a single exercise bout that elicits cellular changes at the gene level leading to cumulative effects of training. The acute effect of exercise results in transient reductions in triglyceride levels, increases in HDL cholesterol level, decreases in blood pressure, reductions in insulin resistance and improvements in glucose control (Thompson *et al.*, 2001). These acute changes underpin the important role that individual exercise sessions have on health status. Thus, single doses of exercise have also a relevant impact on health. Figure 2 summarizes the favourable effects of exercise.

Signalling pathways regulated by exercise in skeletal muscle

The regulation of the cellular functions with exercise are dependent on many stimuli: alterations in metabolite con-

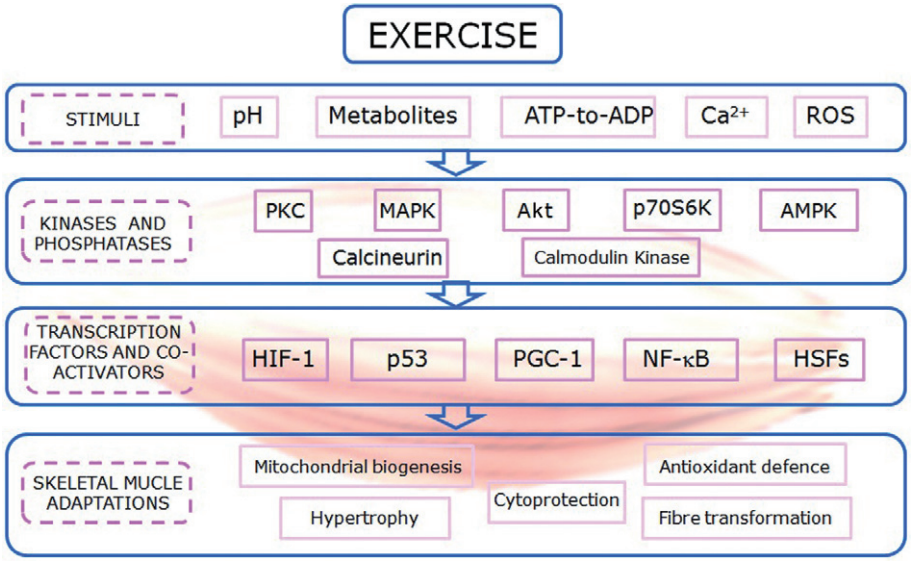


Figure 3
A summary of the signalling pathways regulated by exercise in skeletal muscle.

centrations, a shift in the ATP : ADP ratio, changes in the intracellular concentration of Ca^{2+}, in the intracellular pH, and activations of the oxidative stress-sensitive signalling pathways (Sakamoto and Goodyear, 2002; Ji *et al.*, 2004). To elucidate the molecular signalling mechanisms that enable skeletal muscle to respond to the contractile stimulus and that mediate the adaptations to exercise is of great importance (Sakamoto and Goodyear, 2002). It has been clearly established that physical exercise can activate MAPK signalling, including the ERK1/2 (Goodyear *et al.*, 1996), p38 (Gomez-Cabrera *et al.*, 2005) and JNK pathways (Goodyear *et al.*, 1996). It can also increase the activity of the AMP-activated protein kinase (AMPK), Akt and the p70 S6 kinase (Sakamoto and Goodyear, 2002). In skeletal muscle Ca^{2+}signalling is extensive. In addition to triggering muscle contraction through the troponin system, Ca^{2+} is also involved in the regulation of relevant intracellular proteins such as PKC, calcineurin and calmodulin kinase that mediate cellular signal transduction (Berchtold *et al.*, 2000).

More recently, it has been shown, that low-to-moderate levels of reactive oxygen species (ROS) play multiple regulatory roles in cells such as the control of gene expression, regulation of cell signalling pathways and modulation of skeletal muscle force production (Reid, 2001) (see Figure 3).

ROS and exercise: training as an antioxidant intervention

The role of ROS in the exercise-induced adaptations in skeletal muscle has been extensively studied (Salminen and Vihko, 1983; Gomez-Cabrera *et al.*, 2008b). The idea of the deleterious effects of ROS has been firmly entrenched in the minds of scientists during the last 30 years. However, there is growing evidence that the continued presence of low concentrations of free radicals is, in fact, able to induce the expression of antioxidant enzymes and other defence mechanisms. In this scenario, radicals may be seen as beneficial as they act as signals to enhance defences rather than deleterious as they are when cells are exposed to high levels of these radicals. Animals frequently exposed to chronic exercise have shown less oxidative damage after exhaustive exercise than untrained ones (Salminen and Vihko, 1983). This is largely due to the up-regulation of endogenous antioxidant enzymes such as glutathione peroxidase, mitochondrial superoxide dismutase (MnSOD) and γ-glutamylcysteine synthetase (Salminen and Vihko, 1983). A major conclusion that can be drawn from these results is that exercise itself acts as an antioxidant, because training increases the expression of antioxidant enzymes (Gomez-Cabrera *et al.*, 2008b). Subsequently, we and others have shown that antioxidant supplements prevent the induction of mitochondrial biogenesis, molecular regulators of insulin sensitivity and endogenous antioxidant defence by physical exercise (Gomez-Cabrera *et al.*, 2008a; Ristow *et al.*, 2009). Thus, ROS act as signals in exercise because decreasing their formation prevents activation of important signalling pathways, which cause useful adaptations in cells.

Because of the widespread implications of ROS in almost all important biological functions, it is difficult to define all the pathways and gene targets that are affected by redox signalling during exercise. The following are some of the most relevant signalling pathways modulated by exercise: the PPAR-γ coactivator-1α and β (PGC-1α and PGC-1β) (Gomez-Cabrera *et al.*, 2008a; Ristow *et al.*, 2009), p53 (Borras *et al.*, 2011), hypoxia-inducible factor 1 (HIF-1) (Huang *et al.*, 1996), heat shock factor (HSF) (Palomero *et al.*, 2008), NF-κB and MAPK signalling pathways (Ji *et al.*, 2004; Gomez-Cabrera *et al.*, 2005). Several important adaptations in skeletal muscle such as mitochondrial biogenesis, antioxidant defence, hypertrophy, cytoprotection and fibre transforma-

tion are regulated primarily by these pathways. Thus, its regulation should be tightly controlled (Gomez-Cabrera *et al.*, 2009) (see Figure 3).

Exercise, a psychoactive drug

The effects of exercise training on brain function have received much attention. In the early '80s, exercise was shown to increase β-endorphin in peripheral blood in humans (Bortz *et al.*, 1981; Carr *et al.*, 1981). Elevated serum β-endorphin concentrations induced by exercise have since been linked to a variety of psychological and physiological changes, including mood state changes and 'exercise-induced euphoria', altered pain perception and responses to numerous stress hormones (growth hormone, ACTH, prolactin, catecholamines and cortisol) (Harber and Sutton, 1984).

Exercise training can favourably influence cognitive function (Dishman *et al.*, 2006; Vaynman and Gomez-Pinilla, 2006). Exercise improves learning and memory (van Praag *et al.*, 1999), improves the quality of sleep, counteracts the mental decline that comes with age (Laurin *et al.*, 2001) and facilitates functional recovery from brain injury (Grealy *et al.*, 1999) and depression (Siuciak *et al.*, 1996; Shirayama *et al.*, 2002). Exercise is a very powerful stimulus to the induction of neurogenesis in the adult dentate gyrus (van Praag *et al.*, 1999) that can contribute to remodelling hippocampal synaptic circuits and to enhance cognitive function.

Exercise training can also mitigate the consequences of acute exposure to different types of psychological stress (Dishman *et al.*, 2006) and exercise-induced alterations in the 5-hydroxytryptaminergic and the noradrenergic systems can explain these responses (Dishman *et al.*, 2006). Most of the positive effects of exercise, as mentioned previously, have been related to the induction, in different brain areas, of neurotrophic proteins, including BDNF, glial cell-derived neurotrophic factor (GDNF) and insulin growth factor (IGF). Whether brain metabolic responses to acute physical activity extend beyond regions specifically involved with motor, sensory or cardiovascular autonomic control is not as yet clear (Dishman *et al.*, 2006). Transient increases in local cerebral glucose use and in cerebral blood flow have been reported in the different brain areas in response to acute strenuous treadmill running in rats and in humans (Vissing *et al.*, 1996). Also, the discharge rate of a select pool of hippocampal pyramidal cells increased as running velocity increased (Czurko *et al.*, 1999). Moreover, exercise increased metabolic capacity in the motor cortex and striatum (McCloskey *et al.*, 2001).

The psychoactive effects of exercise that we have just mentioned are not free from risks. Pathological patterns of behaviour in gym clients have been reported (Lejoyeux *et al.*, 2008). As observed in patients with eating disorders, active individuals usually worry about their body shape, put special attention on their eating patterns, show exercise addiction and have a perfectionism personality trait (Freimuth *et al.*, 2011). This body image disorder has been addressed as reverse anorexia, vigorexia or muscle dysmorphia (Lejoyeux *et al.*, 2008). Based on a review of a wide range of studies on exercise addiction, it has been estimated that its prevalence in the general population is close to 3%. Amongst certain groups such as ultra-marathon runners, body builders and sport science students, the percentage is even higher (Freimuth *et al.*, 2011; Sussman and Sussman, 2011).

Contraindications for exercise

The purpose of this section is to discuss why under some circumstances physical exercise does not increase the quality of life.

Although both the heart and the lung benefit significantly from physical activity, there are some contraindications when exercise is performed by patients suffering from heart and pulmonary diseases. Pedersen and Saltin (2006) reviewed the possible contraindications of exercise in most of the diseases in which exercise have shown beneficial effects. For instance in patients with CHD, exercise is contraindicated until the condition has been stable for at least 5 days; dyspnea at rest, aortic stenosis, pericarditis, myocarditis, endocarditis, fever and severe hypertension all are contraindications to exercise (Pedersen and Saltin, 2006). Black *et al.* (1975) were amongst the first to find that strenuous exercise can cause acute injury to coronary plaques, leading to occlusion of coronary arteries. However, years later, it was found that although the risk of primary cardiac arrest was transiently increased during a *single* bout of vigorous exercise, *habitual* vigorous exercise was associated with an overall decrease in this risk (Siscovick *et al.*, 1984; Albert *et al.*, 2000). There are no absolute contraindications to very moderate exercise in chronic obstructive pulmonary disease patients (Pedersen and Saltin, 2006). However, in patients with asthma, a pause in training is recommended when an acute exacerbation occurs. In cases of infection, a pause in training is recommended until the patient has been asymptomatic for a day, where after training can be slowly resumed (Pedersen and Saltin, 2006).

Regarding muscle, bone and joint diseases, for instance, osteoarthritis and rheumatoid arthritis, exercise is contraindicated in cases of acute joint inflammation, if pain worsens after training and in cases of pericarditis and pleuritis (Pedersen and Saltin, 2006). The training of patients with osteoporosis should include activities with a low risk of falling (Pedersen and Saltin, 2006).

In cancer patients being treated with chemotherapy or radiotherapy, exercise is contraindicated when leukocyte concentrations fall below 0.5×10^9 cells L^{-1}, haemoglobin below 100 $g \cdot L^{-1}$, thrombocyte concentration below 20×10^9 cells L^{-1} and temperature above 38°C. Patients with bone metastases should not perform strength conditioning at high load. In cases of infection, a pause in training is recommended until the patient has been asymptomatic for a day, where after training can be slowly resumed (Pedersen and Saltin, 2006). A major concern is whether exercise training influences the anticancer effects of conventional cytotoxic therapy. The potential interaction between exercise and chemotherapy efficacy is biologically plausible. Indeed, earlier preclinical studies have reported both an inhibitory (Baracos, 1989) and augmentary (Thompson *et al.*, 1989) effect of endurance exercise training on mammary tumour growth and progression, although others have reported no association (Jones *et al.*, 2005).

In diabetic patients (both types I and II), exercise should be postponed if blood glucose is >2.5 g·L^{-1} together with ketonuria and >3.0 g·L^{-1} even without ketonuria, in both cases, before it is corrected. In patients with hypertension and active proliferative retinopathy, high-intensity training or training involving Valsalva-like manoeuvres should be avoided. Patients with neuropathy and incipient foot ulcers should refrain from activities entailing the bearing of the patient's own body weight.

In metabolic syndrome-related disorders, such as insulin resistance, dyslipaemia and obesity, there are no general contraindications; but training should take into account any comorbidities (Pedersen and Saltin, 2006). Finally, hypertensive patients with a blood pressure >180/105 should begin pharmacotherapy before regular physical activity is initiated (relative contraindication) (Pescatello *et al.*, 2004). There is no evidence for an enhanced risk of sudden death or stroke in physically active persons with hypertension (Tipton, 1999). The American College of Sports Medicine (ACSM) recommends caution when performing very intensive dynamic exercise or strength conditioning with very heavy weights. Patients with left-sided cardiac hypertrophy should be particularly cautious about heavy strength conditioning. Patients with CHD should refrain from short intensive exercise situations.

It is well known that eccentric muscle contractions cause structural damage to muscle cells or inflammatory reactions within the muscles, as shown by an increase in the plasma activity of cytosolic enzymes and sarcolemma and Z-line disruption (Armstrong *et al.*, 1983). The severity of the damage and the extent of discomfort are exacerbated over time and can last for several days. The damaging effects of eccentric contractions can affect subsequent exercise sessions due to residual muscle pain, restriction of movement and reduced capacity to exercise at an intensity that may be beneficial for the exerciser (Howatson and van Someren, 2008). Thus, caution should be paid in exercise programs which include eccentric contractions especially in recreational or old practitioners.

Exercise mimetics

Despite the clear evidence showing the powerful influence of exercise on health, physical inactivity remains a pressing public health issue. Technology and economic incentives tend to discourage activity: technology by reducing the energy needed for activities of daily living and economics by paying more for sedentary than for physically active work (Haskell *et al.*, 2007). Moreover, endurance exercise can also be unapproachable for most people in whom it might be impractical because of physical limitations or, as mentioned in the previous section, side effects. This fact stimulates the search for exercise mimetics (or 'exercise pills') that mimic exercise and, therefore, it has been the focus of important research over the past decades (Goodyear, 2008).

As mentioned in a previous section, exercise improves performance by activating several pathways that induce genetic changes, particularly in skeletal muscle, to increase aerobic metabolism and vascularisation, to ultimately enhance performance (Narkar *et al.*, 2011). AMPK is activated by exercise and is essential for the exercise-mediated switch to aerobic myofibres in skeletal muscle (Wojtaszewski *et al.*, 2000). AMPK stimulates catabolic and suppresses anabolic pathways in an effort to restore cellular ATP levels and is activated robustly in skeletal muscle by acute as well as by chronic exercise (Winder *et al.*, 2006; Matsakas and Narkar, 2010). Recently, it was reported that 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) can mimic the effects of exercise by increasing GLUT-4 protein, hexokinase activity, resting glycogen content and muscle mitochondria (Narkar *et al.*, 2008). Mitochondrial biogenesis is activated by AMPK (Jorgensen *et al.*, 2007). This may be explained because AMPK is present in a transcriptional complex with PPAR-δ, where it can potentiate receptor activity via direct protein–protein interaction and/or by phosphorylating and activating coactivators such as PGC-1α (Jager *et al.*, 2007). Puigserver *et al.* (1998) identified PGC-1α as the master regulator of mitochondriogenesis and fuel homeostasis in mammalian tissues and Koves *et al.* (2005) observed that PGC-1α mediates metabolic remodelling of skeletal myocytes, mimics exercise and reverses lipid-induced mitochondrial inefficiency. Thus, exercise training and oxidative fibre type are associated with increased mRNA expression of PGC-1α (Lin *et al.*, 2002). Over-expression of PGC-1α in mice induces dramatic changes in skeletal muscle such as increased mitochondrial biogenesis and fibre remodelling (Lin *et al.*, 2005). Moreover, PGC-1α may be activated by the Ca^{2+}-signalling pathway involving both calcineurin and Ca^{2+}/calmodulin-dependent kinase and by p38 MAPK (Schiaffino *et al.*, 2007).

Recently, we have found an age-associated lack of reactivity of PGC-1α in response to exercise (Derbré *et al.*, 2012), as aged rats had the same response as mice with genetic deletion (knockout) of PGC-1α. Our results highlight the role of PGC-1α in the loss of mitochondriogenesis associated with aging and point to this important transcriptional co-activator as a target for pharmacological interventions to prevent age-associated sarcopenia (Derbré *et al.*, 2012). Modulation of PGC-1α levels in skeletal muscle is crucial for the prevention and treatment of age-related disorders (Sandri *et al.*, 2006). Accordingly, we recently proposed several pharmacological, non-hormonal, interventions to prevent the loss of muscle mass and sarcopenia such as PGC-1α activators, angiotensin II receptor antagonists and allopurinol (Sanchis-Gomar *et al.*, 2011a).

On the other hand, activation or over-expression of the transcription factor PPAR-δ in muscle also results in an increase in mitochondrial biogenesis and in the proportion of oxidative muscle fibres (Narkar *et al.*, 2008). This results in increased running endurance and protection against diet-induced obesity and type 2 diabetes. The contrary is also true: muscle-specific knockout of PPAR-δ results in an age-dependent loss of oxidative muscle fibres, running endurance and insulin sensitivity (Schuler *et al.*, 2006). Thus, several potent and selective PPAR-δ agonists such as GW1516 have been also identified and proposed as mimetic drugs for endurance exercise (Narkar *et al.*, 2008).

Resveratrol has also been considered as an exercise mimetic. High doses of resveratrol improve endurance (Lagouge *et al.*, 2006). The deacetylase enzyme Sir2 and its mammalian homologue SIRT1 have been identified as its putative primary targets. Resveratrol also activates AMPK in

cells in culture, and it has been proposed for the prevention of mitochondrial dysfunction (Ungvari *et al.*, 2011) and of the wasting disorders associated with mechanical unloading (Momken *et al.*, 2011).

Remodelling of skeletal muscle by exercise is extremely complex. Many targets are available to mimic muscle adaptations induced by exercise. However, exercise is linked to other multiple physiological adaptations that affect the vast majority of organs. It seems premature to conclude that all these molecules or substances are mimetics of exercise until the effects in other organs have been fully investigated. Applicability of these compounds at this point in time is limited. Studies aimed to find potential drugs that mimic exercise are now being performed.

Concluding remarks

Exercise is so beneficial for health that it should be considered as a drug. As for any other drug, dosing is very important. Otherwise, unfavourable side effects may occur. Some of the favourable effects of exercise apply to the general population. Prominent amongst these are its role in prevention of many diseases and in the promotion of healthy longevity (see Figure 2). But exercise can also be considered as treatment of established diseases. These include commonly occurring conditions such as depression, diabetes or cardiovascular diseases.

