Journal of Hypertension

SYMPATHETIC NEURAL ACTIVITY, METABOLIC PARAMETERS AND CARDIORESPIRATORY FITNESS IN OBESE YOUTH

--Manuscript Draft--

Manuscript Number:	JH-D-16-00213R2
Full Title:	SYMPATHETIC NEURAL ACTIVITY, METABOLIC PARAMETERS AND CARDIORESPIRATORY FITNESS IN OBESE YOUTH
Article Type:	Original Manuscript
Keywords:	Youth; obesity; sympathetic nervous system, metabolic parameters, cardiorespiratory fitness.
Corresponding Author:	Empar Lurbe, Prof. MD. PhD Consorcio Hospital General. University of Valencia and CIBER Fisiopatología Obesidad y Nutrición (CB06/03), Instituto de Salud Carlos III Valencia, SPAIN
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Consorcio Hospital General. University of Valencia and CIBER Fisiopatología Obesidad y Nutrición (CB06/03), Instituto de Salud Carlos III
Corresponding Author's Secondary Institution:	
First Author:	Pau REDÓN
First Author Secondary Information:	
Order of Authors:	Pau REDÓN
	Guido GRASSI
	Josep REDON
	Julio ÁLVAREZ-PITTI
	Empar Lurbe, Prof. MD. PhD
Order of Authors Secondary Information:	
Abstract:	Objective: The main objective of this cross sectional study is to assess the cardiac autonomic neural activity in the presence of abnormally increased body weight in youths and its relationship to metabolic risk factors and cardiorespiratory fitness. Methods: Sixty-four overweight and obese subjects, 9 to 17 years, of both sexes, stratified according to the international body mass index cut-off, were enrolled. Continuous electrocardiogram was recorded during 15 minutes in resting conditions, and the heart rate variability was measured in the time domain, frequency domain and for non-linear dynamics. In addition, cardiometabolic risk factors, and cardiorespiratory fitness in effort conditions were assessed. Results: Among the overweight and obese youths, no significant differences were observed regarding metabolic parameters and heart rate, although cardiorespiratory fitness was the lowest in the severe obese. Likewise, no significant differences were observed in heart rate variability, independent of how it was assessed. A positive and significant relationship, independent of the degree of obesity, pubertal stage and breathing rate under resting conditions, has been observed between sympatho-vagal balance, insulin and the HOMA index. Furthermore, cardiorespiratory fitness assessed by VO2peak was associated with insulin levels ($r=-0.273$; $p<0.05$), the standard deviation of the NN interval (SDNN: $r=0.268$, $p<0.05$) and the long term variation using the Poincaré plot (PS1: $r=0.275$, $p<0.05$; PS2 $r=0.273$, $p<0.05$). Conclusion: The key findings of the present study were the presence of a link between fasting insulin, heart rate variability and cardiorespiratory fitness independent of the degree of obesity, indicating the heterogeneity of obese children and adolescents.

ABBREVIATIONS

ANS - Autonomic nervous system

BMI – Body mass index

BP – Blood pressure

BR - Breathing rate

CRF - Cardiorespiratory fitness

cANS - Cardiac autonomic nervous system

DBP – Diastolic blood pressure

HF - High frequency

HOMA - Homeostatic model assessment

HR – Heart rate

HRV – Heart rate variability

HTN – Hypertension

LF - Low frequency

PSD - Power spectral density

SampEn - Sample entropy

SBP – Systolic blood pressure

SNS - Sympathetic nervous system

VO_2 – Volume oxygen

CONDENSED ABSTRACT

This cross sectional study is to assess the cardiac autonomic neural activity and its relationship with metabolic risk factors and cardiorespiratory fitness in the presence of abnormally increased body weight in youths. The key findings of the present study were the presence of a link between fasting insulin, heart rate variability and cardiorespiratory fitness. Increased fasting insulin is associated with higher sympatho-vagal balance and lower cardiorespiratory fitness independently of the degree of obesity indicating the heterogeneity of obese children and adolescents. Better knowledge of the presence of cardiometabolic risk factors may contribute to the enhancement of the treatment´s efficiency in obese youth and consequently, to improvements in prognosis and life in adulthood.

SYMPATHETIC NEURAL ACTIVITY, METABOLIC PARAMETERS AND CARDIORESPIRATORY FITNESS IN OBESE YOUTH

Running Title: Autonomic Nervous System in Obese Youth

Pau REDÓN, PhD[a,b], Guido GRASSI[c,d], Josep REDON[b,e,f], Julio ÁLVAREZ-PITTI[a,b], Empar LURBE[a,b]

a. Pediatric Department, Consorcio Hospital General, University of Valencia, Spain

b. CIBER Fisiopatología Obesidad y Nutrición (CB06/03), Instituto de Salud Carlos III, Madrid, Spain.

c. Dipartimento di Scienze della Salute, Clinica Medica, Universitá Milano-Bicocca, Milano, Italy

d. IRCCS Multimedica, Sesto San Giovanni, Milano, Italy

e. INCLIVA Research Institute, Valencia, Spain

f. Hypertension Clinic. Department of Internal Medicine, Hospital Clínico de Valencia, University of Valencia, Valencia, Spain

Short Title: *Sympathetic nervous system in obese youths*

Word Count: Text 4109; Tables 3; Figure 1

Correspondence:

