Loneliness in adult people:

Its relationships with stress, health and hypothalamic-pituitary-adrenal axis functioning

PhD Thesis by
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Agraïments

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Aquesta tesi doctoral és el fruit d'anys d'investigació i de moltes experiències que m'han fet aprendre i créixer de la mà de molta gent.

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Abbreviations

Abbreviations

AD: Alzheimer's Disease

APOE: Apolipoprotein E

AUCg: Area Under the Curve with respect to Ground

AUCi: Area Under the Curve with respect to Increase

AUDIT: Alcohol Use Disorder Identification Test

Aβ: Amyloid-Beta

BDI: Beck Depression Inventory

BMI: Body Mass Index

CAR: Cortisol Awakening Respone

CPRS: Comprehensive Psychopathological Rating Scale

CSF: Cerebrospinal Fluid

CTQ: Childhood Trauma Questionnaire

CVD: Cerebrovascular Disease

DCS: Diurnal Cortisol Slope

DSM: Diagnostic and Statistical Manual of Mental Disorders

ELISA: Enzyme Linked Immunosorbent Assay

ELS: Early Life Stress

HPA axis: Hypothalamic-Pituitary-Adrenal axis

IC: Interval of Confidence

KMO: Kaiser-Meyer-Olking

LP: Lumbar Puncture

MADRS: Montgomery-Åsberg Depression Rating Scale

MCI: Mild Cognitive Impairment

MdGini: Mean Decrease of Gini

MMSE: Mini-Mental State Examination

MRI: Magnetic Resonance Imaging

PCR: Polymerase Chain Reaction

PCS3: Pittsburgh Cold Study 3

PET: Positron Emission Tomography

PhD: Doctor of Philosophy

PSS: Perceived Stress Scale

P-tau: Phosphorylated Tau

ReCAPS: Recalled Childhood and Adolescence Perceived Stress

RFQ: Risky Families Questionnaire

R-UCLA: Revised University of California Los Angeles Loneliness Scale

SCD: Subjective Cognitive Decline

SCD-I: Subjective Cognitive Decline Initiative

SD: Standard Deviation

SCCs: Subjective Cognitive Complaints

SNP: Single Nucleotide Polymorphisms

SRB: Synonyms, Reasoning and Block Design Test

TIV: Total Intracranial Volume

WHOQOL: World Health Organization Quality of Life

WMSA: White Matter Signal Abnormalities

Thesis Outline

Thesis outline

Loneliness is one of the main issues in Western societies. Internet advances in recent years have made us a more connected society than ever, but despite this, loneliness is increasing. For survival and advancement, human beings need others to satisfy their needs for affection, belonging, and recognition. Thus, loneliness arises from the discrepancy between desired and actual social and intimate relationships. Research indicates that feelings of loneliness can be understood as a stressor, and it is related to several adverse consequences for physical and psychological health. This feeling can be experienced throughout life, although it is more frequent among the older population. All of this emphasizes the importance of studying loneliness and its influence on the well-being of our society.

In this doctoral thesis, loneliness is addressed in relation to different stressors (Early Life Stress (ELS) and stress during adulthood) and different subjective (psychological, physical, and subjective cognitive decline (SCD)) and objective (basal Hypothalamic-Pituitary-Adrenal axis (HPA axis) functioning measures and biomarkers of Alzheimer's Disease (AD) and Cerebrovascular Disease (CVD) pathology) health indicators in young, middle-aged, and older healthy people.

The *first chapter* is a general introduction to the studies included in this thesis. Briefly, it describes the current literature on loneliness and its relationship with stress and health. In the first place, it presents the relationship between adverse experiences in the early stages of life and feelings of loneliness during adulthood. Likewise, it points out that loneliness can be understood as a stressor, and some studies that report its effects on physical and psychological health are presented. In addition, the literature on the relationship of both ELS and loneliness with the basal HPA axis functioning is reviewed because it is an important endocrine system involved in health and stress. Finally, the

chapter describes the latest research on the possible role of loneliness in the context of SCD, suggested as the earliest preclinical stage of AD, and its relationship with early brain pathology of AD or CVD. The last part of the chapter includes the main aims and hypotheses of this doctoral thesis.

The following three chapters describe the three studies in this doctoral thesis. Each of these chapters contains a brief introduction that describes the most important related literature, the specific methodology used and results found, and a discussion of the main findings.

In the *second chapter,* the first study is explained. This study examines the role of loneliness as a mediator between early life stress and its relationship with the perception of stress and the basal HPA axis functioning during adulthood in healthy young and middle-aged people. In the *third chapter*, the second study is described, which analyzes loneliness and its association with subjective psychological and physical health and basal HPA axis functioning in healthy middle-aged and older people and whether these associations differ depending on sex. Finally, in this chapter, we explore the basal HPA axis functioning as an underlying mechanism in the relationship between loneliness and health. The *fourth chapter* includes the third study. This study investigates the role of loneliness in the context of SCD as a possible early indicator of AD and CVD in cognitively unimpaired older people.

The *fifth chapter* discusses the main findings of the empirical studies mentioned above. The limitations and strengths of these studies are also discussed, as well as directions for future studies. The *Sixth chapter* includes the main conclusions. Finally, the *seventh chapter* consists of a summary of the present doctoral thesis in Valencian.

Chapter I

General Introduction

General Introduction

Relevance of loneliness in well-being

In recent years, mental health has become more relevant and visible in our society. In this line, loneliness is a psychological issue that is receiving a growing amount of attention. Between 10 and 34% of people report feelings of loneliness (Mund et al., 2020; World Health Organization, 2021). Feeling lonely can be experienced by people of all ages, and it is accentuated in aging. Due to the social nature of human beings, the feeling of loneliness is a stressor in itself (Cacioppo et al., 2000; Steptoe et al., 2004; Zawadzki & Gavrilova, 2021), and it is related to serious consequences in broad aspects of health (Courtin & Knapp, 2017; Leigh-Hunt et al., 2017; Richard et al., 2017). In fact, in 2018 the British and Japanese parliaments established a Ministry of Loneliness with the intention of working on its prevention and treatment. In addition, feeling lonely is a classic concern in the psychology field, and it is one of the main topics addressed in existential psychotherapy (May & Yalom, 2005). Given the importance of loneliness, it is necessary to study it in greater depth and investigate its specific relationship with stress and health, in order to improve the well-being of society.

The fact that emotions and feelings can influence our psychological and physical health is well known (see review: Lopez et al., 2018). For example, emotions such as sadness or anxiety have been related to worse immune system functioning (Brod et al., 2014; Lasselin et al., 2016), which can lead to different diseases (Couzin-Frankel, 2010; Slavich, 2015). Similarly, when we feel alone, biological processes related to stress and pain are activated, responding to the threat of rejection by one's social group (Panksepp, 2003; Steptoe et al., 2004).

Loneliness differs from solitude or social isolation because the former refers to an aversive and unpleasant perception of lack of companionship, whereas solitude or social isolation refers to the objective fact of being alone, which may be neutral or even pleasant (Stein & Tuval-Mashiach, 2015; Tillich, 1959). Thus, we can feel accompanied and socially satisfied without being in contact with other people, although loneliness is more likely in people who experience social isolation (Cacioppo et al., 2000, 2015a).

In this regard, loneliness is an emotion associated with the social essence of human beings. Throughout evolution, for survival, we need others to take care of us and protect us, especially in the first years of life. Throughout life, we still need to feel part of the group to provide mutual value and trust (Baumeister & Leary, 1995). Evolutionarily, being rejected by the group increases the possibility of dying; thus, loneliness is understood as an alarm, an unpleasant feeling that pushes the individual to become part of the group again (Cacioppo et al., 2014, 2015a, 2015b). Feelings of loneliness arise from the discrepancy between the desired intimate and social bonds and the real ones, emphasizing the quality of relationships more than the quantity (Peplau & Perlman et al., 1982; Pinquart & Sörensen, 2003; Weiss, 1973). Therefore, loneliness can be experienced within relationships such as family, friendship, or marriage (Cacioppo et al., 2009).

Early life stress (ELS) and its influence on loneliness

Experiences during the early years of life influence the way we are going to relate to others during adulthood, based on the development of attachment styles (Adler, 1996, 1998; Akdoğan, 2017; Bowlby, 1973). Negligence in care or parental abuse during childhood and adolescence is linked to not having the need for affection, belonging, and recognition satisfied by an attachment figure (Bowlby, 1984). These adverse and stressful experiences in the early stages of life, known as ELS, can lead to attachment insecurities,

rejection, distrust, and a tendency to avoid close relationships and contact with others (Bartholomew, 1990; Hazan & Shaver, 1994; Kafetsios & Nezlek, 2002; Shaver & Brennan, 1992). In addition, they can negatively affect the way the individual establishes relationships during adulthood and predispose him/her to feelings of loneliness (Akdoğan, 2017; Thomas, 2016; Weiss, 1973, 1987).

Previous literature suggests that ELS enhances vulnerability to the effects of subsequent stressful events, and it aggravates the health consequences of stressors in adulthood (Hammen et al., 2000; Lähdepuro et al., 2019; McLaughlin et al., 2010). In this line, people who have experienced ELS find it more difficult to face a major stressful situation during adulthood (Hammen et al., 2000; Harkness et al., 2006), which has long-term negative effects on their physical and psychological health (Kessler et al., 2010; Monnat & Chandler, 2015). Related to the above, although these stressful experiences occurred in the early stages of life, their emotional, relational, and neurobiological effects can persist over time (Kessler et al., 2010; Nemeroff, 2016). In this regard, loneliness appears to be a potential mediator between ELS and stress during adulthood, given that people who have experienced ELS report less satisfaction in their relationships and less social support (Beutel et al., 2017; Germine et al., 2015; Repetti et al., 2002). Despite this, to the best of our knowledge, no previous studies have considered how loneliness in adulthood may be related to these early adverse experiences and whether it can contribute to their stress-related consequences. Hence, the purpose of the first study in this thesis is to address the role of loneliness in the relationship between stress in early stages of life and stress in adulthood.

In addition, it is relevant to study the role of ELS and loneliness in HPA axis functioning (Campagne, 2019; Fogelman & Canli, 2018; Hawkley & Cacioppo, 2003) because it is the main endocrine system related to the stress response. Moreover, its basal

functioning is an indicator of the state of health and gives us information about the exposure to chronic stress (Adam et al., 2017; Miller et al., 2007).

Hypothalamic-pituitary-adrenal (HPA) axis functioning, early life stress (ELS) and loneliness

The primary psychobiological system for managing stress is the HPA axis. This endocrine system faces stress through the action of the main glucocorticoid in humans, cortisol. Cortisol is an essential steroid hormone secreted by the cortex of the adrenal gland. In non-stressful or basal situations, the daily cortisol rhythm in healthy people is marked by an increase in the first 30–45 min after awakening, known as the cortisol awakening response (CAR), followed by a constant decrease until nighttime, known as the diurnal cortisol slope (DCS), and reaching the lowest cortisol levels at bedtime (Adam & Kumari, 2009). A dysregulation of this rhythm (i.e., lower awakening cortisol, greater CAR, flatter DCS, or higher bedtime cortisol) has been associated with different adverse health outcomes (Adam et al., 2017; Fries et al., 2009).

According to the literature, both ELS and loneliness could affect the rhythm of cortisol, but the conclusions are not yet clear. A recent meta-analysis reveals that ELS is not related to basal HPA axis functioning (Fogelman & Canli, 2018), although the authors point out the inconsistencies in the results and the heterogeneity in the cortisol measures. In this regard, some studies reported a relationship of ELS with blunted CAR (Li et al., 2015), flatter DCS (Brewer-Smyth & Burges, 2008; Nicolson, 2004), higher overall diurnal cortisol (Franz et al., 2013), enhanced CAR (Lu et al., 2013, 2016), or steeper DCS (Van der Vegt et al., 2009). However, others failed to find any relationship between ELS and overall diurnal cortisol, DCS, or bedtime cortisol (Bublitz & Stroud, 2012; Franz

et al., 2013; Klaassens et al., 2009; Schreuder et al., 2016). In addition, loneliness has been associated with different patterns of basal HPA axis functioning dysregulation in young people, such as attenuated CAR (Lai et al., 2019), flatter DCS (Doane & Adam, 2010), higher overall diurnal cortisol levels (Cacioppo et al., 2000), or steeper DCS (Lai et al., 2018, 2019).

Taken together, these mixed findings highlight the need to shed light on the associations between basal HPA axis functioning and both ELS and loneliness. In addition, given that, on the one hand, ELS can affect relationships with others during adulthood, and on the other hand, both ELS and loneliness can affect the basal HPA axis functioning, the need to study the role of loneliness in the ELS-HPA axis link is clear. Therefore, in the first study of this thesis, loneliness will be addressed as a mediating factor in the relationship between ELS and basal HPA axis functioning.

Loneliness and health in aging

Loneliness can affect people at any stage of life, although this feeling begins to increase in middle age and is especially high in older people (Pinquart & Sörensen, 2003; Yang & Victor, 2011). Age by itself does not explain this increase in loneliness, but factors linked to aging, such as widowhood, retirement, or reduced mobility, make it more common to feel lonely (Mund et al., 2020). Loneliness negatively affects well-being and many areas of psychological and physical health in aging (Ong et al. 2016; Leigh-Hunt et al., 2017; Valtorta et al., 2018; Xia & Li, 2018). Along these lines, feeling alone has been associated with poorer general subjective health (Losada et al., 2012; Richard et al., 2017) and a wide variety of self-reported physical and psychological health problems, such as musculoskeletal pain (Smith et al., 2019), poorer sleep quality (Benson et al., 2021), or

anxiety, depressive mood, and suicidal ideation (Beutel et al., 2017; Ge et al., 2017). Currently, there is no consensus about sex differences in the relationships between loneliness and subjective psychological and physical health (Richard et al., 2017; Zebhauser et al., 2014). Hence, the relationships between loneliness and physical and psychological subjective health will be addressed in the second study of this doctoral thesis, considering the sex of the participants.

Several mechanisms have been proposed to explain health issues related to loneliness. Some authors emphasize that loneliness may affect health through lifestyle factors such as less self-caring, physical inactivity, smoking, alcohol intake, or consuming unhealthy foods (Akerlind & Hörnquist, 1992; Baumeister et al., 2005; Hawkley et al., 2009; Lauder et al., 2006; Richard et al., 2017). In addition, it has been suggested that loneliness acts as a long-term stressor and could dysregulate the HPA axis functioning (Campagne, 2019; Hawkley & Cacioppo, 2003; O'Connor et al., 2021; Steptoe et al., 2004), negatively affecting health (Adam et al., 2017; Fries et al., 2009).

Despite the above, few studies have investigated whether there is a relationship between loneliness and HPA axis basal functioning in middle-aged and older people, with the results for CAR and DCS not being conclusive (Adam et al., 2006; Cole et al., 2007; Schutter et al., 2017, 2021; Steptoe et al., 2004). In a previous study, no significant relationships between loneliness and awakening cortisol, CAR, and DCS were found; however, high loneliness was associated with higher bedtime cortisol levels (Montoliu et al., 2019). Moreover, the role of sex in the relationship between loneliness and basal HPA axis functioning remains unclear (Johar et al., 2021; Montoliu et al., 2019). Therefore, further investigation is necessary to delve deeper into the way loneliness, subjective health, and basal HPA axis functioning are interrelated in aging, considering the possible sex differences. These issues will be addressed in the second study of this doctoral thesis.

Loneliness, subjective cognitive decline (SCD) and brain pathologies in aging

Loneliness has been seen to affect cognitive performance (Boss et al., 2015), and it is associated with an increased risk of dementia (Wilson et al., 2007). There is increasing evidence showing that some neuropsychiatric symptoms may indicate the onset of brain pathology. Perceptions of periods of recurrent forgetfulness or episodes of distraction in daily life, called subjective cognitive complaints (SCCs), are a common experience that increases in older people (Jonker et al. 2000; Ponds et al. 2000). When these SCCs are not accompanied by an objective cognitive impairment (or by other causes explaining this perception), they are known as SCD, which has been suggested as an early stage of AD (Jessen et al., 2014). SCD is associated with the main AD brain pathological changes, amyloid-beta plaques, tau neurofibrillary tangles, and neurodegeneration (Amariglio et al., 2012; Buckley et al. 2017; Cedres et al., 2021; Perrotin et al., 2012). In addition, SCD has been related to biomarkers of CVD (Cedres et al., 2019; 2021; Diaz-Galvan et al., 2021a; Minett et al., 2005).

In recent literature, a current discussion addresses whether SCD not only reflects brain pathologies, but also non-neurodegenerative factors such as depressive symptomatology (Diaz-Galvan et al., 2021a; Jessen et al., 2014, 2020). Although loneliness is very close to depressive symptomatology, its study in the context of SCD is quite incipient. Recently, results showed that people who feel lonely report a greater frequency of SCCs in memory, independently of depressive symptomatology (Montejo et al., 2019). In addition, in recent years, the study of loneliness has explored it as an early neuropsychiatric symptom of AD or CVD. Few studies have investigated the relationship between loneliness and pathological biomarkers of AD (amyloid-beta and tau levels) or CVD (white matter signal abnormalities, WMSA), and although, in general, there seems to be an association between them, not all studies have shown significant results (d'Oleire

Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017; Wilson et al., 2007). Based on this, it is necessary to study the role of loneliness in the context of early stages of AD and CVD brain pathology. It would be important to explore the contribution of loneliness to SCD (and whether its influence is independent of depressive symptomatology) and increase the knowledge about the relationship between loneliness and AD and CVD biomarkers. The third study in this thesis has been proposed to answer these questions.

Aims and Hypothesis

The general objective of this doctoral thesis is to expand the evidence about the role of loneliness in stress and health in adult people, in order to shed light on the current state of the literature. In this regard, loneliness is addressed in relation to different stressors (ELS and stress during adulthood) and different subjective (psychological and physical health and SCD) and objective (basal HPA axis functioning and brain biomarkers associated with AD and CVD) health indicators.

This doctoral thesis includes three studies. The fundamental interest that guides these studies and the specific objectives are described below.

Study 1: In this study, we addressed the role of loneliness in the relationship between ELS and stress experienced in adulthood in young and middle-aged people. Specifically, the aims of this study were to investigate whether ELS was associated with the perception of stress and the basal HPA axis functioning in adulthood, and the mediating role of loneliness in these relationships. For this purpose, we first studied whether ELS and perception of stress, overall diurnal cortisol, DCS, and bedtime cortisol were related, and then we analyzed loneliness as a possible mediator in these associations.

We hypothesized that higher levels of stress in the first years of life would contribute to higher perceived stress in adulthood (Han et al., 2016; Hyman et al., 2007). Because research on the relationship between ELS and cortisol levels is inconclusive, and this was the first study to explore the mediating role of loneliness in the relationship between ELS and perceived stress and basal HPA axis functioning, we did not provide any hypotheses about the existence or direction of the relationship between ELS and basal HPA axis functioning or the mediations, via loneliness, between ELS and perceived stress and basal HPA axis functioning.

Study 2: In this study, we investigated the relationships between loneliness and subjective health and basal HPA axis functioning in middle-aged and older people. First, we aimed to study the associations between loneliness and subjective psychological and physical health and several indicators of HPA axis functioning. Second, we aimed to investigate the role of sex in these relationships. We hypothesized that loneliness would be related to worse subjective psychological and physical health (Beutel et al., 2017; Richard et al., 2017). Regarding the basal HPA axis functioning, based on Montoliu et al. (2019), we expected to confirm the association of loneliness with bedtime cortisol, although not with CAR and DCS. Even though the role of sex in the relationship between loneliness and subjective health and basal HPA axis functioning are unclear, we expected to find that these associations would be stronger in men than in women (Johar et al., 2021; Zebhauser et al., 2014). Finally, we explored basal HPA axis functioning as a mechanism underlying the relationship between loneliness and subjective health.

Study 3: In this study, we addressed the role of loneliness in the context of SCD as an early neuropsychiatric symptom of AD or CVD in cognitively unimpaired older people. The first aim was to explore the relationships of loneliness with depressive symptomatology and biomarkers of AD and CVD pathology. The second aim was to study the associations between SCD and loneliness and biomarkers of AD and CVD pathology, exploring whether they are independent of depressive symptomatology, due to the close relationship between depressive mood and loneliness (Erzen & Çikrikci, 2018) and the co-occurrence between depression and SCD (Diaz-Galvan et al., 2021a; Jessen et al., 2014). We hypothesized that loneliness would be positively associated, not only with depressive symptomatology (Domènech-Abella et al., 2019), but also with AD and CVD biomarkers (d'Oleire-Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017). In addition, we assumed that, independently of depressive symptomatology, SCD would be related to higher loneliness (Montejo et al., 2019) and to higher biomarkers of AD and CVD pathology (Amariglio et al., 2012; Buckley et al., 2017; Cedres et al., 2019, 2021; Diaz-Galvan et al., 2021a). Finally, we investigated whether these associations would differ depending on the type of SCD (memory and non-memory complaints) because recently it has been suggested that different specific SCD could reflect different syndromic profiles (Diaz-Galvan et al., 2021b).