Acknowledgements

We thank Mrs Marilyn Noyes for her kind help in reviewing the manuscript. Our work is supported by grants SAF2008-00270, SAF2009-08334 from the Spanish Ministry of Education and Science; PROMETEO/2010/074 from the Consellería de Educación de la Generalitat Valenciana. ISCIII2006-RED13-027 from the 'Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF)', EU Funded COSTB35 and DPS2008- 06968 from Spanish Ministry of Innovation and Science. Our studies have also been co financed by FEDER funds from the European Union.

Conflict of interest

The authors declare that no conflict of interest exists.

References

American College of Sports Medicine (1978). Position statement on the recommended quantity and quality of exercise for developing and maintaining fitness in healthy adults. Med Sci Sports Exerc 10: vii–x.

Albert CM, Mittleman MA, Chae CU, Lee IM, Hennekens CH, Manson JE (2000). Triggering of sudden death from cardiac causes by vigorous exertion. N Engl J Med 343: 1355–1361.

Armstrong RB, Ogilvie RW, Schwane JA (1983). Eccentric exercise-induced injury to rat skeletal muscle. J Appl Physiol 54: 80–93.

Baracos VE (1989). Exercise inhibits progressive growth of the Morris hepatoma 7777 in male and female rats. Can J Physiol Pharmacol 67: 864–870.

Benito B, Gay-Jordi G, Serrano-Mollar A, Guasch E, Shi Y, Tardif JC *et al.* (2011). Cardiac arrhythmogenic remodeling in a rat model of long-term intensive exercise training. Circulation 123: 13–22.

Berchtold MW, Brinkmeier H, Muntener M (2000). Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 80: 1215–1265.

Black A, Black MM, Gensini G (1975). Exertion and acute coronary artery injury. Angiology 26: 759–783.

Blair SN, Kohl HW 3rd, Paffenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW (1989). Physical fitness and all-cause mortality. A prospective study of healthy men and women. JAMA 262: 2395–2401.

Blair SN, LaMonte MJ, Nichaman MZ (2004). The evolution of physical activity recommendations: how much is enough? Am J Clin Nutr 79: 913S–920S.

Boreham CA, Kennedy RA, Murphy MH, Tully M, Wallace WF, Young I (2005). Training effects of short bouts of stair climbing on cardiorespiratory fitness, blood lipids, and homocysteine in sedentary young women. Br J Sports Med 39: 590–593.

Borras C, Gomez-Cabrera MC, Vina J (2011). The dual role of p53: DNA protection and antioxidant. Free Radic Res 45: 643–652.

Bortz WM 2nd, Angwin P, Mefford IN, Boarder MR, Noyce N, Barchas JD (1981). Catecholamines, dopamine, and endorphin levels during extreme exercise. N Engl J Med 305: 466–467.

Bouchard C, Shephard RJ, Stephens T (1994). Physical Activity, Fitness, and Health: International Proceedings and Consensus Statement: [Second International Consensus Symposium on Physical Activity, Fitness, and Health, Held May 5 to May 9, 1992, in Toronto, Canada]. Human Kinetics Publishers: Champaign, IL; Leeds.

Carr DB, Bullen BA, Skrinar GS, Arnold MA, Rosenblatt M, Beitins IZ *et al.* (1981). Physical conditioning facilitates the exercise-induced secretion of beta-endorphin and beta-lipotropin in women. N Engl J Med 305: 560–563.

Caspersen CJ, Powell KE, Christenson GM (1985). Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep 100: 126–131.

Church TS, Earnest CP, Skinner JS, Blair SN (2007). Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. JAMA 297: 2081–2091.

Czurko A, Hirase H, Csicsvari J, Buzsaki G (1999). Sustained activation of hippocampal pyramidal cells by 'space clamping' in a running wheel. Eur J Neurosci 11: 344–352.

Derbré F, Gomez-Cabrera MC, Nascimento AL, Sanchis-Gomar F, Martinez-Bello VE, Tresguerres JA *et al.* (2012). Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age (Dordr) 34: 669–679.

Dishman RK, Berthoud HR, Booth FW, Cotman CW, Edgerton VR, Fleshner MR *et al.* (2006). Neurobiology of exercise. Obesity (Silver Spring) 14: 345–356.

Drezner JA (2008). Contemporary approaches to the identification of athletes at risk for sudden cardiac death. Curr Opin Cardiol 23: 494–501.

Dunn AL, Trivedi MH, O'Neal HA (2001). Physical activity dose-response effects on outcomes of depression and anxiety. Med Sci Sports Exerc 6 (Suppl.): S587–S597. Discussion 609–610.

Dunstan DW, Daly RM, Owen N, Jolley D, Vulikh E, Shaw J *et al.* (2005). Home-based resistance training is not sufficient to maintain improved glycemic control following supervised training in older individuals with type 2 diabetes. Diabetes Care 28: 3–9.

Fox SM 3rd, Haskell WL (1968). Physical activity and the prevention of coronary heart disease. Bull N Y Acad Med 44: 950–967.

Freimuth M, Moniz S, Kim SR (2011). Clarifying exercise addiction: differential diagnosis, co-occurring disorders, and phases of addiction. Int J Environ Res Public Health 8: 4069–4081.

Gomez-Cabrera MC, Borras C, Pallardo FV, Sastre J, Ji LL, Vina J (2005). Decreasing xanthine oxidase mediated oxidative stress prevents useful cellular adaptations to exercise in rats. J Physiol 567: 113–120.

Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV *et al.* (2008a). Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 87: 142–149.

Gomez-Cabrera MC, Domenech E, Vina J (2008b). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med 44: 126–131.

Gomez-Cabrera MC, Vina J, Ji LL (2009). Interplay of oxidants and antioxidants during exercise: implications for muscle health. Phys Sportsmed 37: 116–123.

Goodyear LJ (2008). The exercise pill – too good to be true? N Engl J Med 359: 1842–1844.

Goodyear LJ, Chang PY, Sherwood DJ, Dufresne SD, Moller DE (1996). Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. Am J Physiol 271 (2 Pt 1): E403–E408.

Grealy MA, Johnson DA, Rushton SK (1999). Improving cognitive function after brain injury: the use of exercise and virtual reality. Arch Phys Med Rehabil 80: 661–667.

Hakim AA, Petrovitch H, Burchfiel CM, Ross GW, Rodriguez BL, White LR *et al.* (1998). Effects of walking on mortality among nonsmoking retired men. N Engl J Med 338: 94–99.

Harber VJ, Sutton JR (1984). Endorphins and exercise. Sports Med 1: 154–171.

Hartley PH, Llewellyn GF (1939). Longevity of oarsmen. Br Med J 1: 657–662.

Haskell WL, Lee IM, Pate RR, Powell KE, Blair SN, Franklin BA *et al.* (2007). Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. Med Sci Sports Exerc 39: 1423–1434.

Holloszy JO, Coyle EF (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol 56: 831–838.

Howatson G, van Someren KA (2008). The prevention and treatment of exercise-induced muscle damage. Sports Med 38: 483–503.

Hu FB, Willett WC, Li T, Stampfer MJ, Colditz GA, Manson JE (2004). Adiposity as compared with physical activity in predicting mortality among women. N Engl J Med 351: 2694–2703.

Huang LE, Arany Z, Livingston DM, Bunn HF (1996). Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 271: 32253–32259.

Jager S, Handschin CS, Pierre J, Spiegelman BM (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 104: 12017–12022.

Ji LL, Gomez-Cabrera MC, Steinhafel N, Vina J (2004). Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. FASEB J 18: 1499–1506.

Jones LW, Eves ND, Courneya KS, Chiu BK, Baracos VE, Hanson J *et al.* (2005). Effects of exercise training on antitumor efficacy of doxorubicin in MDA-MB-231 breast cancer xenografts. Clin Cancer Res 11: 6695–6698.

Jorgensen SB, Treebak JT, Viollet B, Schjerling P, Vaulont S, Wojtaszewski JF *et al.* (2007). Role of AMPKalpha2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. Am J Physiol Endocrinol Metab 292: E331–E339.

Koves TR, Li P, An J, Akimoto T, Slentz D, Ilkayeva O *et al.* (2005). Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency. J Biol Chem 280: 33588–33598.

Kujala UM (2011). Born to be rich, physically active, fit and healthy? Scand J Med Sci Sports 20: 367.

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F *et al.* (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127: 1109–1122.

Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K (2001). Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 58: 498–504.

Lee DC, Artero EG, Sui X, Blair SN (2010). Mortality trends in the general population: the importance of cardiorespiratory fitness. J Psychopharmacol 24 (4 Suppl.): 27–35.

Lee IM (2007). Dose-response relation between physical activity and fitness: even a little is good; more is better. JAMA 297: 2137–2139.

Lee IM, Skerrett PJ (2001). Physical activity and all-cause mortality: what is the dose-response relation? Med Sci Sports Exerc 33 (6 Suppl.): S459–S471. Discussion S493–S454.

Lejoyeux M, Avril M, Richoux C, Embouazza H, Nivoli F (2008). Prevalence of exercise dependence and other behavioral addictions among clients of a Parisian fitness room. Compr Psychiatry 49: 353–358.

Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O *et al.* (2002). Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature 418: 797–801.

Lin J, Handschin C, Spiegelman BM (2005). Metabolic control through the PGC-1 family of transcription coactivators. Cell Metab 1: 361–370.

Lyons AS, RJja P (1978). Medicine: An Illustrated History. H. N. Abrams: New York.

Macera CA, Hootman JM, Sniezek JE (2003). Major public health benefits of physical activity. Arthritis Rheum 49: 122–128.

Mandroukas K, Krotkiewski M, Hedberg M, Wroblewski Z, Bjorntorp P, Grimby G (1984). Physical training in obese women. Effects of muscle morphology, biochemistry and function. Eur J Appl Physiol Occup Physiol 52: 355–361.

Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO (2009). Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980–2006. Circulation 119: 1085–1092.

Matsakas A, Narkar VA (2010). Endurance exercise mimetics in skeletal muscle. Curr Sports Med Rep 9: 227–232.

McCloskey DP, Adamo DS, Anderson BJ (2001). Exercise increases metabolic capacity in the motor cortex and striatum, but not in the hippocampus. Brain Res 891: 168–175.

Mittleman MA, Maclure M, Tofler GH, Sherwood JB, Goldberg RJ, Muller JE (1993). Triggering of acute myocardial infarction by heavy physical exertion. Protection against triggering by regular exertion. Determinants of Myocardial Infarction Onset Study Investigators. N Engl J Med 329: 1677–1683.

Momken I, Stevens L, Bergouignan A, Desplanches D, Rudwill F, Chery I *et al*. (2011). Resveratrol prevents the wasting disorders of mechanical unloading by acting as a physical exercise mimetic in the rat. FASEB J 25: 3646–3660.

Moorstein B (1968). Life expectancy of Ivy League rowing crews. JAMA 205: 106.

Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J *et al*. (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 34: 267–273.

Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW (1953a). Coronary heart-disease and physical activity of work. Lancet 265: 1111–1120; concl.

Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW (1953b). Coronary heart-disease and physical activity of work. Lancet 265: 1053–1057; contd.

Murphy MH, Blair SN, Murtagh EM (2009). Accumulated versus continuous exercise for health benefit: a review of empirical studies. Sports Med 39: 29–43.

Myers J, Kaykha A, George S, Abella J, Zaheer N, Lear S *et al*. (2004). Fitness versus physical activity patterns in predicting mortality in men. Am J Med 117: 912–918.

Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E *et al*. (2008). AMPK and PPARdelta agonists are exercise mimetics. Cell 134: 405–415.

Narkar VA, Fan W, Downes M, Yu RT, Jonker JW, Alaynick WA *et al*. (2011). Exercise and PGC-1alpha-independent synchronization of type I muscle metabolism and vasculature by ERRgamma. Cell Metab 13: 283–293.

Neeper SA, Gomez-Pinilla F, Choi J, Cotman C (1995). Exercise and brain neurotrophins. Nature 373: 109.

Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Res 726: 49–56.

O'Donnell MP (1986). Definition of health promotion: Part II: Levels of programs. Am J Health Promot 1: 6–9.

O'Donovan G, Blazevich AJ, Boreham C, Cooper AR, Crank H, Ekelund U *et al*. (2010). The ABC of Physical Activity for Health: a consensus statement from the British Association of Sport and Exercise Sciences. J Sports Sci 28: 573–591.

Paffenbarger RS, Hale WE (1975). Work activity and coronary heart mortality. N Engl J Med 292: 545–550.

Paffenbarger RS Jr, Brand RJ, Sholtz RI, Jung DL (1978). Energy expenditure, cigarette smoking, and blood pressure level as related to death from specific diseases. Am J Epidemiol 108: 12–18.

Palomero J, Broome CS, Rasmussen P, Mohr M, Nielsen B, Nybo L *et al*. (2008). Heat shock factor activation in human muscles following a demanding intermittent exercise protocol is attenuated with hyperthermia. Acta Physiol (Oxf) 193: 79–88.

Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C *et al*. (1995). Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA 273: 402–407.

Pedersen BK, Saltin B (2006). Evidence for prescribing exercise as therapy in chronic disease. Scand J Med Sci Sports 16 (Suppl. 1): 3–63.

Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA, Ray CA (2004). American College of Sports Medicine position stand. Exercise and hypertension. Med Sci Sports Exerc 36: 533–553.

Physical activity and cardiovascular health. NIH Consensus Development Panel on Physical Activity and Cardiovascular Health (1996). JAMA 276: 241–246. Review.

Polednak AP (1972). Longevity and cause of death among Harvard College athletes and their classmates. Geriatrics 27: 53–64.

van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 96: 13427–13431.

Prout C (1972). Life expectancy of college oarsmen. JAMA 220: 1709–1711.

Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92: 829–839.

Reid MB (2001). Invited Review: redox modulation of skeletal muscle contraction: what we know and what we don't. J Appl Physiol 90: 724–731.

Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M *et al*. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A 106: 8665–8670.

Ruiz JR, Moran M, Arenas J, Lucia A (2010). Strenuous endurance exercise improves life expectancy: it's in our genes. Br J Sports Med 45: 159–161.

Sakamoto K, Goodyear LJ (2002). Invited review: intracellular signaling in contracting skeletal muscle. J Appl Physiol 93: 369–383.

Salminen A, Vihko V (1983). Endurance training reduces the susceptibility of mouse skeletal muscle to lipid peroxidation in vitro. Acta Physiol Scand 117: 109–113.

Sanchis-Gomar F, Gomez-Cabrera MC, Vina J (2011a). The loss of muscle mass and sarcopenia: non hormonal intervention. Exp Gerontol 46: 967–969.

Sanchis-Gomar F, Olaso-Gonzalez G, Corella D, Gomez-Cabrera MC, Vina J (2011b). Increased average longevity among the 'Tour de France' cyclists. Int J Sports Med 32: 644–647.

Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH *et al.* (2006). PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc Natl Acad Sci U S A 103: 16260–16265.

Schiaffino S, Sandri M, Murgia M (2007). Activity-dependent signaling pathways controlling muscle diversity and plasticity. Physiology (Bethesda) 22: 269–278.

Schuler M, Ali F, Chambon C, Duteil D, Bornert JM, Tardivel A *et al.* (2006). PGC1alpha expression is controlled in skeletal muscles by PPARbeta, whose ablation results in fiber-type switching, obesity, and type 2 diabetes. Cell Metab 4: 407–414.

Shephard RJ (2001). Absolute versus relative intensity of physical activity in a dose-response context. Med Sci Sports Exerc 33 (6 Suppl.): S400–S418. Discussion S419–S420.

Shephard RJ, Futcher R (1997). Physical activity and cancer: how may protection be maximized? Crit Rev Oncog 8: 219–272.

Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22: 3251–3261.

Siscovick DS, Weiss NS, Fletcher RH, Lasky T (1984). The incidence of primary cardiac arrest during vigorous exercise. N Engl J Med 311: 874–877.

Siuciak JA, Boylan C, Fritsche M, Altar CA, Lindsay RM (1996). BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. Brain Res 710: 11–20.

Speed C, Jaques R (2010). High-performance sports medicine: an ancient but evolving field. Br J Sports Med 45: 81–83.

Sussman S, Sussman AN (2011). Considering the definition of addiction. Int J Environ Res Public Health 8: 4025–4038.

Teramoto M, Bungum TJ (2010). Mortality and longevity of elite athletes. J Sci Med Sport 13: 410–416.

Thompson HJ, Ronan AM, Ritacco KA, Tagliaferro AR (1989). Effect of type and amount of dietary fat on the enhancement of rat mammary tumorigenesis by exercise. Cancer Res 49: 1904–1908.

Thompson PD, Crouse SF, Goodpaster B, Kelley D, Moyna N, Pescatello L (2001). The acute versus the chronic response to exercise. Med Sci Sports Exerc 33 (6 Suppl.): S438–S445. Discussion S452–S433.

Thompson PD, Franklin BA, Balady GJ, Blair SN, Corrado D, Estes NA 3rd *et al.* (2007). Exercise and acute cardiovascular events placing the risks into perspective: a scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism and the Council on Clinical Cardiology. Circulation 115: 2358–2368.

Tipton CM (1999). Exercise training for the treatment of hypertension: a review. Clin J Sport Med 9: 104.

Ungvari Z, Sonntag WE, de Cabo R, Baur JA, Csiszar A (2011). Mitochondrial protection by resveratrol. Exerc Sport Sci Rev 39: 128–132.

Vaynman S, Gomez-Pinilla F (2006). Revenge of the 'sit': how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. J Neurosci Res 84: 699–715.

Vissing J, Andersen M, Diemer NH (1996). Exercise-induced changes in local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 16: 729–736.

Wannamethee SG, Shaper AG, Walker M (1998). Changes in physical activity, mortality, and incidence of coronary heart disease in older men. Lancet 351: 1603–1608.

Warburton DE, Nicol CW, Bredin SS (2006a). Health benefits of physical activity: the evidence. CMAJ 174: 801–809.

Warburton DE, Nicol CW, Bredin SS (2006b). Prescribing exercise as preventive therapy. CMAJ 174: 961–974.

Wen CP, Wai JP, Tsai MK, Yang YC, Cheng TY, Lee MC *et al.* (2011). Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. Lancet 378: 1244–1253.

Westerlind KC (2003). Physical activity and cancer prevention – mechanisms. Med Sci Sports Exerc 35: 1834–1840.

Winder WW, Taylor EB, Thomson DM (2006). Role of AMP-activated protein kinase in the molecular adaptation to endurance exercise. Med Sci Sports Exerc 38: 1945–1949.

Wojtaszewski JF, Nielsen P, Hansen BF, Richter EA, Kiens B (2000). Isoform-specific and exercise intensity-dependent activation of 5′-AMP-activated protein kinase in human skeletal muscle. J Physiol 528 (Pt 1) 221–226.