Empar Lurbe

Pediatrics Department

Consorcio Hospital General Universitario de Valencia

Avenida Tres Cruces s/n

Valencia, 46014

Spain

Tel: 0034 963 131 800

E-mail: empar.lurbe@uv.es

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ABSTRACT

Objective: The main objective of this cross sectional study is to assess the cardiac autonomic neural activity in the presence of abnormally increased body weight in youths and its relationship to metabolic risk factors and cardiorespiratory fitness. **Methods**: Sixty-four overweight and obese subjects, 9 to 17 years, of both sexes, stratified according to the international body mass index cut-off, were enrolled. Continuous electrocardiogram was recorded during 15 minutes in resting conditions, and the heart rate variability was measured in the time domain, frequency domain and for non-linear dynamics. In addition, cardiometabolic risk factors, and cardiorespiratory fitness in effort conditions were assessed. **Results**: Among the overweight and obese youths, no significant differences were observed regarding metabolic parameters and heart rate, although cardiorespiratory fitness was the lowest in the severe obese. Likewise, no significant differences were observed in heart rate variability, independent of how it was assessed. A positive and significant relationship, independent of the degree of obesity, pubertal stage and breathing rate under resting conditions, has been observed between sympatho-vagal balance, insulin and the HOMA index. Furthermore, cardiorespiratory fitness assessed by VO_{2peak} was associated with insulin levels ($r=-0.273$; $p<0.05$), the standard deviation of the NN interval (SDNN: $r=0.268$, $p<0.05$) and the long term variation using the Poincaré plot (P_{S1}: $r=0.275$, $p<0.05$; P_{S2} $r=0.273$, $p<0.05$).

Conclusion: The key findings of the present study were the presence of a link between fasting insulin, heart rate variability and cardiorespiratory fitness independent of the degree of obesity, indicating the heterogeneity of obese children and adolescents.

KEYWORDS: Youth; obesity; sympathetic nervous system, metabolic parameters, cardiorespiratory fitness.

INTRODUCTION

Recent years have seen a rapid worldwide increase in obesity, especially among children and young people [1]. The adverse effects of weight gain on metabolic and cardiovascular function and the association of weight gain with a higher incidence of health problems later in life represent major issues in health care which have generated great concern over the last few years [2-5]. Indeed, considering the increasing tendency for obesity to appear during childhood and to track, to some extent, into adult life [6-8], as well as the firmly established relationships among obesity, type 2 diabetes, and hypertension (HTN) in adults, obese children appear to be at particularly high risk of becoming diabetic and hypertensive as they age.

Hyperinsulinemia, high blood pressure (BP) and reduction in cardiovascular fitness are all associated with overweight and obesity. In a recent study [9], with 611 obese youths, 39%, 16.5% and 2.8% of this population presented 1, 2 or 3 risk factors, respectively, with hyperinsulinemia being the most prevalent, followed by lipid abnormalities and high BP. Moreover, obesity is inversely related to cardiorespiratory fitness (CRF), and when expressed by oxygen consumption during the effort test (VO_{2peak}), it is a marker of cardiovascular morbidity and mortality [10].

The association between obesity and cardiometabolic risk factors, however, differs among obese patients. While some subjects with a mild degree of obesity have an increment in risk, others do not, even though they have a higher degree of obesity so, as a consequence, the concept of “healthy” obese has been proposed [11]. The challenge is to find out whether altered underlying mechanisms are present in order to

optimize interventions. Therefore, identifying these potential intermediate mechanisms for the development of cardiometabolic abnormalities may define a high risk group in obese youth.

One of the potential mechanisms which could contribute to the cardiometabolic alterations is the imbalance in the activity of the autonomic nervous system (ANS). Experimental and clinical studies with overweight and obese populations have related autonomic nervous activity to obesity, mainly the sympathetic nervous system (SNS) [12-16]. The role of the autonomic imbalance in the over activity of the SNS, on BP values [15], hyperinsulinemia [14] and cardiorespiratory fitness has been studied in the adult population [17]; however, studies in children are scarce [18]. The main objective of this cross sectional study is to assess the cardiac ANS activity in the presence of abnormally increased body weight in youths and its relationship with metabolic risk factors BP and CRF.

SUBJECTS AND METHODS

Sixty-Four overweight and obese, 9 to 17 years old, of both sexes, from those who underwent an assessment of overweight/obesity in the Pediatric Department of the Consorcio Hospital General de Valencia (Spain) were enrolled. None of them had secondary obesity syndromes suffered from acute or chronic disease or had enrolled in weight loss programmes. Data about physical activity was obtained from physician-patient clinical interviews. Anthropometric parameters, BP measurements and metabolic profile were assessed. In addition, heart rate (HR) and respiratory rate (BR) were measured in resting and effort conditions. While HRV was performed exclusively

under resting conditions from the recorded ECG signal, the CRF was evaluated as oxygen consumption during the effort test. The protocol was approved by the Ethical Committee of the Consorcio Hospital General de Valencia and a consent form was signed by the parents and the participants.

Anthropometrics:

Body weight was recorded to the nearest 0.1 kg using a standard beam balance scale with the individuals wearing light indoor clothing and no shoes. Height was recorded to the nearest 0.5 cm using a standardized wall-mounted height board. Overweight and obesity have been qualified based on the recently published extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity in children [19]. In this case, BMI values for age and sex between 25 and 30 are overweight, between 30 and 35 are obese and above 35 are morbid obese. Waist circumference was measured at the midpoint between the iliac crest and the costal margin in the mid-axillary line in the standing position at the end of a gentle expiration. Height and waist ratio was calculated.

Blood pressure measurement and metabolic assessment

Following the published recommendations of the European Society of Hypertension Guidelines [20], trained nurses measured the BP of each individual three times consecutively in the seated position at five minute intervals. Office BP was taken as the mean of the three measurements. Hypertension was defined when SBP and/or DBP were persistently higher than the 95^{th} percentile specific for age, sex and height [20].