Chapter II

Study 1: Importance of loneliness in the relationship between early life stress and perceived stress and HPA axis functioning in adulthood

The main results of this study have been published in:

Crespo-Sanmiguel, I., Zapater-Fajarí, M., Pulopulos, M. M., Hidalgo, V., & Salvador, A. (2021). Loneliness Mediates the Relationship Between Early Life Stress and Perceived Stress but not Hypothalamic-Pituitary-Adrenal Axis Functioning. *Frontiers in Psychology*, *12*, 647265.

Introduction

ELS is usually operationalized as a wide variety of adverse experiences that occur in the first stages of the individual's development, and they include negligence, socioeconomic disadvantage, physical or psychological maltreatment, or early parental loss, among others (Fogelman & Canli, 2018). Although this exposure to stress takes place during childhood and/or adolescence, the relational, emotional, and neurobiological consequences may persist throughout life (Kessler et al., 2010; Lähdepuro et al., 2019; Nemeroff, 2016). In fact, as the stress-sensitization model proposes, the ELS-related negative effects may enhance vulnerability to several stress-related psychopathological conditions (McLaughlin et al., 2010), such as posttraumatic stress disorder (Kiser et al., 1991; Yehuda et al., 2010), anxiety (Heim & Nemeroff, 2001; Lähdepuro et al., 2019), depression (Colman & Ataullahjan, 2010; Gallo et al., 2017), eating disorders (Su et al., 2016), psychosis (Read et al., 2005), bipolar disorder (Post et al., 2015), and substance abuse (Keyes et al., 2012; Scheller-Gilkey et al., 2004).

This predisposition could be due to the fact that having experienced trauma during childhood or adolescence increases vulnerability to the effects of subsequent stressful events, which aggravates the health consequences of stressors in adulthood (Hammen et al., 2000; McLaughlin et al., 2010). That is, ELS influences the capability to manage stress in adulthood, which would act as an important factor related to health. In this context, studies have shown that adults who experienced overall childhood maltreatment express more difficulties when facing a stressful episode (Han et al., 2016), which could be explained by poor management in the response to stress, as in the use of less adaptive coping strategies (Hyman et al., 2007). Likewise, it has been observed that having suffered from overall early maltreatment is associated with a greater perception of stress during adulthood in different types of populations, such as individuals with cocaine

dependence in periods of abstinence (Hyman et al., 2007), women inmates (Brewer-Smyth & Burgess, 2008), and breast cancer (Han et al., 2016) or coronary artery (Bossé et al., 2018) patients, although fewer studies have been carried out in the general population (Betz et al., 2021).

Moreover, ELS has been investigated in relation to the physiological stress system via HPA axis functioning (Baes et al., 2014; Juruena et al., 2020; Schalinski et al., 2015), with inconsistencies in the results reported (Fogelman & Canli, 2018). In healthy individuals, the daily cortisol rhythm is characterized by a marked increase the first 30–45min after awakening, followed by a constant decrease until nighttime (Clow et al., 2004, 2010; Elder et al., 2014). An HPA axis dysregulation, reflected in higher overall diurnal cortisol secretion and a flattened DCS, has been related to different health outcomes (Adam et al., 2017; Miller et al., 2007). Although many studies have investigated the association between ELS and the CAR (Heim et al., 2009; Meinlschmidt & Heim, 2005; Wielaard et al., 2018) or its relationship with baseline stress and reactivity to a psychosocial stressor (Andreotti et al., 2015; Cărnuţă et al., 2015; Flory et al., 2009; Janusek et al., 2017), little is known about ELS in relation to overall diurnal cortisol, DCS, and bedtime cortisol levels.

Specifically, in the relationship between these three cortisol indexes and ELS, studies that include the period of childhood and adolescence have mostly found mixed results, depending on the cortisol index employed and the type of ELS. Thus, considering overall diurnal cortisol, men who have experienced parental loss presented higher values on this index than men with temporary parental separation (Nicolson, 2004). Although childhood economic and social adversities are not related to overall diurnal cortisol in the general population (Karlamangla et al., 2019), in a prospective study with men twins, a positive relationship was found (Franz et al., 2013). Furthermore, no relationship was

found between physical and emotional abuse and neglect and overall diurnal cortisol in healthy women (Klaassens et al., 2009) or between parental bipolar disorder and overall diurnal and bedtime cortisol levels (Schreuder et al., 2016). Regarding DCS, a prospective study reported that adoptees who experienced severe neglect before the adoption presented a flatter DCS, whereas adoptees who experienced severe abuse presented a steeper DCS (Van der Vegt et al., 2009). Childhood sexual abuse has been associated with flatter DCS in women prison inmates (Brewer-Smyth & Burgess, 2008) and in women with chronic pain (Nicolson et al., 2010); however, no associations between sexual or other types of abuse and the DCS were found in pregnant women (Bublitz & Stroud, 2012). Moreover, Franz et al. (2013) showed that childhood disadvantage does not affect the DCS in men. In this context, it is important to further investigate the relationships and factors that might explain the association between ELS and stress indicators during adulthood.

It is also worth noting that ELS is a risk factor that can affect the individual's social functioning, given that individuals who have experienced ELS report less social support (Beutel et al., 2017; Germine et al., 2015) or fewer benefits of this support, perhaps because ELS experiences negatively affect the ability to be interested in and conserve interpersonal affective ties, leading to unsatisfactory social relationships (Repetti et al., 2002). This implies a lack of companionship, emotional and instrumental support, and expressions of positive affect by others (Barrera, 1986), all of which translate into greater feelings of loneliness.

Loneliness is a complex concept that involves the subjective and painful experience of perceiving a deficient quantity and quality of desired social relationships (Peplau & Perlman, 1982). Loneliness has been associated with high perceived stress and several symptoms related to it, such as sleep disorders or chronic interpersonal stress

(Doane & Adam, 2010; Doane & Thurston, 2014; Matthews et al., 2019; Yaacob et al., 2009; Yarcheski et al., 2011). Moreover, previous research observed a relationship between loneliness and HPA axis functioning, specifically, higher diurnal cortisol levels (Campagne, 2019; Lai et al., 2018, 2019), a flattened DCS (Doane & Adam, 2010; Johar et al., 2021), and a steeper DCS (Lai et al., 2019). Therefore, loneliness could be a factor that plays an important role in the relationship between ELS and both the perception of stress and HPA axis functioning in adulthood.

Based on the above, the aims of this study were to investigate the association between ELS and adult perceived stress and HPA axis functioning, and whether loneliness mediates these relationships. Specifically, we expected that higher ELS would be related to higher perceived stress (Han et al., 2016; Hyman et al., 2007). Moreover, given the heterogeneity in the results on the relationship between ELS and HPA axis functioning, and because this is the first study to investigate loneliness as a mediator between ELS and perceived stress and HPA axis indicators, our aims were to explore the existence and directionality of these relationships. Additionally, we aimed to test whether a 3-item non-standardized questionnaire on overall ELS (Recalled Childhood and Adolescence Perceived Stress, ReCAPS) is a valid tool to measure ELS. To do this, in addition to using the Risky Family Questionnaire to assess ELS, the analyses will be replicated using the ReCAPS Questionnaire.

Materials and Methods

Participants

The final sample in our study was composed of 187 healthy volunteers (108 men and 79 women). The data were collected by the Laboratory for the Study of Stress, Immunity, and Disease (2016) at Carnegie Mellon University under the directorship of Sheldon Cohen, PhD, and they were accessed via the Common Cold Project website (www.commoncoldproject.com; grant number NCCIH AT006694). The participants were recruited from Pennsylvania metropolitan areas through newspaper advertisements, as part of the Pittsburgh Cold Study 3 (PCS3), a prospective viral challenge study with data collected from 2007–2011. The participants' ages ranged from 18 to 55 years, with a mean of 30.39 ± 10.98. Table I.1 shows the characteristics of the study sample.

Two hundred and thirteen participants were recruited for the entire research project. Of the exclusion criteria for participating in the entire study protocol (www.commoncoldproject.com), for the present study, we considered the following: women who were currently lactating (breast-feeding) or pregnant; people who were currently taking sleeping pills, tranquilizers, steroids, immunosuppressants, or other regular medication regimens; individuals diagnosed with a psychiatric disorder treated within the past year or psychiatric hospitalization within the past 5 years; and individuals with a history of a cardiovascular (heart) disorder, diabetes, or another chronic illness. These exclusion criteria, along with demographic and clinical data information, were evaluated in an interview held in the Children's Hospital of Pittsburgh in a first screening session. Of the 213 participants, 26 were excluded from the data analyses in the current study because their cortisol indexes could not be calculated due to missing data.

Table I.1 Sample characteristics and descriptive statistics

	Mean/n	SE
Age (years)	30.39	10.98
Sex		
Women	79	
Men	108	
Educational level reached in years	14.15	1.84
Ethnicity		
White/Caucasian	130	
Black, African-American	48	
Native American, Eskimo, Aleut	1	
Asian or Pacific Islander	3	
Hispanic, Latino	3	
Other	2	
Body mass index (kg/m²)	27.34	6.34
Loneliness	5.34	1.92
Perceived stress (PSS)	11.87	5.75
RFQ	27.58	10.14
ReCAPS	3.05	1.26
Cortisol indexes		
AUCg	3.68	.20
DCS	−.04	1.83
Bedtime levels	.39	.40

Note. RFQ = Risky Family Questionnaire; ReCAPS = Recalled Childhood and Adolescence Perceived Stress; AUCg = Area Under the Curve with respect to Ground; DCS = Diurnal Cortisol Slope; SE = Standard Error

Procedure

Participants provided informed consent and received $1,000 for their participation in the whole protocol. The study was approved by the Carnegie Mellon University and University of Pittsburgh institutional review boards. The protocol for the whole project lasted between 14 and 16 weeks, and 10 to 12 weeks after the beginning of the study, the

participants were infected with a virus to investigate susceptibility to the common cold. In the current study, we focus on the data available for the assessment of ELS, diurnal cortisol levels, current perceived stress, loneliness, and sociodemographic information. Although the objective of PCS3 was to observe the effect of a virus inoculation, this does not affect the psychosocial variables we investigated in the current study because they were evaluated during the visits to the hospital before the inoculation, using the self-reported questionnaires detailed below. The whole protocol is described in detail at the Common Cold Project website (www.commoncoldproject.com). Below we present the factors considered in the current study.

Measures

Early Life Stress (ELS)

The ELS was evaluated by two questionnaires: Risky Family Questionnaire (RFQ) and ReCAPS.

Risky Family Questionnaire

This questionnaire refers to adverse environmental, physical, emotional, and mental abuse or a neglectful home, among others. It was adapted (Taylor et al., 2004) from an instrument originally created to evaluate the association between family stress and health outcomes in adulthood (Felitti et al., 1998). It was composed of 13 items rated on a 5-point Likert scale (from 1 = not at all to 5 = very often). Examples of items are as: (1) "How often did a parent or other adult in the household push, grab, shove, or slap you?"; (2) "Would you say you were neglected while you were growing up, left on your own to fend for yourself?"; and (3) "In your childhood, did you live with anyone who was a

problem drinker or alcoholic, or who used street drugs?". Internal consistency of this scale for the study sample had a Cronbach's $\alpha = 0.90$.

Recalled Childhood and Adolescence Perceived Stress

To assess overall ELS, participants completed the ReCAPS scale, which was created by the experimenters for the original study (PCS3). Participants were asked to rate their level of overall stress compared to other people with similar ages, using the same item three times: "For this age, indicate your level of overall stress compared to other people your age," with reference to the ages of 5, 10, and 15 years old. The participants had to answer using a 6-point Likert rating scale (from 1 = much less stress to 6 = much more stress). The outcome used was the mean of the three periods evaluated. Internal consistency of this scale for the study sample had a Cronbach's $\alpha = 0.78$.

Loneliness

Loneliness was measured using the Short Loneliness Scale (Hughes et al., 2004). This scale has three items rated on a 4-point Likert scale (from 1 = never to 4 = very often). These items are as: (1) "In general, how often do you feel that you lack companionship?"; (2) "In general, how often do you feel left out?"; and (3) "In general, how often do you feel isolated from others?". Internal consistency of this scale had a Cronbach's $\alpha = 0.80$ for the study sample.

Perceived Stress

The degree to which people perceived their lives as stressful, uncontrollable, unpredictable, and overloaded was measured using the 10-item perceived stress scale (PSS; Cohen et al., 1983; Cohen & Janicki-Deverts, 2012). The respondents had to answer using a 5-point Likert scale (from 0 = never to 4 = very often). Sample items were as: "In

the last month, how often have you felt you were unable to control the important things in your life?" and "In the last month, how often have you felt nervous and 'stressed'?". Internal consistency of this scale had a Cronbach's $\alpha = 0.70$ for the study sample.

Cortisol Measurements

Fourteen salivary samples were collected to assess participants' cortisol levels using Salivettes (Sarstedt, Rommelsdorf, Germany). The saliva samples were collected 1, 2, 4, 7, 9, 11, and 14h after awakening on two non-consecutive days in their natural environment and while carrying out their usual daily activities. Participants were told to place the cotton roll in their mouth, chew on it until it became saturated, place it in the inner vial of the Salivette, and then tightly cap the outer tube. They were instructed not to eat, smoke, or brush their teeth during the 30min before the collection. Volunteers were taught and given written instructions about how and when to perform the saliva samples and the number of Salivettes. Additionally, they received a pre-programmed handheld device that identified each sample and provided a unique alphanumeric code for each. Subjects recorded this code and added the exact date and time of the samples. Moreover, they were given saliva collection records to complete after collecting the last sample on each evaluation day. They were instructed to seal and store their samples in the refrigerator until they brought them to the researchers on the baseline day of the quarantine for virus inoculation. These storage conditions ensure the stability of saliva cortisol concentrations (Garde & Hansen, 2005; Nalla et al., 2015). Cortisol levels were processed by the laboratory of Dr. Clemens Kirschbaum in Dresden (Germany), and they were determined by using time-resolved fluorescence immunoassay with a cortisol-biotin conjugate as a tracer (Dressendörfer et al., 1992). Intra- and inter- assay variabilities were each less than 12%. For each cortisol sample, there was a time window within which the samples were collected. These windows were between 45min and 90min after waking for

the first sample, and between one hour before and after the established collection time for the rest of the samples. As a control measure, and to homogenize the cortisol concentrations, all the samples used were collected within these time windows, and participants who collected saliva samples outside these time windows were not included in this study. Saliva samples were adjusted according to the waking time to control for differences in cortisol levels due to variations in the awakening time. We computed three indexes, AUCg, DCS, and bedtime levels, using the average of the saliva samples on the two days. If the participant only had the samples from one day (N = 8 for AUCg, N = 38 for DCS, and N = 24 for bedtime cortisol), we only used the cortisol value that could be obtained. The AUCg reflects the overall diurnal cortisol secretion. AUCg was computed using all the saliva samples, and it was calculated using the trapezoidal formula proposed by Pruessner et al. (2003). The DCS reflects the decrease in cortisol levels during the day, and it was calculated by regressing the cortisol values from the second sample to the last sample for each participant. In the case of the DCS, a larger value indicates a flatter slope (less cortisol decline throughout the day), whereas a smaller value indicates a steeper slope (greater diurnal decline). Finally, bedtime cortisol was calculated as the mean cortisol before going to sleep on the two days.

Statistical Analyses

Figure I.1 shows the diurnal cortisol profile of each day using raw data. First, because the cortisol levels did not follow a normal distribution, they were log transformed. To verify that the cortisol data really reflected the baseline functioning of the HPA axis, correlation analyses were performed between the two days at each collection time, which allowed us to use the average cortisol levels ($p \leq 0.047$). To determine the covariates that would be included in the regression and mediation analyses, Pearson's correlations were performed

between all the variables included in the current study. Only the sociodemographic variables that were significantly related to the main factors of the study (i.e., ELS, loneliness, perceived stress, and cortisol indexes) were included as covariates (see Table I.2). Thus, sex was associated with the three cortisol indexes, with men having higher cortisol levels. Therefore, sex was a covariate in all the regression and mediation analyses that included cortisol indexes. Years of education was positively associated with the ReCAPS, and so it was included as a covariate in all the regression and mediation analyses that included the ReCAPS.

Figure I.1 Diurnal cortisol profile of each day (raw data)

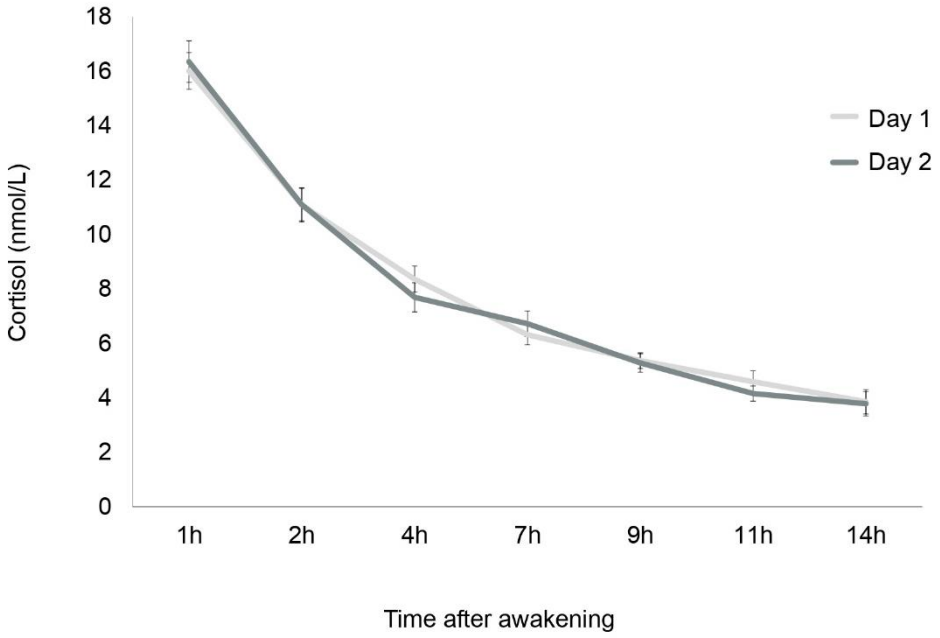

Table I.2 Pearson's correlations between all variables studied

	Sex	Education	Ethnicity	BMI	Loneliness	PSS	RFQ	ReCAPS	AUCg	DCS	Bedtime
Age	.060	−.023	.153*	.312**	.130	−.102	−.144	.034	.063	.102	.143
Sex		.089	.139	.176*	.030	−.032	.016	.133	−.166*	−.233**	−.182*
Education			−.269**	−.233**	.054	.067	−.055	.237**	−.059	−.065	−.043
Ethnicity				.275*	.005	.005	.101	−.105	−.024	.041	.082
BMI					−.015	.005	.075	−.044	−.030	.019	.016
Loneliness						.430**	.299**	.333**	−.064	.040	−.007
PSS							.201**	.193*	−.029	−.016	−.049
RFQ								.438**	−.025	−.009	.003
ReCAPS									−.075	−.054	−.084
AUCg										.738**	.487**
DCS											.549**

Note. BMI = Body Mass Index; PSS = Perceived Stress Scale; RFQ = Risky Family Questionnaire; ReCAPS = Recalled Childhood and Adolescence Perceived Stress; AUCg = Area Under the Curve with respect to Ground; DCS = Diurnal Cortisol Slope. *p <.05; **p <.01

We used PROCESS 3.4 for SPSS to test mediation effects. It makes possible to estimate the indirect effect of ELS on PSS and on the cortisol indexes (AUCg, DCS, and bedtime levels) via loneliness, which is equivalent to the difference between the total effect (relationship between ELS and PSS/cortisol indexes, not controlling for loneliness) and the direct effect of the independent factor (relationship between ELS and PSS/cortisol indexes, controlling for loneliness). To determine the significance of the indirect effect, PROCESS uses bootstrapped bias-corrected 95% confidence intervals (Hayes, 2017) of the interaction effect with 5,000 bootstrapped samples. We interpret that there is a significant indirect effect when this confidence interval does not contain zero. The mediation analyses were performed including the covariates.

Post-hoc statistical power analyses were performed with de G*Power program, estimating a power > 0.80 with an alpha level $p = 0.05$ and an N = 187 for regression analyses between ELS and PSS and cortisol indexes. Only the relationship between ELS and AUCg cortisol index has statistical power of 0.70. The mediation analyses use bootstrapping technique that draws random sample of a fixed sample size with replacement from the dataset, which increases the statistical power. The sample size is considered and this statistical approach controls for this factor in the analyses (Hayes, 2017).

Tolerance values indicate that there are no collinearity issues for the factors included in the model (i.e., tolerance > 0.01). In this study, we used the multivariate outliers, and the number of outliers for each regression and mediation analysis is indicated for each analysis in Tables I.3, I.4, and I.5, respectively. We considered the values outliers when they differed by more than ± 3 SD and, thus, were eliminated from the regression and mediation analyses. Statistical analyses were carried out using SPSS v.24 (IBMS

Statistics, Chicago, IL, United States). All *p* values were two tailed, and the level of significance was taken as $p < 0.05$.

Additionally, we performed moderated regression analyses between ELS (i.e., RFQ and ReCAPS) and perceived stress and the three cortisol indexes with sex as a moderating factor to explore possible sex differences. The sex factor did not moderate any association (all $p > 0.158$).