Clin Chem Lab Med 2012;50(8):1287–1295 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/cclm-2011-0795

Review

Mitochondria as sources and targets of damage in cellular aging

Mari Carmen Gomez-Cabrera*, Fabian Sanchis-Gomar, Rebeca Garcia-Valles, Helios Pareja-Galeano, Juan Gambini, Consuelo Borras and Jose Viña

Department of Physiology, University of Valencia, Fundacion Investigacion Hospital Clinico Universitario/ INCLIVA, Valencia, Spain

Abstract

Mitochondria are considered as the most important cellular sources and targets of free radicals. They are also a source of signalling molecules that regulate cell cycle, proliferation, and apoptosis. Denham Harman postulated the free radical theory of aging in 1956. Previously Rebecca Gershman showed that radiation toxicity could be attributed to free radical damage. Subsequently, Jaime Miquel formulated the mitochondrial free radical theory of aging. We have shown that mitochondrial size, membrane potential, inner membrane mass and peroxide production is altered inside cells in old animals. These result in an increase in the oxidative damage to mitochondrial DNA with aging that can be prevented by antioxidant supplementation. Aging is also associated with a lower renewal of mitochondria. This is mainly due to the lack of reactivity of proliferator-activated receptor-γ (PPAR-γ) coactivator 1α (PGC-1α) in old animals. PGC-1α acts as a master regulator of energy metabolism and mitochondrial biogenesis and recent evidence shows that it interacts with p53 and telomerase. The promotion of mitochondriogenesis is critical to prevent aging. In skeletal muscle it has relevance to prevent sarcopenia and frailty.

Keywords: antioxidants; DNA damage; longevity; oxidative stress; mitochondrial biogenesis; PGC-1.

Mitochondrial as a source of free radicals

Mitochondria have been classically recognized as the organelle that produce the energy required to drive the endergonic processes of cell life thought the respiratory chain, but now they are also considered as the most important cellular source of free radicals, as the main target for free radical regulatory and toxic actions, and as a source of signalling molecules that regulate cell cycle, proliferation and apoptosis (1). The occurrence of free radicals in biological processes is widely accepted (2). Over 95% of all the oxygen we breathe undergoes a reduction to produce water in the mitochondrial electron transport chain. Cytochrome oxidase is the terminal electron acceptor in the chain and must release its reducing equivalents to allow continued electron transport and ATP production (1). Although the mitochondrial electron transport chain is a very efficient system it predisposes each electron carrier to side reactions with molecular oxygen. If an atom/molecule contains one or more unpaired electrons and is capable of independent existence, it is referred to as a "free radical" (3). The primary free radicals generated in cells are superoxide ($O_2^{\bullet -}$) and nitric oxide (NO) (4). The mitochondrial generation of these free radicals, as well as H_2O_2 (hydrogen peroxide) and peroxynitrite ($ONOO^-$), represent the major intracellular source of reactive oxygen species (ROS) under physiological conditions (1). Mitochondria seem to be (quantitatively) the most important cellular site of $O_2^{\bullet -}$ and H_2O_2 production in mammalian organs, and the steady state concentration of $O_2^{\bullet -}$ in the mitochondrial matrix is about five- to ten-fold higher than that in the cytosol and nucleus (1). Thus, mitochondrial components are exposed to a high flux of free radicals. These cause damage to mitochondrial components and initiate degradative processes. Helmut Sies first coined the term *oxidative stress* in 1985 as "a disturbance in the prooxidant-antioxidant balance in favour of the former" (5, 6). Although this definition has been widely used for over two decades, the definition of oxidative stress will likely undergo modifications in the future. In an effort to refine the meaning of oxidative stress, Dean Jones has proposed that this term should be redefined as "a disruption of redox signalling and control" (7).

Mitochondrial free radical theory of aging

The toxic reactions associated to oxidative stress constitute the central dogma of the free radical theory of aging. Aging is associated with an overall loss of function at the level of the whole organism that has origins in cellular deterioration. Bernard Strehler defined aging by means of four postulates: aging is universal, must be intrinsic, progressive and

*Corresponding author: Mari Carmen Gomez-Cabrera, PhD, Department of Physiology, Faculty of Medicine, University of Valencia, Av. Blasco Ibañez, 15 46010 Valencia, Spain
Phone: +(34) 96 386 46 50, Fax: +(34) 96 386 46 42,
E-mail: carmen.gomez@uv.es
Received October 30, 2011; accepted December 25, 2011; previously published online February 1, 2012

deleterious (8). Aging causes a loss in the capacity to maintain the internal milieu of the old animal/person when faced by changes in the external atmosphere. Thus, the individual loses the capacity to maintain homeostasis and almost all physiological functions lose efficiency with aging (9).

The mitochondria require continuous recycling throughout the lifespan and are particularly susceptive to damage over time as they are the major bioenergetic machinery and source of oxidative stress in cells (10). The free radical theory of aging is one of the most prominent theories to explain aging. It was proposed by Denam Harman in 1956 (11) following initial observations and suggestions by Rebecca Gershman (12). The findings in the laboratory of Britton Chance that approximately 2% of oxygen consumed by mitochondria in state 4 is converted to hydrogen peroxide, underpinned the role of mitochondria in ROS production (13). These experiments led Jaime Miquel to refine the free radical theory of aging and in the 1970s he formulated the mitochondrial free radical theory of aging. The main contributions of Miquel were: emphasized the importance of mitochondrial DNA as a target of oxidants produced during aging, and pointing out that mitochondriogenesis might be impaired in aging (14). Brand and colleagues (15) have recently concluded that the upper estimate of the total fraction of oxygen utilized by the mitochondria that forms superoxide is approximately 0.15%; this value is significantly lower than the original estimate of 2%–5%. This low rate of superoxide production may include a role for uncoupling proteins in the protection of mitochondria against oxidative damage (4).

The mitochondrial theory of aging, although recently questioned (16), has been tested in various laboratories and there are many published papers in support of this theory. For instance, data from our own research group have shown that mitochondria from old animals produce more ROS than those from young ones (17, 18). Moreover, there is an inverse relationship between mitochondrial peroxide production and longevity in mammals (19, 20). These results support the hypothesis that the rate of the oxidant generation by mitochondria is a critical factor in aging (21). In fact the rate of peroxide generation increases with age. Corbisier and Remacle postulated that the mitochondria are involved in cell degeneration by microinjecting isolated mitochondria from fibroblasts of old rats into cells of young ones. They found that the cells who had received ‘old’ mitochondria rapidly entered senescence (22). Thus, the continuous free radical generation by mitochondria during the whole life span causes a chronic oxidative stress that plays a critical role in aging. In addition, the rate of oxidant production by mitochondria from short-lived species is much higher than that of longer-lived ones (20). It appears, therefore, that the rate of ROS production by mitochondria is a key determinant of maximal life span potential at least at the cellular level.

Mitochondria are damaged inside cells in the process of aging

In the 1970s, Jaime Miquel demonstrated the involvement of the mitochondria in the loss of functional properties associated with aging using basically histological studies (23). In 1996, we studied the involvement of mitochondria in aging using whole liver cells and not isolated mitochondria (24). Until then, the mitochondrial damage leading to aging had only been tested using isolated mitochondria. Subsequently, other research groups showed age-related changes in mitochondrial respiratory function and transport systems using this experimental approach (25, 26). We determined the rate of gluconeogenesis and ketogenesis, which critically depend on mitochondrial function, in the liver of old and young animals. We also determined the mitochondrial size, peroxide production and membrane potential in whole liver cells using flow cytometry (24). Our results showed, for the first time in intact cells, a correlation between age-associated impairment of cell metabolism and specific changes in mitochondrial function and morphology. This was almost simultaneously confirmed by the group of Bruce Ames (27). Subsequently, we observed that respiratory activity of mitochondria not only decreases with age in liver but also in other tissues such as muscle and brain.

Mitochondrial DNA damage correlates with oxidative stress in aging and can be prevented by antioxidant administration

The rate of oxidant production by mitochondria is difficult to study (9). Consequently, studying biomarkers of oxidative stress appears more adequate than studying the rate of oxidant production. The mitochondrial gene *16S rRNA*, for instance, can be considered as a biomarker of cellular aging. This RNA molecule is highly susceptible to oxidative stress (28), and its rate of transcription decreases with age and in parallel with the survival curve of Drosophila (29). Damage to different macromolecules such as lipids, proteins and DNA can also be studied as biomarkers of aging. Many of the post-translational modifications found in old cells are due to the deleterious effects of free radicals (30). Lipid peroxidation, for instance, is associated with the pathogenesis of a number of age-associated diseases and can be studied using different biomarkers such as malodialdehyde and isoprostanes (4, 31). Regarding protein oxidation, another interesting biomarker of oxidative stress, it has been found that some enzymes are more susceptible to damage than others with aging. In fact, some key amino acids such as arginine, lysine or proline are more susceptible to yield carbonyl derivatives (32). As previously mentioned, DNA may be the most critical target molecule for age associated oxidative stress (33). It has been calculated that ROS modify approximately 10,000 bases of DNA per cell (34). DNA repairing enzymes are able to repair the vast majority of these lesions, but not all. Therefore, DNA lesions that go unrepaired accumulate with age. It is well known that mitochondrial DNA is much more oxidized with age than nuclear DNA (21). Our group found that oxidative damage to mitochondrial DNA was increased in aging and that it could be prevented by antioxidant supplementation (35). Thus, the initial prediction of Jaime Miquel highlighting the importance of mitochondrial DNA as a target of oxidants produced during aging could be experimentally proved.

Mitochondrial renewal is impaired in aging causing accumulation of damage to mitochondria

Mitochondrial biogenesis includes the cellular processes involved in the synthesis and degradation of the organelle (36). The prediction that aging was associated with a lower renewal of mitochondria was also postulated by Jaime Miquel (33). However, it took several years to establish the relationship between the mitochondria decay in different tissues with aging and the low mitochondriogenesis (37, 38). The major reason is that the number of mitochondria in the cell is difficult to assess. To solve this problem, the elucidation of the mitochondriogenic pathway was required. Mitochondrial biogenesis is a complex process. It involves changes in the expression of more than one thousand genes, the cooperation of two genomes, and alters the level of approximately 20% of cellular proteins (39). Importantly, in addition to the nuclear genes (which encode the major number of mitochondrial proteins), mitochondriogenesis requires the participation of the mitochondrial genome, which is responsible for the synthesis of proteins of the electron transport chain, as well as mitochondrial tRNAs and rRNAs.

Thus, the precise synchronization of the transcription affecting both nuclear and mitochondrial genomes (located in separate subcellular compartments) must be essential in order to produce new mitochondria (40).

Despite the complexity of the various signalling pathways that regulate mitochondrial biogenesis, they all seem to share proliferator-activated receptor-γ (PPAR-γ) coactivator 1α (PGC-1α) family of transcription factors. PGC-1α was initially identified as a cold-inducible coactivator for PPAR-γ in brown fat and skeletal muscle (41). PGC-1α appears to act as a master regulator of energy metabolism and mitochondrial biogenesis by coordinating the activity of multiple transcription factors (41). PGC-1α strongly co-activates nuclear respiratory factor-1 (NRF-1) and NRF-1 regulatory responsive elements have been found in the promoter of nuclear genes, such as *mitochondrial transcription factor A (Tfam), cytochrome c and aminolevulinate synthase* (42). Tfam can be considered the most important mammalian transcription factor for mtDNA because it stimulates mitochondrial DNA transcription and replication.

Low mitochondrial biogenesis is associated with the lack of reactivity of PGC-1α, a master regulator of mitochondrial biogenesis

There is a very significant decline in lean body mass associated with aging (43). The mechanisms controlling muscle loss are very relevant in medicine because muscle contraction is involved in the prevention of chronic diseases. Thus, understanding the signalling that regulates muscle mass may provide potential therapeutic targets (44). Muscle aging is a key component of the increase in frailty in human and animal populations (45). Frailty is a syndrome highly prevalent with increasing age and consists of decreased reserve and resistance to stressors, weakness, decline in activity, weight loss and vulnerability to adverse health outcomes (46). One of the main components of frailty is sarcopenia. This is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcomes such as physical disability, poor quality of life and death (43, 47). The promotion of mitochondriogenesis is critical to prevent aging in skeletal muscle. A functional muscle that has not lost the capacity to synthesise healthy mitochondria is an important contributor to the prevention of frailty (48, 49). As shown in Figure 1, aging has been associated, in skeletal muscle, with reductions in mitochondrial oxidative phosphorylation activity (50), mitochondrial DNA mutations (51), reductions in mitochondrial DNA content (52), decreased activities of the mitochondrial electron transport chain (53), altered apoptotic signalling and an increase in the mitochondrial release of free radicals (54). Several strategies have been developed to stimulate mitochondrial biogenesis. Among them different compounds, such as pyrroloquinoline quinone, resveratrol, genistein, hydroxy-tyrosol, GW1516 (PPARδ agonists), 5-aminoimidazole-4- carboxamide-1-β -D-ribofuranoside (AICAR), and quercetin have been reported to improve mitochondrial respiratory control or stimulate mitochondrial biogenesis (55–57). In vivo and in vitro studies have shown that PGC-1α levels stimulate mitochondrial proliferation in skeletal muscle (58). Increased PGC-1α levels in skeletal muscle prevents muscle wasting by reducing autophagy, proteasome degradation and apoptosis (59). Autophagy is used to define the controlled recycling and degradation of intracellular structures (dysfunctional organelles and protein aggregates) to replenish nutrient stores and ensure the integrity of the cell and its survival (60). The non-functional mitochondria accumulation in aged individuals could be counteracted by removing damaged mitochondria by an autophagic process called “mitophagy” (61).

In a recent study by Henriette Pilegaard’s group it has been shown that PGC-1α is required for the beneficial effects of moderate exercise training at an advanced age to maintain mitochondrial metabolic and antioxidant capacity (62). These studies suggest that the modulation of PGC-1α levels in skeletal muscle present an avenue for the prevention and treatment of age-related disorders. In 2011 we studied the mechanism by which mitochondriogenesis is decreased in aging and tried to determine to which extent it could be prevented by exercise training (63). As endurance training is known to up-regulate PGC-1α expression in young skeletal muscle (64), we postulated that the modulation of PGC-1α levels by endurance training in aged skeletal muscle should be a very effective strategy for the prevention and treatment of sarcopenia. For our purpose we used old rats and compared them with PGC-1α KO mice. Our results showed that muscle from old rats present a marked loss in mitochondriogenesis and that this may be due to a lack of induction of PGC-1α (63). We found a striking similarity between the response to exercise training in PGC-1α KO mice and in old rats. In young rats, PGC-1α was activated in skeletal muscle not only by training but also by cold exposure or triiodothyronine. However, in the old animals we found an

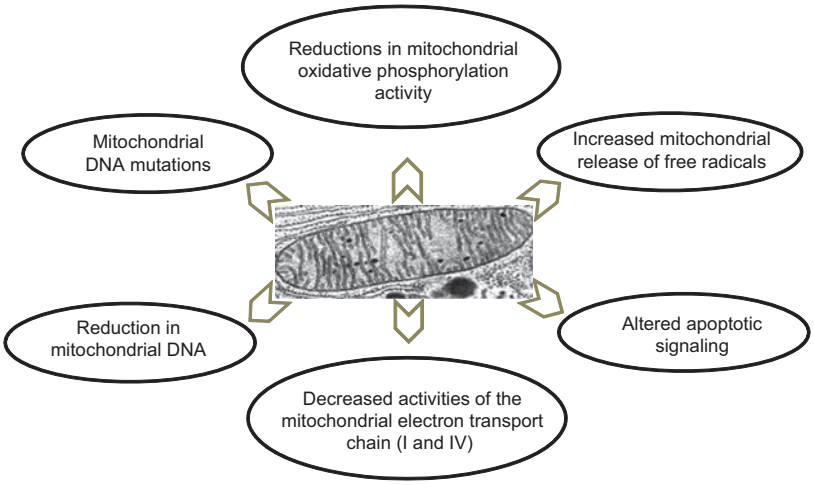


Figure 1 Mitochondrial alterations associated to aging in skeletal muscle.

age-associated lack of expression of PGC-1α in response to exercise training or to any of the other stimuli tested in rat skeletal muscle (Figure 2). Our study highlighted the importance of maintaining a normal PGC-1α responsiveness to maintain normal muscle function.

A unified theory of aging involves the interaction between telomerase, p53, and mitochondrial free radical production

There are more than 300 theories of aging (65). Several researchers have worked in the development of a unified theory, which includes all the phenomena associated with aging. A new theory that unifies the interaction between p53, telomerase and mitochondria has been published recently (66). Telomeres are nucleoprotein complexes at chromosome ends that preserve chromosomal integrity and are involved in age-related disorders (66). Telomerase is a cellular enzyme capable of compensating the progressive shortening of telomeres (67). Telomerase is required for telomere maintenance, as well as its importance for cancer and aging (68). In this regard, in the absence of telomerase, continued cell division results in telomere shortening and p53 activation (69). The tumour suppressor p53 is a transcriptional factor that responds to a multitude of stresses and play a central part in the detection and elimination of cellular damage (70). Thus, p53 mediates cellular checkpoints of growth arrest, senescence and apoptosis in cells (66). Interestingly, Nakano et al. determined that exists a threshold level of p53 for the initiation of apoptosis in cells (71). Moreover, it has been established that p53 regulates mtDNA content in skeletal muscle (72). Finally, Sahin et al. (73) established that telomere dysfunction-induced p53 represses PGC-1α and PGC-1β, linking telomeres to mitochondrial biology, oxidative defence, and metabolism. Therefore, when telomeres have shortened down to a critical length they are recognized as DNA damage, activating a p53-mediated DNA damage signalling response (68). Decisively,

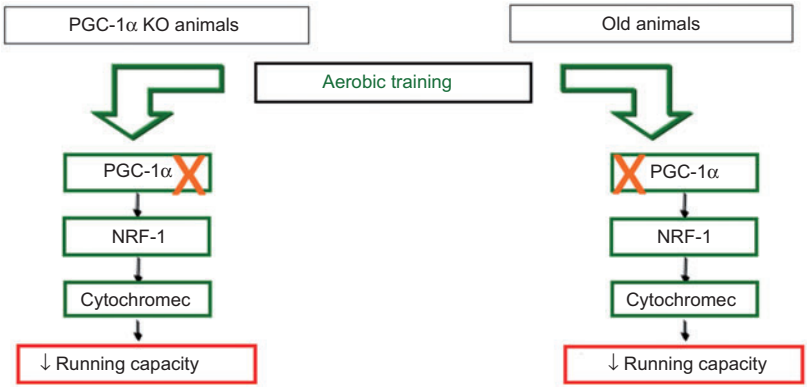


Figure 2 PGC-1α is not functional in the aged skeletal muscle and it can be involved in the decrease in mitochondrial biogenesis during aging.
An old animal behaves as a knock-out mouse for PGC-1α.

telomeres shorten with age, limiting the proliferative capacity and thus contributing to organism aging.