Individuals were considered normotensive when both SBP and DBP were less than the 90^{th} for age, sex and height.

Metabolic assessment was performed under fasting conditions in the early morning. Peripheral blood samples were obtained to measure glucose by the glucose oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, CA, USA) [21], insulin (Pharmacia Insulin RIA kit; Uppsala, Sweden) [22] and lipid profile. Homeostatic model assessment for insulin resistance (HOMA-IR) index was calculated by dividing the product of insulin (microunits per milliliter) and glucose (millimoles per liter) by 22.5 [23]. Hyperinsulinism was defined from norms for pubertal stage [4,24,25]. Abnormal fasting lipids were defined from normative data [26].

Monitoring Heart Rate Variability and Cardio Respiratory Fitness in resting and effort conditions

The heart rate, BR and ECG signal, under resting conditions, were continuously measured and recorded using a TIPS shirt. The procedure lasted 15 minutes in which the patient, in fasting conditions, remained lying down in a quiet room at 23ºC temperature, following the recommendations summarized in Guixeres, et al. [27].

Cardiorespiratory fitness was measured according to a modified version of the Balke protocol for treadmills which consisted of two phases: an initial 3-min warm-up and the effort stage which lasted until the subject reaches 90% of the maximum theoretical HR. The $(HR)_{peak}$, the $(BR)_{peak}$ and the $(VO_2)_{peak}$ are the corresponding values when the stopping criterion was reached. All the participants reached the stopping criterion of

the effort test. In addition, the BP [$(\text{Systolic BP})_{peak}$ and $(\text{Diastolic BP})_{peak}$] were measured [28].

Heart rate variability assessment

The HRV of all the participants was analysed from the continuous 15 minute ECG signal recorded under resting conditions following the recommendations previously published [29-31]. These analyses have been performed in the time and frequency domain as well as in that regarding non-linear dynamics.

Time domain analyses include statistical and geometric methods directly calculated from the inter-beat interval time-dependant series. Special attention was paid to the standard deviation of the NN interval series (SDNN). *Frequency domain analyses* used to calculate the total power spectral density (PSD), as well as the power density corresponding to low frequency (LF) and high frequency (HF), were performed using the Lomb-Scargle method. Both absolute (aLF, aHF and aTotal), as well as normalized values (nLF, nHF and LF/HF) are presented. It is worth noting that the intervals considered for LF and HF are 0.04-0.15 and 0.15-1, respectively.

Non-linear dynamics were studied from the ECG signal using entropy base measures (sample entropy), Poincaré plot (PS) and fractal base measures (Detrended Fluctuation Analyses, DFA). While the first of them calculates the complexity of the whole signal, the other two differentiate between short and long term variation. To distinguish between them, a subscript 1 or 2 following the name of the procedure is used (i.e: P_{S1}:

short term variation according to the Poincaré plot procedure, P_{S2}: long term variation according to the Poincaré Plot).

Statistical analysis

The results were statistically analysed using SPSS version 16 (SPSS Inc., Chicago, Illinois, USA). Shapiro-Wilk and normal Q-Q plots were used to analyse the degree of normal distribution of the study population. One-way ANOVA was used to compare quantitative variables, followed by Bonferroni's correction to control for multiple comparisons. Bi-variate partial correlation tests were performed to assess relationships among the different variables. Obesity degree, BR and Pubertal stage were considered as confounders. Those *p*-values lower than 0.05 were considered statistically significant.

RESULTS

General characteristics of the study population

A total of 64 young Caucasian individuals were included in the study, of whom 14 (22%) were overweight, 33 (51%) were moderately obese and 17 (27%) severely obese. No differences in age and sex distribution among groups were detected. Six subjects were hypertensive. All youth included in this cohort manifested a lack of physical activity other than the mandatory school activities. The general characteristics of the study population grouped by overweight and degree of obesity have been summarized in Table 1.

No significant differences were observed in insulin, HOMA-IR, fasting glucose, lipid profile, breath rate or in BP and HR under resting conditions. Despite the absence of statistical significance, severely obese subjects tend to have the highest values of insulin, HOMA-IR and systolic BP. Under effort conditions, however, significant differences in $VO_{2peak,}$ were observed between obese and overweight, BR and diastolic BP for the obese groups.

Heart rate variability,

Heart rate variability in the time and frequency domain and its non-linear components were analysed. All these parameters are summarized for each of the groups in Table 2. Significant differences were observed in the total power spectral density -calculated using the Lomb-Scargle method - between the moderate and severe obese. The rest of the parameters do not seem to follow well-defined trends. This suggests that obesity by itself is not responsible for altering cANS activity.

Heart rate variability, metabolic profile and CRF

The association of HRV parameters with metabolic profile, BP values and CRF were assessed by Pearson´s correlation coefficients in subjects excluding the hypertensives (Table 3). Obesity degree, pubertal stage and BR at rest were considered cofounders. Only normotensive subjects were included because of the bias HTN may introduce to the results of HRV assessment.

Fasting insulin and HOMA-IR have presented significant positive correlations with the sympatho-vagal balance (LS_LF/HF: $r=0.281$, $p<0.05$; $r=0.304$, $p<0.05$, respectively);

however, no significant relationship was observed with BP at rest or under effort conditions.

Cardiorespiratory fitness, assessed by $VO_{2peak,}$ was associated with the standard deviation of the NN interval (SDNN: r=0.268, p<0.05) and with the long-term variation using the Poincaré plot (P_{S1}: r=0.275, p<0.05; P_{S2} r=0.273, p<0.05). These are in agreement with the fact that higher ECG signal complexity is expected in subjects with a lower degree of obesity. Finally, VO_{2peak} was significantly associated with insulin (r=-0.273; p<0.05), although with HOMA-IR it shows a trend (r=-0.236; p<0.09) when adjusted by degree of obesity, pubertal stage and BR in effort.