Results

Correlation analyses

The Pearson's correlation between the RFQ and ReCAPS showed that the two questionnaires were positively and strongly related [r (185) = 0.438, $p < 0.001$]. After controlling for years of education, due to its positive relationship with ReCAPS, the partial correlation showed the same statistical result [r (184) = 0.465, $p < 0.001$].

Table I.2 shows Pearson's correlations between all the variables used in the study. Results showed that the PSS was positively related to the RFQ [r (185) = 0.201, $p = 0.006$] and ReCAPS [r (185) = 0.193, $p = 0.008$]. However, RFQ was not related to any cortisol index [AUCg: r (185) = −0.025, $p = 0.735$; DCS: r (185) = −0.009, $p = 0.902$; Bedtime levels: r (185) = 0.003, $p = 0.971$] and neither ReCAPS [AUCg: r (185) = −0.075, $p = 0.311$; DCS: r (185) = −0.054, $p = 0.460$; Bedtime levels: r (185) = −0.084, $p = 0.250$]. In addition, loneliness was positively related to RFQ [r (185) = 0.299, $p < 0.001$], ReCAPS [r (185) = 0.333, $p < 0.001$], and PSS [r (185) = 0.430, $p < 0.001$], but not to the cortisol outputs [AUCg: r (185) = −0.064, $p = 0.383$; DCS: r (185) = 0.040, $p = 0.586$; Bedtime levels: r (185) = −0.007, $p = 0.921$].

Regression analyses

Regression analyses showed that the RFQ was positively associated with the PSS (B = 0.203, p = 0.006), but not with any cortisol indexes (all $p > 0.749$). When the regression analyses were re-analyzed using ReCAPS as an indicator of ELS, results showed similar significance. That is, ReCAPS was positively associated with PSS ($B = 0.181$, $p = 0.014$), but no associations were found between ReCAPS and any cortisol indexes (all $p > 0.356$; Table I.3).

Table I.3 Adjusted regression analyses with RFQ and ReCAPS as predictors and PSS and cortisol indexes as dependent variable. The analyses were controlled by educational level for the analyses that include ReCAPS and by sex for the analyses that include the cortisol indexes

		PSS	AUCg	DCS	Bedtime
RFQ	Change R^2	.041	.001	.000	.000
	Adj. R^2	.036	.021	.058	.032
	Beta (standardized)	.203	−.023	.014	−.017
	p	.006**	.749	.840	.814
	Outliers	1	2	3	1
ReCAPS	Change R^2	.033	.003	.000	.004
	Adj. R^2	.027	.024	.058	.036
	Beta (standardized)	.181	−.059	.018	−.067
	p	.014*	.423	.809	.356
	Outliers	1	2	3	1

Note. RFQ = Risky Family Questionnaire; ReCAPS = Recalled Childhood and Adolescence Perceived Stress; PSS = Perceived Stress Scale; AUCg = Area Under the Curve with respect to Ground; DCS = Diurnal Cortisol Slope. *$p < .05$; **$p < .01$

Mediation analyses between ELS and perceived stress via loneliness

Mediation analyses revealed that higher RFQ scores were associated with greater loneliness (*B* = 0.299, IC 95% [0.160, 0.437]). Moreover, people who showed greater loneliness had higher scores on the PSS (*B* = 0.401, IC 95% [0.268, 0.534]). The indirect effect (i.e., effect of RFQ on PSS via loneliness) was statistically significant (*B* = 0.120, IC 95% [0.048, 0.209]). In addition, the total effect (i.e., effect of RFQ on PSS, without considering loneliness) was significant (*B* = 0.196, IC 95% [0.058, 0.335]). However, the direct effect (i.e., effect of RFQ on PSS, controlling for loneliness) was not significant (*B* = 0.077, IC 95% [−0.056, 0.210]; Table I.4).

Results of the mediation analyses using ReCAPS showed that higher ReCAPS scores were associated with greater loneliness (*B* = 0.339, IC 95% [0.197, 0.481]). Moreover, people who showed greater loneliness had higher scores on the PSS (*B* = 0.411, IC 95% [0.276, 0.547]). The indirect effect (i.e., effect of ReCAPS on PSS via loneliness) was statistically significant (*B* = 0.140, IC 95% [0.068, 0.225]). In addition, the total effect (i.e., effect of ReCAPS on PSS, without considering loneliness) was significant (*B* = 0.174, IC 95% [0.030, 0.317]). However, the direct effect (i.e., effect of ReCAPS on PSS, controlling for loneliness) was not significant (*B* = 0.034, IC 95% [−0.105, 0.173]; Table I.5).

Table I.4 Adjusted mediation models of the relationship between ELS (measured by RFQ) as predictor and perceived stress and cortisol levels (AUCg, DCS and bedtime) as dependent variables via loneliness. The analyses were controlled by sex for the analyses that include the cortisol indexes

	Dependent variable: PSS (1 outlier)					
	Effect	SE	*t*	*p*	LLCI	ULCI
RFQ to loneliness	.299	.070	4.245	<.001**	.160	.437
Loneliness to PSS	.401	.067	5.951	<.001**	.268	.534
Indirect effect	.120	.042	-	-	.048	.209
Total effect	.196	.070	2.805	.006**	.058	.335
Direct effect	.077	.067	1.140	.256	−.056	.210
	Dependent variable: AUCg (2 outliers)					
	Effect	SE	*t*	*p*	LLCI	ULCI
RFQ to loneliness	.299	.071	4.221	<.001**	.159	.439
Loneliness to AUCg	−.038	.072	−.523	.601	−.180	.104
Indirect effect	−.011	.022	-	-	−.058	.028
Total effect	−.022	.069	−.320	.749	−.158	.114
Direct effect	−.011	.072	−.149	.882	−.153	.132
	Dependent variable: DCS (3 outliers)					
	Effect	SE	*t*	*p*	LLCI	ULCI
RFQ to loneliness	.296	.071	4.171	<.001**	.156	.436
Loneliness to DCS	.101	.113	.887	.376	−.123	.324
Indirect effect	.030	.031	-	-	−.027	.096
Total effect	.022	.108	.202	.840	−.192	.235
Direct effect	−.008	.113	−.070	.944	−.232	.216

(Continue on next page)

Continuation of **Table I.4**

Dependent variable: Bedtime levels (1 outlier)

	Effect	SE	*t*	*p*	LLCI	ULCI
RFQ to loneliness	.293	.071	4.153	<.001**	.154	.433
Loneliness to bedtime	−.022	.074	−.302	.763	−.168	.123
Indirect effect	−.007	.019	-	-	−.043	.032
Total effect	−.017	.070	−.236	.814	−.155	.122
Direct effect	−.010	.074	−.137	.892	−.155	.135

Note. PSS = Perceived Stress Scale; RFQ = Risky Family Questionnaire; AUCg = Area Under the Curve with respect to Ground; DCS = Diurnal Cortisol Slope; SE = Standard Error.
**$p < .01$

Table I.5 Adjusted mediation models of the relationship between ELS (measured by ReCAPS) as predictor and perceived stress and cortisol levels (AUCg, DCS and bedtime) as dependent variables via loneliness. All analyses were controlled by educational level and for the analyses that include the cortisol indexes, in addition to educational level, sex

Dependent variable: PSS (1 outlier)

	Effect	SE	*t*	*p*	LLCI	ULCI
ReCAPS to loneliness	.339	.072	4.720	<.001**	.197	.481
Loneliness to PSS	.411	.069	6.003	<.001**	.276	.547
Indirect effect	.140	.040	-	-	.068	.225
Total effect	.174	.073	2.389	.018*	.030	.317
Direct effect	.034	.071	.485	.629	−.105	.173

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Continuation of **Table I.5**

	Dependent variable: AUCg (2 outliers)					
	Effect	SE	*t*	*p*	LLCI	ULCI
ReCAPS to loneliness	.343	.073	4.711	<.001**	.199	.486
Loneliness to AUCg	−.026	.073	−.357	.721	−.170	.118
Indirect effect	−.009	.025	-	-	−.063	.035
Total effect	−.050	.071	−.704	.482	−.191	.090
Direct effect	−.041	.076	−.545	.587	−.191	.108
	Dependent variable: DCS (3 outliers)					
	Effect	SE	*t*	*p*	LLCI	ULCI
ReCAPS to loneliness	.339	.073	4.661	<.001**	.196	.483
Loneliness to DCS	.101	.115	.879	.380	−.126	.328
Indirect effect	.034	.038	-	-	−.042	.111
Total effect	.022	.112	.193	.847	−.200	.243
Direct effect	−.013	.119	−.107	.915	−.248	.222
	Dependent variable: Bedtime levels (1 outlier)					
	Effect	SE	*t*	*p*	LLCI	ULCI
ReCAPS to loneliness	.339	.072	4.695	<.001**	.196	.481
Loneliness to bedtime	−.004	.075	−.056	.956	−.152	.143
Indirect effect	−.001	.020	-	-	−.043	.037
Total effect	−.065	.073	−.892	.374	−.208	.079
Direct effect	−.063	.077	−.822	.412	−.215	.089

Note. PSS = Perceived Stress Scale; ReCAPS = Recalled Childhood and Adolescence Perceived Stress; AUCg = Area Under the Curve with respect to Ground; DCS = Diurnal Cortisol Slope; SE = Standard Error. *$p < .05$; **$p < .01$

Mediation analyses between ELS and cortisol via loneliness

Mediation analyses revealed that higher RFQ scores were associated with greater loneliness in the analyses with the three cortisol indexes (for AUCg: *B* = 0.299, IC 95% [0.159, 0.439], for DCS: *B* = 0.296, IC 95% [0.156, 0.436], and for bedtime: *B* = 0.293, IC 95% [0.154, 0.433]). However, people who showed more loneliness did not show significant results on any cortisol index (for AUCg: *B* = −0.038, IC 95% [−0.180, 0.104], for DCS: *B* = 0.101, IC 95% [−0.123, 0.324], and for bedtime: *B* = −0.022, IC 95% [−0.168, 0.123]). The indirect effects (i.e., effect of RFQ on cortisol levels via loneliness) were not significant (for AUCg: *B* = −0.011, IC 95% [−0.058, 0.028] for DCS: *B* = 0.030, IC 95% [−0.027, 0.096], and for bedtime: *B* = −0.007, IC 95% [−0.043, 0.032]). Neither the total effect (i.e., effect of RFQ on cortisol levels, without considering loneliness; for AUCg: *B* = −0.022, IC 95% [−0.158, 0.114] for DCS: *B* = 0.022, IC 95% [−0.192, 0.235], and for bedtime: *B* = −0.017, IC 95% [−0.155, 0.122]) nor the direct effects (i.e., effect of RFQ on cortisol levels, controlling for loneliness; for AUCg: *B* = −0.011, IC 95% [−0.153, 0.132] for DCS: *B* = −0.008, IC 95% [−0.232, 0.216], and for bedtime: *B* = −0.010, IC 95% [−0.155, 0.135]) were significant either (Table I.4).

Mediation analyses using ReCAPS showed that higher ReCAPS scores were associated with greater loneliness in the analyses with the three cortisol indexes (for AUCg: *B* = 0.343, IC 95% [0.199, 0.486], for DCS: *B* = 0.339, IC 95% [0.196, 0.483], and for bedtime: *B* = 0.339, IC 95% [0.196, 0.481]). However, people who showed more loneliness did not show significant results on any cortisol index (for AUCg: *B* = −0.026, IC 95% [−0.170, 0.118], for DCS: *B* = 0.101, IC 95% [−0.126, 0.328], and for bedtime: *B* = −0.004, IC 95% [−0.152, 0.143]). The indirect effects (i.e., effect of ReCAPS on cortisol levels via loneliness) were not significant (for AUCg: *B* = −0.009, IC 95% [−0.063, 0.035], for DCS: *B* = 0.034, IC 95% [−0.042, 0.111], and for bedtime: *B* =

−0.001, IC 95% [−0.043, 0.037]). Neither the total effects (i.e., effect of ReCAPS on cortisol levels, without considering loneliness; for AUCg: $B = -0.050$, IC 95% [−0.191, 0.090], for DCS: $B = 0.022$, IC 95% [−0.200, 0.243], and for bedtime: $B = -0.065$, IC 95% [−0.208, 0.079]) nor the direct effects (i.e., effect of ReCAPS on cortisol levels, controlling for loneliness; for AUCg: $B = -0.041$, IC 95% [−0.191, 0.108], for DCS: $B = -0.013$, IC 95% [−0.248, 0.222], and for bedtime: $B = -0.063$, IC 95% [−0.215, 0.089]) were significant either (Table I.5).

Discussion

The aims of this study were to investigate whether ELS was associated with the current perception of stress and the HPA axis functioning in adulthood, and the mediating role of loneliness in these relationships. In addition, we wanted to check whether the ReCAPS is an adequate and useful tool to measure overall ELS. The results showed that ELS was related to perceived stress, but not to HPA axis functioning. Moreover, loneliness mediated the relationship between ELS and perceived stress, but not the relationship with the HPA axis. Additionally, RFQ and ReCAPS were strongly associated, and similar results were found with both questionnaires.

Regarding the relationship between ELS and perceived stress, the results indicated that ELS, evaluated as emotional and physical abuse and neglect, was associated with a higher perception of stress in adulthood. Despite the different types of samples studied, this result agrees with previous studies that reported significant relationships between childhood maltreatment (assessed by the Childhood Trauma Questionnaire; CTQ) and perceptions of stress during adulthood (Betz et al., 2021; Bossé et al., 2018; Han et al., 2016; Hyman et al., 2007). Research has shown that a history of ELS can influence the

way stress is perceived and managed in adulthood because it can lead to vulnerability and low tolerance to stressors in later life (Hammen et al., 2000), as well as poor adaptive stress coping strategies (Hyman et al., 2007). In this regard, the relationship with attachment figures, such as parents, who are the source for learning emotional management and self-regulation in stressful situations, is important (Bowlby, 1982; Schore, 2000; Schore & Schore, 2008). However, among people who experience ELS, these relationships are neglected or disorganized, making it difficult for them to learn to self-regulate in stressful situations from an early age and into adulthood. For example, breast cancer patients who had experienced ELS had more perceived stress during the disease (Han et al., 2016). This greater perception of stress could be due to the effects of ELS on lower self-efficacy and greater helplessness as different facets of stress perception. Experiences of abandonment before the age of 18 can lead to more perceived stress and, specifically, lower perceived self-efficacy (Betz et al., 2021). In this study, we added a possible mediating factor to contribute to explaining the relationship between early and current perceived stress and HPA axis functioning, as well as the discrepant results reported by several studies.

As our results suggest, loneliness may be a mediator between ELS and the current perception of stress in adulthood. The analyses demonstrate that individuals who have experienced more ELS referred to higher scores on loneliness and, in turn, presented higher levels of perceived stress. This association between ELS and loneliness is in line with previous studies that found that individuals with ELS experiences reported less social support (Beutel et al., 2017; Germine et al., 2015), a situation that has been associated with a greater perception of loneliness (Matthews et al., 2019). This result might be explained by the fact that some adverse events, such as parental divorce or interpersonal traumas, are related to poor/inadequate representations and abilities in

relationships with others (Crowell et al., 2009). These inadequate learnings are reflected in the type of interpersonal bonds established throughout life (Fonagy & Luyten, 2018; Repetti et al., 2002). In addition, our results support previous studies that report the same relationship between loneliness and perceived stress (Yaacob et al., 2009; Yarcheski et al., 2011). Two explanations can be suggested for this association. First, because the social network would act as a buffer of stressors, people who feel lonely or present poor quality social ties may suffer more from daily stressors or be more sensitive to their impact (Hawkley et al., 2008). Second, people who feel lonely tend to interpret social interactions as more threatening, due to hypersensitivity in this type of interaction. For this reason, loneliness would also be understood as a stressor in itself (Cacioppo & Hawkley, 2009).

Regarding HPA axis functioning, ELS was not directly or indirectly associated with diurnal cortisol. This finding agrees with studies that failed to find a direct relationship between ELS and overall diurnal cortisol (Karlamangla et al., 2019; Klaassens et al., 2009; Schreuder et al., 2016), DCS (Bublitz & Stroud, 2012; Franz et al., 2013), or bedtime levels (Schreuder et al., 2016), employing different questionnaires and indexes to measure ELS, such as the CTQ (Franz et al., 2013; Klaassens et al., 2009; Nicolson et al., 2010; Schreuder et al., 2016), Early Trauma Inventory (Klaassens et al., 2009), Adverse Childhood Experiences (Bublitz & Stroud, 2012), or composite indexes (Franz et al., 2013; Karlamangla et al., 2019; Nicolson, 2004; Van der Vegt et al., 2009). Although in our study an association was found between ELS and loneliness, the association between loneliness and the HPA axis was not significant, which differs from studies that found a significant association (Campagne, 2019; Doane & Adam, 2010; Johar et al., 2021; Lai et al., 2018, 2019). However, these studies did not control the potential effects of ELS. Moreover, this lack of relationship could suggest that the

allostatic load approach to basal hormone levels used in our study may not be the most appropriate indicator of the effects of ELS on HPA axis functioning. Perhaps, we should focus on another type of measure, such as the dynamic range of the system given that, in individuals with ELS, lower morning cortisol peak levels or a compression of the diurnal dynamic range of cortisol have been observed (Karlamangla et al., 2019; Meinlschmidt & Heim, 2005). Thus, a range that includes the morning cortisol peak and its difference from minimum levels at rest might be more appropriate. The contradictory results can also be explained by methodological differences, such as the relatively large compliance window for cortisol collection. In addition, the ELS severity of the sample in our study was low and may be sufficient to affect the perception of stress as a long-life bias, but not severe enough to affect the HPA axis. If individuals with clinical diagnoses had been included in the study, the relationship between ELS and the HPA axis might have yielded significant results, as reported in individuals diagnosed with psychosis (Faravelli et al., 2017).

The ReCAPS questionnaire was strongly associated with the RFQ. In addition, results of the regression and mediation analyses using ReCAPS to investigate the relationship between ELS and perceived stress and HPA axis functioning and the role of loneliness in both relationships obtained the same statistical conclusions as the RFQ. These results suggest that ReCAPS could be an adequate brief tool, complementary to other ELS questionnaires, such as the RFQ used in the current study or the widely used CTQ (Bernstein & Fink, 1998). Although different types of abuse and neglect are collected in the RFQ items, the score used is an overall ELS score, without differentiating between types of stressors. A similar self-report measure, the Global Perceived Early Life Stress Scale (Carpenter et al., 2004; consisting of a 6-point Likert scale, responded to in relation to what they consider normal for their peer cohort) was developed to measure

ELS in adults, and it has shown sensitivity on measures of HPA axis functioning. To the best of our knowledge, longitudinal studies investigating the effect of ELS on the stress system are not comparable with our results due to the disparity in the evaluation of both ELS and cortisol (Doom et al., 2014; Trickett et al., 2010). Therefore, our results should be confirmed in future longitudinal studies. Finally, it is worth mentioning that we found a positive relationship between ReCAPS and years of education, whereas the literature on this topic reports a relationship in the opposite direction (Fors et al., 2009; Zielinski, 2009). For future research, it would be interesting to study mediating effects that explain this relationship.

In addition to the important findings, some limitations of this study must be considered. First, the cross-sectional data of the study make it impossible to reach conclusions about causal relationships. Second, both questionnaires employed to assess ELS are general and retrospective measures and, consequently, could be affected by recall bias. Prospective and longitudinal studies would greatly improve this research area, as well as the combination of general and specific measurements because there is evidence suggesting that different forms of early adversity can lead to different clinical outcomes (Bentall et al., 2012). Moreover, the recall of ELS could be affected by stressful situations during adulthood that were not explored in the current study, given that adverse events in childhood increase the risk of experiencing more stressful life events during adulthood (Shapero et al., 2014), which is associated with worse clinical results (Cotter et al., 2016; Pearlin et al., 1981; Shevlin et al., 2008; Stevens et al., 2017). However, RFQ is considered a valid instrument and has been employed in several investigations (Benedetti et al., 2011; Collazzoni et al., 2017, 2020; Counts et al., 2018; Poletti et al., 2020). Although the use of non-consecutive days for cortisol sample collection reduces the replicability of the data, the pattern of sample times used (seven samples per day) provides

a large number of samples throughout the day, thus allowing a valid evaluation of diurnal HPA axis functioning. In addition, the fact that there were two collection days increases the reliability of the data (Kraemer et al., 2006).

In sum, loneliness appears to be a mediating factor between ELS and perceived stress, but not HPA axis functioning (as measured by saliva diurnal cortisol levels). Our results highlight the importance of intervening in young people who have suffered from ELS, in order to reduce the perception of loneliness and promote the quality of social network support and significant emotional ties. Loneliness interventions could also be useful to reduce the perception of stress produced by the daily stress of having fewer strategies to cope with ELS experiences and feelings of loneliness and improve the person's state of health. These are novel findings, although more research is needed to address how loneliness mediates the association between ELS and perceived stress. In this line, unhelpful metacognitive beliefs, such as "worrying about threats means I can be prepared" or "if I continue to worry, I will lose my mind," can arise during childhood as an attempt to manage early emotional abuse (Myers & Wells, 2015). These metacognitive beliefs may be influencing negative cognitive and emotional consequences of ELS during adulthood (Mansueto et al., 2019), which suggests that they could be an important factor to study in the relationship between ELS and feelings related to loneliness or stress. Thus, future research is needed to determine this specific pathway and others such as attachment styles, through which early adverse experiences affect psychological processes related to loneliness and stress in adulthood.