A new group of proteins, called sestrins (SESNs), are targets of p53 (74) and stress-inducible. SESNs have dual biochemical functions, as antioxidants that control the activity of peroxiredoxins (a family of thioredoxin-dependent peroxidases which scavenge free radicals) and as inhibitors of target of rapamycin complex 1 (TORC1) signalling (75). Both free radicals accumulation (76) and TORC1 activation (77) are associated with accelerated aging and development of age-associated pathologies in diverse organs and organisms, implicating SESNs as anti-aging agents. SESNs may act as suppressors of aging that are responsive to stressful stimuli and insults that can accelerate the aging process. By activating AMPK and inhibiting TORC1, SESNs can reprogram cells to adapt to stressful conditions by attenuating anabolism and enhancing autophagic catabolism (78). By up regulating SESNs expression, p53 is able to induce a strong anti-apoptotic response whose physiological implications have been elucidated only recently (79). In the absence of severe stresses, relatively low p53 levels are sufficient for up regulation of several antioxidant genes including *GPX, SESN1* and *SESN2* correlating with a decrease in intracellular ROS levels (79). In contrast, elevated ROS production is only observed in heavily stressed cells upon induction of the p53 targets Bax and Puma that, however, might only be a consequence of cells dying rather than its cause (79). Thus, the antioxidant function of p53 represents an important component of its tumour suppressor activity, which decreases the probability of genetic alterations and assists the survival and repair of cells with minor injuries. Therefore, we also can view SESNs as physiological brakes that can attenuate stress-dependent acceleration of aging (80).

During the last decades, many studies have been confirming and settling this "unified theory of aging". Telomerase-deficient mice developed premature aging pathologies (81). In addition, increased lifespan is observed in mouse models that overexpress telomerase, although the probabilities of initiating a tumour are higher (82, 83). However, in mice that overexpressed telomerase, p53, p16 and p19ARF, cancer appearance and degenerative lesions are significantly delayed while symptoms of aging are also attenuated (84). Tumour suppressor p53 limits the reprogramming of cells with different kinds of DNA damage due to short telomeres, deficiencies in their DNA repair systems or exogenously inflicted DNA damage (85). Thus, Matheu et al. observed that increasing the activity of p53 in mice produces both cancer resistance and delayed aging (86). It was also observed that a single extra gene-dose of p53 protects mice against cancer (87, 88). Likewise, p53 is known to enhance mitochondrial function (89). In fact, p53 activation results in the direct suppression of PGC-1 genes in telomere-deficient mice and reducing p53 levels in these animals reverses PGC-1 suppression associated with telomere deficiency (73). Reduction of mitochondrial function would seem a deleterious response and the loss of PGC-1 function can lead to the generation of ROS, which can damage mitochondrial DNA. Therefore, dysfunctional mitochondria may trigger cell injury and death.

To conclude, telomerase and telomere maintenance are considered to be rate limiting for longevity. Telomere shortening continues throughout the adult life and it is proposed to be a major cause of aging. p53 also integrates and responds to a multitude of stresses, such as cancer or physiological process of aging. Finally, mitochondria are the chief energy-producing cells. Mitochondrial deficiency increase ROS production and DNA damage. Thus, all the mechanisms above mentioned conform a DNA-repair machinery that seems to be connected. Consequently, a new "unified theory of aging" may be postulated.

Acknowledgments

We thank Mrs Marilyn Noyes for her kind help in reviewing the manuscript. Our work is supported by grants SAF2008-00270, SAF2009-08334 from the Spanish Ministry of Education and Science; PROMETEO/ 2010/074 from the Consellería de Educación de la Generalitat Valenciana. ISCIII2006-RED13-027 from the "Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF)", EU Funded COSTB35 and DPS2008- 06968 from Spanish Ministry of Innovation and Science. Our studies have also been co-financed by FEDER funds from the European Union.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 2000;29:222–30.
2. Commoner B, Townsend J, Pake GE. Free radicals in biological materials. Nature 1954;174:689–91.
3. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford, UK: Oxford University Press, 2007.
4. Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 2008;88:1243–76.
5. Sies H. Oxidative stress. London: Academic Press, 1985.
6. Sies H, Cadenas E. Oxidative stress: damage to intact cells and organs. Philos Trans R Soc Lond B Biol Sci 1985;311:617–31.
7. Jones DP. Redefining oxidative stress. Antioxid Redox Signal 2006;8:1865–79.
8. Strehler BL. Aging: a challenge to science, society, and the individual. Clin Geriatr Med 1985;1:5–13.
9. Vina J, Borras C, Miquel J. Theories of ageing. IUBMB Life 2007;59:249–54.
10. Miquel J, de Juan E, Sevila I. Oxygen-induced mitochondrial damage and aging. EXS 1992;62:47–57.
11. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956;11:298–300.

12. Gershman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and X irradiation: a mechanism in common. Science 1954;119:623–6.
13. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979;59:527–605.
14. Miquel J, Economos AC, Fleming J, Johnson JE Jr. Mitochondrial role in cell aging. Exp Gerontol 1980;15:575–91.
15. St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD. Topology of superoxide production from different sites in the mitochondrial electron transport chain. J Biol Chem 2002;277:44784–90.
16. Yang W, Hekimi S. A mitochondrial superoxide signal triggers increased longevity in Caenorhabditis elegans. PLoS Biol;8:e1000556.
17. Sohal RS, Orr WC. Relationship between antioxidants, prooxidants, and the aging process. Ann N Y Acad Sci 1992;663:74–84.
18. García de la Asunción J, Millan A, Pla R, Bruseghini L, Esteras A, Pallardo FV, et al. Mitochondiral glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. FASEB J 1996;10:333–8.
19. Sohal RS, Sohal BH. Hydrogen peroxide release by mitochondria increases during aging. Mech Ageing Dev 1991;57:187–202.
20. Barja G, Cadenas S, Rojas C, Perez-Campo R, Lopez-Torres M. Low mitochondrial free radical production per unit O_2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds. Free Radic Res 1994;21:317–27.
21. Richter C, Park JW, Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci USA 1988;85:6465–7.
22. Corbisier P, Remacle J. Involvement of mitochondria in cell degeneration. Eur J Cell Biol 1990;51:173–82.
23. Rockstein M, Chesky J, Philpott DE, Takahashi A, Johnson JE Jr, Miquel J. An electron microscopic investigation of age-dependent changes in the flight muscle of Musca domestica L. Gerontologia 1975;21:216–23.
24. Sastre J, Pallardo FV, Pla R, Pellin A, Juan G, O'Connor JE, et al. Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. Hepatology 1996;24:1199–205.
25. Yen TC, Chen YS, King KL, Yeh SH, Wei YH. Liver mitochondrial respiratory functions decline with age. Biochem Biophys Res Commun 1989;165:944–1003.
26. Tummino PJ, Gafni A. A comparative study of succinate-supported respiration and ATP/ADP translocation in liver mitochondria from adult and old rats. Mech Ageing Dev 1991;59:177–88.
27. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proc Natl Acad Sci USA 1994;91:10771–8.
28. Crawford DR, Wang Y, Schools GP, Kochheiser J, Davies KJ. Down-regulation of mammalian mitochondrial RNAs during oxidative stress. Free Radic Biol Med 1997;22:551–9.
29. Calleja M, Pena P, Ugalde C, Ferreiro C, Marco R, Garesse R. Mitochondrial DNA remains intact during Drosophila aging, but the levels of mitochondrial transcripts are significantly reduced. J Biol Chem 1993;268:18891–7.
30. Stadtman ER. Protein oxidation and aging. Science 1992;257: 1220–4.
31. Vladimirov YA. Free radical lipids peroxidation in biomembranes: Mechanism, regulation and biological consequences. In: Johnson JE, Walford R, Harman D, Miquel J, Liss AR, editors. Free radicals, aging and degenerative diseases, New York: Liss, 1986:141–95.
32. Vitorica J, Satrustegui J. Involvement of mitochondria in the age-dependent decrease in calcium uptake of rat brain synaptosomes. Brain Res 1986;378:36–48.
33. Miquel J. An update on the mitochondrial-DNA mutation hypothesis of cell aging. Mutat Res 1992;275:209–16.
34. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915–22.
35. Pallardo FV, Asensi M, Garcia de la Asuncion J, Anton V, Lloret A, Sastre J, et al. Late onset administration of oral antioxidants prevents age-related loss of motor co-ordination and brain mitochondrial DNA damage. Free Radic Res 1998;29:617–23.
36. Holloszy JO. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. J Biol Chem 1967;242:2278–82.
37. Lopez-Lluch G, Irusta PM, Navas P, de Cabo R. Mitochondrial biogenesis and healthy aging. Exp Gerontol 2008;43:813–9.
38. Vina J, Gomez-Cabrera MC, Borras C, Froio T, Sanchis-Gomar F, Martinez-Bello VE, et al. Mitochondrial biogenesis in exercise and in ageing. Adv Drug Deliv Rev 2009;61:1369–74.
39. Ryan MT, Hoogenraad NJ. Mitochondrial-nuclear communications. Annu Rev Biochem 2007;76:701–22.
40. Scarpulla RC. Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. Gene 2002;286:81–9.
41. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998;92:829–39.
42. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999;98:115–24.
43. Rosenberg IH. Sarcopenia: origins and clinical relevance. J Nutr 1997;127:990S–1S.
44. Wolfe RR. The underappreciated role of muscle in health and disease. Am J Clin Nutr 2006;84:475–82.
45. Vanitallie TB. Frailty in the elderly: contributions of sarcopenia and visceral protein depletion. Metabolism 2003;52:22–6.
46. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 2001;56:M146–56.
47. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. Age Ageing 2010;39:412–23.
48. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 1994;330:1769–75.
49. Gill TM, Gahbauer EA, Han L, Allore HG. Trajectories of disability in the last year of life. N Engl J Med 2010;362:1173–80.
50. Drew B, Phaneuf S, Dirks A, Selman C, Gredilla R, Lezza A, et al. Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. Am J Physiol Regul Integr Comp Physiol 2003;284:R474–80.
51. McKiernan SH, Colman RJ, Lopez M, Beasley TM, Aiken JM, Anderson RM, et al. Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle. Exp Gerontol 2011;46:23–9.
52. Cassano P, Sciancalepore AG, Lezza AM, Leeuwenburgh C, Cantatore P, Gadaleta MN. Tissue-specific effect of age and caloric restriction diet on mitochondrial DNA content. Rejuvenation Res 2006;9:211–4.

53. Navarro A, Boveris A. The mitochondrial energy transduction system and the aging process. Am J Physiol Cell Physiol 2007;292:C670–86.
54. Marzetti E, Wohlgemuth SE, Lees HA, Chung HY, Giovannini S, Leeuwenburgh C. Age-related activation of mitochondrial caspase-independent apoptotic signaling in rat gastrocnemius muscle. Mech Ageing Dev 2008;129:542–9.
55. Chowanadisai W, Bauerly KA, Tchaparian E, Wong A, Cortopassi GA, Rucker RB. Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression. J Biol Chem 2010;285:142–52.
56. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 2006;127:1109–22.
57. Narkar VA, Downes M, Yu RT, Embler E, Wang YX, Banayo E, et al. AMPK and PPARdelta agonists are exercise mimetics. Cell 2008;134:405–15.
58. Hood DA, Irrcher I, Ljubicic V, Joseph AM. Coordination of metabolic plasticity in skeletal muscle. J Exp Biol 2006;209:2265–75.
59. Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT. Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. Proc Natl Acad Sci USA 2009;106:20405–10.
60. Saleem A, Carter HN, Iqbal S, Hood DA. Role of p53 within the regulatory network controlling muscle mitochondrial biogenesis. Exerc Sport Sci Rev;39:199–205.
61. Mammucari C, Rizzuto R. Signaling pathways in mitochondrial dysfunction and aging. Mech Ageing Dev 2010;131:536–43.
62. Leick L, Lyngby SS, Wojtasewski JF, Pilegaard H. PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. Exp Gerontol 2010;45:336–42.
63. Derbre F, Gomez-Cabrera MC, Nascimento AL, Sanchis-Gomar F, Martinez-Bello VE, Tresguerres JA, et al. Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1alpha to exercise training. Age (Dordr) 2011.
64. Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, et al. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 2008;87:142–9.
65. Medvedev ZA. An attempt at a rational classification of theories of ageing. Biol Rev 1990;65:375–98.
66. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature 2010;464:520–8.
67. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985;43:405–13.
68. Donate LE, Blasco MA. Telomeres in cancer and ageing. Philos T Roy Soc B 2011;366:76–84.
69. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell 1997;91:25–34.
70. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature 2004;432:307–15.
71. Nakano H, Yonekawa H, Shinohara K. Threshold level of p53 required for the induction of apoptosis in X-irradiated MOLT-4 cells. Int J Radiat Oncol 2007;68:883–91.
72. Park JY, Wang PY, Matsumoto T, Sung HJ, Ma W, Choi JW, et al. p53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content. Circ Res 2009;105:705–12.
73. Sahin E, Colla S, Liesa M, Moslehi J, Muller FL, Guo M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 2011;470:359–65.
74. Budanov AV, Shoshani T, Faerman A, Zelin E, Kamer I, Kalinski H, et al. Identification of a novel stress-responsive gene Hi95 involved in regulation of cell viability. Oncogene 2002;21:6017–31.
75. Budanov AV, Sablina AA, Feinstein E, Koonin EV, Chumakov PM. Regeneration of peroxiredoxins by p53-regulated sestrins, homologs of bacterial AhpD. Science 2004;304:596–600.
76. Lee JH, Budanov AV, Park EJ, Birse R, Kim TE, Perkins GA, et al. Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies. Science 2010;327:1223–8.
77. Kapahi P, Zid B. TOR pathway: linking nutrient sensing to life span. Sci Aging Knowledge Environ 2004;2004:PE34.
78. Budanov AV, Lee JH, Karin M. Stressin' Sestrins take an aging fight. EMBO Mol Med 2010;2:388–400.
79. Sablina AA, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant function of the p53 tumor suppressor. Nat Med 2005;11:1306–13.
80. Lee JH, Bodmer R, Bier E, Karin M. Sestrins at the crossroad between stress and aging. Aging 2010;2:369–74.
81. Gonzalez-Suarez E, Samper E, Flores JM, Blasco MA. Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. Nat Genet 2000;26:114–7.
82. Serrano M, Blasco MA. Cancer and ageing: convergent and divergent mechanisms. Nat Rev Mol Cell Biol 2007;8:715–22.
83. Gonzalez-Suarez E, Geserick C, Flores JM, Blasco MA. Antagonistic effects of telomerase on cancer and aging in K5-mTert transgenic mice. Oncogene 2005;24:2256–70.
84. Tomas-Loba A, Flores I, Fernandez-Marcos PJ, Cayuela ML, Maraver A, Tejera A, et al. Telomerase reverse transcriptase delays aging in cancer-resistant mice. Cell 2008;135:609–22.
85. Marion RM, Strati K, Li H, Murga M, Blanco R, Ortega S, et al. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. Nature 2009;460:1149–53.
86. Matheu A, Maraver A, Klatt P, Flores I, Garcia-Cao I, Borras C, et al. Delayed ageing through damage protection by the Arf/p53 pathway. Nature 2007;448:375–9.
87. Garcia-Cao I, Garcia-Cao M, Martin-Caballero J, Criado LM, Klatt P, Flores JM, et al. "Super p53" mice exhibit enhanced DNA damage response, are tumor resistant and age normally. EMBO J 2002;21:6225–35.
88. Matheu A, Pantoja C, Efeyan A, Criado LM, Martin-Caballero J, Flores JM, et al. Increased gene dosage of Ink4a/Arf results in cancer resistance and normal aging. Genes Dev 2004;18:2736–46.
89. Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, et al. p53 regulates mitochondrial respiration. Science 2006;312:1650–3.

Advanced Drug Delivery Reviews 61 (2009) 1369–1374

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Mitochondrial biogenesis in exercise and in ageing ☆

Jose Viña [a,*], Mari Carmen Gomez-Cabrera [a], Consuelo Borras [a], Teresa Froio [b], Fabian Sanchis-Gomar [a], Vladimir E. Martinez-Bello [a], Federico V. Pallardo [a]

[a] Department of Physiology. University of Valencia, School of Medicine, Spain
[b] Department of Clinical and Biological Sciences, University of Turin, Italy

ARTICLE INFO

Article history:
Received 12 June 2009
Accepted 22 June 2009
Available online 27 August 2009

Keywords:
PGC-1α
NRF1
TFAM
Sarcopenia
Skeletal and cardiac muscle

ABSTRACT

Mitochondrial biogenesis is critical for the normal function of cells. It is well known that mitochondria are produced and eventually after normal functioning they are degraded. Thus, the actual level of mitochondria in cells is dependent both on the synthesis and the degradation. Ever since the proposal of the mitochondrial theory of ageing by Jaime Miquel in the 70's, it was appreciated that mitochondria, which are both a target and a source of radicals in cells, are most important organelles to understand ageing. Thus, a common feature between cell physiology of ageing and exercise is that in both situations mitochondria are critical for normal cell functioning.

Mitochondrial synthesis is stimulated by the PGC-1α–NRF1–TFAM pathway. PGC-1α is the first stimulator of mitochondrial biogenesis. NRF1 is an intermediate transcription factor which stimulates the synthesis of TFAM which is a final effector activating the duplication of mitochondrial DNA molecules. This pathway is impaired in ageing. On the contrary, exercise, particularly aerobic exercise, activates mitochondriogenesis in the young animal but its effects on mitochondrial biogenesis in the old animal are doubtful. In this chapter we consider the interrelationship between mitochondrial biogenesis stimulated by exercise and the possible impairment of this pathway in ageing leading to mitochondrial deficiency and eventually muscle sarcopenia.

Contents

1. Introduction

Mitochondria are one of the most intensely studied organelles in the cell. It has, of course, the classical function of producing energy through the respiratory chain. However, many more functions have emerged particularly related to the role of these organelles in cellular signalling. For instance, mitochondria generate signals which are essential for the onset of the "mitochondrial pathway of apoptosis". This is unleashed by the release of cytochrome *c* under suitable conditions, which leads to activation of caspase-3 and eventually cell death [1]. Much more recently, experimental evidence has come from various laboratories that proteins which a few years ago were thought to be completely cytosolic, enter mitochondria and regulate their function. A very important one is the MAP kinase JNK which enters the mitochondria and regulates the critical enzyme pyruvate dehydrogenase by phosphorylation [2,3]. Very recently, telomerase has also been shown to accumulate in mitochondria and in fact to serve as an

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Mitochondrial Medicine and Therapeutics, Part II".