Multivariant analysis relating the three components, metabolic (insulin levels), HRV (LS_LF/HF) and the CRF (VO_{2peak}) and confounders has been performed. Insulin levels were independently related to LF/HF (p=0.003), VO_{2peak} (p=0.028), pubertal stage (p=0.014) and BR at exercise (p=0.016). These factors explain 35% of the variance. Figure 1 summarizes how CRF, HRV and insulin are significantly related to each other.

DISCUSSION

In a pediatric population with a wide age range and BMI distribution, from overweight to severely obese, the activity of cANS was assessed through the HRV analysis of the ECG signal recorded under resting conditions. The key findings of the present study were the presence of a link between fasting insulin, HRV and CRF. Increased fasting insulin was associated with a higher sympatho-vagal balance and lower CRF,

independent of the degree of obesity and puberty, indicating the heterogeneity of obese children and adolescents.

The study was performed in a cohort of overweight and obese individuals recruited from an obesity clinic and may not be representative of the general population. Six subjects (10%) were diagnosed with HTN. The overweight and obese population has been qualified following the new extended international body mass index cut-offs (19). Even though this reformulation is expected to lead to minor changes in existing thresholds, the main benefits among others are, its capability to be compared directly with the international and WHO cut-offs and the fact that they are exclusively for children and are not linked to the corresponding adult BMI values.

Obesity is an increasingly prevalent condition which predisposes to the presence of cardiometabolic risk factors, hyperinsulinemia, HTN and lipid abnormalities [9]. It is important to reinforce that the present study was performed in a pediatric population in which comorbidities were not yet fully established. Data show that insulin levels correlate with cANS activity, measured in the frequency domain, independent of the degree of obesity and the pubertal stage. This finding coupled with non-significant differences of insulin levels between overweight and obese subjects, points out the potential role of cANS activity in the dysmetabolic state. While some studies [32-34] have concluded that insulin may trigger sympathetic excitatory effects on central adrenergic neural outflow, in others an increased adrenergic state has shown per se to favour the development and progression of a hyperinsulinemic state [35].

In addition to the evaluation of traditional parameters, the effort test, CRF, has been added and assessed by oxygen consumption, (VO_{2peak}. In the evaluation of overweight/obese children and adolescents the assessment of CRF is regularly not included, even though the information gained can be relevant when categorizing the risk of obese subjects [10]. The effort test was performed using a modified Balke protocol suited for children and adolescents in which the speed and the inclination profile were modified. The main purpose of these modifications was to assure a more secure environment for the subjects participating in the study and to avoid reaching extenuation. Despite them, the procedure retains its discriminatory effect as described in Guixeres et al. [27]. During the exercise, lower (VO_{2peak} was observed in obese subjects as compared with overweight ones, indicating the poorest CRF performance is most evident in the severely obese.

Studies performed in adults [36] and recently in non-obese children [37] have related low (VO_{2peak} with life expectancy in adults and metabolic abnormalities, like insulin resistance in children, respectively. In the present research, this finding has also been demonstrated, but in this case in the obese pediatric population. The low (VO_{2peak} is inversely associated with fasting insulin, indicating that the lower the CRF the higher the risk for insulin resistance at this early stage of life and foreseeing the future risk to develop metabolic syndrome and type 2 diabetes. The concurrence of low CRF, high levels of insulin and diastolic BP values indicates the presence of subtle derangements of the cardiometabolic status, predicting a future increment of cardiovascular risk. Consequently, CRF is a relevant clinical parameter that could be included into the routine assessment of obese subjects.

Autonomic nervous system imbalance, assessed in the heart, may not only contribute to the development or stabilization of obesity (38,39) but it is also associated with cardiovascular mortality (40). Therefore, the study of the ANS function in the presence of obesity is of considerable clinical interest. Currently, several procedures [41-43] have been developed to assess the ANS activity. Nowadays, HRV has become one of the most popular methods (cANS), because it is simple, reliable and non-invasive. The HRV assessment is based on the NN interval duration in ECG, reflecting the complex interactions between the SNS and the parasympathetic one.

In general, time and frequency domain analyses have been applied to assess the activity of ANS [41]. Moreover, an analysis performed by non-linear dynamics is becoming more frequent as complementary information [43,44]. The increment of cANS in those subjects with elevated insulin levels suggests that obesity itself does not alter the ECG signal variability in contrast with metabolic factors. These data are partially in agreement with the study of Baum et al. in which an ANS dysfunction was described in obese children and adolescents [45]. These data are partially in agreement with those of the study by Baum et al. in which an ANS dysfunction was described in obese children and adolescents [45]. Autonomic nervous system dysfunction, was characterized by both parasympathetic and sympathetic activity reduction, although the LF/LH ratio was positively related to body weight. Similarly, cANS abnormalities were observed in pre-obese and obese young adults [46].

Non-linear dynamics evaluated with the Poincaré plot method have shown a positive correlation with VO_{2peak}. The fact that both short and long term variations are positively related highlights the relationship between signal complexity and CRF [44]. Several studies have associated these variations with parasympathetic activity (PS1) and sympathetic modulation (PS2) [47] or as an indicator of vagal modulation (PS1) [48]. In either case, higher activity is associated with healthier subjects which at the end reach higher VO_{2peak} values reflecting better CRF conditions. This is confirmed by the positive correlation between SDNN and VO_{2peak}. The coherence of these findings highlights the robustness of the Poincaré plot procedure in analysing non-linear dynamics under resting conditions and its application for predicting CRFs in an overweight and obese youth.