Chapter III

Study 2: Loneliness and health indicators in aging. Importance of the sex

The main results of this study have been published in:

Crespo-Sanmiguel, I., Zapater-Fajarí, M., Garrido-Chaves, R., Hidalgo, V., & Salvador, A. (2022). Loneliness and Health Indicators in Middle-Aged and Older Females and Males. *Frontiers in Behavioral Neurosciences*, *16*, 809733.

Introduction

Humans are essentially social beings. From birth, we need an attachment figure in order to develop, survive, and understand how the social universe and personal ties work (Bowlby, 1973, 1980; Fonagy & Luyten, 2018). During the rest of life, we have the need to be integrated into a social network (Baumeister & Leary, 1995). When this need is not fulfilled, loneliness appears, the painful feeling that accompanies the perception of a lack of desired personal and social relationships (Peplau & Perlman, 1982). Hence, loneliness can be understood as a potent psychosocial stressor (Cacioppo et al., 2003; Hawkley & Cacioppo, 2003). In addition, loneliness increases with age and is usually associated with adverse health outcomes (Cohen-Mansfield et al., 2016; Holt-Lunstad et al., 2015; Luo et al., 2012; Richard et al., 2017; Victor & Yang, 2012).

Thus, loneliness has been related to self-reported psychological and physical health issues, such as poor health and low life satisfaction (Losada et al., 2012; Tomstad et al., 2017), subjective memory complaints (Montejo et al., 2019), lower self-esteem (Wagner et al., 2015), depression (Aylaz et al., 2012; Ge et al., 2017), mobility characteristics (Van Den Berg et al., 2016), sleep disorders (Shankar, 2020), or more doctor visits (Beutel et al., 2017; Richard et al., 2017). In addition, loneliness plays a mediating role between early life stress and perceived stress in adulthood (Crespo-Sanmiguel et al., 2021). These health issues can be explained by the fact that loneliness acts as a stressor, activating the stress response via HPA axis functioning through the action of cortisol (Hawkley & Cacioppo, 2003; O'Connor et al., 2021; Steptoe et al., 2004). In this regard, a dysregulation of this axis has been associated with different harmful health issues (Adam et al., 2017; Fries et al., 2009).

Several studies have investigated the relationship between loneliness, CAR, and DCS in aging, but with inconsistent results. In middle-aged adults, loneliness was found to be associated with CAR (Adam et al., 2006; Okamura et al., 2011; Steptoe et al., 2004), but no differences were found in CAR or DCS based on loneliness in older men and women (Schutter et al., 2017, 2021). However, in older people, a flatter DCS was found in lonely people in comparison with non-lonely individuals (Cole et al., 2007). In a previous study, we found that loneliness was positively associated with bedtime cortisol levels, but not with awakening cortisol or the DCS (Montoliu et al., 2019). As far as we know, our previous study was the first one to study the possible association between loneliness and bedtime cortisol levels. However, we could not test subjective health in relation to loneliness. Thus, the association between loneliness, cortisol indexes, and subjective health requires further research.

Sex differences have been found in some research on loneliness and its effects on subjective health and HPA axis functioning. Specifically, Zebhauser et al. (2014) reported that loneliness had a higher impact on subjective psychological health in men than in women. Furthermore, a dysregulation of the HPA axis, with a diminished CAR and flatter DCS, has been found in lonely married men, but not in their women counterparts (Johar et al., 2021). However, other studies did not find sex differences in the relationship between loneliness and subjective health (Richard et al., 2017), the DCS, or bedtime cortisol (Montoliu et al., 2019).

Given that loneliness can be understood as a stressor, we first aimed to study whether loneliness is associated with subjective psychological and physical health and HPA axis functioning in late middle-aged and early older people (older people henceforth). Second, we also aimed to explore whether these results differ depending on sex. Finally, we investigated whether HPA axis functioning is a mechanism underlying

the relationship between loneliness and subjective health. We hypothesized that there would be a negative association between loneliness and subjective psychological and physical health (Beutel et al., 2017; Richard et al., 2017), and we expected to confirm the association between loneliness and cortisol indexes found previously (Montoliu et al., 2019). Finally, despite the heterogeneity in the findings, we expected to find clearer relationships in men than in women.

Materials and Methods

Participants

Participants were recruited from a study program for people over 55 years old at the University of Valencia (Spain). This program, called "La Nau Gran," is a three-year education program with basic modules from one of the different official degrees. The students are not issued an official degree, and they do not share the subjects with the regulated degree students. However, this program allows middle-aged and older people who want to learn and continue to grow to access the university as students. Two researchers from the laboratory came to these lessons to offer students the chance to participate in the research. Information on the type of data collected, the duration of the session, and the location of the laboratory was provided. Interested students provided their contact information. Participants who were not excluded, based on the exclusion criteria in a telephone interview, were given an appointment to attend an individual session at the Laboratory of Social Cognitive Neuroscience, of the University of Valencia.

Exclusion criteria were: being outside the age range from 55 to 75 years old; having diseases and disorders that interfere with daily wellbeing, such as endocrine (e.g., Type II diabetes), neurological (e.g., epilepsy), psychiatric (e.g., personality and

psychotic disorders or depression), or other diseases (cancer, CVD or chronic pain); using a medication such as glucocorticoids, anxiolytics, antidepressants, or other medications that can interfere directly with emotional, endocrinological, or cognitive functioning; having been under general anesthesia in the past 12 months; smoking more than 10 cigarettes a day, alcohol or other drug abuse; and the presence of a stressful life event during the past year, such as the death of the spouse, the appearance of a major disease, or any other event that had affected them in a significant way.

Procedure

The current study follows an observational and cross-sectional design. Experimental sessions, which lasted approximately 1h, had three different schedules (at 10 a.m, 12 p.m, and 4 p.m). Both the schedule and the sex of the participants were counterbalanced, so that the number of participants and the number of men and women who attended each session were similar. During the session, a general questionnaire about sociodemographic information and questionnaires about loneliness, subjective health, social relationships, depressive symptomatology, and stress perception were filled out. Furthermore, weight and height measurements were taken to calculate the body mass index (BMI). The experimenter explained verbally to the participants how and when they had to collect salivary cortisol samples at home. In addition, written instructions were given to participants, attached to a diary where they could provide the collection times of the salivary samples. Within three days after the session, participants returned to the laboratory to bring the samples. The protocol was approved by the Research Ethics Committee of the University of Valencia (Code: 1034878) and written according to the Declaration of Helsinki. All participants read and signed the informed consent. The period of the data collection was between January 2018 and January 2020.

Questionnaires

Socioeconomic Status

We assessed the socioeconomic status with the nine-rung social ladder (Adler & Stewart, 2007). A ladder with nine rungs is presented by explaining that the highest rungs would contain the people in our country with the highest standing, that is, with a lot of money, a good education, and the best jobs. In contrast, the lowest rungs would contain the poorest people with less education and worse jobs or no job. Participants are asked the following question: Where would you place yourself on this ladder?

Loneliness

We used the Spanish adaptation (Vázquez Morejón & Jiménez García-Bóveda, 1994) of the revised UCLA loneliness scale (R-UCLA) (Russell et al., 1980) to assess loneliness. This scale contains 20 items rated on a 4-point Likert scale ranging from 1 (never) to 4 (often), obtaining a total score ranging from 20 (low) to 80 (high). The Cronbach's α in our study was 0.887.

Subjective Psychological and Physical Health and Social Relationships

We used the three domains of the Spanish version of the World Health Organization Quality of Life Short Form Survey (Carrasco, 1998), created by the WHOQOL Group (1998), to assess the perception of: (i) psychological health (e.g., self-esteem, positive and negative feelings, or capacity for concentration); (ii) physical health (e.g., mobility, activities of daily living, sleep and rest, or pain and discomfort); and (iii) social relationships (e.g., social support or sexual activity). The subscales have 6, 7, and 3 items, respectively, with a 5-point Likert response scale ranging from 1 to 5. The scores were transformed to a scale from 0 to 100, where higher scores represent perceptions of better

health. The internal consistency (Cronbach's α) for the psychological health scale was 0.763, for the physical health scale 0.623, and for the social relationships scale 0.708.

Depressive Symptomatology

We used the Spanish version (Sanz et al., 2003) of the Beck Depression Inventory-II (BDI-II; Beck et al., 1996) to assess depressive symptomatology. This test consists of 21 items, with a response scale ranging from 0 to 3, that evaluate the symptoms of depression (emotional, cognitive, somatic, and motivational) in the past month. Scores range from 0 to 63, where higher scores are interpreted as higher symptomatology. The internal consistency (Cronbach's α) of this scale was 0.868.

Perceived Stress

The degree to which people perceived their lives as stressful and overloaded was evaluated using the Spanish version (Remor, 2006) of the 14-item Perceived Stress Scale (PSS-14; Cohen et al., 1983). Participants had to answer using a 5-point Likert scale ranging from 0 (never) to 4 (very often), and total scores range from 0 to 56, with higher scores indicating higher stress perception. The evaluation refers to the past month. The internal consistency (Cronbach's α) of this scale in this study was 0.801.

Cortisol Measurements

Ten salivary samples were collected to assess participants' diurnal cortisol levels using Salivettes (Sarstedt, Rommelsdorf, Germany). The saliva samples were collected by participants immediately, + 15, + 30 and + 45 min after awakening and before sleep on two consecutive days in their natural environment and without disturbing their usual daily activities. Participants were instructed to keep the cotton in their mouth for exactly 2 min and then store it in the refrigerator until they took it to the laboratory. They returned the

samples as soon as possible, with 3 days after their collection being the maximum time. Once in the laboratory, the saliva samples were centrifuged for 15 min at 4.000 rpm, resulting in a clear supernatant of low viscosity that was stored at −80°C until analyses were performed. The ELISA kit from Salimetrics (Newmarket, United Kingdom) was used to determine the cortisol concentrations.

For each participant, all samples were measured in duplicate and analyzed in the same trial. The assay sensitivity and the inter- and intra- assay variation coefficients of raw densities were below 10%. Salivary cortisol levels were determined in the Laboratory of Social Cognitive Neuroscience (Valencia, Spain).

Statistical Analyses

Because the cortisol levels did not follow a normal distribution, they were log transformed. We used three cortisol indexes: (i) the CAR, a dynamic measure of post-awakening cortisol secretion, calculated from cortisol samples taken 0, + 15, + 30, and + 45 min after awakening following the trapezoidal formula for the area under the curve with respect to the increase (AUCi; see Pruessner et al., 2003); (ii) the DCS, which is calculated as awakening cortisol minus bedtime cortisol and reflects the diurnal decline in cortisol levels; and (iii) bedtime cortisol, which reflects cortisol levels immediately before going to sleep. To calculate each cortisol index, the mean for both days was used, and for participants who had missing cortisol data from one of the 2 days (CAR: n = 24, DCS: n = 7, bedtime: n = 6), we used the data from the day they were available. To study the effect of CAR compliance, we reran the analysis, excluding 12 participants (15.19%) who were outside the recommended strict time window, that is, with a 5-min delay (Stalder et al., 2016). These results are reported in Tables II.3.1, II.4.1 and II.5.1.

To evaluate sex differences, Student's t-tests for independent samples were performed for age, socioeconomic status, BMI, loneliness, psychological and physical health, cortisol indexes, social relationships, depressive symptomatology, perceived stress, and hours spent with their children. In addition, χ^2 were performed for educational level, marital status, and number of children, and Z analysis was performed for the statistically significant results of χ^2. To investigate whether loneliness was related to subjective psychological and physical health and HPA axis functioning, linear regression analyses were performed. In these analyses, loneliness was the independent variable, and subjective psychological and physical health and the cortisol indexes were the dependent variables. Additionally, we tested whether these relationships varied depending on sex by performing moderation analyses. Thus, loneliness was the independent variable, sex was the moderator variable, and subjective health (psychological and physical) and endocrine indicators (cortisol indexes) were the dependent variables. Both linear regression and moderation analyses were performed separately for each dependent variable. Finally, we tested whether the HPA axis mediates the relationship between loneliness and subjective psychological and physical health by performing mediation analyses. Thus, loneliness was the independent variable, the cortisol indexes were the mediators, and the two types of subjective health (psychological and physical) were the dependent variables.

Because loneliness has been widely related to depressive symptomatology (Erzen & Çikrikci, 2018) and most of the studies on loneliness and cortisol control depression in the analyses (Schutter et al., 2017; Montoliu et al., 2019; Johar et al., 2021), we included depressive symptomatology as covariate in the regression, moderation, and mediation analyses. Moreover, for the regression, moderation, and mediation analyses that include the CAR, we used time of awakening and cortisol levels immediately after awakening as covariates, as in Stalder et al. (2016). Furthermore, Pearson's correlations were performed

between the sociodemographic variables and loneliness, subjective psychological and physical health, and the cortisol indexes. The sociodemographic variables that were significantly related to these factors were used as covariates in the main analyses (regression, moderation, and mediation analyses). Thus, in all the main analyses, in addition to depressive symptomatology, age was controlled because it was positively related to loneliness in our sample. Likewise, socioeconomic status was a covariate in the main analyses that included subjective physical health, due to their relationship. BMI was a covariate in the main analyses that included bedtime cortisol, due to their relationship. In addition, due to the sex differences in marital status and in BMI, these variables were included as covariates in all the moderation analyses.

Multivariate outliers were considered those that deviated from the mean (± 3 SD), and standardized residuals were used to detect them. Specifically, there was an outlier in the main analyses that included the CAR. No collinearity issues were detected for the variables included in the main analyses, indicated by tolerance values > 0.1. One piece of data was missing for BMI and for level of studies, three for CAR and DCS, two for bedtime cortisol, thirteen for the time of awakening, and five for the hours per week spent with their children. Consequently, the number of participants in the different analyses varies.

We estimated a sample size of N = 55 to obtain a medium effect size ($f^2 = 0.15$, $\alpha = 0.05$ and power = 0.80), calculated using the G*Power (Faul et al., 2007). Thus, our sample size (N = 79) is adequate because in the recruitment we anticipated possible missing data. The bootstrap technique used for moderation and mediation analysis uses the original sample size as a miniature representation that is randomly replaced and resampled, which increases the statistical power and solves the problem of having a relatively small sample (Hayes, 2017).

To carry out all the statistical analyses, version 25.0 of SPSS was used. All *p*-values were two-tailed, and the level of significance was taken as $p < 0.05$. To test moderated and mediated regression effects, we used PROCESS 3.4 for SPSS (Model 1) and Z scores. PROCESS uses bootstrapped bias-corrected 95% confidence intervals with 5,000 bootstrapped samples in order to determine the significance of the interaction effect in the moderation analysis and the significance of the indirect effect in the mediation analysis. When the confidence interval for the interaction effect (moderation analysis) or the indirect effect (mediation analysis) did not include zero, it was interpreted that there was a significant interaction/indirect effect (Hayes, 2017).

Results

Descriptive analyses

The sessions contained 82 participants, but three participants were eliminated due to missing data on the loneliness, depression, or health questionnaires. The final sample was composed of 79 participants (39 men, 40 women) with ages ranging from 55 to 75 years old (M = 64.481, SD = 5.563). Participants reported a medium-high socioeconomic status, medium-high levels of satisfaction with their social relationships, and low levels of depressive symptomatology and perceived stress. More than half the participants (57.7%) had university studies, and 52 (65.8%) were married. All the women were post-menopausal. There were no sex differences in loneliness [t (77) = 1.273, p = 0.207], subjective psychological [t (77) = 1.042, p = 0.301] or physical health [t (77) = −0.898, p = 0.372], or the cortisol indexes [CAR: t (64) = −0.041, p = 0.968; DCS: t (74) = 0.094, p = 926; bedtime: t (75) = 0.600, p = 0.551]. Significant differences were only found in BMI [t (76) = 3.999 $p < 0.001$] and marital status [χ^2 (4) = 9.691, p = 0.046], with a higher

proportion of married men compared to married women and of widowed women compared to widowed men. Sample characteristics are described in Table II.1.

Pearson's correlation analyses

In our sample, loneliness increased with age (p = 0.001) and was negatively related to subjective psychological health (p = 0.011) and physical health (p = 0.038), but it was not significantly associated with the CAR (p = 0.710), DCS (p = 0.433), and bedtime (p = 0.950) cortisol indexes. Moreover, loneliness was negatively related to satisfaction with their relationships (p < 0.001) and to the hours per week they spent with their children (p = 0.006). However, loneliness was not significantly related to depressive symptomatology (p = 0.096) (Table II.2).

Table II.1 Characteristics of the total sample and for men and women

	Total (N=79)	Men (N=39)	Women (N=40)	*t (p) / X² (p)*
Age	64.48 (5.56)	65.39 (5.68)	63.60 (5.37)	1.435 (.155)
Educational level:				6.047 (.302)
Primary School or less	10.3	7.9	12.5	
Secondary School	32.1	23.7	40.0	
Graduate	56.4	65.7	47.5	
PhD	1.3	2.6	0	
Marital status:				9.691 (.046)
Single	8.9	2.6	15.0	
Married	65.8	76.9	55.0	
Divorced	15.2	18.0	12.5	
Widower	10.1	2.6	17.5	
SES	6.11 (1.58)	6.21 (1.59)	6.03 (1.58)	.505 (.615)
BMI	26.66 (4.04)	28.33 (3.59)	24.99 (3.79)	3.999(<.001)

(Continue on next page)

Continuation of **Table II.1**

	Total (N=79)	Men (N=39)	Women (N=40)	*t (p) / X² (p)*
Loneliness	35.81 (7.17)	36.85 (7.58)	34.80 (6.69)	1.273 (.207)
Psychological health	65.72 (10.77)	67.00 (11.38)	64.48 (10.14)	1.042 (.301)
Physical health	69.89 (11.71)	68.69 (9.13)	71.05 (13.78)	−.894 (.372)
Cortisol indexes				
CAR	.30 (.57)	.29 (.57)	.30 (.58)	−.041 (.968)
DCS	.67 (.33)	.68 (.29)	.67 (.36)	.094 (.926)
Bedtime levels	.11 (.24)	.13 (.20)	.09 (.28)	.600 (.551)
Depressive symptomatology	6.03 (6.01)	5.05 (4.53)	6.98 (7.09)	−1.433(.156)
Perceived stress	17.62 (6.78)	16.67 (7.32)	18.55 (6.16)	−1.239(.219)
Social relationships	62.38 (16.77)	62.77 (15.73)	62.00 (17.91)	.203 (.840)
Number of sons:				2.508 (.643)
0	6.3	2.6	10.0	
1	12.7	12.8	12.5	
2	63.3	69.2	57.5	
3	12.7	10.3	15.0	
4	5.1	5.1	5.0	
Time with sons[a]	9.04 (11.11)	8.16 (10.57)	9.97 (11.74)	−.700 (.486)

Note. SES = Subjective Socioeconomic Status; BMI = Body Mass Index; [a] = measured in hours per week. Sex differences in educational level, marital status and number of sons are expressed as percentages and analyzed with Chi-square tests. The means and standard deviations of other variables are shown and were analyzed with Student-t tests

Table II.2 Pearson's correlations

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Age	.213	.247*	−.044	.376**	−.046	−.119	.110	−.018	−.106	.100	.027	−.222*	.190	−.329**
2. Educational level		.234*	.048	.168	.031	.048	−.021	−.087	.130	−.269*	−.006	−.257*	−.138	.058
3. SES			−.040	.056	.083	.238*	.109	−.051	.042	−.148	−.112	−.028	.233*	−.014
4. BMI				.044	.032	−.129	−.096	−.161	.235*	−.108	.065	.023	.027	.012
5. Loneliness					−.284*	−.234*	.047	−.091	.007	.189	.139	−.484**	−.039	−.315**
6. Psychological health						.354**	.069	−.069	−.047	−.464**	−.262*	.355**	.046	.337**
7. Physical health							.054	−.041	.114	−.374**	−.348**	.325**	.058	.000
8. CAR								−.424**	−.038	.039	−.143	−.129	.003	.099
9. DCS									−.679**	.179	.005	.114	.012	.010
10. Bedtime										−.159	.039	−.040	.019	.015
11. Depressive sympt.											.453**	−.245*	−.045	−.101
12. Perceived stress												−.220	−.276*	.013
13. Social relationships													.814	−.006
14. Number of sons														−.056
15. Time sons[a]														

Note. SES = Subjective Socioeconomic Status; BMI = Body Mass Index; CAR = Cortisol Awakening Response; DCS = Diurnal Cortisol Slope; Depressive sympt. = Depressive symptomatology; [a] = measured in hours per week. *$p < .05$; **$p < .01$

Adjusted regression analyses

Results of adjusted regression analyses confirmed the significant negative relationship between loneliness and subjective psychological health ($p = 0.034$). However, when covariates were included, the relationship between loneliness and subjective physical health became non-significant ($p = 0.167$)[1]. The relationship between loneliness and the cortisol indexes was not significant either (all $p > 0.291$) (Table II.3).