* Corresponding author. Department of Physiology Faculty of Medicine Blasco Ibañez, 15 Valencia 46010, Spain. Tel.: +34 96 386 46 46; fax: +34 96 386 46 42.
E-mail address: jose.vina@uv.es (J. Viña).

0169-409X/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2009.06.006

antioxidant in this organelle [4]. Thus, the relevance of mitochondria in cell function continues to go and it is the subject of critical scrutiny.

Mitochondria are involved in two physiological situations of great importance, namely physical exercise and ageing [5]. The fact that physical training, in particular aerobic training, is a very clear-cut stimulus for mitochondriogenesis is beyond doubt. Animals or persons who train show an increased number of mitochondria with the subsequent increased capability of oxygen utilisation [5–7].

Mitochondria are also very relevant to ageing. In fact, the free radical theory of ageing first postulated by Harman in 1956 [8] together with the findings by Boveris and Sies in the laboratory of Britton Chance that 2% of oxygen consumed by mitochondria in state 4 is converted to hydrogen peroxide [9], led Denham Harman to postulate that mitochondria might be critical in the generation of radicals which are responsible for damage associated with ageing. This was further refined by Jaime Miquel who in the 70's formulated the mitochondrial free radical of ageing. Two critical contributions of Miquel were: underpinning the importance of mitochondrial DNA as a target of oxidants produced during ageing and pointing out that mitochondriogenesis might be impaired in ageing and that in fact a lower rate of the renewal of mitochondria was one of the critical events which led to damage associated with ageing [10]. The majority of the studies performed by Jaime Miquel in the United States in the 70's were basically histological [11]. Later on in the 90's, we reported that mitochondria are damaged inside cells [12,13]. This was almost simultaneously confirmed by the group of Bruce Ames [14]. Moreover, we found that oxidative damage to mitochondrial DNA was increased in ageing and this could prevent it by antioxidant supplementations [15]. So the initial prediction of the free radical theory of ageing i.e., that mitochondrial DNA was a key target of damage associated with ageing could be experimentally proved. However, the prediction that ageing was associated with the lower renewal of mitochondria in cells took much longer. The major reason was that it was (and still is) difficult to assess the amount of mitochondria in a cell, particularly in a dynamic way, i.e. whether interventions accelerate or decrease the rate of mitochondriogenesis. To solve this problem, the elucidation of the mitochondriogenic pathway was required.

2. The mitochondriogenic pathway

Mitochondria are continuously produced in the cell, and in fact old or damaged mitochondria are also continuously removed from the cellular compartments. It is therefore critical to understand the mechanisms by which mitochondria are synthesised.

Of paramount importance in this pathway was the discovery of PGC-1α. This is a co-activator of PPARγ [16]. It has a very wide variety of functions. For instance, it is a master regulator of the antioxidant defence. In this situation, PGC-1α is able to control the cellular response to oxidative stress [17]. In a similar fashion, it activates the production of NRF-1 and NRF-2. These are nuclear respiratory factors which are involved in mitochondriogenesis. In fact, these two factors and most importantly NRF-1 are potent stimulators of the expression of TFAM which is a potent stimulator of the duplication of mitochondrial DNA [16]. Thus, when an active mitochondriogenesis is required, PGC-1α is activated, and this leads to an activation of NRF-1 and a subsequent increase in synthesis of TFAM and an increased duplication of mitochondrial DNA. This in turn leads to an increased number of copies of DNA present in the cell and eventually to duplication of mitochondria.

Thus, we now are able to dissect the whole mitochondriogenic pathway in our attempts to study the rate of synthesis of mitochondria and its regulation in the various physiological conditions (see Fig. 1).

3. Mitochondriogenesis is impaired in ageing

As stated above, changes in mitochondriogenesis were key postulates of Miquel's mitochondrial free radical theory of ageing. Critical to any theory of ageing is that its proposition should give us

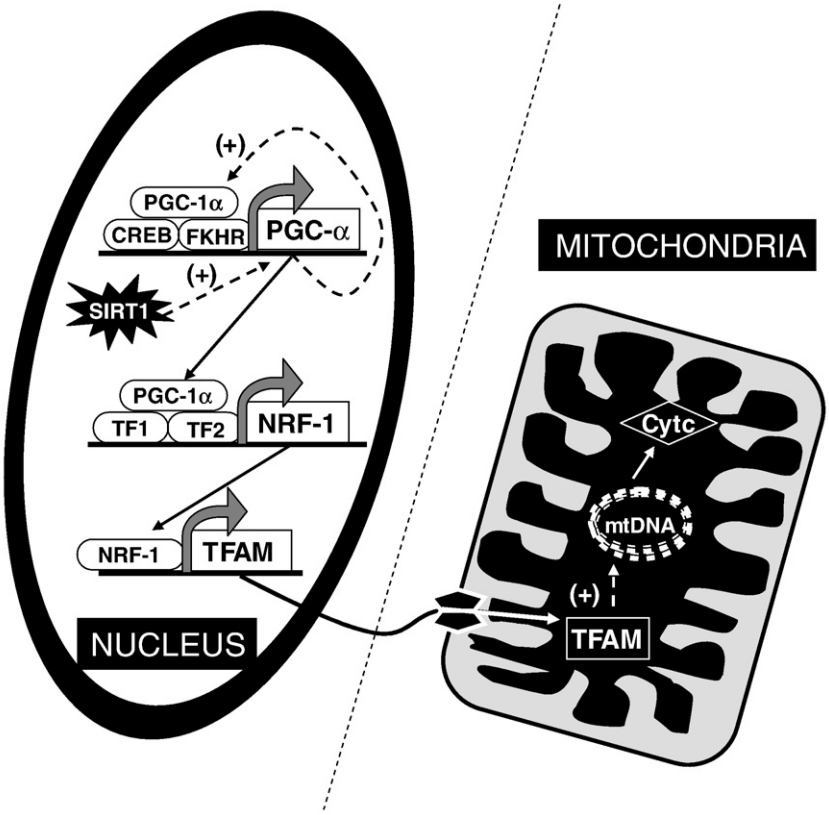


Fig. 1. Schematic representation of the regulation of synthesis of mitochondria.

room for intervention. Therefore, if one studies the loss of mitochondriogenic stimuli in ageing one can hope to promote the synthesis of these microorganelles by interventions such as exercise (see below). Thus, we studied the activity of the mitochondriogenic pathway by measuring the expression PGC-1α, of nuclear respiratory factor 1 [16] and finally we estimated the amount of mitochondria by determining the expression of cytochrome *c*. The problem of the synthesis of mitochondria in ageing was already experimentally attacked by the group of Gadaleta in Bari over a decade ago [18]. Now, as stated above, we have been able to dissect the mitochondriogenic pathway and to determine the expression of the various components of this pathway. We have used heart because it contains mainly post-mitotic cells and because of the importance of this organ in ageing. Fig. 2a shows the expression of PGC-1α in the hearts of young and old animals. As seen in Fig. 2a, the expression in old animals is very significantly lower than that of young ones. In fact, we find an expression of PGC-1α in old animals of approximately one third of that of the young ones. Downstream in the pathway of mitochondriogenesis is NFR-1. Again, according to our prediction, the heart of old animals expressed approximately 25% of NRF-1 when compared with that of young ones (Fig. 2b). The difference is highly significant. Therefore, both factors studied which critically activate the synthesis of mitochondria in myocytes are very much depressed in the old. An expected result would be that mitochondrial proteins are significantly lower in the heart of old animals when compared with young ones and this is indeed the case as shown in Fig. 2c. We have measured cytochrome *c* because of its importance in the mitochondria itself and because of its critical role in the regulation of apoptosis. Cytochrome *c* is indeed significantly lower in old animals than in young ones (see Fig. 2c). Thus, our results indicate that the regulatory factors which control mitochondriogenesis as well as its constituent proteins are significantly lower in the old than in the young animal. Moreover, the fact that there is significantly less mitochondria in tissues which have to be as active, and as aerobic as the heart, provides bases to understand, at least partially, senile cardiomyopathy.

Of course, the fact that aerobic training is so dependent on mitochondriogenesis, gave us a common base to try and understand both mechanisms, the loss of mitochondriogenesis in ageing and its possible prevention by exercise. The next paragraph refers to our experiments on mitochondriogenesis in aerobic training.

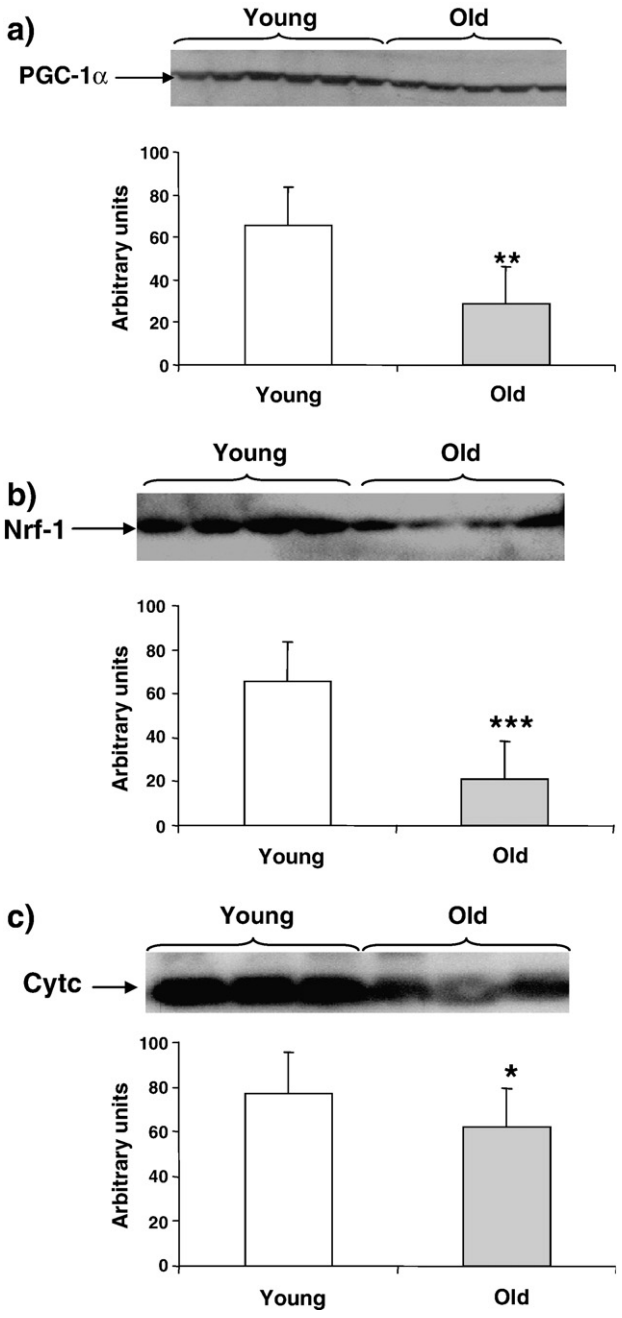


Fig. 2. Cardiac mitochondriogenesis is impaired in ageing.

4. Mitochondriogenesis in physical exercise is controlled by reactive oxygen species

The fact that PGC-1α is a master regulator of the cellular response to oxidative stress [19] and therefore that it may be a good sensor for

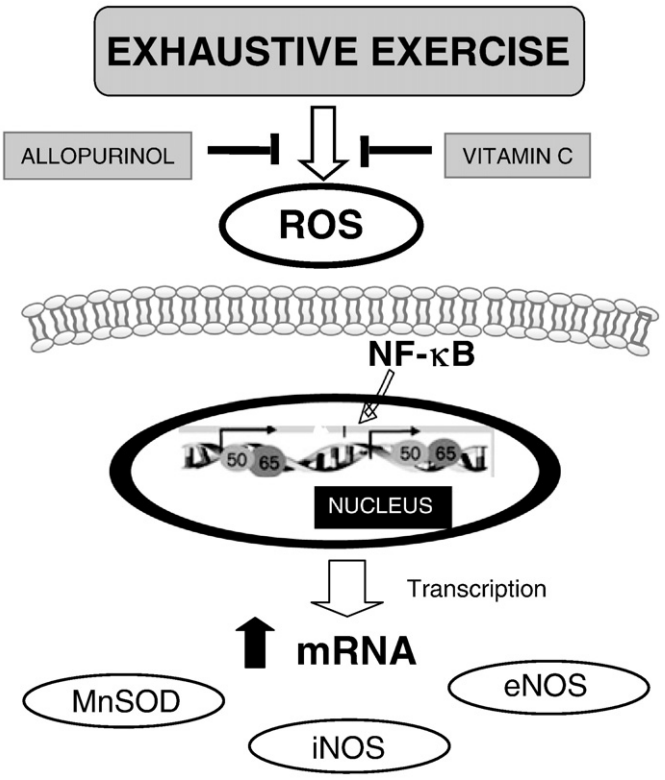


Fig. 3. Prevention, by allopurinol and vitamin C administration, of the NF-κB activation induced by exercise in skeletal muscle and the subsequent up-regulation of gene expression of Mn-SOD, iNOS and eNOS.

the response of the cells to free radicals led us to postulate the idea that free radicals generated in exercise could be signals to increase mitochondriogenesis [20]. In fact, pioneer work by Kelvin Davies in the laboratory of Lester Packer already suggested this idea. When these authors first reported unequivocal evidence that free radicals were produced by muscle in exercise, they suggested that mitochondriogenesis could be a result of the action of these radicals [21]. However, in the early 80's the idea could not be tested experimentally. We took this idea almost twenty years later and studied the role of oxidants and antioxidants in the mitochondriogenesis associated with physical exercise.

Our previous studies had shown that xanthine oxidase is a most important source of reactive oxygen species associated with exhaustive exercise. We tested this hypothesis experimentally both in animals [22,23] and in humans [24,25]. Allopurinol is a very effective inhibitor of xanthine oxidase which is used continuously in clinical practise. Thus, we administered allopurinol to animals subjected to physical exercise and determined the activation of very relevant signalling pathways involved in muscle adaptations to exercise (MAPKs and NF-κB) [23,26]. Fig. 3 shows that allopurinol or vitamin C (which counteracts ROS by different mechanisms) are able to prevent the MAPKinases and NF-κB activation induced by exercise in skeletal muscle and therefore the subsequent up-regulation of gene expression of Mn-SOD, iNOS and eNOS. But the effects we report in Fig. 3 might be due to allopurinol itself and not to its antioxidant effects. We confirmed that vitamin C which acts by a different mechanism to allopurinol, had the same effects on the NF-κB–MAPKs pathway.

Fig. 4 shows a very clear effect of vitamin C in lowering the mitochondriogenic response to exercise (e.g. PGC-1α, NRF-1, TFAM and cytochrome *c*). Both the RNA transcription and the protein expression are decreased when animals are treated with vitamin C. Thus, inhibiting the effect of antioxidants is able to hamper the mitochondriogenic response to exercise [20].

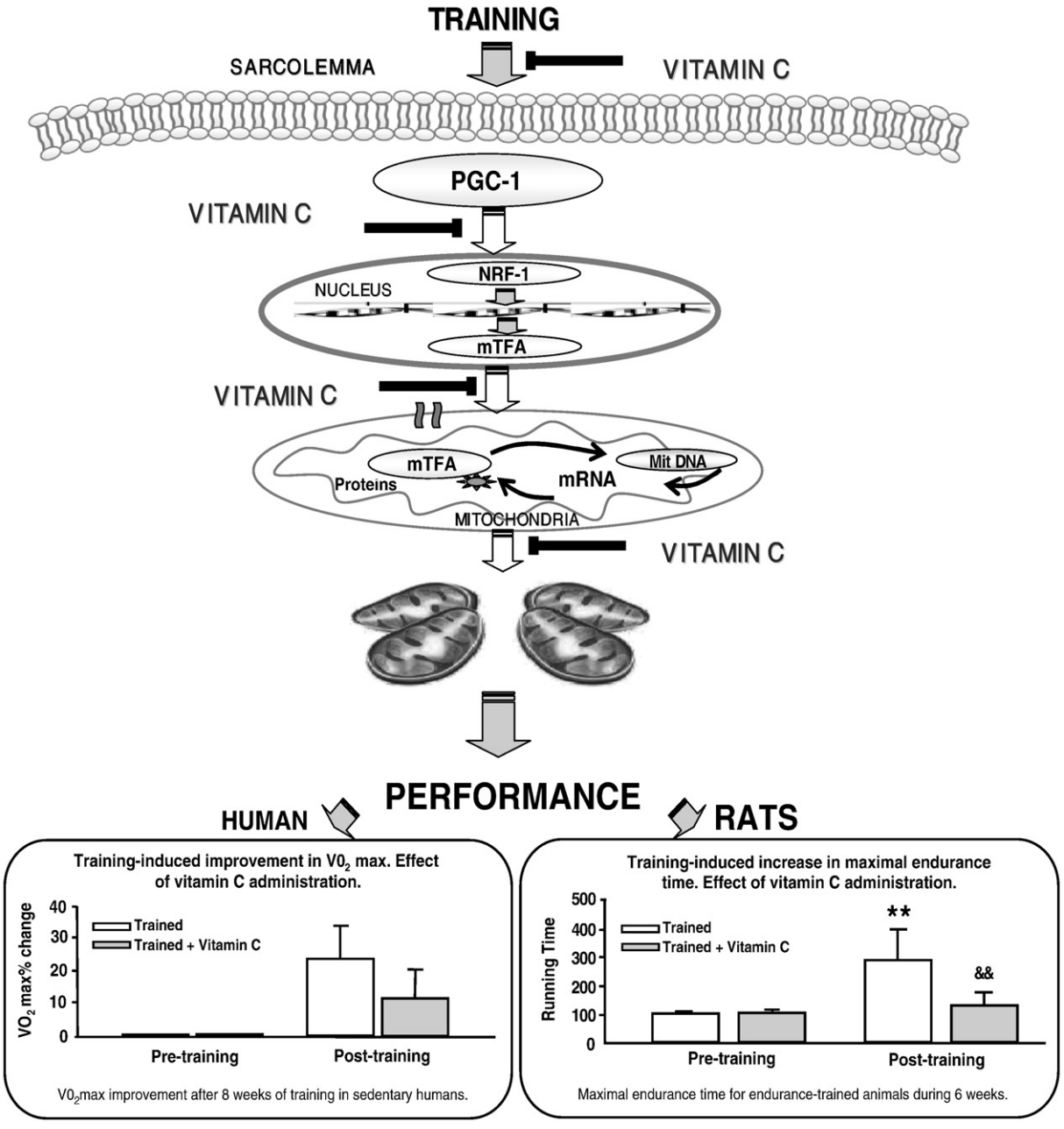


Fig. 4. Summary of the role of reactive oxygen species in signalling of cell adaptations during training and their effect in exercise performance.