Strengths and limitations of the present research need to be considered. Being a cross-sectional study with a limited sample size does not allow for casual relationships among the parameters tested. Data from clinical samples may not be representative of the general population, and selection and referral bias was present. Moreover, no information about the duration of obesity was available, a factor that can influence cANS. The lack of physical activity, other than that mandatory at school was common for all subjects. Combining the information of traditional parameters with those coming from the assessment of cANS in a cohort of young people with a wide BMI distribution and heterogeneous metabolic abnormalities contributes relevant information to the link between the cANS activity, metabolic status and CRF, strengthening the relevance of the study.

The present work deals with an under investigated area in pediatric patients, adding significant insights into the links among metabolic parameters, CRF and HRV in the presence of abnormally increased body weight. In addition, it reflects the heterogeneity of obese subjects with autonomic imbalance and the association of this latter one with cardiometabolic risk factors. Better knowledge of the presence of cardiometabolic risk factors may contribute to an enhancement of treatment efficiency in obese youth, and consequently improve prognosis and quality of life in adulthood. Longitudinal studies should be performed to determine whether or not cANS imbalance can be a cause or a consequence of the metabolic and cardiovascular alterations linked to obesity.

CONFLICTS OF INTEREST AND SOURCE OF FUNDING

The study was partially funded by grant number PI14/01781, Instituto de Salud Carlos III, Spain and CIBER Fisiopatología Obesidad y Nutrición (CB06/03), FEDER funds, Instituto de Salud Carlos III, Spain.

The authors declare no conflicts of interest.

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LEGEND OF FIGURES

Figure 1. Pearson´s correlation coefficients (*p<0.05*) showing the link between insulin, heart rate variability and cardiorespiratory fitness. All correlations were adjusted by degree of obesity, pubertal stage and BR (baseline when HRV and in effort when VO_{2peak} was considered, respectively).

$^{\Omega}$ *r* denotes the relationship between LS_LF/HF and fasting insulin

$^{¥}$ *r* denotes the relationship between SDNN, Poincaré S1 and S2

* *r* denotes the relationship between insulin and VO_{2peak}

See text for details

Reviewer #1:
The manuscript has improved, but still has several inaccuracies.

** The purpose of the study is "to assess the cardiac ANS activity in the presence of abnormally increased body weight in youths and its relationship with metabolic risk factors, BP and CRF". Results demonstrate that insulin and HOMA are associated positively and independently of obesity degree, puberty and breathing rate with HRV and insulin is negatively associated with CRF. However, the association between CRF and insulin is not reported in the Abstract (only in condensed abstract). Furthermore, the relation between CRF and HOMA is not described and the negative correlation of insulin with CRF shown in Figure 1 does not seem to be adjusted for puberty and obesity (which is essential). This association must be also reported in the text of Results.*

The information requested has been added in the abstract **"…VO_{2peak}, was associated with insulin levels (r=-0.273; p<0.05)…**" On page 12, first paragraph: **"Finally, VO_{2peak} was significantly associated with insulin (r=-0.273; p<0.05), although with HOMA-IR it shows a trend (r=-0.236; p<0.09) when adjusted by degree of obesity, pubertal stage and BR in effort.**"

The caption for Figure 1 has been revised to explain the confounders included. "**Figure 1. Pearson´s correlation coefficients (*p<0.05*) showing the link between insulin, heart rate variability and cardiorespiratory fitness. All correlations were adjusted by degree of obesity, pubertal stage and BR (baseline when HRV and in effort when VO_{2peak} was considered, respectively).**"

** In the revised version of the manuscript, subjects are categorized into hyperinsulinemic, hypertensive and perhaps in the original intention of the authors in dyslipidemic (in Methods is mentioned a category of "abnormal lipids" that however is not reported in Results). If the main result of the study is the association of insulin with cardiac autonomic neural activity, why the authors did not assess whether the autonomic dysfunction is more pronounced in hyperinsulinemia subjects? In any case, I would suggest to identify the "high insulin" levels using the reference values of insulin in a large population of children aged 7-20 years (Pediatrics 2012; 129, e1020).*

Data for the lipid profile were included as a baseline variable to exclude extreme dyslipemic subjects, such as familial hypercholesterolemia or other genetic causes, but they were not relevant for the purpose of the study.

Hyperinsulinemia was defined following the criterion used in reference 24. In addition, we added the sources for the reference values (Goran and Gower, 2001; Alberti and Zimmet, 1998). The use of other criteria, such as those recommended by the reviewer, does not add any relevant issues. To the contrary, the criteria used in the present study are more accepted by the scientific community. This is reflected by the number of the citations. Those recommended by the reviewer have been poorly quoted in the literature (referenced on only 20 occasions). In contrast, the criteria used in the present study have been widely quoted (358 references, 15 in the last six months).

** Reference 24 for hyperinsulinism is incorrect and should be removed. The reference for adult WHO criteria of hyperinsulinism (>20 mU/l) is missing.*

Reference 24 has been replaced by Goran and Gower, 2001 (24); for adult criteria, Alberti and Zimmet, 1998 (25) has been added.

** HOMA is incorrectly defined "homeostatic model assessment index" rather than HOMA-IR "homeostatic model assessment for insulin resistance".*

This semantic issue has been resolved.

** The ref 20 should be updated with the latest 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents*

The authors of the manuscript are quite aware of the new guidelines; however, the present research was undertaken well before their publication. They differ from the previous one in the definition of hypertension in subjects 16 years or older. That notwithstanding, use of the 2016 guidelines resulted in no changes to the classification of the subjects. The reference of the Guidelines has been updated.