Table II.3 Adjusted regression analyses with loneliness as a predictor and the psychological and physical health and cortisol indexes as dependent variables

			Loneliness		
	R^2 adjusted	R^2 change	Beta	*p*	N
Psychological health	.232	.046	−.235	.034*	79
Physical health	.171	.021	−.158	.167	79
CAR	.332	.011	−.110	.319	65
DCS	.009	.015	−.135	.291	76
Bedtime cortisol	.029	.004	.066	.602	76

Note. CAR = Cortisol Awakening Response; DCS = Diurnal Cortisol Slope. Controlled by depressive symptomatology and age. In addition, socioeconomic status for physical health, time of awakening and cortisol levels immediately after awakening for CAR and body mass index for bedtime levels. *$p < .05$

[1] To study in more detail which covariate is influencing the change in the significance of the relationship between loneliness and physical health, we performed a stepwise regression including the covariates (age, socioeconomic status, and depressive symptomatology). Depressive symptomatology was the only covariate that remained in the model, and so it is the one that is modifying the statistical conclusion.

Table II.3 Adjusted regression analyses with loneliness as a predictor and the psychological and physical health and cortisol indexes as dependent variables

	Loneliness				
	R^2 adjusted	R^2 change	Beta	*p*	N
CAR	.222	.029	−.187	.169	54

Note. CAR = Cortisol Awakening Response. Controlled by depressive symptomatology and age, time of awakening and cortisol levels immediately after awakening

Moderation analyses

Results of the moderation analyses showed a significant interaction effect of sex in the relationship between loneliness and subjective psychological health ($p < 0.001$). Thus, loneliness was negatively related to subjective psychological health in men ($p < 0.001$), but not in women ($p = 0.263$). No interaction effect of sex was found in the relationships between loneliness and subjective physical health ($p = 0.697$). Additionally, sex did not moderate the relationship between loneliness and the cortisol indexes (all $p > 0.274$) (Table II.4).

Table II.4 Adjusted moderation analyses with loneliness as a predictor and psychological and physical health and cortisol indexes as dependent variables in men and women

Dependent variable: Psychological health

ΔR^2 interaction = .130; F = 15.361; df(1,2) = 1, 70; *p* <.001; LLCI = .183; ULCI = .563						
Sex	Effect	SE	*t*	*p*	LLCI	ULCI
Men	−.581	.136	− 4.284	<.001**	−.851	−.310
Women	.162	.143	1.129	.263	−.124	.447

Dependent variable: Physical health

ΔR^2 interaction = .002; F = .153; df(1,2) = 1, 69; *p* = .697; LLCI = −.173; ULCI = .258						
Sex	Effect	SE	*t*	*p*	LLCI	ULCI
Men	−.177	.154	−1.155	.252	−.484	.129
Women	−.093	.162	−.576	.567	−.417	.230

Dependent variable: CAR (1 outlier)

ΔR^2 interaction = .011; F = 1.038; df(1,2) = 1, 54; *p* = .313; LLCI = −.638; ULCI = .208						
Sex	Effect	SE	*t*	*p*	LLCI	ULCI
Men	.008	.153	.051	.960	−.299	.315
Women	−.207	.152	−1.360	.180	−.513	.098

Dependent variable: DCS

ΔR^2 interaction = .001; F = .039; df(1,2) = 1, 67; *p* = .844; LLCI = −.255; ULCI = .209						
Sex	Effect	SE	*t*	*p*	LLCI	ULCI
Men	−.093	.165	−.559	.578	−.423	.238
Women	−.138	.176	−.783	.436	−.489	.214

(Continue on next page)

Continuation of **Table II.4**

Dependent variable: Bedtime

ΔR^2 interaction = .016; F = 1.219; df(1,2) = 1, 68; *p* = .274; LLCI = −.362; ULCI = .104						
Sex	Effect	SE	*t*	*p*	LLCI	ULCI
Men	.185	.166	1.116	.269	−.146	.517
Women	−.071	.177	−.399	.691	−.424	.283

Note. CAR = Cortisol Awakening Response; DCS = Diurnal Cortisol Slope. ***p* < .01. Values for quantitative moderators are the mean and plus/minus one SD from mean

Table II.4.1 Adjusted moderation analysis with loneliness as a predictor and CAR as dependent variable in men and women excluding participants outside the time window

Dependent variable: CAR (N = 53)

ΔR^2 interaction = .013; F = .853; df(1,2) = 1, 43; *p* = .361; LLCI = −.708; ULCI = .263						
Sex	Effect	SE	*t*	*p*	LLCI	ULCI
Men	−.079	.169	−.469	.641	−.420	.261
Women	−.302	.190	−1.588	.120	−.685	.082

Note. CAR = Cortisol Awakening Response. Values for quantitative moderators are the mean and plus/minus one SD from mean

Mediation analyses

Mediation analyses revealed that the cortisol indexes did not mediate the relationship between loneliness and subjective health. Regarding the analyses with subjective psychological health as dependent variable, the indirect effect (i.e., effect of loneliness on subjective psychological health via cortisol indexes) was not significant for any cortisol indexes: CAR (IC 95% [−0.025, 0.076]), DCS (IC 95% [−0.026, 0.058]), and bedtime

(IC 95% [−0.047, 0.030]) (Table II.5). In the analysis with subjective physical health as dependent variable, the indirect effect (i.e., effect of loneliness on subjective physical health via cortisol indexes) was not significant for any of the cortisol indexes: CAR (IC 95% [−0.082, 0.016]), DCS (IC 95% [−0.038, 0.038]), and bedtime (IC 95% [−0.023, 0.050]) (Table II.6).

Table II.5 Adjusted mediation models of the relationship between loneliness as predictor and psychological health as dependent variables via cortisol indexes (CAR, DCS, and bedtime)

Mediating variable: CAR (1 outlier)

	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to CAR	−.109	.109	−1.005	.319	−.327	.108
CAR to psychological health	−.106	.136	−.776	.441	−.378	.167
Indirect effect	.012	.026	-	-	−.025	.076
Total effect	−.258	.113	−2.275	.027*	−.484	−.031
Direct effect	−.269	.115	−2.349	.022*	−.498	−.040

Mediating variable: DCS

	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to DCS	−.133	.125	−1.064	.291	−.381	.116
DCS to psychological health	−.013	.106	−.125	.901	−.225	.198
Indirect effect	.002	.020	-	-	−.026	.058
Total effect	−.245	.112	−2.197	.031*	−.468	−.023
Direct effect	−.247	.113	−2.180	.033*	−.473	−.021

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Continuation of **Table II.5**

	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to bedtime	.065	.123	.523	.602	−.181	.311
Bedtime to psychological health	−.106	.108	−.981	.330	−.322	.110
Indirect effect	−.007	.018	-	-	−.047	.030
Total effect	−.241	.112	−2.146	.035*	−.466	−.017
Direct effect	−.234	.113	−2.081	.041*	−.459	−.010

Note. CAR = Cortisol Awakening Response; DCS = Diurnal Cortisol Slope. *$p < .05$

Table II.5.1. Adjusted mediation models of the relationship between loneliness as predictor and psychological and physical health as dependent variables via CAR excluding participants outside the time window

Mediating variable: CAR (N = 54)

	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to CAR	−.187	.134	−1.397	.169	−.456	.082
CAR to psychological health	−.125	.134	−.930	.357	−.394	.145
Indirect effect	.023	.042	-	-	−.023	.139
Total effect	−.347	.124	−2.793	.008**	−.596	−.097
Direct effect	−.370	.127	−2.918	.005**	−.625	−.115
	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to CAR	−.169	.134	−1.260	.214	−.439	.101
CAR to physical health	.183	.141	1.295	.202	−.101	.467
Indirect effect	−.031	.043	-	-	−.147	.023
Total effect	−.230	.131	−1.761	.085	−.493	.033
Direct effect	−.199	.132	−1.511	.138	−.465	.066

Note. CAR = Cortisol Awakening Response. **$p < .01$

Table II.6 Adjusted mediation models of the relationship between loneliness as predictor and physical health as dependent variables via cortisol indexes (CAR, DCS, and bedtime)

	Mediating variable: CAR (1 outlier)					
	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to CAR	−.098	.107	−.915	.364	−.312	.116
CAR to physical health	.163	.143	1.138	.260	−.123	.448
Indirect effect	−.016	.025	-	-	−.082	.016
Total effect	−.096	.117	−.826	.412	−.330	.137
Direct effect	−.080	.117	−.687	.495	−.315	.154
	Mediating variable: DCS					
	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to DCS	−.133	.126	−1.058	.294	−.383	.118
DCS to physical health	.011	.110	.097	.923	−.208	.229
Indirect effect	−.001	.018	-	-	−.038	.038
Total effect	−.136	.115	−1.182	.241	−.366	.094
Direct effect	−.135	.117	−1.152	.253	−.368	.099
	Mediating variable: Bedtime					
	Effect	SE	*t*	*p*	LLCI	ULCI
Loneliness to bedtime	.065	.124	.523	.602	−.182	.312
Bedtime to physical health	.091	.111	.823	.414	−.130	.313
Indirect effect	.006	.018	-	-	−.023	.050
Total effect	−.126	.115	−1.098	.276	−.355	.103
Direct effect	−.132	.115	−1.145	.256	−.362	.098

Note. CAR = Cortisol Awakening Response; DCS = Diurnal Cortisol Slope

Discussion

The aims of this study were, first, to test whether loneliness was related to subjective psychological and physical health indicators and HPA axis functioning (CAR, DCS, and bedtime cortisol) and, second, to analyze the role of sex in these relationships. Finally, we investigated whether HPA axis functioning was a mediator in the relationship between loneliness and subjective health. In the total sample, loneliness was correlated with psychological and physical health, but when these relationships were analyzed in more detail (including the pertinent covariates), loneliness did not appear to be associated with subjective physical health or the cortisol indexes, and the only relationship that remained was between loneliness and psychological health in men, but not in women.

Men with higher loneliness scores showed lower subjective psychological health. This finding is in line with a previous study showing that men tend to experience greater effects of loneliness on mental health (Zebhauser et al., 2014). These authors, based on Stevens (1995), proposed that the sex differences could be due to the fact that women have more settings where they can obtain social support, whereas men seek more social contact in the public spheres of organizations, where it is more difficult to find close personal contacts. However, this explanation is not supported by our results because there are no sex differences in satisfaction with social relationships. Although there are no sex differences in loneliness in previous literature (Maes et al., 2019) or in our sample, the association between loneliness and subjective psychological health could be explained by sex differences in experiences in interdependent relationships. It has been observed that women focus more on intimate and dyadic attachments (Baumeister & Sommer, 1997; Gardner & Gabriel, 2004) in which they can share their most personal and intimate experiences and, thus, strengthen their psychological wellbeing. In contrast, men focus more on the group (Hoza et al., 2000), where there is no space to share concerns. These

differences are often related to masculinity (ideals and rules about what it means to be a man), manifested as difficulty in expressing their needs (Wide et al., 2011), a tendency to solve problems independently (Roy et al., 2017), and being less likely to request psychological support through services (Ogrodniczuk et al., 2016). These traits could influence the way loneliness affects older people differently depending on their sex. Therefore, men would benefit less from their relationships in terms of mental health. However, more research is needed to understand the underlying mechanisms that associate loneliness with psychological health in men but not in women.

Previous studies found that lonely people were more likely to report factors related to poor physical health, such as visits to medical doctors, more chronic diseases, or poor subjective health, among others (Richard et al., 2017). In our sample, loneliness was associated with subjective physical health, although the inclusion of depressive symptomatology as a covariate made the relationship non-significant. This could be explained by the fact that depressive symptomatology can aggravate the way people perceive their health (Gaynes et al., 2002; Wells et al., 1989). In line with this, depressive symptomatology is associated with more difficulty sleeping (Koffel & Watson, 2009) and a wide variety of somatic complaints, which also share biological pathways and neurotransmitters with depression (Bair et al., 2003). Our results are not consistent with Richard et al. (2017), who found an association between loneliness and self-reported physical health. However, Richard et al. (2017) included participants with non-specified chronic illnesses, and this worse state of health may have corresponded to lower subjective physical health (Benyamini et al., 2014; Benyamini & Idler, 1999; Elran-Barak et al., 2019; Idler & Benyamini, 1997). In the current study, due to the restrictive exclusion criteria followed, participants did not have any important diseases that seriously interfered with their wellbeing, and they did not take any medications that could indicate

an initial phase of illness. These characteristics could explain the non-relationship found between loneliness and subjective physical health.

Regarding the lack of association between loneliness and the HPA axis indexes, this result agrees with a previous study that did not find a relationship between loneliness and CAR (Schutter et al., 2017), although Johar et al. (2021) found a diminished CAR in lonely married men. However, in this latter study, the relationship between CAR and loneliness in men was no longer significant when adjusting for sociodemographic covariates, depression, or awakening time. Therefore, our results are in line with those of Johar et al. (2021), suggesting that loneliness and CAR are not associated. Furthermore, although in the regressions separated by sex, the relationship was significant for men, the sex interaction of the relationship between loneliness and CAR was not significant.

We also failed to find a relationship between loneliness and DCS. This result agrees with other previous studies (Montoliu et al., 2019; Schutter et al., 2017, 2021), although it contrasts with studies that found a flattened DCS in married participants (Johar et al., 2021) and in a selected sample with extremely high loneliness scores (Cole et al., 2007). In both of these studies, the sample was composed of people who could suffer from alcohol abuse, smoking, or diabetes, which can influence the DCS (Adam et al., 2017). These health issues alone could contribute to the dysregulation of the HPA axis functioning and skew the association between loneliness and cortisol patterns. In addition, in Cole et al. (2007), although the saliva samples were collected reliably (on three consecutive days and at three different time points due to the longitudinal study design), the procedure used to classify the groups and the small number of participants in each group could explain the disparity in the results. Specifically, the groups were composed of 14 participants who consistently scored in the top 15% of the loneliness distribution throughout the study (high-lonely group; N = 6) and in the bottom 15% (low-lonely; N =

8). Thus, the analyses of group differences were performed with extreme scores and small samples.

Regarding the lack of relationship between loneliness and bedtime cortisol, the current results did not confirm our previous study (Montoliu et al., 2019), which found a positive association between them. Although it is true that participants in both studies showed similar loneliness scores, there were protective factors that we added in the current study and had not tested before. The characteristics of our participants represent optimal aging: no chronic diseases, a high socioeconomic level, low depressive symptomatology, low perceived stress, children they see frequently, and high satisfaction with social relationships. These circumstances could be acting as a buffer against stressors (Hawkley et al., 2008) such as loneliness and its endocrine effects, or they could even keep feelings of loneliness from appearing (Teater et al., 2021).

Finally, the results of the mediation analyses showed that the direct relationship between loneliness and psychological health was negative, confirming the regression analyses. However, the indirect effect that indicates whether HPA axis functioning is an underlying mechanism between loneliness and health was not found, contrary to what other authors suggested (Hawkley & Cacioppo, 2003; Steptoe et al., 2004). Considering all this, it is worth noting that loneliness is an experience with potentially adverse effects on psychological health, although more research is needed on what factors could influence the way loneliness affects subjective health in older people without severe perceptions of loneliness.

Some limitations should be considered when interpreting the results of this study. First, the cross-sectional design of the study makes it impossible to draw conclusions about causal relationships. Second, the internal consistency of the physical health scale shows low values, and so the results that include this scale should be confirmed in further

studies. Moreover, to control possible confounders, this study had restrictive exclusion criteria, and only participants who were in good general health were included. Our participants have healthy characteristics and behaviors, and perhaps due to this, in our study loneliness was not related to the perception of physical health or to HPA axis indicators. Therefore, in future studies, these associations could be tested using longitudinal designs, other middle and older age ranges, and people with greater loneliness, for example, due to social circumstances or chronic diseases, such as older people with diabetes who report higher feelings of loneliness (Hackett et al., 2020).

Our findings support the view that loneliness is not associated with HPA dysregulation, but it is associated with subjective health, specifically psychological health in men. Subjective health is an important health measure because it predicts the evolution of health and life expectancy as well as or even better than objective health examinations (Helmer et al., 1999; Miilunpalo et al., 1997). Finally, men appeared to be more vulnerable to loneliness because loneliness was related to their subjective health, and so they could benefit from prevention procedures and follow-ups to avoid more severe psychological difficulties.

Chapter IV

Study 3: Loneliness, subjective cognitive decline, and biomarkers of brain pathology in cognitively unimpaired older people

The main results of this study are being prepared for submission:

Crespo-Sanmiguel, I., Cedres, N., Zapater-Fajarí, M., Rydberg Sterner, T., Rydén, L., Sacuiu, S., Waern, M., Zettergren, A., Zetterberg, H., Blennow, K., Kern, S., Hidalgo, V., Salvador, A., Westman, E., Skoog, I. & Ferreira, D., The role of loneliness as an early neuropsychiatric symptom of AD and CVD pathology in subjective cognitive decline in cognitively unimpaired older people

Introduction

Among the older population, the experience of SCCs has been gaining importance. If this perception of cognitive decline is not accompanied by objective impairments in cognition or daily functioning, it is known as SCD (Jessen et al., 2014). SCD has been suggested as the earliest clinical stage of AD, related to the long-term development of dementia (Dardenne et al., 2017; Jessen et al., 2014; Mitchell et al., 2014). SCD has been associated with AD pathological changes, including amyloid-beta plaques, tau neurofibrillary tangles, and neurodegeneration (Amariglio et al., 2012; Buckley et al. 2017; Cedres et al., 2021; Perrotin et al., 2012), as well as CVD (Cedres et al., 2019; 2021; Diaz-Galvan et al., 2021a; Diniz et al., 2013; Minett et al., 2005). Therefore, SCD is gaining interest as an early indicator of various brain pathologies that can be diagnosed before the onset of objective impairments in cognition.

In recent years, the focus has been on whether SCD not only reflects brain pathologies, but also other non-neurodegenerative factors such as depressive symptomatology, given that SCD and depressive symptomatology usually co-occur (Diaz-Galvan et al., 2021a; Jessen et al., 2014, 2020). An emerging concept here is loneliness, which increases with age (Yang & Victor, 2011) and has been related to a greater risk of dementia (Sundström et al., 2020; Sutin et al., 2020) and adverse health outcomes, increasing all-cause mortality (Leigh-Hunt et al., 2017; Richard et al., 2017). Although loneliness and depressive mood are closely related symptoms (Dahlberg et al., 2014; Domènech-Abella et al., 2019; Erzen & Çikrikci, 2018; VanderWeele et al., 2011), only one previous study has investigated the role of loneliness in SCD, suggesting that memory complaints are more frequent in older people with loneliness (Montejo et al., 2019). This finding demonstrates the presence of loneliness in individuals at risk of dementia. Furthermore, it is estimated that around a third of dementia patients feel lonely

(Victor et al., 2020). Nevertheless, few studies have investigated the association between loneliness and biomarkers of brain pathology. The Harvard Aging Brain Study showed that loneliness is related to cortical amyloid burden and higher tau binding in positron emission tomography (PET) in sites of early tau accumulation (d'Oleire Uquillas et al., 2018; Donovan et al., 2016).

Another common finding on aging, SCD, and dementia is the presence of CVD, which can be observed in magnetic resonance imaging (MRI) scans. Interestingly, an in-vivo longitudinal study showed an association between loneliness and an increased volume of WMSA (Duan et al., 2017), a CVD neuroimaging marker that increases the risk of cognitive impairment and dementia (Au et al., 2006; Debette et al., 2010). However, a post-mortem study showed that loneliness was not related to CVD or AD pathology (Wilson et al., 2007). Together, these findings highlight the importance of elucidating the role of loneliness in AD and CVD, as well as in SCD.

The main goal of this study was to investigate loneliness in the context of SCD, depressive symptomatology, and brain pathologies in a relatively large population-based cohort of cognitively unimpaired individuals. First, we investigated associations between loneliness and depressive symptomatology and biomarkers of AD and CVD pathology (levels of amyloid-beta and tau and WMSA). We hypothesized that loneliness would be related to depressive symptomatology (Domènch-Abella et al., 2019; Erzen & Çikrikci, 2018), but also to biomarkers of AD and CVD pathology (d´Oleire Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017). The second aim was to investigate the association between SCD and loneliness and biomarkers of AD and CVD pathology. We wanted to ascertain whether the potential association between loneliness and SCD was independent of depressive symptomatology, due to the known relationship of depressive symptomatology with SCD and loneliness (Diaz-Galvan et al., 2021a; Erzen & Çikrikci,

2018). We hypothesized that SCD would be positively related to the presence of loneliness (Montejo et al., 2019) and increased biomarkers of AD and CVD pathology (Amariglio et al., 2012; Buckley et al. 2017; Cedres et al., 2019, 2021), independently of depressive symptomatology. In addition, there is recent interest in further describing the role of specific subjective complaints, e.g. memory *vs.* non-memory. Different cognitive complaints may reflect different syndromic profiles and patterns of brain atrophy (Diaz-Galvan et al., 2021b). Thus, we investigated whether the associations proposed would differ for memory and concentration complaints.