5. Reactive oxygen species, antioxidants and performance

The fact that mitochondriogenesis is so much inhibited by allopurinol or vitamin C led us to think that treatment with antioxidants during training might not be advantageous for the efficiency of training. This was an important question because more than 40% of the exercising population in the United States and in Europe regularly take antioxidant supplements [27]. Since an active mitochondriogenesis is imperative during aerobic training, then antioxidant treatment could hamper the efficiency of training. And this was indeed the case. A blind study (in the case of animals) and a double blind study (in the case of humans) indicated that administration of antioxidants lowers the efficiency of training. Fig. 4 (low panels) indicates that indeed the increase in running time to exhaustion of rats after eight weeks of training was much less pronounced when rats were administered vitamin C than in controls. Likewise, administration of 1 g of vitamin C daily to humans decreased the increase in VO_{2max} which occurs after aerobic training. Thus, our theoretical studies on the rate of the mitochondriogenic pathway in exercise led us to a practical conclusion and this is that one should not give antioxidants to athletes when they are training. And then, we are left with the open question, should we give antioxidant supplements to any athlete at all? Our current view is that antioxidant supplementation may be useful when one is likely to perform very exhaustive exercise which causes muscle damage (i.e. competition). However, one should not give antioxidants when the athletes are training and preparing themselves for competition because it may hamper the beneficial adaptations due to training.

These results have been recently confirmed and extended to the healthy effect of exercise (and not only performance) by Michael Ristow and coworkers. They have demonstrated that the exercise-induced oxidative stress ameliorates insulin resistance and causes an adaptive response, promoting endogenous antioxidant defence capacity. Supplementation with antioxidants (vitamins C and E) precludes these health promoting effects of exercise in humans [28].

In summary, we recommend administration of antioxidants to exercise practitioners when they are competing (and therefore they are likely to perform very exhaustive exercise) but not when they are training and they need the reactive oxygen species produced in exercise to attain maximal muscle adaptations.

Our studies further support the view that reactive oxygen species are not just damaging to cells but they have very important, indeed critical, role as signals to adapt the cell to various physiological demands [29,30].

6. Concluding remarks

Mitochondria are key components in cells providing energy to maintain normal cell function. The rate of mitochondriogenesis is very important for the cell to maintain a normal number and mass of mitochondria. Elucidation of the pathway of the mitochondriogenesis which involves PGC-1α, NRF-1, and TFAM to activate duplication of mitochondrial DNA has given us tools to understand changes in mitochondriogenesis both in young and in old animals. Exercise is an intervention which, when practised aerobically, critically depends on the activity of mitochondria. In fact, exercise training activates the mitochondriogenic pathway. It is well known that aerobic capacity is in fact a better predictor of longevity than age itself [31]. Since a critical factor in the ability of the organism to utilise oxygen is the full activity of mitochondria, it is reasonable to speculate that activation of mitochondriogenesis by exercise may delay the impairment in mitochondrial activity with ageing. We have observed that essentially the same mechanisms control mitochondria during ageing and in exercise. We have also reviewed here experiments which indicate that exercise activates mitochondriogenesis mediated by reactive oxygen species and therefore that these may be signals to activate mitochondrial activity, at least in young animals. Research in the future will have to deal with the possibility of activating the impaired rate of mitochondrial synthesis with ageing by aerobic training, in the hope of preventing senile sarcopenia, an area of research which deserves much attention.

Acknowledgements

This work was supported by grants: BFU 2007-65803/BFI and DPS2008-06968 to J.V., 24/UPB20/07 and 35/UPB20/08 to M.C. G-C and by grant (ISCIII2006-RED13-027) from the "Red Temática de investigación cooperativa en envejecimiento y fragilidad (RETICEF). Instituto de Salud Carlos III".

References

[1] K. Kuida, T.F. Haydar, C.Y. Kuan, Y. Gu, C. Taya, H. Karasuyama, M.S. Su, P. Rakic, R.A. Flavell, Reduced apoptosis and cytochrome *c*-mediated caspase activation in mice lacking caspase 9, Cell 94 (1998) 325–337.
[2] H. Schroeter, C.S. Boyd, R. Ahmed, J.P. Spencer, R.F. Duncan, C. Rice-Evans, E. Cadenas, c-Jun N-terminal kinase (JNK)-mediated modulation of brain mitochondria function: new target proteins for JNK signalling in mitochondrion-dependent apoptosis, Biochem. J. 372 (2003) 359–369.
[3] Q. Zhou, P.Y. Lam, D. Han, E. Cadenas, Activation of c-Jun-N-terminal kinase and decline of mitochondrial pyruvate dehydrogenase activity during brain aging, FEBS Lett. 583 (2009) 1132–1140.
[4] S. Ahmed, J.F. Passos, M.J. Birket, T. Beckmann, S. Brings, H. Peters, M.A. Birch-Machin, T. von Zglinicki, G. Saretzki, Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress, J. Cell Sci. 121 (2008) 1046–1053.
[5] J.O. Holloszy, Adaptations of skeletal muscle mitochondria to endurance exercise: a personal perspective, Exerc. Sport Sci. Rev. 32 (2004) 41–43.
[6] J.O. Holloszy, F.W. Booth, Biochemical adaptations to endurance exercise in muscle, Annu. Rev. Physiol. 38 (1976) 273–291.
[7] J.O. Holloszy, E.F. Coyle, Adaptations of skeletal muscle to endurance exercise and their metabolic consequences, J. Appl. Physiol. 56 (1984) 831–838.
[8] D. Harman, Aging: a theory based on free radical and ratiation chemistry, J. Gerontol. 11 (1956) 298–300.
[9] B. Chance, H. Sies, A. Boveris, Hydroperoxide metabolism in mammalian organs, Physiol. Rev. 59 (1979) 527–605.
[10] J. Miquel, A.C. Economos, J. Fleming, J.E. Johnson Jr., Mitochondrial role in cell aging, Exp. Gerontol. 15 (1980) 575–591.
[11] M. Rockstein, J. Chesky, D.E. Philpott, A. Takahashi, J.E. Johnson Jr., J. Miquel, An electron microscopic investigation of age-dependent changes in the flight muscle of *Musca domestica* L, Gerontologia 21 (1975) 216–223.
[12] J.G. de la Asuncion, A. Millan, R. Pla, L. Bruseghini, A. Esteras, F.V. Pallardo, J. Sastre, J. Vina, Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA, Faseb J. 10 (1996) 333–338.
[13] J. Sastre, F.V. Pallardo, R. Pla, A. Pellin, G. Juan, J.E. O'Connor, J.M. Estrela, J. Miquel, J. Vina, Aging of the liver: age-associated mitochondrial damage in intact hepatocytes, Hepatology 24 (1996) 1199–1205.
[14] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Oxidative damage and mitochondrial decay in aging, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 10771–10778.
[15] J. Sastre, A. Millan, J. Garcia de la Asuncion, R. Pla, G. Juan, Pallardo, E. O'Connor, J.A. Martin, M.T. Droy-Lefaix, J. Vina, A *Ginkgo biloba* extract (EGb 761) prevents mitochondrial aging by protecting against oxidative stress, Free Radic. Biol. Med. 24 (1998) 298–304.
[16] P. Puigserver, Z. Wu, C.W. Park, R. Graves, M. Wright, B.M. Spiegelman, A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis, Cell 92 (1998) 829–839.
[17] J. St-Pierre, S. Drori, M. Uldry, J.M. Silvaggi, J. Rhee, S. Jager, C. Handschin, K. Zheng, J. Lin, W. Yang, D.K. Simon, R. Bachoo, B.M. Spiegelman, Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators, Cell 127 (2006) 397–408.
[18] M.N. Gadaleta, A. Cormio, V. Pesce, A.M. Lezza, P. Cantatore, Aging and mitochondria, Biochimie 80 (1998) 863–870.
[19] Y. Olmos, I. Valle, S. Borniquel, A. Tierrez, E. Soria, S. Lamas, M. Monsalve, Mutual dependence of Foxo3a and PGC-1{alpha} in the induction of oxidative stress genes, J. Biol. Chem. 284 (2009) 14476–14484.
[20] M.C. Gomez-Cabrera, E. Domenech, M. Romagnoli, A. Arduini, C. Borras, F.V. Pallardo, J. Sastre, J. Vina, Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance, Am. J. Clin. Nutr. 87 (2008) 142–149.
[21] K.J. Davies, A.T. Quintanilha, G.A. Brooks, L. Packer, Free radicals and tissue damage produced by exercise, Biochem. Biophys. Res. Commun. 107 (1982) 1198–1205.
[22] J. Vina, M.C. Gomez-Cabrera, A. Lloret, R. Marquez, J.B. Minana, F.V. Pallardo, J. Sastre, Free radicals in exhaustive physical exercise: mechanism of production, and protection by antioxidants, IUBMB Life 50 (2000) 271–277.
[23] M.C. Gomez-Cabrera, C. Borras, F.V. Pallardo, J. Sastre, L.L. Ji, J. Vina, Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats, J. Physiol. 567 (2005) 113–120.

[24] M.C. Gomez-Cabrera, F.V. Pallardo, J. Sastre, J. Vina, L. Garcia-del-Moral, Allopurinol and markers of muscle damage among participants in the Tour de France, Jama 289 (2003) 2503–2504.
[25] M.C. Gomez-Cabrera, A. Martinez, G. Santangelo, F.V. Pallardo, J. Sastre, J. Vina, Oxidative stress in marathon runners: interest of antioxidant supplementation, Br. J. Nutr. 96 (Suppl 1) (2006) S31–33.
[26] L.L. Ji, M.C. Gomez-Cabrera, N. Steinhafel, J. Vina, Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle, Faseb J. 18 (2004) 1499–1506.
[27] J.N. Hathcock, A. Azzi, J. Blumberg, T. Bray, A. Dickinson, B. Frei, I. Jialal, C.S. Johnston, F.J. Kelly, K. Kraemer, L. Packer, S. Parthasarathy, H. Sies, M.G. Traber, Vitamins E and C are safe across a broad range of intakes, Am. J. Clin. Nutr. 81 (2005) 736–745.
[28] M. Ristow, K. Zarse, A. Oberbach, N. Kloting, M. Birringer, M. Kiehntopf, M. Stumvoll, C.R. Kahn, M. Bluher, Antioxidants prevent health-promoting effects of physical exercise in humans, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 8665–8670.
[29] L.L. Ji, M.C. Gomez-Cabrera, J. Vina, Exercise and hormesis: activation of cellular antioxidant signaling pathway, Ann. N. Y. Acad. Sci. 1067 (2006) 425–435.
[30] J. Vina, C. Borras, M.C. Gomez-Cabrera, W.C. Orr, Part of the series: from dietary antioxidants to regulators in cellular signalling and gene expression. Role of reactive oxygen species and (phyto)oestrogens in the modulation of adaptive response to stress, Free Radic. Res. 40 (2006) 111–119.
[31] J. Myers, M. Prakash, V. Froelicher, D. Do, S. Partington, J.E. Atwood, Exercise capacity and mortality among men referred for exercise testing, N. Engl. J. Med. 346 (2002) 793–801.

AGE (2012) 34:669–679
DOI 10.1007/s11357-011-9264-y

Age associated low mitochondrial biogenesis may be explained by lack of response of PGC-1α to exercise training

Frederic Derbré · Mari Carmen Gomez-Cabrera · Ana Lucia Nascimento · Fabian Sanchis-Gomar · Vladimir Essau Martinez-Bello · Jesus A. F. Tresguerres · Teresa Fuentes · Arlette Gratas-Delamarche · Maria Monsalve · Jose Viña

Received: 22 December 2010 / Accepted: 28 April 2011 / Published online: 18 May 2011

Abstract Low mitochondriogenesis is critical to explain loss of muscle function in aging and in the development of frailty. The aim of this work was to explain the mechanism by which mitochondriogenesis is decreased in aging and to determine to which extent it may be prevented by exercise training. We used aged rats and compared them with peroxisome proliferator-activated receptor-γ coactivator-1α deleted mice (PGC-1α KO). PGC-1α KO mice showed a significant decrease in the mitochondriogenic pathway in muscle. In aged rats, we found a loss of exercise-induced expression of PGC-1α, nuclear respiratory factor-1 (NRF-1), and of cytochrome C. Thus muscle mitochondriogenesis, which is activated by exercise training in young animals, is not in aged or PGC-1α KO ones. Other stimuli to increase PGC-1α synthesis apart from exercise training, namely cold induction or thyroid hormone treatment, were effective in young rats but not in aged ones. To sum up, the low mitochondrial biogenesis associated with aging may be due to the lack of response of PGC-1α to different stimuli. Aged rats behave as PGC-1α KO mice. Results reported here highlight the role of PGC-1α in the loss of mitochondriogenesis associated with aging and point to this important transcriptional coactivator as a target for pharmacological interventions to prevent age-associated sarcopenia.

Frederic Derbré and Mari Carmen Gomez-Cabrera contributed equally to this work.

F. Derbré · A. Gratas-Delamarche
Laboratory "Movement Sport and Health Sciences",
University Rennes,
Rennes, France

M. C. Gomez-Cabrera · A. L. Nascimento ·
F. Sanchis-Gomar · V. E. Martinez-Bello · J. Viña
Department of Physiology, University of Valencia,
Fundacion Investigacion Hospital Clinico
Universitario/INCLIVA,
Valencia, Spain

J. A. F. Tresguerres
Department of Physiology,
University Complutense of Madrid,
Madrid, Spain

T. Fuentes
Department of Physical Education,
University of Las Palmas de Gran Canaria,
Canary Island, Spain

M. Monsalve
Fundación Centro Nacional de Investigaciones
Cardiovasculares Carlos III,
Madrid, Spain

J. Viña (✉)
Department of Physiology, Faculty of Medicine,
University of Valencia,
Av. Blasco Ibañez, 15,
Valencia 46010, Spain
e-mail: jose.vina@uv.es

Keywords Sarcopenia · Exercise · Oxidative stress · Aging · Cytochrome C · Gene knockout

Introduction

Recent advances in medical care as well as in basic gerontology have led to a significant increase in longevity of populations (life span). However, the remarkable increase in life span has also led to an important increase in frailty and dependency (Gill et al. 2002). It is clear now that we must aim at increasing health span. Muscle aging is a key component of the increase in frailty in human and animal populations (Vanitallie 2003). Physical exercise is an obvious anti-aging mechanism and it is intended to serve as a prevention of cardiovascular aging but also as a prevention of sarcopenia as well as loss in muscle functionality (Fiatarone et al. 1994).

Early work by Miquel et al. proposed that loss of mitochondriogenesis was critical in the fundamental process of aging (Miquel et al. 1980; Miquel 1992). Later, in the 1990s, we reported that mitochondrial damage is an early event in cellular aging (Sastre et al. 1996). This was independently confirmed by the group of Bruce Ames (Hagen et al. 1997). In 2003, it was shown in skeletal muscle that age causes a decrease in ATP content and production by approximately 50% in isolated rat mitochondria (Drew et al. 2003). Since the promotion of mitochondriogenesis is critical to prevent aging, an obvious approach was to try and enhance it by physical exercise (Holloszy and Booth 1976; Davies et al. 1982). Researches have identified the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) as the master regulator of mitochondriogenesis in mammalian tissues (Puigserver and Spiegelman 2003; Wu et al. 1999; Puigserver et al. 1998). In vivo and in vitro studies have shown that PGC-1α levels stimulate mitochondrial proliferation in skeletal muscle (Hood et al. 2006). Increased PGC-1α levels in skeletal muscle by using transgenic MCK-PGC-1α mice (PGC-1α driven by a muscle creatine kinase promoter), prevents muscle wasting by reducing apoptosis, autophagy, and proteasome degradation (Wenz et al. 2009). Moreover, in a recent study by Henriette Pilegaard's group, it has been shown that PGC-1α is required for the beneficial effects of moderate exercise training at advanced age to maintain mitochondrial metabolic and antioxidant capacity (Leick et al. 2010). These studies suggest that the modulation of PGC-1α levels in skeletal muscle present an avenue for the prevention and treatment of age-related disorders.

The aim of our work was to explain the mechanism by which mitochondriogenesis is decreased in aging and to determine to which extent it may be prevented by exercise training. As endurance training is known to upregulate PGC-1α expression in young skeletal muscle (Gomez-Cabrera et al. 2008a), modulation of PGC-1α levels by endurance training in aged skeletal muscle may be a very effective strategy for the prevention and treatment of sarcopenia. For our purpose, we used aged rats and compared them with PGC-1α knockout (KO) mice.

Our results show that muscle from old rats present a marked loss in mitochondriogenesis and that this may be due to a lack of induction of PGC-1α (Puigserver et al. 1998). We find a striking similarity between the response to exercise training in PGC-1α KO mice and in old rats. In young rats, PGC-1α is activated in skeletal muscle not only by training but also by cold exposure or triiodothyronine (T3). We report here that there is an age-associated lack of expression of PGC-1α in response to exercise training or to any of the other stimuli tested in rat skeletal muscle.

Material and methods

Rats For the exercise training experiments, 24 male Wistar rats were randomly divided into four experimental groups: young untrained (n=6), young trained (n=6), aged untrained (n=6), and aged trained (n=6). For the cold induction experiments, 16 male Wistar rats were randomly divided into four experimental groups: young control (n=4), young exposed to cold (n=4), aged control (n=4), and aged exposed to cold (n=4). For the thyroid hormone experiments, 16 male Wistar rats were randomly divided into four experimental groups: young control (n=4), young treated with T3 (n=4), aged control (n=4), and aged treated with T3 (n=4). In all the experimental models, the aged animals were 24 months old and the young ones were 3 months old. We chose 24-month-old rats because previous studies have reported that sarcopenia is evident at this age in this species (Hopp 1993).

Mice The generation and phenotype of PGC-1α KO mice have been described previously (Lin et al. 2004). The genotype of the offspring was assessed by determining the presence of either a wild type (WT)- or KO-specific DNA fragment after extraction of DNA from a tail piece by the phenol-chloroform/isoamyl method, amplification of fragments by PCR using specific primers and separation on an agarose gel. Analysis of the PGC-1α expression revealed that its mRNA was absent in the skeletal muscle of the PGC-1α KO mice. We also wanted to check the PGC-1α protein levels in the skeletal muscle of the KO mice. For this purpose and in order to prevent unspecific cross-reactivity of PGC-1α antibody in the PGC-1α KO mice, we immunoprecipitated the samples of the KO and WT animals (Fig. 1a). Immunoprecipitation was performed with Dynabeads protein A (Invitrogen) according to the manufacturer's instructions. The incubation of the antibody (anti-PGC-1, Cayman) with the beads and the incubation of the extract with antibody cross-linked to the beads were both carried out overnight. The PGC-1 IP fractions were then analyzed by Western Blotting. The band of PGC-1α (~92 KDa) was clearly present in the skeletal muscle of the wild-type animals and was absent in the skeletal muscle of the PGC-1α KO mice. Although a faint band was present with a molecular weight over 100 KDa in the PGC-1α KO muscles, taking into account its molecular weight, we do not consider that this band represents PGC-1α. PGC-1β is a very close homolog of PGC-1α and shares extensive sequence identity (Lin et al. 2002). In addition to their similarities, PGC-1α and PGC-1β share common protein binding partners and the regulation of certain gene programs in skeletal muscle (Handschin et al. 2007). This is why we consider that this band could be PGC-1β. However and to further test the effect of PGC-1α deletion on muscle structure, we performed an electron microscopy analysis of soleus muscle from WT and PGC-1α KO animals. Soleus muscle was dissected and fixed overnight in 2% glutaraldehyde, 1% paraformaldehyde, and 0.08% sodium cacodylate buffer. The tissues were post-fixed in 1% osmium tetroxide, dehydrated in graded ethanol, embedded in Poly Bed plastic resin, and sectioned for electron microscopy. Electron microscopic analysis revealed fewer and smaller mitochondria in soleus muscle of PGC-1α KO mice compared to sex- and age-matched WT ones (see Fig. 2).