** The reference 26 does not seem appropriate*

We are not in agreement with this comment. The paper is relevant and related to the topic of the present work. Moreover, the paper is included in Pubmed free for download and with impact factor.

** The obesity index reported in Table 1 (height/waist ratio) is incorrect because the commonly used index of obesity is the waist/height ratio (for which there are reference data).*

This has been rectified.

** Values in Table 1 and 2 should be adjusted for age and puberty (better if also for gender). Tanner stage (at least the % of prepubertal) should be included in Table 1.*

In our opinion, the suggested adjustments add no relevance, particularly when no differences in age or gender exist.

The percentage of the prepubertal stage has been included.

** Figure 1 is confusing because the length of the sides of the triangle does not correspond to the r value. It should include the statistical significance. A multivariate analysis with HRV as dependent variable and CRF, insulin, w/h and Tanner stage (or with insulin as dependent variable) should clarify the relationships among these factors*

Figure 1 is conceptual and not a mathematical expression. Multivariant analysis relating the three components, metabolic (insulin levels), HRV (LS_LF/HF) and the CRF ($\mathbf{VO_{2peak}}$) has been performed. Insulin levels were independently related to LF/HF (p=0.003), $\mathbf{VO_{2peak}}$ (p=0.028), pubertal stage (p=0.014) and BR at exercise (p=0.016). These factors explain 35% of the

variance.

Multiple regression analysis table: **factors related to insulin levels**

	Beta coefficient	**P value**	**R2**
			0.35
LF/HF	0.393	0.003	
VO_{2peak}	-0.293	0.028	
Pubertal stage	0.309	0.014	
BR at exercise	0.319	0.016	

This data has been included on **Page 12, paragraph two. "Multivariant analysis relating the three components, metabolic (insulin levels), HRV (LS_LF/HF) and the CRF (VO_{2peak}) and confounders, has been performed. Insulin levels were independently related to LF/HF (p=0.003), VO_{2peak} (p=0.028), pubertal stage (p=0.014) and BR at exercise (p=0.016). These factors explain 35% of the variance."**

** In the Discussion, page 12 line 6, "independently of obesity degree and puberty" instead of "independently of obesity degree"; page 12 line 9 eliminate "and non-diabetic individuals were present"; the last 6 lines of page 12 should be deleted. Page 13 line 4 "hyperinsulinemia" should be replaced with "insulin levels". Page 14 "Autonomic nervous system imbalance, assessed in the heart, may not only contribute to the development or stabilization of obesity (add reference) but is also associated with cardiovascular mortality (add reference)". Page 15 line 4 the description of Baum et al results should be expanded (her results are not completely in accord with the results of this study).*

The suggested changes have been made:

page 13 line 1, "**independent of the degree of obesity and of puberty**" instead of "independently of obesity degree."

page 13 line 4, eliminate "and non-diabetic individuals were present."

The last 6 lines of the first paragraph in Discussion should be deleted: We believe that they clarify concepts to readers, not experts in the field.

Page 13 line 14, "hyperinsulinemia" should be replaced with "**insulin levels**."

Page 15 The references requested have been added: "Autonomic nervous system imbalance, assessed in the heart, may not only contribute to the development or stabilization of obesity (**Bahler L, Molenaars RJ, Verberne HJ, Holleman F. Role of the autonomic nervous system in activation of human brown adipose tissue: A review of the literature. Diabetes Metab. 2015;41:437-445; Thorp AA, Schlaich MP. Relevance of Sympathetic Nervous System Activation in Obesity and Metabolic Syndrome. J Diabetes Res. 2015;2015:341583**), but it is also associated with cardiovascular mortality (**Wulsin LR, Horn PS, Perry JL, Massaro JM, D'Agostino RB. Autonomic Imbalance as a Predictor of**

Metabolic Risks, Cardiovascular Disease, Diabetes, and Mortality. J Clin Endocrinol Metab. 2015;100:2443-2448)."

Page 15 line 4 the description of Baum et al. results has been expanded. **Page 15, second paragraph, "These data are partially in agreement with those of the study by Baum et al. in which an ANS dysfunction was described in obese children and adolescents [45]. Autonomic nervous system dysfunction was characterized by both parasympathetic and sympathetic activity reduction, although the LF/LH ratio was positively related to body weight. Similarly, cANS abnormalities were observed in pre-obese and obese young adults [46].**

** Several sentences of the manuscript are incomplete or disconnected (third last line of page 5, last sentence of page 6, first line of page 7)*

The manuscript has been reviewed for the incomplete or disconnected sentences:

"…even though they have a higher degree of obesity, and as a consequence the concept of "healthy" obese has been proposed [11]."

"While HRV was performed exclusively under resting conditions from the recorded ECG signal, the CRF was evaluated as oxygen consumption during the effort test."

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Prof. Alberto Zanchetti,
Editor-in-Chief
Journal of Hypertension,

Valencia, 20th September 2016

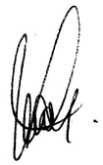
Dear Prof. Zanchetti,

For the consideration of the Editorial Board of *Journal of Hypertension,* please find enclosed the review of our Original Article entitled “SYMPATHETIC NEURAL ACTIVITY, METABOLIC PARAMETERS AND CARDIORESPIRATORY FITNESS IN OBESE YOUTH” according to the recommendation of the reviewers.

All authors have read and approved the submission of the manuscript to Journal of Hypertension. The manuscript has not been published and is not being considered for publication elsewhere.

Thank you in advance for your consideration.