Material and Methods

Participants and Procedure

The sample for the current study was collected from a population-based study conducted from 2014 to 2016 in Gothenburg (Sweden). The sample was composed of 1203 seventy-year-old participants from the Gothenburg H70 Birth Cohort 1944 Study. Full details on tests and procedures are reported in Rydberg et al. (2019). From these participants, we selected the 297 individuals who had available cerebrospinal fluid (CSF) biomarkers via a lumbar puncture (LP) in combination with an MRI scan that included T1-weighted and T2-weighted sequences.

Inclusion criteria for the current study were in accordance with the leading international SCD initiative (SCD-I) working group:

I) Normal cognition: Dementia was excluded based on a clinical diagnosis of dementia using DSM-III-R criteria, a Mini-Mental State examination (MMSE) score < 24, or a Clinical Dementia Rating > 0.5. Mild cognitive impairment (MCI) was excluded according to the criteria proposed by Jak et al. (2009) and Molinuevo et al. (2017), which

are based on a comprehensive neuropsychological protocol (using age, sex, and education adjusted norms). Participants were classified as MCI if at least one of the following two criteria were met: a) Impaired scores (< 16 percentile) on two tests in at least one of the following four cognitive domains: *Memory*, assessed with Thurstone's Picture Memory 10-word list, and remembering 12 objects; *Speed/executive function*, assessed with the Digit Span Forward and Backward test and the Figure Logic of the Synonyms, Reasoning, and Block Design Test (SRB 2); *Verbal fluency*, assessed with a semantic verbal task (animals); and *Visuospatial capacities,* assessed with Block Design (Koh's Block Test). b) Impaired scores (< 16 percentile) on three independent tests in three out of the four cognitive domains covered by the neuropsychological protocol (memory, speed/executive function, verbal fluency, and visuospatial capacities). When criterion 'a' could not be met because the domain was evaluated by one test, criterion 'b' was considered. Although Jak et al. (2009) and Molinuevo et al. (2017) formulated their proposals based on the minus one standard deviation (-1SD) cut-off point, we opted for the 16th percentile (which reflects -1SD on the normal curve), due to the asymmetrical distribution of the neuropsychological test data in our cohort.

II) No abnormal findings of large infarcts or tumors on the brain in MRI according to a neuroradiologist and no history of stroke or transient ischemic attack.

III) No medical history of neurological or psychiatric disorders (e.g., major depression), systemic diseases, or head trauma.

IV) No history of substance or alcohol abuse, based on a clinical interview and a score < 20 on the alcohol use disorder identification test (AUDIT) (Bergman et al., 1994).

From the initial 297 participants, 79 were excluded because they failed to satisfy some of the inclusion criteria specified above. In addition, three participants were excluded due to missing data on loneliness, SCD, or civil status. Thus, the final sample in the current study was composed of 215 participants.

Measures

Loneliness

A self-perceived feeling of loneliness was assessed with the question "Do you feel lonely?", rated on a four-point Likert scale from 1 (never) to 4 (very often). Responses were dichotomized into a "non-loneliness group" (response 1) and a "loneliness group" (responses 2-4) for statistical analyses (see below).

Subjective cognitive decline (SCD)

To assess SCD, we used two questions from the semi-structured interview CPRS (Åsberg et al., 1978), which covers subjective concentration and memory complaints. Complaints referred to self-perceived difficulties with concentration or memory, compared to previous ability, during the past month. Both complaints were rated on a seven-point Likert scale ranging from 0 (no difficulties) to 6 (severe difficulties), with intermediate options. Based on clinical experience and considerations of the Likert scale, the presence of subjective complaints was determined by the cut-off point of ≥ 2. Participants who scored ≥ 2 on concentration complaints were classified as having SCD in concentration (SCD concentration complaints group), and those who scored ≥ 2 on memory complaints were classified as having SCD in memory (SCD memory complaints group). Participants who scored ≤ 1 on these CPRS questions were classified as not having SCD in

concentration (non-SCD-concentration complaints group) and not having SCD in memory (non-SCD-memory complaints group). We chose the dichotomous SCD variable due to the nature of our statistical analyses (see below).

Depressive Symptomatology

Depressive symptomatology was assessed using *the Montgomery-Åsberg Depression Rating Scale* (MADRS) (Montgomery & Åsberg, 1979), which was derived from the CPRS. Items are responded to on a seven-point Likert scale (ranging from 0 to 6), with higher scores indicating a higher degree of depressive symptomatology.

Cerebrospinal Fluid (CSF) biomarkers and APOE ε4 genotype

AD pathology can be measured *in vivo* through CSF biomarkers. In this study, we used the amyloid-beta 42/40 (Aβ 42/40) ratio biomarker to reflect amyloid-beta pathology and the phosphorylated tau (p-tau) biomarker to reflect tau pathology (Blennow & Zetterberg, 2018). A LP in the L3/L4 or L4/L5 inter-space was performed in the morning to collect the CSF samples. A total of 10ml of CSF was collected in a polypropylene tube and immediately transported to the laboratory and centrifuged (1800g at 20°C for 10 minutes). A sandwich enzyme-linked immunosorbent assay (ELISA) (INNOTEST® β-amyloid1-42) specifically constructed to measure amyloid-beta peptides starting at amino acid 1 and ending at amino acid 42 (Andreasen et al., 1999) was used to measure the concentration of CSF Aβ 42. The CSF Aβ 42/40 was measured using the V-PLEX amyloid-beta peptide panel 1 (6E10) kit (Meso Scale Discovery, Rockville, MD) (Andreasson et al., 2018; Kern et al., 2018). The supernatant was gently mixed to avoid possible gradient effects, aliquoted in polypropylene tubes, and stored at -70°C. The sandwich ELISA (INNOTEST® htau Ag and PHOSPHO_TAU (181P) (Fujirebio, Ghent

Belgium) was used to determine the concentrations of tau phosphorylated at threonine 181 (p-tau) (Blennow et al., 1995; Vanmechelen et al., 2000). This variable was treated continuously in the main analyses. For the characterization of the sample, the cut-off proposed by Samuelsson et al. (2021) was used to dichotomize the CSF Aβ 42/40 ratio and p-tau biomarker levels into normal and abnormal: CSF Aβ 42/40 ratio ≤ .082 and p-tau of ≥ 80 pg/mL. To determine the *APOE* ε4 genotype, the KASPar PCR (polymerase chain reaction) SNP (single nucleotide polymorphisms) genotyping system was used (LGC Genomics, Hoddesdon, Herts, UK), as described in Skoog et al. (2021). Participants with at least one *APOE* allele ε4 were classified as *APOE* ε4 carriers.

Magnetic resonance imaging (MRI) biomarkers of cerebrovascular disease (CVD)

CVD can be measured with MRI (Wardlaw et al., 2013) in the form of hypointense WMSA. Hypointense WMSA are related to poorer white matter integrity and reflect chronic white matter damage (Riphagen et al., 2018). The neurodegenerative CVD is usually chronic and insidious. Therefore, in this study, we selected hypointense WMSA as a proxy for CVD in this population, as in other previous studies that investigated WMSA in the context of SCD (Cedres et al., 2019, 2021). Participants' MRI data were acquired in a 3.0T Philips Achieva system (Philips Medical Systems). For estimations of hypointense WMSA, we used a three-dimensional T1-weighted Turbo Field Echo (TFE) sequence (repetition time = 7.2 ms., echo time = 3.2 ms., flip angle = 9°, number of slices = 160, matrix size = 250x250 mm, field of view = 256 × 256, slice thickness = 1.0 mm).

Hypointense WMSA were automatically segmented with FreeSurfer 6.0.0. Briefly, The T1-weighted images were processed with the FreeSurfer 6.0.0 image analyses suite (http://surfer.nmr.mgh.harvard.edu/). FreeSurfer detects white matter hypointensities and automatically labels them using a probabilistic procedure (Fischl et al., 2002). The sensitivity of this procedure in assessing white matter damage has been

demonstrated in both healthy individuals and AD patients (Leritz et al., 2014; Salat et al., 2010). WMSA volumes in millimeters (ml) were adjusted by the total intracranial volume (TIV) obtained from FreeSurfer. This adjustment was performed by dividing the WMSA volume by the TIV of each participant (Voevodskaya et al., 2014), and the TIV-adjusted WMSA measures were used for statistical analyses.

Following Cedres et al. (2020), we classified WMSA into low and high hypointense WMSA burden using the cut-off value of 0.00321, which resembles low and high Fazekas visual rating scale WMSA burden (Fazekas et al.,1987). Henceforth, when we refer to WMSA, we are referring to hypointense WMSA. This variable was treated continuously in the main analyses, but for the characterization of the sample, the measure was categorized as high and low to describe the degree of pathology. All MRI data were managed and processed through the Hive DB system (Muehlboeck et al., 2014).

Statistical Analyses

Pearson´s Chi-square test was used to investigate group differences across categorical variables, including sex, marital and living status, concentration and memory complaints, Aβ 42/40 ratio, p-tau, WMSA, and *APOE* ε4 genotype. Student's t-test was used for group differences when variables were continuous, including age, years of education, income, MMSE, and depressive symptomatology. Box-Cox transformations were performed for the continuous variables that did not follow a normal distribution (Aβ 42/40 ratio, p-tau, and WMSA) (Osborne, 2010).

Random forest classification analyses were performed to investigate the contribution of biomarkers, depressive symptomatology, and loneliness to a dichotomous outcome (loneliness in Aim 1 and SCD in Aim 2) using the oversampling approach. We

assessed the variables' importance by using the mean decrease in the Gini (mdGini) parameter, which reflects the decrease in the mean of the model's discriminative classification capacity when a predictor is excluded from the model. To address Aim 1, we performed a random forest model with depressive symptomatology and biomarkers as the predictors and loneliness as the dependent variable. For Aim 2, we performed two random forest models, one for concentration complaints and one for memory complaints (with the SCD group as a dichotomous dependent variable). Finally, we performed logistic regression analysis to investigate the partial effect of each predictor (biomarkers, depressive symptomatology, and loneliness) on a dichotomous outcome (loneliness in Aim 1 and SCD in Aim 2). Logistic regression was preceded by a factorial analysis to reduce the number of predictors due to the small size of the loneliness group (n = 30) and the concentration complaints group (n = 23). The factorial analysis was based on the depressive symptomatology, Aβ 42/40 ratio, p-tau, and WMSA variables, with direct oblimin rotation. We inverted inverse variables, which means that, for all the variables included in the factorial analysis, higher scores could be interpreted as higher pathology.

All statistical analyses were performed using SPSS v.27 (IBM Statistics, Chicago, IL, USA) and the R programming language (R, version 3.5; R Foundation for Statistical Computing, Vienna, Austria). All *p* values were two-tailed, and the level of significance was taken as $p < .05$.

Results

Key characteristics of the cohort

Table III.1 shows the characteristics of the cohort (N = 215). Based on the study design, all individuals were 70 years old. Fifty-three percent were women, and the average number of years of education was 13±4 years. Regarding biomarkers, 31% of the individuals had abnormal Aβ 42/40 ratio levels, 6% had abnormal p-tau levels, and 14% had abnormal WMSA burden.

Table III.1 Characteristics of cohort

	Total sample (N = 215)	Not lonely (N = 185)	Lonely (N = 30)	*t (p) / X² (p)*
Age (years)	70.54 ± .26	70.54 ± .27	70.56 ± .26	.358 (.720)
Sex (%/N Women)	53/114	48.6/ 90	80/24	10.186 (.001)
Without a partner (%/N)	26.2/56	19/35	70/21	34.696 (<.001)
Living alone (%/N)	34/73	28.1/52	70/21	20.201 (<.001)
Years of education	13.30 ± 4.10	13.13 ± 3.98	14.53 ± 4.64	1.754 (.081)
Income	17300 ± 7852	17607 ± 8192	15518 ± 5252	−1.255 (.211)
MMSE	29.19 ± 1.07	29.18 ± 1.08	29.23 ± 1.01	.235(.815)
SCD Memory complaints (%/N)	55.3/119	54.6 /101	60/18	.305 (.581)
SCD Concentration complaints (%/N)	10.7/23	8.1/15	26.7/8	9.307 (.002)
Depressive symptomatology M (SD)	3 ± 3.67	2.34 ± 2.89	7.03 ± 5.19	4.833 (<.001)

(Continue on next page)

Continuation of **Table III.1**

	Total sample (N = 215)	Not lonely (N = 185)	Lonely (N = 30)	*t (p) / X^2 (p)*
Aβ 42/40 ratio (abnormal levels) (%/N)	30.8/66	28.8/53	43.3/13	2.553(.110)
p-Tau (abnormal levels) (%/N)	6/13	6.5/12	3.3/1	.452 (.501)
WMSA (high burden) (%/N)	14.4/31	13/24	23.3/7	2.245 (.134)
APOE ε4 carriers (%/N)	32.2/68	32.6/59	30/9	.079 (.778)

Note. MMSE = Mini-Mental State Examination; SCD = Subjective Cognitive Decline; Aβ 42/40 = Amyloid-beta 42/40 ratio; p-tau= Phosphorylated tau; WMSA = White Matter Signal Abnormalities; *APOE*-ε4 = participants with at least one *APOE* ε4 allele; χ^2= Chi-square

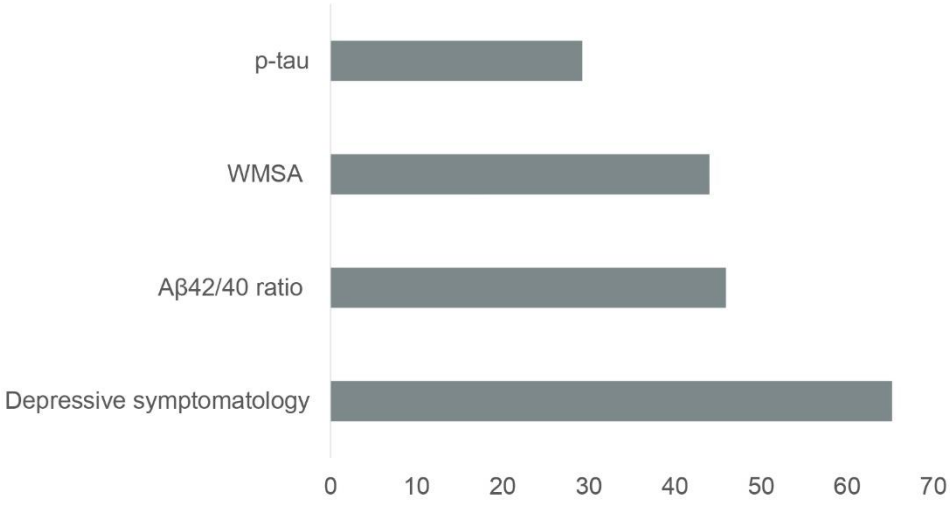
Aim 1 – Association of depressive symptomatology and biomarkers with loneliness

Fourteen percent (n = 30) of the individuals endorsed feelings of loneliness, whereas 86% (n = 185) did not endorse any feelings of loneliness. These two groups will be referred to as the loneliness *vs.* non-loneliness groups, respectively. The univariate analysis showed that the loneliness group included individuals who were more often women, did not have a partner, lived alone, had concentration complaints, and had higher depressive symptomatology (Table III.1).

The multivariate random forest analysis with the loneliness group as the dependent variable (dichotomous) and depressive symptomatology, Aβ 42/40 ratio, p-tau, and WMSA as the predictors (continuous) had an excellent performance

(classification error: 2%, Figure III.1). Depressive symptomatology was the most important variable in the classification (mdGini = 65), followed by the Aβ 42/40 ratio, WMSA, and p-tau (all with a mdGini < 46) (Figure III.1). To investigate whether all these predictors could explain partial variance in loneliness, the next step was to conduct a logistic regression model. Due to the small size of the loneliness group (n = 30), we used factorial analysis to reduce the number of predictors prior to logistic regression.

Figure III.1 Mean Decrease Gini on Loneliness



The factorial analysis provided two factors that explained a total variance of 56%. Factor 1 included depressive symptomatology and the Aβ 42/40 ratio, and Factor 2 included p-tau and WMSA (27% and 29% of the variance, respectively) (Kaiser-Meyer-Olking (KMO) = .454, Bartlett' sphericity test; $\chi^2(6) = 8.915$, p =.178). Although these models did not reach significance, it served our purpose of reducing the number of predictors to two orthogonal factors for the logistic regression model. These two factors

were included as predictors in the logistic regression model, with the loneliness groups as a dichotomous outcome. Table III.2 shows the results of this logistic regression model. We found that the depressive symptomatology and Aβ42/40 ratio factor was associated with an increased odds ratio of endorsing loneliness feelings. In contrast, the WMSA and p-tau factor was not significant. Because there is no association between depressive symptomatology and the Aβ 42/40 ratio in our cohort (r = .109, p = .113), we conducted another logistic regression model to further clarify whether they could both explain partial variance in loneliness. Depressive symptomatology and the Aβ 42/40 ratio were thus included as independent predictors in this new logistic regression model, with the loneliness groups as the dichotomous outcome. The logistic regression model showed that depressive symptomatology was the only significant predictor associated with an increased odds ratio of loneliness (Table III.2).

Table III.2 Logistic regression with loneliness as a dichotomous outcome

Predictors: Factor 1 (depressive symptomatology and Aβ42/40 ratio) and Factor 2 (p-tau and WMSA)

$\chi^2(2) = 33.481$; $p < .001$; $R^2 = .261$ (Nagelkerke)

Predictor	OR	Wald	B	SE	*p*
Factor 1	3.099	24.674	1.131	.228	<.001**
Factor 2	.776	1.430	−.253	.212	.232

(Continue on next page)

Continuation of **Table III.2**

Predictors: depressive symptomatology and Aβ42/40 ratio

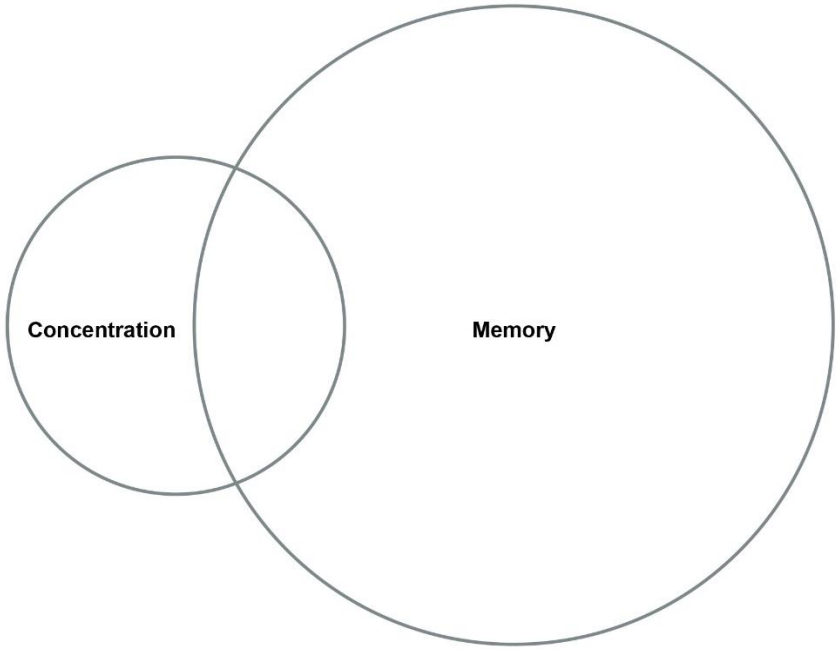
$\chi^2(2) = 35.821$; $p < .001$; $R^2 = .278$ (Nagelkerke)					
Predictor	OR	Wald	B	SE	*p*
Depressive symptomatology	1.323	26.040	.280	.055	<.001**
Aβ42/40 ratio	.008	2.146	− 4.866	3.321	.143

Note. Aβ 42/40 = Amyloid-beta 42/40 ratio; p-tau = Phosphorylated tau; WMSA = White Matter Signal Abnormalities; OR = Odds ratio; SE = Standard Error. ** $p < 0.01$

Aim 2 – Association of depressive symptomatology, biomarkers, and loneliness with concentration and memory complaints

A total of 87 individuals did not endorse any subjective complaints. 128 individuals did endorse subjective complaints; 23 endorsed concentration complaints, 119 endorsed memory complaints and 14 of these two groups endorsed both concentration and memory complaints (Figure III.2).

Figure III.2 Distribution of SCD concentration complaints and SCD memory complaints



Concentration complaints

The multivariate random forest analysis with concentration complaints as the outcome variable (dichotomous: SCD concentration complaints group *vs.* non-SCD concentration complaints group) and the Aβ 42/40 ratio, p-tau, WMSA, depressive symptomatology, and loneliness as the predictors had an excellent performance (classification error: 3%). Depressive symptomatology was the most important variable in the classification (mdGini = 68), followed by the Aβ 42/40 ratio, WMSA, p-tau, and loneliness (all mdGini < 56) (Figure III.3). Next, we conducted a logistic regression model to investigate whether all these predictors could explain partial variance in concentration complaints. Due to the small size of the group of individuals with concentration complaints (n = 23), we set up a simplified logistic regression model with loneliness and the two factors from the

factorial analysis described above (Factor 1: depressive symptomatology and the Aβ 42/40 ratio; and Factor 2: WMSA and p-tau) as the predictors and the concentration complaints groups as the dichotomous outcome. The logistic regression model showed that the depressive symptomatology and Aβ 42/40 ratio factor was associated with an increased odds ratio of concentration complaints (Table III.3). Hence, we demonstrated that loneliness is not significantly associated with concentration complaints when depressive symptomatology and the Aβ 42/40 ratio are in the same model (Table III.3).