Twenty-seven male mice (14 wild-type and 13 PGC-1α KO) were randomly divided into four experimental groups: WT untrained (n=6), WT trained (n=8), KO untrained (n=7), and KO trained (n=6). The animals were kindly provided from the Centro Nacional de Investigaciones Cardiovasculares Carlos III (Madrid. Spain). The animals were 5–6 months old at the beginning of the experimental protocol.

All animals were fed an ad libitum laboratory diet (Global diet 2,014 l; Harlan Teklad, Madison, WI, USA) and were maintained at 23°C under a light/dark cycle of 12/12 h. The experimental protocol was approved by the Committee on Ethics in Research of the Faculty of Medicine of the University of Valencia, Spain.

Training protocols Endurance-trained young and aged rats were exercised 5 day/week on an animal treadmill (Model 1050 LS Exer3/6; Columbus Instruments, Columbus, OH, USA) at a relative intensity of 75% VO_{2max}. The treadmill grade and velocity for each experimental group were chosen based on previous studies performed in young and aged rats (Powers et al. 1994; Lawler et al. 1993; Patch and Brooks 1980). During all the experiments, the grade of the treadmill corresponded to 15% for young rats and 5% for aged rats. We followed a modification of the protocol of Davies et al. (1981). The young animals were required to run, the first training session, for 25 min at a speed of 26.8 $m \times min^{-1}$. The old animals were required to run, the first training session, at a speed of 15 $m \times min^{-1}$ for 15 min. The duration an intensity of each work period was increased progressively. The last day of the training week 3, young animals were running for 1 h at a speed of 30 $m \times min^{-1}$ and the aged ones were running for 45 min at a speed of 18 $m \times min^{-1}$. It has been shown that young untrained rats require approximately 75% of their VO_{2max} to run at 26.8 $m \times min^{-1}$ (15% grade) on a treadmill (Patch and Brooks 1980) (Davies et al. 1981). Aged untrained animals require approximately 75% of their VO_{2max} to run at 15 $m \times min^{-1}$ (5% grade) on a treadmill (Lawler et al. 1993). Exercise motivation was provided for all rodents by means of an electronic shock grid at the treadmill rear. However, the electric shock was used sparingly

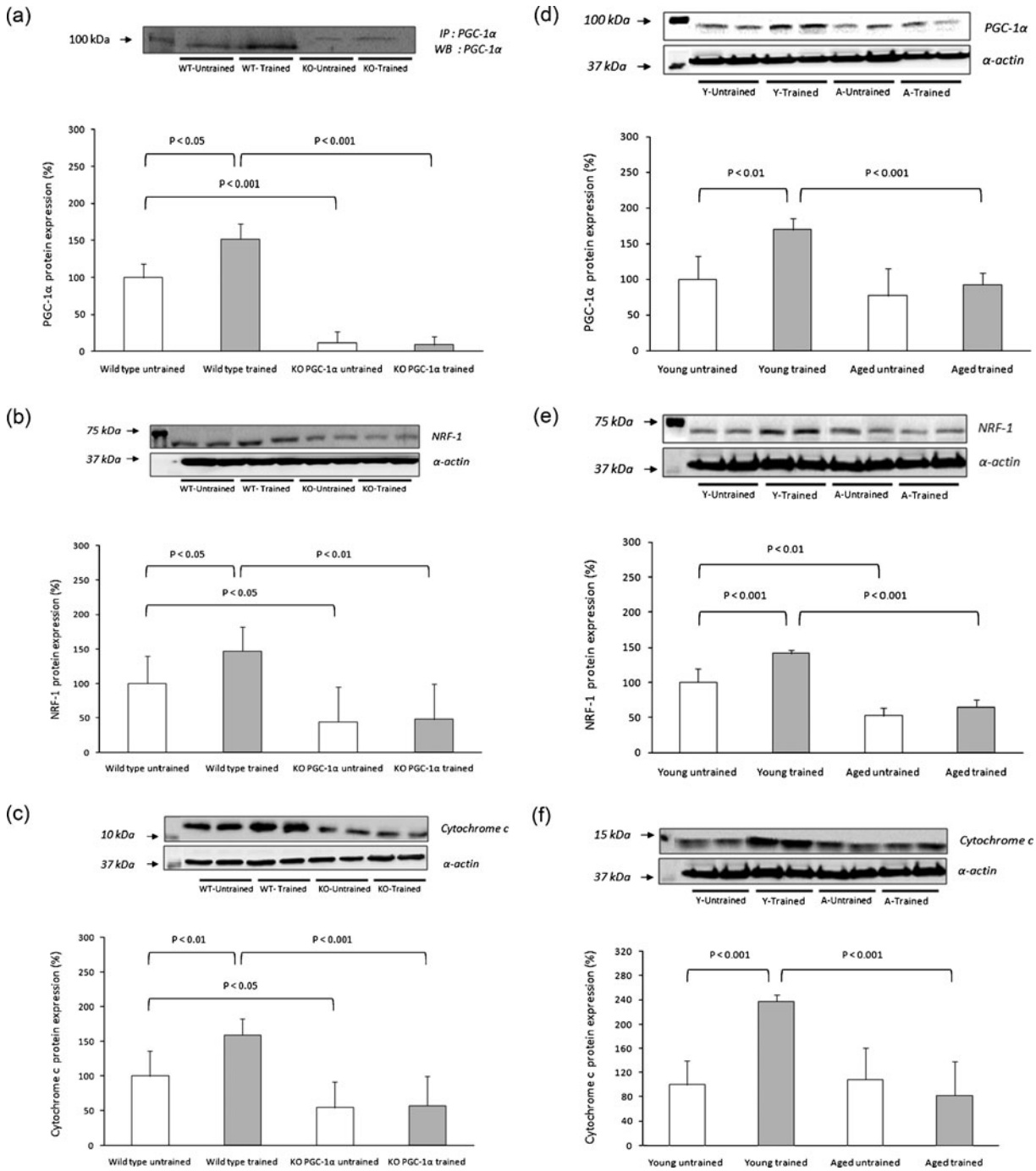


Fig. 1 Exercise-induced activation of the mitochondrial biogenesis pathway in skeletal muscle. Western blotting analysis to detect PGC-1α (**a** and **d**), NRF-1 (**b** and **e**), and cytochrome C (**c** and **f**) in PGC-1α KO mice and aged rats. Twenty-seven male mice were randomly divided into four experimental groups: WT untrained (n=6), WT trained (n=8), KO untrained (n=7), and KO trained (n=6). Twenty-four male Wistar rats were randomly divided into four experimental groups: young untrained (n=6), young trained (n=6), aged untrained (n=6), and aged trained (n=6). Representative blots are shown. For the densitometric analysis of the results, values are shown as mean (±SD).The content of α-actin, a housekeeping protein marker in skeletal muscle, was determined in all the experimental groups

during training and during the endurance capacity test. The untrained groups were exercised at the same speeds for only 10 min every 3 days for the entire 3-week period. Endurance capacity was assessed, before and after the training period, during a run to exhaustion at 26.8 $m \times min^{-1}$ at a grade of 15% for young rats and at 15 $m \times min^{-1}$ at a grade of 5% for aged rats (Davies et al. 1982; see Table 1a).

PGC-1α KO and WT male mice were randomly allocated to either a training group or a control group. The training groups completed 4 weeks of treadmill exercise training five times per week and progressively increased until 60 min at 20 $m \times min^{-1}$ (10% slope) at the end of the second week. Endurance capacity was assessed, before and after the training period, during a run to exhaustion at 20 $m \times min^{-1}$ at a grade of 10% (sees Table 1b). After the tests, the animals were given 48 h of complete rest before being sacrificed.

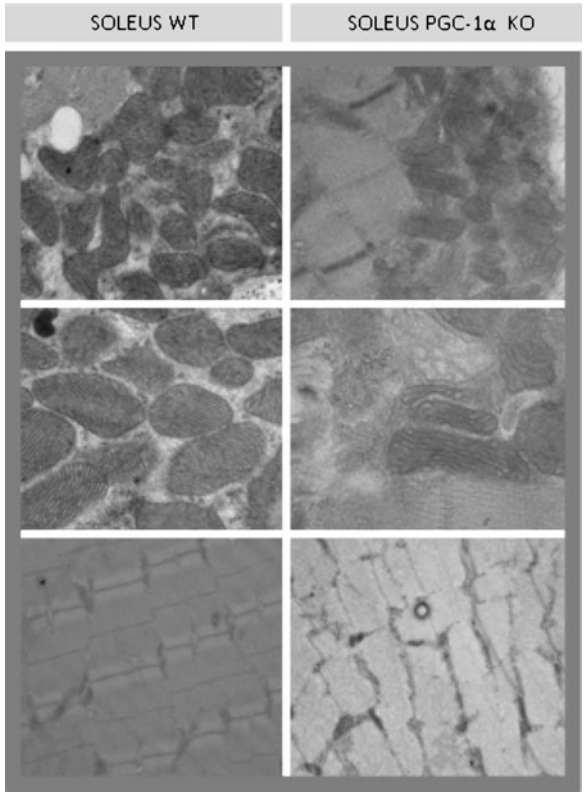


Fig. 2 Electron microscopy analysis of soleus muscle from WT and PGC-1α KO animals. Smaller mitochondria were found in the muscle of PGC-1α KO mice compared to sex- and age-matched WT

Cold exposure protocol After an acclimatization period (1 week), young and aged Wistar male rats were randomly divided into two groups: cold-exposed animals (4±1°C for 24 h) and control animals (24±1°C; Puigserver et al. 1998). Skeletal muscles were removed immediately after the end of the cold exposure.

T3 treatment Young and aged Wistar male rats were injected intraperitoneally one dose with either T3 (0.4 $mg \times kg^{-1}$) or vehicle (0.9% NaCl-propylene glycol; 40:60 vol/vol). Skeletal muscles were removed 6 h after the injections (Irrcher et al. 2003). Gastrocnemius and soleus muscles were removed quickly, freeze-clamped immediately, and stored at −80°C. All the animals were sacrificed by an overdose of sodium pentobarbital.

SDS-PAGE and Western Blotting Aliquots of muscle lysates (Ji et al. 2004) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The whole gastrocnemius was used to ensure homogeneity. Proteins were then transferred to nitrocellulose membranes, which were incubated overnight at 4°C with appropriate primary antibodies: anti-PGC-1 (1:1,000, Cayman), anti-NRF-1 (1:200, Santa Cruz Biotechnology Inc.), anti-cytochrome C (1:1,000, Santa Cruz Biotechnology Inc.) and anti-α-actin (1:700, Sigma Aldrich). Thereafter, membranes were incubated with a secondary antibody for 1 h at room temperature. Specific proteins were visualized by using the enhanced chemiluminescence procedure as specified by the manufacturer (Amersham Biosciences, Piscataway, NJ, USA). Autoradiographic signals were assessed by using a scanning densitometer (BioRad, Hercules, CA, USA).

Protein carbonylation Oxidative modification of total proteins was assessed by immunoblot detection of protein carbonyl groups using the "OxyBlot" protein oxidation kit (Intergen) as previously described (Romagnoli et al. 2010).

Statistics Results are expressed as mean±SD. Normality of distribution was checked with the Kolmogorov test and homogeneity of variance was tested by Levene's statistics. For endurance capacity, a repeated measures two-factor analysis of variance was performed. Repeated measures were performed for training (before training compared with after training); the second factor was the status of animals (control or trained). The

Table 1 Training-induced improvement in maximal endurance time in PGC-1α KO and aged rats

	Untrained	Trained	Untrained	Trained
Endurance capacity (min)[a]	Young rats		Aged rats	
Before	36.8±4.6	42.0±12.1	13.2±4.8	14.7±5.8
After 3 weeks	38.5±4.0	115.7±18.2*, **	19.7±7.5	46.3±6.5*, **
Endurance capacity (min)[b]	Wild-type mice		PGC-1α KO mice	
Before	37.0±24.5	39.3±26.2	12.0±6.9	13.2±8.8
After 4 weeks	41.3±27.8	158.8±53.3***, ****,*****	21.2±3.6	58.0±16.4***, ****

[a] Means (±SD) results of maximal endurance time before and after endurance training in young and aged rats. Twenty-four male Wistar rats were randomly divided into four experimental groups: young untrained (n=6), young trained (n=6), aged untrained (n=6), and aged trained (n=6)

[b] Means (±SD) results of maximal endurance time before and after endurance training in WT and PGC-1α KO mice. Twenty-seven male mice were randomly divided into four experimental groups: WT untrained (n=6), WT trained (n=8), KO untrained (n=7), and KO trained (n=6)

*p<0.05 when compared to values before training

**p<0.05 when compared to untrained groups

***p<0.05 when compared to values before training

****p<0.05 when compared to the untrained group

*****p<0.05 when compared to the KO trained mice

main effect of training was tested with Newmann–Keuls post hoc test. For Western Blot analysis, we used a two-way analysis of variance and Bonferroni's post hoc test (Sigma Stats, version 3.11). Results were considered statistically significant for p<0.05.

Results

Effect of aging or PGC-1α deletion on endurance response to training Running time to exhaustion during an endurance capacity test was approximately 63% lower in aged than in young rats (Table 1a). Similarly, running time to exhaustion was ~65–70% lower in the PGC-1α KO mice than in WT animals (Table 1b). These results are in accordance with previous studies demonstrating that endurance capacity was lower in PGC-1α KO animals than in WT (Leick et al. 2008). Table 1a also shows that the intensity and duration of the training regimen followed by young and aged rats was enough to induce a significant improvement in maximal endurance capacity (~200% and ~135%, respectively). Similarly, the training protocol induced an increase in the endurance capacity both in WT and PGC-1α KO mice (~284% and ~173%, respectively; Table 1b). It cannot be ruled out that the differences found on the endurance capacity test, between the young and old rats, may be explained by the different durations of the exercise training protocols, although this is unlikely. Both groups improved their endurance capacity following a similar exercise intensity training protocol (~75% of their VO_{2max}). The different durations of the training periods might explain, in part, some of our results (see "Material and methods" section).

Muscle mitochondriogenesis in PGC-1α KO and aged animals Figure 1 shows that muscle mitochondriogenesis is considerably impaired in PGC-1α-deficient mice. Figure 1a show that training caused an increase in PGC-1α content in WT mice. As expected, we did not find any PGC-1α protein levels in sedentary or in trained PGC-1α KO animals. Although the band corresponding to the PGC-1α protein was absent in our KO mice (92 kDa), a faint band, which is likely to be PGC-1β, appeared in the Western Blotting over 100 kDa (see "Material and methods" section).

Figure 1b shows that training increased nuclear respiratory factor-1 (NRF-1), a critical intermediate of the mitochondriogenic pathway in WT animals but not in PGC-1α KO ones. It is also shown that sedentary PGC-1α KO animals have considerably

less NRF-1 than controls. Mitochondrial content can be measured directly, using morphometric estimates of organelle volume in relation to total cellular volume. More commonly, it is estimated by the change in maximal activity, measured under optimal conditions in vitro, of a typical "marker enzyme" such as citrate synthase, or by the change in content of a single protein-like cytochrome C (Hood 2001; Terjung 1979). Several authors have used cytochrome C content as a marker of mitochondrial mass (Hood et al. 2006; Leick et al. 2008, 2010) and this is the methodology that we have followed in the present work. Training caused an increase in cytochrome C content in WT but not in PGC-1α KO mice. Cytochrome C content in PGC-1α KO animals was also lower than in WT (Fig. 1c). To sum up, deficiency in PGC-1α resulted in a hampered mitochondriogenic responsiveness to exercise training. In aged rats, we found very similar results, i.e., a loss of exercise-induced increase in the protein levels of PGC-1α, NRF-1 and cytochrome C (Fig. 1d–f). The main idea reported here is that old age resembles PGC-1α deficiency in terms of lack of responsiveness to training. Indeed, young animals showed an increase in the protein levels of PGC-1α after training which did not occur in aged ones (Fig. 1d). In Fig. 1e, we show that NRF-1 content was increased in muscle of young rats after training. This effect was lost when we studied aged animals. Finally, Fig. 1f shows that training increased cytochrome C content in muscle of young rats but again this effect was also lost in aged rats.

Oxidative stress and training in aged animals and PGC-1α KO As apparent in Fig. 1, many of the adaptations of the skeletal muscle to exercise training in aging are similar to those found in PGC-1α KO animals. Thus, we measured the effect of exercise training on skeletal muscle protein oxidation status in young and aged animals and in WT and PGC-1α KO animals. In Fig. 3, we show protein oxidation in PGC-1α KO (Fig. 3a) and in aged animals (Fig. 3b). PGC-1α KO animals show an increase in resting protein oxidation (Fig. 3a). The same happens in aged animals (Fig. 3b). No effects of training were found in any experimental group.