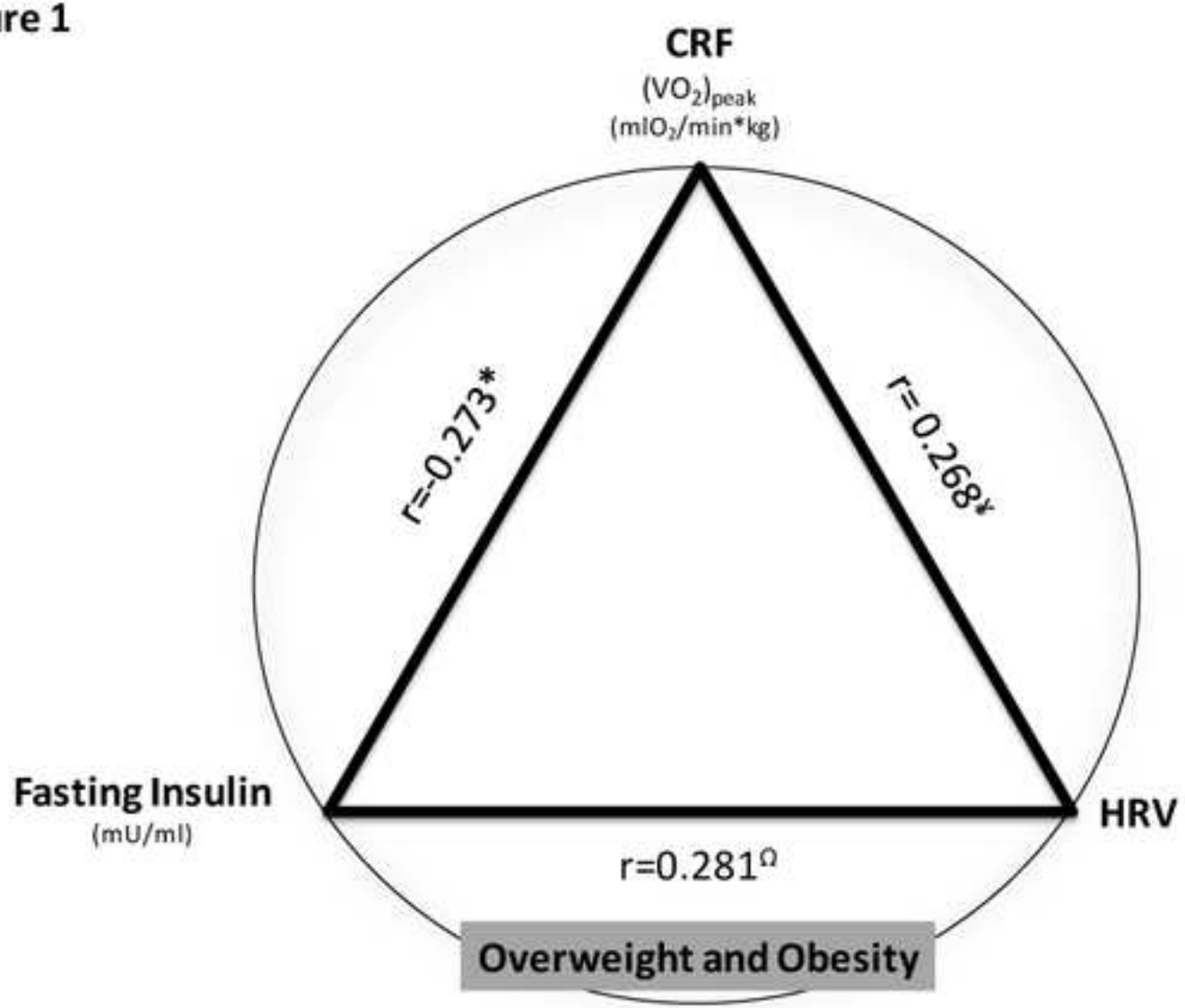
Sincerely yours,



Empar Lurbe, MD, PhD, FAHA

Figure

Figure 1



Table

Table 1. General characteristics of the study population grouped by overweight and degree of obesity

Parameter	Overweight	Moderate Obese	Severe Obese	p-value
Subjects, n (%)	14 (22)	33 (51)	17 (27)	
Sex (M/F)	10/4	18/15	13/4	0.244
Age (years)	12.8 ± 1.1	12.0 ± 2.0	11.7 ± 2.4	0.260
Weight (kg)	65.2 ± 9. 9	66.8 ± 15.0	84.3 ± 18.1¥Ω	0.000*
Height (cm)	162.4 ± 11.0	152.6 ± 10.8 Ω	157.6 ± 11.8	0.024*
BMI (kg/m2)	24.6 ± 1.2	28.2 ± 3.0 Ω	33.5 ± 3.5¥Ω	0.000*
Waist (cm)	90.0 ± 6.3	93.2 ± 12.0 Ω	103.9 ± 8.2¥Ω	0.000*
Waist/Height	0.56 ± 0.03	0.61 ± 0.06 Ω	0.66 ± 0.0.4¥Ω	0.000*
Prepubertal stage, n (%)	5 (36)	16 (48)	11 (65)	
Hypertension, n	1	3	2	
Metabolic				
Fasting glucose (mg/dl)	84.6 ± 6.3	85.4 ± 6.3	87.0 ± 6.5	0.564
Fasting insulin (mU/ml)	10.0 ± 4.2	13.3 ± 12.0	17.0 ± 8.0	0.154
HOMA Index	2.1 ± 1.0	2.8 ± 2.8	3.2± 2.0	0.258
LDL cholesterol (mg/dl)	96.7 ± 29.0	93.3 ± 24.3	98.1 ± 18.4	0.773
HDL cholesterol(mg/dl)	47.5 ± 10.9	47.0 ± 9.6	45.8 ± 9.1	0.872
Triglycerides (mg/dl)	100.3 ± 48.0	83.5 ± 32.0	95.7 ± 40.3	0.317
Resting Conditions				
Breath rate (brpm)	13.0 ± 4.9	14.1 ± 6.1	13.7 ± 6.6	0.863
SBP (mmHg)	108.1 ± 8.7	110.6 ± 11.4	114.1 ± 10.6	0.293
DBP (mmHg)	63.7 ± 9.9	65.7 ± 8.8	66.6 ± 20.4	0.825
Heart rate (bpm)	75.0 ± 11.0	73.3 ± 23.1	81.8 ± 20.2	0.392
Effort Conditions				
VO_2 (mlO_2/min*kg)	31.3 ± 7.1	26.9 ± 3.8 Ω	24.5 ± 5.3¥	0.001*
Breath rate (brpm)	41.7 ± 13.5	41.4 ± 8.7	50.0 ± 14.0¥	0.037*
SBP (mmHg)	130.4 ± 12.9	130.1 ± 13.8	133.1 ± 14.9	0.758
DBP (mmHg)	68.4 ± 5.7	72.8 ± 8.4	78.3 ± 8.0¥	0.003*