Figure III.3 Mean Decrease Gini on Concentration Complaints

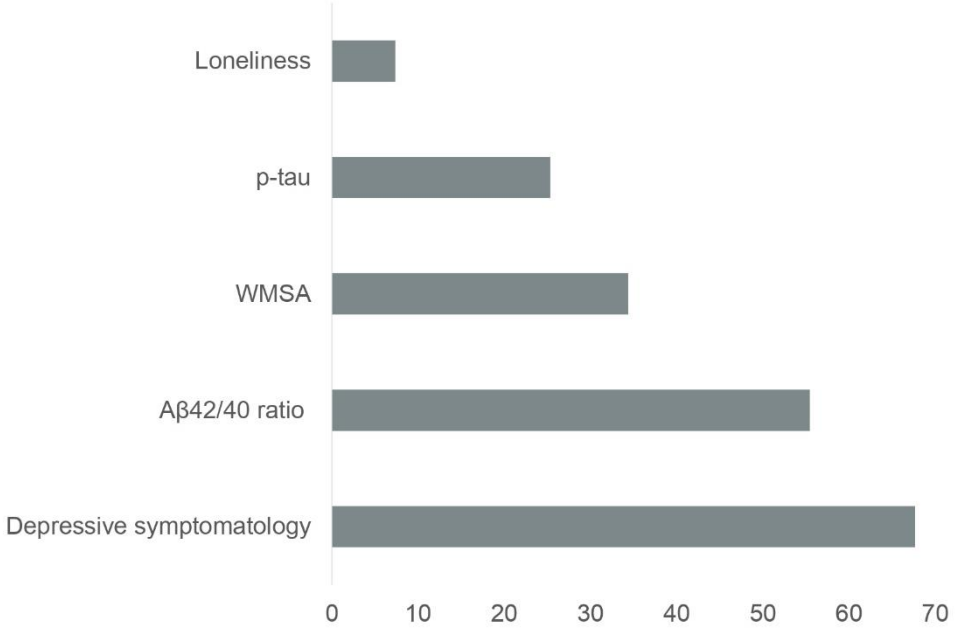


Table III.3 Logistic regression with Factor 1 (depressive symptomatology and Aβ42/40 ratio) and Factor 2 (p-tau and WMSA) and loneliness as predictors and SCD subtypes as a dichotomous outcome

Dependent variable: SCD concentration complaints					
$\chi^2(3) = 32.214$; $p < .001$; $R^2 = .283$ (Nagelkerke)					
Predictor	OR	Wald	B	SE	*p*
Factor 1	3.517	18.717	1.258	.291	<.001**
Factor 2	1.060	.064	.058	.230	.800
Loneliness	1.028	.002	.028	.641	.966
Dependent variable: SCD memory complaints					
$\chi^2(3) = 1.224$; $p = .747$; $R^2 = .008$ (Nagelkerke)					
Predictor	OR	Wald	B	SE	*p*
Factor 1	1.134	.674	.125	.153	.412
Factor 2	1.065	.205	.063	.139	.651
Loneliness	1.085	.034	.081	.439	.853

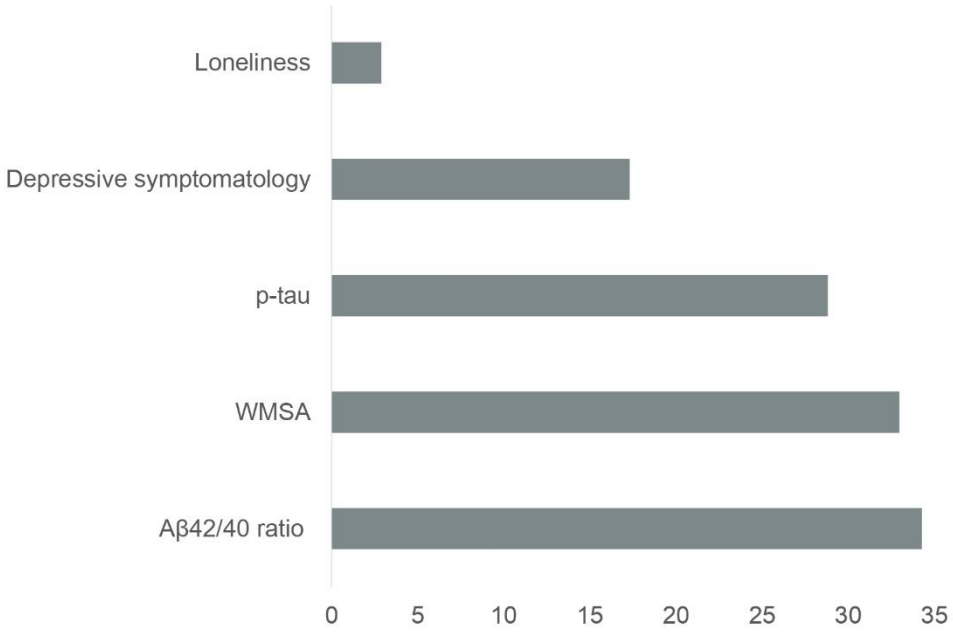
Note. Aβ 42/40 = Amyloid-beta 42/40 ratio; p-tau = Phosphorylated tau; WMSA = White Matter Signal Abnormalities; SCD = Subjective Cognitive Decline; OR = Odds ratio; SE = Standard Error. **$p < 0.01$

Memory complaints

The multivariate random forest analysis with memory complaints as the outcome variable (dichotomous: SCD memory complaints group *vs.* non-SCD memory complaints group) and the Aβ 42/40 ratio, p-tau, WMSA, depressive symptomatology, and loneliness as the predictors had a good performance (classification error: 23%). The Aβ 42/40 ratio (mdGini = 34) was the most important variable in the classification, followed by WMSA,

p-tau, depressive symptomatology, and loneliness (all mdGini < 33) (Figure III.4). We conducted a logistic regression model to investigate whether all these predictors could explain partial variance in memory complaints. The logistic regression model included loneliness and the two factors from the factorial analysis described above as the predictors and the memory complaints groups as the dichotomous outcome. This logistic regression model for memory complaints was not significant (Table III.3).

Figure III.4 Mean Decrease Gini on Memory Complaints



Discussion

To the best of our knowledge, this is the first study to investigate loneliness in the context of SCD, depressive symptomatology, and AD and CVD biomarkers. We found that increased brain pathology and, especially, increased depressive symptomatology contributed to discriminating individuals with loneliness. Moreover, loneliness contributed to discriminating SCD individuals who endorsed concentration complaints, although this was not independent of depressive symptomatology and amyloid-beta levels. In addition, different patterns were observed for concentration and memory complaints: the main variable that contributed to concentration complaints was a psychological factor (depressive symptomatology), whereas the main variables that contributed to memory complaints were biomarkers of AD and CVD pathology.

Biomarkers of AD and CVD pathology showed a contribution to the presence of loneliness, although this association was no longer significant when depressive symptomatology was included in the logistic regression model as a competitive predictor. This result differs from previous studies showing that loneliness is a neuropsychiatric symptom associated with brain pathology in preclinical stages of AD or CVD, independently of depression (d'Oleire Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017). There are different factors that can influence the stronger relationship found by d'Oleire Uquillas. (2018) and Donovan et al. (2016). First, the age of the participants could be influencing the results because AD pathology increases with age (Marks et al., 2017; Spires-Jones et al., 2017). In the Harvard Aging Brain Study that reported an association between loneliness and AD biomarkers, the participants had a mean age of 76 years and included participants between 68 and 89 years old. Thus, most of the

participants were older than our participants, who are all 70 years old (d'Oleire Uquillas et al., 2018; Donovan et al., 2016). Moreover, considering that the entorhinal cortex is an initial site of tau pathology in both healthy aging and early AD (Braak et al., 2011), the association between loneliness and tau reported by d'Oleire Uquillas et al. (2018) could be due to the regional PET measure used, in contrast to the global measure used in our current study. All these findings together could suggest that in younger elderly people, loneliness may be mostly related to emotional factors, whereas in older ages, loneliness could be a marker of an incipient neuropathology and have a weaker association with depressive symptomatology.

Regarding CVD biomarkers, WMSA burden contributed to discriminating individuals with loneliness. However, WMSA burden did not explain loneliness beyond the effects of depressive symptomatology. A previous longitudinal study suggested that loneliness is related to higher risk of late-life dementia, although the study did not show associations with brain pathology at autopsy (Wilson et al., 2007). The lack of an association between loneliness and CVD and AD pathology in Wilson et al. (2007) could be explained by the fact that participants were in a more advanced stage (post-mortem measurements) than in our cohort (in-vivo measurements) and that their more heterogeneous sample included people with and without dementia. Another longitudinal study showed that loneliness and depression were independently related to an increased volume of WMSA over time (Duan et al., 2017). We found a weak association between WMSA burden and loneliness, with loneliness mostly being explained by depressive symptomatology. Nevertheless, our data are cross-sectional, and a longitudinal approach may show different results. Thus, more research is needed to address the role of loneliness as an indicator of CVD.

The fact that loneliness was explained by brain pathology, in addition to depressive symptomatology, opens the door to questions about disease mechanisms and the directionality between loneliness and brain pathology. On the one hand, loneliness has been related to impaired social skills (Cacioppo et al., 2006) as well as a smaller gray matter volume in brain areas related to social processing (Kanai et al., 2012). Therefore, loneliness could be reflected in less developed neural networks underlying social processing and less cognitive and brain reserve. Therefore, it would be related to less capacity to compensate for the damage of other neural networks affected by age-related factors or neuropathologies. On the other hand, neuropathology could be affecting important neural networks related to socioemotional processing and, thus, to loneliness, as suggested by d'Oleire Uquillas et al. (2018). This explanation is in line with Duan et al. (2017), who suggested changes in brain structure related to white matter or synaptic density as a mechanism underlying loneliness. Our current cohort is rather healthy, and we cannot exclude that a higher level of neuropathology or loneliness may be necessary to find the associations reported in previous studies in our competitive effects models (logistic regression). More research is needed to delve deeper into the causality among loneliness, depression, and brain pathology, ranging from low levels of pathology to advanced stages of the disease.

Additionally, we investigated the role of loneliness in SCD. We found that loneliness, depressive symptomatology, and biomarkers of AD and CVD pathology contributed to both concentration and memory complaints. To the best of our knowledge, only one previous study investigated the association between loneliness and SCD (Montejo et al., 2019), concluding that loneliness was associated with a higher frequency of memory complaints. In contrast, we found that loneliness was not an important

predictor of complaints when depressive symptomatology and biomarkers of brain pathology were included in the models. In fact, the factor composed of depressive symptomatology and amyloid-beta was the only one that explained SCD concentration complaints. This finding was in line with a recent study showing that depressive symptomatology and early biomarkers co-occur in SCD (Diaz-Galvan et al., 2021a). Moreover, it is worth highlighting the order of importance of the variables that explain each type of SCD. Depressive symptomatology had more importance than biomarkers in explaining concentration complaints. In contrast, biomarkers had more importance than depressive symptomatology in explaining memory complaints. This is consistent with recent studies that found that different subtypes of SCD may reflect different syndromic profiles (Amariglio et al., 2012; Diaz-Galvan et al., 2021b).

This study has some limitations. We investigated associations using cross-sectional data, and so we cannot draw conclusions about causality in our findings. Our tau biomarker was based on CSF samples, whereas tau PET can provide regional information, and perhaps tau uptake in the entorhinal cortex could be more sensitive in cognitively unimpaired individuals (d'Oleire Uquillas et al., 2018). In addition, the fact that all the participants are 70 years old makes the results difficult to extrapolate to other ages and populations, although it increases the homogeneity of the sample. Finally, measures of loneliness based on a direct single item are widely used and validated (Dyal et al., 2015; Shiovitz-Ezra & Ayalon, 2012). Despite this, the negative connotations associated with the word loneliness in direct measures could lead people who feel lonely to not report it due to the stigma involved (Pinquart & Sörensen, 2001), which might partially influence the results. In this scenario, a broad assessment of loneliness might be more appropriate.

In conclusion, loneliness may hold promise to detect risk of cognitive impairment due to brain pathologies. However, given the close association between loneliness and depressive symptomatology, more research is needed to fully clarify the role of loneliness in the context of brain pathologies, particularly in early stages of the disease such as SCD. We also suggest that future studies use direct simple items about loneliness versus more elaborated indirect measures of loneliness.

Chapter V

General Discussion

General discussion

The general objective of this doctoral thesis was to study the role of loneliness in stress and health in young, middle-aged, and older healthy people. Specifically, in Study 1 we explored whether loneliness mediates the relationship between ELS and both stress perception and basal HPA axis functioning during adulthood in young and middle-aged healthy people. In Study 2, we tested the relationship between loneliness, psychological and physical subjective health, and basal HPA axis functioning, considering possible sex differences, in middle-aged and older healthy people. Finally, in Study 3, we investigated the associations between loneliness and biomarkers of AD and CVD pathology and SCD in healthy older people.

What follows is a short description of the main results of the three studies and then a global reflection on their implications.

Summary of main findings

In *Study 1,* we analyzed loneliness, ELS, perceived stress, and cortisol levels on two days in 187 healthy people between 18 and 55 years old from the metropolitan area of Pennsylvania (USA). These data were collected by the Laboratory of the Study of Stress, Immunity and Disease (Carnegie Mellon University) as part of the Common Cold Project, and we accessed them through its website. Basal HPA axis functioning was measured with seven saliva cortisol samples per day, and overall diurnal cortisol (AUCg), DCS, and bedtime cortisol levels were obtained. Results of lineal regression and mediation analyses showed that ELS was associated with higher perceived stress during adulthood, with loneliness mediating this relationship. In this regard, people who experienced higher ELS felt greater loneliness and, in turn, reported higher perceived stress. However, we

did not find a significant association between ELS or loneliness and basal HPA axis functioning in adulthood. The results coincide with previous studies that observe worse management and an increase in the perception of stress in people who have experienced ELS (Betz et al., 2021; Bossé et al., 2018; Han et al., 2016; Hyman et al., 2007). In addition, they are in line with studies that found that people with an experience of ELS feel less satisfaction in social contacts in adulthood (Beutel et al., 2017; Germine et al., 2015; Repetti et al., 2002), which, in turn, has been related to a higher perception of stress (Matthews et al., 2019).

Results could be related to the role of attachment figures in early stages, through whom we learn to manage emotions and stress and understand how the world of relationships works (Bowlby, 1982). In adverse experiences such as negligence or abuse by parents, the attachment figure might not be available for this relational learning, and so inadequate schemes for coping with emotions, stress, and bonds with others are internalized (Fonagy & Luyten, 2018). This would influence the interpersonal relationships formed throughout life (Crowell et al., 2009; Pilkington et al., 2021; Repetti et al., 2002), leading to experiencing greater loneliness and perception of stress. Finally, the lack of relationships of ELS and loneliness with basal HPA axis functioning could be explained by methodological aspects. The cortisol measurements used might not be sensitive enough to capture these associations. Moreover, the low levels of ELS in our participants could be expressed psychologically, but not biologically via basal HPA axis functioning. In sum, our results suggest that early stressful experiences hinder relationships with others, increasing loneliness and accentuating the perception of stress during adulthood. In this regard, the results of this study highlight the importance of social or psychotherapeutic loneliness interventions in people who have experienced ELS with

the aim to promote significant healthy bonds with others and reduce loneliness and its negative associated effects.

In *Study 2*, we carried out an evaluation of 39 men and 40 women between 55 to 75 years old from the Nau Gran, an education program of the University of Valencia (Spain). We measured loneliness, psychological and physical subjective health, and depressive symptomatology as the main variables. In addition, basal HPA axis functioning was assessed through five saliva samples on two days, which allowed us to obtain the following cortisol indexes: CAR, DCS and bedtime cortisol. Lineal regression, moderation and mediation analyses were performed. Results indicated that loneliness was related to subjective psychological health in men, but not in women. Loneliness was not related to subjective physical health. In addition, basal HPA axis functioning was not related to loneliness and did not appear as a mechanism underlying the association between loneliness and subjective health. These results suggest that sex is a critical factor in the relationship between loneliness and subjective psychological health in middle-aged and older people. This is in line with a previous study that observed a more pronounced effect of loneliness on psychological health in men than in women (Zebhauser et al., 2014).

These sex differences could be explained by the variability in experiences with interpersonal relationships in men and women. On the one hand, women tend to move in intimate and dyadic relationships (Baumeister & Sommer, 1997; Gardner & Gabriel, 2004), which makes it easier for them to find a safe space to express themselves, strengthening their psychological health. On the other hand, men tend to move in larger groups where it is more difficult to share their emotions and concerns (Hoza et al., 2000). In addition, these differences are usually linked to values related to masculinity, such as self-sufficiency or difficulty asking for psychosocial support (Ogrodniczuk et al., 2016;

Roy et al., 2017). These reasons could explain why loneliness is related to worse psychological health in men and not in women. Overall, the lack of relationship between loneliness and subjective physical health and basal HPA axis functioning suggests the important role of protective factors for loneliness. We surmise that characteristics that represent the optimal aging of our participants (no chronic illnesses, attending classes at the university, high socioeconomic level, low stress and depressive symptoms) could compensate for factors related to higher loneliness in aging (natural losses or retirement) or at least delay their harmful effects, acting as a buffer against the loneliness stressor (Hawkley et al., 2008; Teater et al., 2021). However, it seems that men have more vulnerability to loneliness than women, showing worse psychological health. Therefore, these sex differences should be taken into account in loneliness interventions.

In *Study 3*, we analyzed 215 seventy-year-old cognitively unimpaired participants from the Gothenburg H70 Birth Cohort 1944, obtained through collaboration with a research stay at the Karolinska Institutet (Sweden). Measures of loneliness, SCD, and depressive symptomatology were analyzed. In addition, biomarkers of AD pathology (Aβ42/40 ratio and p-tau) were assessed through CSF levels, and a biomarker of CVD pathology (WMSA) was assessed using MRI. Random forest classification analyses were performed to investigate the contribution of predictors to loneliness and SCD, and logistic regression analyses were performed to investigate the partial effect of each predictor on loneliness and SCD. Results showed that biomarkers of AD and CVD pathology and, especially, depressive symptomatology contributed to discriminate individuals who endorsed feelings of loneliness. Although loneliness was associated with SCD, it showed a low capacity to discriminate the SCD group. Depressive symptomatology and amyloid-beta were the main factors that contributed to discriminating individuals who endorsed SCD concentration complaints. Biomarkers of AD and CVD pathology were the main

factors that contributed to discriminating individuals who endorsed SCD memory complaints.

Results add new evidence to the study of the association between biomarkers of AD and CVD pathology and loneliness (d'Oleire-Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017), and they suggest that this relationship is not independent of depression levels. Furthermore, these results reinforce the idea of considering loneliness as an early neuropsychiatric symptom of AD and CVD. It is important to delve into how loneliness and biomarkers are related, the directionality, and the underlying mechanisms between them. On the one hand, people who feel lonely seem to have poorer social skills (Cacioppo et al., 2006) and smaller gray matter volume in brain areas related to social processing (Kanai et al., 2012). Therefore, loneliness could be reflected in less elaborate neural systems involved in social functioning, with reduced cognitive and neural reserve. This would imply less capacity to compensate for neural damage in other neural systems (due to age-related factors or brain pathology). On the other hand, brain pathology could be affecting neural networks related to socioemotional processing and, thus, to loneliness. Regarding the relationship between loneliness and SCD, results showed that loneliness, depressive symptomatology, and biomarkers of AD and CVD pathology contribute to both subtypes of SCD. However, loneliness does not play an important role in explaining SCD when depressive symptomatology and biomarkers of brain pathology are included in a competitive model. In conclusion, this study suggests that loneliness holds promise in detecting risk of cognitive impairment due to brain pathologies. The association between loneliness and depressive symptomatology needs to be further elucidated, particularly in the context of AD and CVD pathologies and early stages of these diseases such as SCD.

In general, this doctoral thesis adds relevant evidence to the literature focusing on the study of loneliness in relation to different stressors and health factors in young, middle-aged and older healthy people. We have seen that loneliness is a feeling that can be related to adverse experiences in early stages in life, and it can lead to a greater perception of stress in adulthood. In middle-aged and older people, there seem to be sex differences in the relationship between loneliness and worse subjective psychological health because this association appears only in men. Loneliness seems to not be related to worse subjective physical health and a dysregulation of the basal HPA axis functioning based on the findings of our studies, suggesting that other possible resilient factors may be acting as a buffer. Finally, the potential of loneliness as an early neuropsychiatric symptom of preclinical AD and CVD should be highlighted, although more research is needed to better understand its relationship with depressive symptomatology, SCD and brain pathologies related to AD and CVD.