Lack of activation of PGC-1α by cold exposure or by thyroid hormone treatment in the aged animal The experiments reported above showing that the muscle of aged animals did not upregulate the expression of PGC-1α in response to exercise training, led us to think that aging could result in a lack of responsiveness of PGC-1α to other physiological stimuli. Two of the key stimulators of PGC-1α are thyroid hormones (Irrcher et al. 2003) and cold exposure (Puigserver et al. 1998). Figure 4a shows that young animals, when exposed to cold, upregulated the

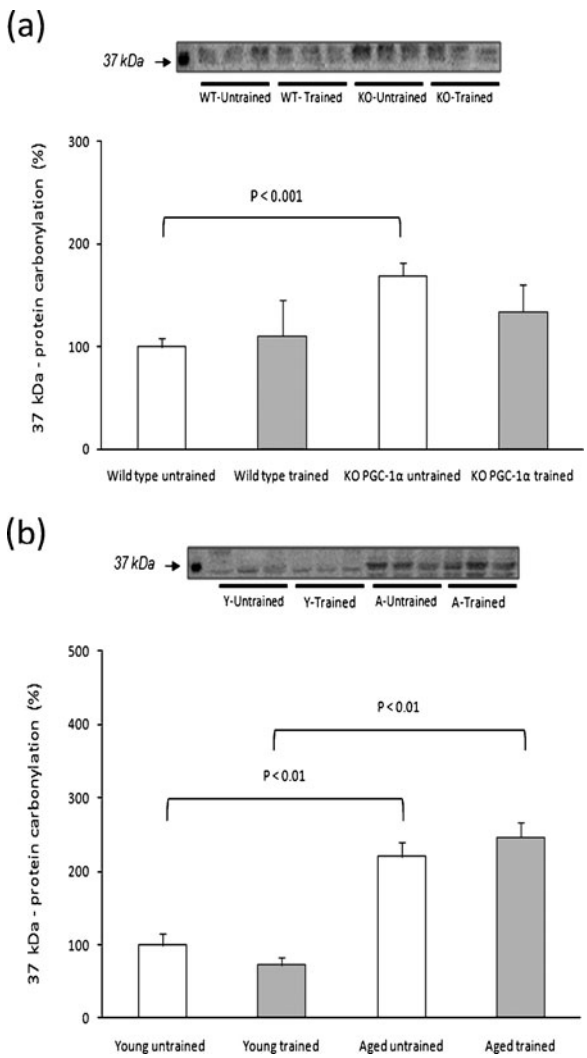


Fig. 3 Muscle protein oxidation in untrained and trained animals. Western blotting analysis to detect carbonylated proteins (MW: 37 kDa). Representative experiments are shown. For the densitometric analysis of the results values are shown as mean (±SD) of **a** WT untrained (n=6), WT trained (n=8), PGC-1α KO untrained (n=7), and PGC-1α KO-trained animals (n=6); **b** young untrained (n=6), young trained (n=6), aged untrained (n=6), and aged-trained animals (n=6)

expression of PGC-1α threefold but aged animals did not. The same happens with triiodothyronine (T3; Fig. 4b).

Discussion

The major idea in this paper is that aging causes a lack of response of PGC-1α to various stimuli, the most important being exercise training (see Fig. 1), but also to cold exposure or thyroid hormone treatment (see Fig. 4).

The role of mitochondria as key generator of oxidative stress and also target of the damage associated with reactive oxygen species (ROS) production was postulated by Miquel in the 1970s (Johnson et al. 1975). Independent work from our laboratory (Sastre et al. 1996) and that from Bruce Ames' (Hagen et al. 1997) using both metabolic and flow cytometric approaches provided such evidence. We showed that mitochondria are damaged within cells instead of being frail and damaged during the isolation procedure. A functional muscle that has not lost the capacity to synthesize healthy mitochondria is an important contributor to the prevention of frailty, a major problem in medicine, particularly in geriatrics (Fiatarone et al. 1994; Gill et al. 2010). Thus, understanding the molecular mechanisms of mitochondriogenesis in aging has both theoretical and practical importance. It has been reported that the mitochondrial function is adapted in response to calorie restriction and this adaptation is critically involved in lifespan extension (Anderson et al. 2008). Calorie restriction has been shown to activate PGC-1α (Nisoli et al. 2005; Anderson et al. 2008; Anderson and Weindruch 2009) and it may be an effective strategy in delaying aging-induced cellular phenotypes in skeletal muscle (McKiernan et al. 2010). PGC-1α is critical for the adaptation of muscle mitochondriogenesis to exercise which activates the expression of NRF-1 which in turn, activates TFAM, a factor required for the duplication of mitochondrial DNA (Hood 2001). This led us to think that mitochondriogenesis might be impaired in aging because of the impaired response of PGC-1α in old animals when compared with young ones. To understand the role of the redox-sensitive PGC-1α in the regulation of mitochondriogenesis in muscle, we used animals which were KO for PGC-1α.

We found a striking similarity in the molecular response of exercise in the mitochondriogenic pathway in mice which are deleted of PGC-1α and in the old rats. The whole pathway involving PGC-1α→NRF-1 and finally cytochrome C (an indicator of mitochondrial mass) responded positively to exercise training in young rats, but failed to do so in old ones. This was precisely the same behavior as that in PGC-1α-deleted mice.

It could be argued that the intensity of the exercise training was not enough to onset the mitochondrial biogenesis in PGC-1α KO mice or in old rats. However, Table 1 shows that the intensity and duration of the training regimen followed by our animals was enough

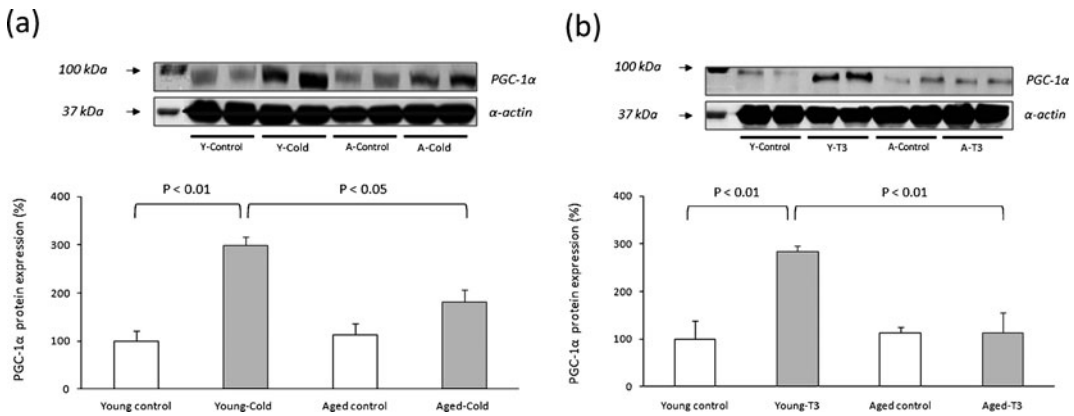


Fig. 4 PGC-1α protein levels in skeletal muscle of animals exposed to cold (4±1°C) or treated with triiodothyronine (0.4 mg × kg^{-1}). Representative experiments are shown. For the densitometric analysis of the results values are shown as mean (±SD) of **a** young control (n=4), young exposed to cold (n=4), aged control (n=4), and aged-exposed to cold (n=4); **b** young control (n=4), young treated with T3 (n=4), aged control (n=4), aged treated with T3 (n=4). The content of α-actin, a housekeeping protein marker in skeletal muscle, was determined in all the experimental groups

to induce a significant improvement in maximal endurance capacity.

PGC-1α protects against skeletal muscle atrophy (Sandri et al. 2006) and very recently it has been shown to be required for training-induced prevention of age associated decline in mitochondria (Leick et al. 2010). Moreover, relevance of PGC-1α in sarcopenia and metabolic diseases during aging has been also suggested (Wenz et al. 2009). Transgenic MCK-PGC-1α animals have preserved mitochondrial function, neuromuscular junctions, and muscle integrity during aging. Moreover, increased PGC-1α levels in skeletal muscle prevent muscle wasting by reducing apoptosis, autophagy, and proteasome degradation (Wenz et al. 2009).

Our studies in exercise adaptations to aging, as stated above, led us to the conclusion that PGC-1α was not responding to exercise training in old animals. We suspected that PGC-1α might not be induced by any kind of stimulus in old animals. To test this hypothesis, we used two well-known stimuli of PGC-1α in young and old rats, namely thyroid hormone stimulation and cold acclimation (Irrcher et al. 2003; Puigserver et al. 1998). Figure 4 shows that both T3 treatment or cold acclimatization caused a very pronounced activation of PGC-1α in young animals. However, there was a striking lack of activation of PGC-1α by any of the stimuli tested when we were using old animals.

It has been reported that the health benefits of chronic exercise may be, at least partially, due to a reduction in mitochondrial oxidant production (Judge et al. 2005). These data question the very well-established idea that exercise generates free radicals. This was first established by the group of Packer (Davies et al. 1982) who showed that ROS are generated during muscle contraction. In that seminal paper, the authors already postulated that the exercise-induced mitochondriogenesis might be stimulated by ROS (Davies et al. 1982). We provided the first clear-cut evidence that exercise generates oxidative stress only when it is exhaustive (Sastre et al. 1992; Gomez-Cabrera et al. 2003). However, physical exercise can be considered as a double edge sword: when practiced strenuously it causes oxidative stress and cell damage but when practiced with moderation, it increases the expression of antioxidant enzymes and thus should be considered as an antioxidant (Gomez-Cabrera et al. 2008b). Here, we have measured the effect of exercise training on skeletal muscle protein oxidation status in young and aged rats and in WT and PGC-1α KO mice. We have found that skeletal muscle from aged and KO PGC-1α animals exhibit oxidative stress, i.e., an increase in protein carbonylation, in resting conditions. The protein oxidation was not significantly increased after training in any experimental group which is in accordance with our idea that exercise training does not increase oxidative stress (Gomez-Cabrera et al. 2008b). Previous work from our laboratory as well as from others has demonstrated that interfering with free radical production with antioxidants may hamper mitochondriogenic activation by exercise (Gomez-Cabrera et al. 2008a; Strobel et al. 2010; Ristow et al. 2009). In the case of aging, we outline a different scenario. Our data show that there are chronic high levels of ROS in the skeletal muscle of old and PGC-1α KO animals. We consider that under these circumstances, the response to exercise of the redox-sensitive cell signaling pathways may be hampered. However, this hypothesis needs to be confirmed in future investigations.

PGC-1α is currently identified as a new therapeutic target for treatment of age-related mitochondrial dysfunction in skeletal muscle, and more generally for sarcopenia (Sandri et al. 2006). Moreover, recently, an interesting paper has underpinned the critical role of PGC-1α to link nuclear and mitcochondrial changes in aging (Kelly 2011). Our study highlights the importance of maintaining a normal PGC-1α responsiveness (which we show here is lost in aging) to maintain normal muscle function, and certainly this problem deserves more research. A schematic interpretation of our results is in Fig. 5.

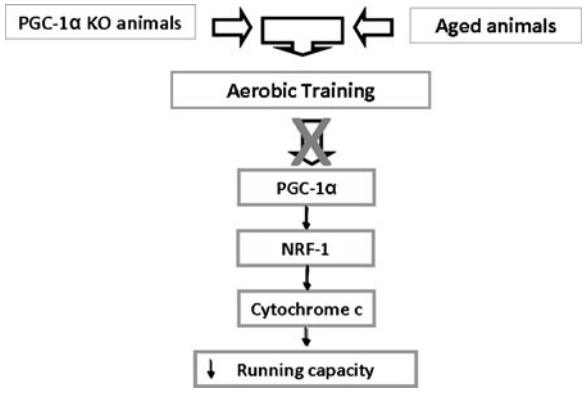


Fig. 5 PGC-1α is not functional in the aged skeletal muscle and it can be involved in the decrease in mitochondrial biogenesis during aging. Proposed mechanism

Acknowledgements This work was supported by grants SAF2008-00270, SAF2009-08334, and BFU2007-65803/BFI from the Spanish Ministry of Education and Science; PROMETEO/2010/074 from the Consellería de Educación de la Generalitat Valenciana. ISCIII2006-RED13-027 from the "Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF)", EU Funded COSTB35 and DPS2008-06968 from Spanish Ministry of Innovation and Science. This study has been cofinanced by FEDER funds from the European Union.

References

Anderson RM, Weindruch R (2009) Metabolic reprogramming, caloric restriction and aging. Trends Endocrinol Metab 21 (3):134–141

Anderson RM, Barger JL, Edwards MG, Braun KH, O'Connor CE, Prolla TA, Weindruch R (2008) Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. Aging Cell 7(1):101–111

Davies KJ, Packer L, Brooks GA (1981) Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. Arch Biochem Biophys 209 (2):539–554

Davies KJ, Quintanilha AT, Brooks GA, Packer L (1982) Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107(4):1198–1205

Drew B, Phaneuf S, Dirks A, Selman C, Gredilla R, Lezza A, Barja G, Leeuwenburgh C (2003) Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. Am J Physiol Regul Integr Comp Physiol 284(2):R474–R480

Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, Nelson ME, Roberts SB, Kehayias JJ, Lipsitz LA, Evans WJ (1994) Exercise training and nutritional supplementation for physical frailty in very elderly people. N Engl J Med 330(25):1769–1775

Gill TM, Baker DI, Gottschalk M, Peduzzi PN, Allore H, Byers A (2002) A program to prevent functional decline in physically frail, elderly persons who live at home. N Engl J Med 347(14):1068–1074

Gill TM, Gahbauer EA, Han L, Allore HG (2010) Trajectories of disability in the last year of life. N Engl J Med 362 (13):1173–1180

Gomez-Cabrera MC, Pallardo FV, Sastre J, Vina J, Garcia-del-Moral L (2003) Allopurinol and markers of muscle damage among participants in the Tour de France. Jama 289(19):2503–2504

Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, Sastre J, Vina J (2008a) Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 87(1):142–149

Gomez-Cabrera MC, Domenech E, Vina J (2008b) Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med 44(2):126–131

Hagen TM, Yowe DL, Bartholomew JC, Wehr CM, Do KL, Park JY, Ames BN (1997) Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. Proc Natl Acad Sci USA 94(7):3064–3069

Handschin C, Chin S, Li P, Liu F, Maratos-Flier E, Lebrasseur NK, Yan Z, Spiegelman BM (2007) Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals. J Biol Chem 282(41):30014–30021

Holloszy JO, Booth FW (1976) Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38:273–291

Hood DA (2001) Invited review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. J Appl Physiol 90(3):1137–1157

Hood DA, Irrcher I, Ljubicic V, Joseph AM (2006) Coordination of metabolic plasticity in skeletal muscle. J Exp Biol 209(Pt 12):2265–2275

Hopp JF (1993) Effects of age and resistance training on skeletal muscle: a review. Phys Ther 73(6):361–373

Irrcher I, Adhihetty PJ, Sheehan T, Joseph AM, Hood DA (2003) PPARgamma coactivator-1alpha expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. Am J Physiol Cell Physiol 284(6): C1669–C1677

Ji LL, Gomez-Cabrera MC, Steinhafel N, Vina J (2004) Acute exercise activates nuclear factor (NF)-kappaB signaling pathway in rat skeletal muscle. FASEB J 18(13):1499–1506

Johnson JE, Mehler WR, Miquel J (1975) A fine structural study of degenerative changes in the dorsal column nuclei of aging mice. Lack of protection by vitamin E. J Gerontol 30(4):395–411

Judge S, Jang YM, Smith A, Selman C, Phillips T, Speakman JR, Hagen T, Leeuwenburgh C (2005) Exercise by lifelong voluntary wheel running reduces subsarcolemmal and interfibrillar mitochondrial hydrogen peroxide production in the heart. Am J Physiol Regul Integr Comp Physiol 289 (6):R1564–R1572

Kelly DP (2011) Cell biology: ageing theories unified. Nature 470:342–343

Lawler JM, Powers SK, Visser T, Van Dijk H, Kordus MJ, Ji LL (1993) Acute exercise and skeletal muscle antioxidant and metabolic enzymes: effects of fiber type and age. Am J Physiol 265(6 Pt 2):R1344–R1350

Leick L, Wojtaszewski JF, Johansen ST, Kiilerich K, Comes G, Hellsten Y, Hidalgo J, Pilegaard H (2008) PGC-1alpha is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle. Am J Physiol Endocrinol Metab 294(2):E463–E474

Leick L, Lyngby SS, Wojtasewski JF, Pilegaard H (2010) PGC-1alpha is required for training-induced prevention of age-associated decline in mitochondrial enzymes in mouse skeletal muscle. Exp Gerontol 45(5):336–342

Lin J, Puigserver P, Donovan J, Tarr P, Spiegelman BM (2002) Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. J Biol Chem 277(3):1645–1648

Lin J, Wu PH, Tarr PT, Lindenberg KS, St-Pierre J, Zhang CY, Mootha VK, Jager S, Vianna CR, Reznick RM, Cui L, Manieri M, Donovan MX, Wu Z, Cooper MP, Fan MC, Rohas LM, Zavacki AM, Cinti S, Shulman GI, Lowell BB, Krainc D, Spiegelman BM (2004) Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. Cell 119(1):121–135

McKiernan SH, Colman RJ, Lopez M, Beasley TM, Aiken JM, Anderson RM, Weindruch R (2010) Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle. Exp Gerontol 46(1):23–29

Miquel J (1992) An update on the mitochondrial-DNA mutation hypothesis of cell aging. Mutat Res 275(3–6):209–216

Miquel J, Economos AC, Fleming J, Johnson JE Jr (1980) Mitochondrial role in cell aging. Exp Gerontol 15(6):575–591

Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO (2005) Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science 310(5746):314–317

Patch LD, Brooks GA (1980) Effects of training on VO2 max and VO2 during two running intensities in rats. Pflugers Arch 386(3):215–219

Powers SK, Criswell D, Lawler J, Ji LL, Martin D, Herb RA, Dudley G (1994) Influence of exercise and fiber type on antioxidant enzyme activity in rat skeletal muscle. Am J Physiol 266(2 Pt 2):R375–R380

Puigserver P, Spiegelman BM (2003) Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocr Rev 24(1):78–90

Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92(6):829–839

Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, Bluher M (2009) Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci USA 106 (21):8665–8670

Romagnoli M, Gomez-Cabrera MC, Perrelli MG, Biasi F, Pallardo FV, Sastre J, Poli G, Vina J (2010) Xanthine oxidase-induced oxidative stress causes activation of NF-kappaB and inflammation in the liver of type I diabetic rats. Free Radic Biol Med 49(2):171–177

Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL, Spiegelman BM (2006) PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc Natl Acad Sci USA 103(44):16260–16265

Sastre J, Asensi M, Gasco E, Pallardo FV, Ferrero JA, Furukawa T, Vina J (1992) Exhaustive physical exercise causes oxidation of glutathione status in blood: prevention by antioxidant administration. Am J Physiol 263(5 Pt 2): R992–R995

Sastre J, Pallardo FV, Pla R, Pellin A, Juan G, O'Connor JE, Estrela JM, Miquel J, Vina J (1996) Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. Hepatology 24(5):1199–1205

Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS, Wadley GD (2010) Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. Med Sci Sports Exerc. doi:10.1249/MSS.0b013e318203afa3

Terjung RL (1979) The turnover of cytochrome C in different skeletal-muscle fibre types of the rat. Biochem J 178 (3):569–574

Vanitallie TB (2003) Frailty in the elderly: contributions of sarcopenia and visceral protein depletion. Metabolism 52 (10 Suppl 2):22–26

Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT (2009) Increased muscle PGC-1alpha expression protects from sarcopenia and metabolic disease during aging. Proc Natl Acad Sci USA 106(48):20405–20410

Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98(1):115–124