Values are average ± standard deviation. BMI body mass index, HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; VO_2, oxygen consumption¸ SBP systolic blood pressure; DBP Diastolic Blood Pressure; brpm breath per minute; bpm beat per minute; $P < 0.05$.

*denotes significant differences among groups

Ω denotes significant differences with overweight

¥ denotes significant differences with moderate obese

Table

Table 2. Heart rate variability parameters grouped by overweight and obesity degree (n=64)

		Obese		
Parameters	**Overweight**	**Moderate**	**Severe**	**p-value**
Number	14	33	17	
Time domain				
SDNN (ms)	178.63 ± 156.22	211.38 ± 201.06	136.21 ± 86.22	0.303
Frequency domain				
LS_LF(ms2)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.814
LS_HF(ms2)	0.04 ± 0.04	0.02 ± 0.01	0.06 ± 0.04 ¥	0.005*
LS_Total(ms2)	0.06 ± 0.04	0.05 ± 0.02	0.07 ± 0.05	0.062
LS_LF (n.u)	0.28 ± 0.11	0.32 ± 0.12	0.25 ± 0.16	0.171
LS_HF (n.u)	0.72 ± 0.11	0.68 ± 0.11	0.75 ± 0.16	0.171
LS_LF/HF	0.43 ± 0.23	0.50 ± 0.35	0.47 ± 0.53	0.849
Non-linear dynamics				
Poincare S1 (ms)	153.19 ± 159.76	178.02 ± 188.14	103.49 ± 74.95	0.272
Poincare S2 (ms)	198.67 ± 155.64	238.69 ± 214.89	160.85 ± 99.06	0.315
Sample entropy	1.40 ± 0.45	1.33 ± 0.52	1.36 ± 0.39	0.916
DFA short term	0.74 ± 0.21	0.74 ± 0.16	0.75 ± 0.21	0.977
DFA long term	0.71 ± 0.17	0.77 ± 0.17	0.73 ± 0.21	0.548

SDNN standard deviation of the NN interval series; ms miliseconds
LS denotes the Lomb-Scargle method to calculate frequency domain parameters
LF low frequency; HF high frequency
ms2 power; n.u normalized values
DFA detrended fluctuation analyses
*denotes significant differences among groups
¥ denotes significant differences with moderate overweight

Table

Table 3. Pearson´s correlation coefficients among heart rate variability parameters, insulin and cardiorespiratory fitness in normotensives, adjusted by confounders (n=58)

	Time domain	Frequency domain						Non linear dynamics				
	SDNN (ms)	LS_LF (ms2)	LS_HF (ms2)	LS_Total (ms2)	LS_LF (n.u)	LS_HF (n.u)	LS_LF/HF	Poincare S1 (ms)	Poincare S2 (ms)	Sample entropy	DFA α1	DFA α2
Adjusted by obesity degree, pubertal stage and baseline respiration rate												
Metabolic profile												
Insulin (mU/ml)	-0.009	0.099	0.086	0.096	0.131	-0.131	0.281*	-0.000	-0.013	-0.054	0.109	-0.181
HOMA index	-0.010	0,145	0.079	0.094	0.162	-0.162	0.304*	-0.005	-0.012	-0.079	0.172	-0.138
Resting conditions												
Systolic BP (mmHg)	-0.208	0.072	0.040	0.044	0.086	-0.086	-0.132	-0.210	-0.208	0.117	0.181	0.128
Diastolic BP (mmHg)	-0.058	0.185	0.064	0.105	0.012	-0.012	-0.054	-0.065	-0.045	-0.053	0.273	0.082
Adjusted by obesity degree and effort respiration rate												
Effort test												
Systolic BP (mmHg)	-0.240	0.022	-0.061	-0-074	-0.089	0.089	-0.084	-0.243	-0.238	0.211	0.091	0.184
Diastolic BP (mmHg)	0.081	-0.065	-0.170	-0.149	-0.088	0.088	-0.161	0.101	0.061	0.094	-0.026	0.078
$(VO_2)_{peak}$	0.268*	0.015	-0.140	-0.094	0.150	-0.150	0.138	0.275*	0.273*	-0.198	-0.098	0.080

BP blood pressure. HOMA homeostatic model assessment

SDNN standard deviation of the NN interval series; ms miliseconds

LS denotes the Lomb-Scargle method to calculate frequency domain parameters LF low frequency; HF high frequency

ms^2 power; n.u normalized values

DFA detrended fluctuation analyses *denotes statistical significant ($p<0.05$)