Limitations and strengths

Specific limitations have been detailed and discussed in each of the studies, and here some of the general aspects to consider are presented. First, the cross-sectional design of the studies makes it impossible to draw conclusions about causal relationships. Second, in the three studies, we controlled the main potentially confounding factors described in the literature, but other extraneous variables could play a relevant role, and alternative explanations for the results are possible. Third, the participants in the three studies were healthy people because we excluded many potential participants by applying rigorous exclusion criteria based on medication intake and general health. This allowed us to obtain homogeneous samples in which diseases and factors related to health status were

not confounding variables, although at the same time, it makes it difficult to generalize the results.

Among the strengths of this doctoral thesis, it is important to note the relevance of studying loneliness and its relationships with specific stressors and health factors, given that it is a mental health issue in our society that has been receiving more attention in recent years. We studied loneliness in people with a wide age range, which allows us to have broader information about how this feeling affects us in different stages of our lives. In addition, people from different nationalities were included in this doctoral thesis, which facilitates a greater understanding of the influence of loneliness in different cultures. Regarding methodology, we were strict about the collection and/or selection of saliva samples in order to obtain mainly unbiased cortisol levels (Adam et al., 2017; Stalder et al., 2016). Likewise, we used complex and sophisticated statistical analyses, such as mediation or moderation regression analyses through bootstrapping techniques or random forest classification analyses, allowing us to achieve a greater depth of analysis and cover our objectives more concisely.

Future directions

The results of this doctoral thesis add evidence to the current state of the literature and open the door to new studies in this emerging field of research.

There is a need for longitudinal studies starting at very early stages to further address the association between adverse experiences and feelings of loneliness, including the effect and evolution of attachment styles. We have seen that loneliness was not related to subjective physical health or HPA axis functioning, contrary to what we expected. We suggest expanding the study of these relationships in order to identify resilience factors

influencing these non-associations. Furthermore, different forms of socialization in men and women, as well as values related to masculinity, could be studied in order to explain how they can contribute to the relationship between loneliness and psychological health. Regarding the cortisol samples, given the relatively stable nature of loneliness, it would be worth studying loneliness with a stable measure of long-term cumulative cortisol. In this regard, hair cortisol samples would be a valid option because they can reflect integrated cortisol secretion over several weeks or months (Iob & Steptoe, 2019; Stalder & Kirschbaum, 2012). Finally, more research and longitudinal studies are needed to completely understand the role of loneliness in SCD and AD and CVD pathology, considering its relationship with depressive symptomatology.

Chapter VI

Main Conclusions

Main Conclusions

1) ELS appears to be related to perceived stress, but not to basal HPA axis functioning (i.e., overall diurnal cortisol, DCS and bedtime cortisol), in adulthood in young and middle-aged people.
2) Loneliness mediates the relationship between ELS and perceived stress in adulthood in young and middle-aged people.
3) Loneliness does not mediate the relationship between ELS and basal HPA axis functioning (i.e., overall diurnal cortisol, DCS and bedtime cortisol) in young and middle-aged people.
4) Loneliness is associated with subjective psychological health, but not with subjective physical health in middle-aged and older people. This association only occurs in men.
5) Loneliness is not related to basal HPA axis functioning (i.e., CAR, DCS and bedtime cortisol) in middle-aged and older people.
6) Basal HPA axis functioning (i.e., CAR, DCS and bedtime cortisol) does not play a relevant role as a mechanism underlying the relationship between loneliness and subjective psychological and physical health in middle-aged and older people.
7) Loneliness reflects AD and CVD pathology and, especially, depressive symptomatology in cognitively unimpaired seventy-year-old people.
8) Loneliness contributes to SCD, although it is not independent of depressive symptomatology and amyloid-beta levels in cognitively unimpaired seventy-year-old people.

Chapter VII

General Summary in Valencian

Introducció general

Rellevància de la solitud en el benestar

En els darrers anys, la salut mental és cada vegada més rellevant i més visible a la nostra societat. En aquesta línia, la solitud és un problema psicològic que rep una atenció creixent. Entre el 10 i el 34% de les persones reporten sentiments de solitud (Mund et al., 2020; Organització Mundial de la Salut, 2021). Sentir-se sol pot ser experimentat per persones de totes les edats i s'accentua amb l'envelliment. A causa de la naturalesa social de l'ésser humà, el sentiment de solitud és un factor estressant en si mateix (Cacioppo et al., 2000; Steptoe et al., 2004; Zawadzki i Gavrilova, 2021), i està relacionat amb greus conseqüències en amplis aspectes de la salut (Courtin i Knapp, 2017; Leigh-Hunt et al., 2017; Richard et al., 2017). Tant és així que, l'any 2018 el parlament britànic i també el japonès van establir un Ministeri per a la Solitud amb la intenció de treballar-ne la prevenció i el tractament. A més, sentir-se sol és una preocupació clàssica en l'àmbit de la psicologia i és un dels principals temes tractats en la psicoteràpia existencial (May i Yalom, 2005). Donada la importància de la solitud, cal aprofundir en ella, i investigar específicament la seua relació amb l'estrès i la salut, per millorar el benestar de la societat.

El fet que les emocions i els sentiments poden influir en la nostra salut psicològica i física és ben conegut (Lopez et al., 2018). Per exemple, emocions com la tristesa o l'ansietat s'han relacionat amb un pitjor funcionament del sistema immunitari (Brod et al., 2014; Lasselin et al., 2016), la qual cosa pot provocar diferents malalties (Couzin-Frankel, 2010; Slavich, 2015). De la mateixa manera, quan ens sentim sols, s'activen processos biològics relacionats amb l'estrès i el dolor, responent a l'amenaça d'un rebuig per part del propi grup social (Panksepp, 2003; Steptoe et al., 2004).

La solitud o soledat difereix de l'aïllament social, ja que el primer fa referència a una percepció aversiva i desagradable de la manca de companyia, i l'aïllament social es refereix al fet objectiu d'estar sol, que pot ser neutre o fins i tot agradable (Stein i Tuval-Mashiach, 2015; Tillich, 1959). En aquesta línia, podem sentir-nos acompanyats i socialment satisfets sense estar en contacte amb altres persones, encara que la solitud és més probable entre persones que experimenten aïllament social (Cacioppo et al., 2000, 2015a).

En aquest sentit, la solitud és una emoció associada a l'essència social de l'ésser humà. Al llarg de l'evolució, per sobreviure, necessitem d'un altre que ens cuide i ens protegisca, sobretot els primers anys de vida. Al llarg de la vida, seguim necessitant sentir-nos part del grup per oferir valor i confiança mutus (Baumeister i Leary, 1995). Evolutivament, ser rebutjat pel grup augmenta la possibilitat de morir, per tant, la solitud s'entén com una alarma, un sentiment desagradable que empeny l'individu a tornar a formar part del grup (Cacioppo et al., 2014, 2015a, 2015b). Els sentiments de solitud sorgeixen de la discrepància entre els vincles íntims i socials desitjats i els reals, posant més èmfasi en la qualitat que en la quantitat de les relacions (Peplau i Perlman et al., 1982; Pinquart i Sörensen, 2003; Weiss, 1973). Per tant, la solitud es pot viure dins de relacions com ara la família, l'amistat o el matrimoni (Cacioppo et al., 2009).

L'estrès als primers anys de vida i la seua influència en la solitud

Les experiències durant els primers anys de vida influeixen en la manera com ens relacionarem amb els altres durant l'edat adulta, basat en el desenvolupament dels estils d'aferrament (Adler, 1996, 1998; Akdoğan, 2017; Bowlby, 1973). La negligència en les cures o el maltractament parental durant la infància i l'adolescència està lligada a la

insatisfacció amb les necessitats d'afecte, pertinença i reconeixement per part de la figura d'aferrament (Bowlby, 1984). Aquestes experiències adverses i estressants en les primeres etapes de la vida, conegudes com a estrès als primers anys de vida (ELS, per les seues sigles en anglès), poden conduir a inseguretats en l'aferrament, rebuig, desconfiança i tendència a evitar les relacions properes i el contacte amb els altres (Bartholomew, 1990; Hazan i Shaver, 1994; Kafetsios i Nezlek, 2002; Shaver i Brennan, 1992). A més, podrien afectar negativament la manera com l'individu establirà relacions durant l'edat adulta i predisposar a la sensació de solitud (Akdoğan, 2017; Thomas, 2016; Weiss, 1973, 1987).

La literatura prèvia suggereix que l'ELS augmenta la vulnerabilitat als efectes dels esdeveniments estressants posteriors, agreujant les conseqüències per a la salut dels estressors en l'edat adulta (Hammen et al., 2000; Lähdepuro et al., 2019; McLaughlin et al., 2010). En aquesta línia, les persones que han experimentat ELS tenen més dificultats per afrontar una situació d'estrès important durant l'edat adulta (Hammen et al., 2000; Harkness et al., 2006), mostrant efectes negatius a llarg termini sobre la seua salut física i psicològica (Kessler et al., 2010; Monnat i Chandler, 2015). Relacionat amb l'anterior, malgrat que aquestes experiències estressants ocorren en les primeres etapes de la vida, els seus efectes emocionals, relacionals i neurobiològics poden persistir al llarg del temps (Kessler et al., 2010; Nemeroff, 2016). En aquest sentit, la solitud apareix com un potencial mediador entre l'ELS i l'estrès durant l'edat adulta, ja que les persones que han experimentat ELS reporten menys satisfacció en les seues relacions i menys suport social (Beutel et al., 2017; Germine et al., 2015; Repetti et al., 2002). Malgrat això, fins el que nosaltres sabem, no hi ha estudis previs que consideren com la solitud en l'edat adulta pot estar relacionada amb aquestes experiències adverses primerenques i si pot contribuir a les conseqüències relacionades amb l'estrès. Per tant, l'objectiu del primer estudi d'aquesta

tesi serà abordar el paper de la solitud en la relació entre l'estrès en etapes primerenques de la vida i l'estrès en l'edat adulta.

A més, és important estudiar el paper de l'ELS i la solitud en el funcionament de l'eix hipotàlem-hipòfisi-adrenal (HPA, per les seues sigles en anglès) (Campagne, 2019; Fogelman i Canli, 2018; Hawkley i Cacioppo, 2003), ja que és el principal sistema endocrí relacionat amb la resposta a l'estrès. A més, el seu funcionament basal és un indicador de l'estat de salut i ens dóna informació sobre l'exposició a l'estrès crònic (Adam et al., 2017; Miller et al., 2007).

El funcionament de l'eix HPA, l'estrès als primers anys de vida i la solitud

El sistema psicobiològic primari per gestionar l'estrès és l'eix HPA. Aquest sistema endocrí s'enfronta a l'estrès per l'acció del principal glucocorticoide en humans, el cortisol. El cortisol és una hormona esteroide essencial, secretada per l'escorça de la glàndula suprarenal. En situacions no estressants o basals, el ritme diari del cortisol en persones sanes està marcat per un augment en els primers 30-45 minuts després del despertar, conegut com a resposta matutina de cortisol (CAR, per les seues sigles en anglès), seguit d'una disminució constant fins a la nit, coneguda com a pendent diürna de cortisol (DCS, per les seues sigles en anglès) i assoleix els nivells de cortisol més baixos a l'hora d'anar a dormir (Adam i Kumari, 2009). Una desregulació d'aquest ritme (és a dir, un cortisol al despertar més baix, una CAR més gran, una DCS més plana o un cortisol més alt a l'hora d'anar a dormir) s'ha associat amb diferents resultats adversos per a la salut (Adam et al., 2017; Fries et al., 2009).

Segons la literatura, tant l'ELS com la solitud podrien afectar el ritme del cortisol, però les conclusions encara no estan clares. Una metaanàlisi informa que l'ELS no està relacionat amb el funcionament basal de l'eix HPA (Fogelman i Canli, 2018), tot i que els autors assenyalen les inconsistències en els resultats i l'heterogeneïtat en les mesures de cortisol. En aquest sentit, alguns estudis van informar d'una relació de l'ELS amb una CAR atenuada (Li et al., 2015), una DCS més aplanada (Brewer-Smyth i Burges, 2008; Nicolson, 2004), un cortisol diürn general més elevat (Franz et al., 2013), una CAR augmentada (Lu et al., 2013, 2016) o una DCS més empinada (Van der Vegt et al., 2009). Tanmateix, altres no van trobar cap relació entre l'ELS i el cortisol diürn general, la DCS o el cortisol abans d'anar a dormir (Bublitz i Stroud, 2012; Franz et al., 2013; Klaassens et al., 2009; Schreuder et al., 2016). A més, la solitud s'ha associat a diferents patrons de desregulació del funcionament basal de l'eix HPA en els joves, com ara una CAR atenuada (Lai et al., 2019), una DCS més aplanada (Doane i Adam, 2010), uns nivells de cortisol diürn general més alts (Cacioppo et al., 2000) o una DCS més empinada (Lai et al., 2018, 2019).

En conjunt, aquestes troballes mixtes posen de manifest la necessitat d'aclarir les associacions entre el funcionament de l'eix HPA i ambdós, l'ELS i la solitud. A més, atès que, d'una banda, l'ELS pot afectar les relacions amb els altres durant l'edat adulta, i que d'altra banda, tant l'ELS com la solitud poden afectar el funcionament basal de l'eix HPA, és evident la necessitat d'estudiar el paper de la solitud a la relació entre l'ELS i l'eix HPA. Per tant, en el primer estudi d'aquesta tesi, s'abordarà la solitud com a factor mediador en la relació entre l'ELS i el funcionament basal de l'eix HPA.

Solitud i salut en l'envelliment

La solitud pot afectar a les persones en qualsevol etapa de la vida, tot i que aquest sentiment comença a augmentar des de la mitjana edat, sent especialment elevat entre la gent gran (Pinquart i Sörensen, 2003; Yang i Victor, 2011). L'edat per si sola no explica aquest augment de la solitud, però factors vinculats a l'envelliment com la viduïtat, la jubilació o la mobilitat reduïda fan que siga més habitual sentir-se sol (Mund et al., 2020). La solitud afecta negativament el benestar i moltes àrees de la salut psicològica i física en l'envelliment (Ong et al. 2016; Leigh-Hunt et al., 2017; Valtorta et al., 2018; Xia i Li, 2018). En aquesta línia, sentir-se sol s'ha associat a una salut subjectiva general més pobra (Losada et al., 2012; Richard et al., 2017) i a una gran varietat de problemes autoinformats de salut física i psicològic com el dolor musculoesquelètic (Smith et al., 2019), pitjor qualitat del son (Benson et al., 2021) o ansietat, estat d'ànim depressiu i ideació suïcida (Beutel et al., 2017; Ge et al., 2017). Actualment, no hi ha consens sobre les diferències de sexe en les relacions entre la solitud i la salut física i psicològica subjectiva (Richard et al., 2017; Zebhauser et al., 2014). Per tant, les relacions entre la solitud i la salut subjectiva física i psicològica seran abordades en el segon estudi d'aquesta tesi doctoral tenint en compte el sexe dels participants.

S'han proposat diversos mecanismes per explicar els problemes de salut relacionats amb la solitud. Alguns autors subratllen que la solitud pot afectar la salut a través de factors de l'estil de vida com ara menys autocura, inactivitat física, tabaquisme, ingesta d'alcohol o consum d'aliments poc saludables (Akerlind i Hörnquist, 1992; Baumeister et al., 2005; Hawkley et al., 2009; Lauder et al., 2006; Richard et al., 2017). A més, s'ha suggerit que la solitud actua com un factor estressant a llarg termini i podria desregular el funcionament basal de l'eix HPA (Campagne, 2019; Hawkley i Cacioppo,

2003; O'Connor et al., 2021; Steptoe et al., 2004) afectant negativament la salut (Adam et al., 2017; Fries et al., 2009).

Malgrat l'anterior, hi ha pocs estudis que investiguen si existeix una relació entre la solitud i el funcionament basal de l'eix HPA en persones de mitjana edat i edat avançada, sent els resultats sobre CAR i DCS no concloents (Adam et al., 2006; Cole et al., 2007; Schutter et al., 2017, 2021; Steptoe et al., 2004). En un estudi anterior, no es van trobar relacions significatives entre la solitud i el cortisol al despertar, la CAR ni la DCS; tanmateix, una major solitud es va associar amb nivells més elevats de cortisol abans d'anar a dormir (Montoliu et al., 2019). A més, el paper del sexe en les relacions entre la solitud i el funcionament basal de l'eix HPA encara no està clar (Johar et al., 2021; Montoliu et al., 2019). Per tant, cal investigar més per aprofundir en la manera en què la solitud, la salut subjectiva i el funcionament basal de l'eix HPA estan interrelacionats en l'envelliment, tenint present les possibles diferències de sexe. Aquestes qüestions seran tractades en el segon estudi d'aquesta tesi doctoral.

Solitud, declivi cognitiu subjectiu i patologia cerebral en l'envelliment

S'ha vist que la solitud afecta el rendiment cognitiu (Boss et al., 2015) i s'associa a un augment del risc de demència (Wilson et al., 2007). Cada vegada hi ha més evidències que mostren que els símptomes neuropsiquiàtrics poden indicar l'aparició de patologia cerebral. Les percepcions de períodes d'oblit recurrent o episodis de distracció a la vida diària, anomenades queixes cognitives subjectives (SCCs, per les seues sigles en anglès), són una experiència habitual que augmenta entre les persones grans (Jonker et al. 2000; Ponds et al. 2000). Quan aquestes SCCs no van acompanyades d'un deteriorament cognitiu objectiu (o d'altres causes que expliquen aquesta percepció), es coneix com a

declivi cognitiu subjectiu (SCD, per les seues sigles en anglès) que s'ha suggerit com una etapa primerenca de la Malaltia d'Alzheimer (AD, per les seues sigles en anglès) (Jessen et al., 2014). L'SCD s'associa amb els principals canvis patològics cerebrals de l'AD, plaques beta-amiloide, capdells neurofibril·lars tau i neurodegeneració (Amariglio et al., 2012; Buckley et al. 2017; Cedres et al., 2021; Perrotin et al., 2012). A més, l'SCD s'ha relacionat amb biomarcadors de la Malaltia Cerebrovascular (CVD, per les seues sigles en anglès) (Cedres et al., 2019; 2021; Diaz-Galvan et al., 2021a; Minett et al., 2005).

A la literatura recent, una discussió actual és si l'SCD no només reflecteix patologies cerebrals, sinó també factors no neurodegeneratius com la simptomatologia depressiva (Diaz-Galvan et al., 2021a; Jessen et al., 2014, 2020). Tot i que la solitud és un concepte molt proper a la simptomatologia depressiva, el seu estudi en el context de l'SCD és molt incipient. Recentment, s'ha vist que les persones que se senten soles reporten més freqüència d'SCCs en memòria, independentment de la simptomatologia depressiva (Montejo et al., 2019). A més, en els últims anys, l'estudi de la solitud com a símptoma neuropsiquiàtric primerenc de l'AD o la CVD està començant. Encara hi ha pocs estudis que hagen investigat la relació entre la solitud i els biomarcadors de la patologia de l'AD (nivells beta-amiloide i tau) o la CVD (anomalies del senyal de la substància blanca), i encara que en general sembla haver una associació entre ells, no tots els estudis van mostrar resultats significatius (d'Oleire Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017; Wilson et al., 2007). Basant-nos en això, cal estudiar el paper de la solitud en el context de les primeres etapes de l'AD i la CVD. Seria important explorar la contribució de la solitud a l'SCD (i si la seua influència és independent de la simptomatologia depressiva) i ampliar el coneixement sobre la relació entre la solitud i els biomarcadors de la patologia de l'AD i la CVD. El tercer estudi d'aquesta tesi s'ha proposat per donar resposta a aquestes preguntes.

Objectius i hipòtesi

L'objectiu general d'aquesta tesi doctoral és ampliar l'evidència sobre el paper de la solitud en l'estrès i la salut en persones adultes, per tal de donar llum a l'estat actual de la literatura. En aquest sentit, s'aborda la solitud en relació amb diferents factors estressants (ELS i estrès durant l'edat adulta), i diferents indicadors subjectius de salut (salut psicològica i física i SCD) i indicadors objectius de salut (funcionament basal de l'eix HPA i biomarcadors de la patologia de l'AD i la CVD).

Aquesta tesi doctoral inclou tres estudis. A continuació es descriuen l'interès fonamental que orienta aquests estudis i els objectius específics.

Estudi 1: En aquest estudi, vam abordar el paper de la solitud en la relació de l'ELS amb l'estrès experimentat a l'edat adulta en persones joves i de mitjana edat. Concretament, els objectius d'aquest estudi eren investigar si l'ELS estava associat amb la percepció de l'estrès i el funcionament basal de l'eix HPA en l'edat adulta, i el paper mediador de la solitud en aquestes relacions. Per a això, primer es va estudiar si l'ELS i la percepció de l'estrès, el cortisol diürn general, la DCS i el cortisol abans d'anar a dormir estaven relacionats, i després es va analitzar la solitud com a possible mediador d'aquestes associacions. Vam plantejar la hipòtesi que nivells més alts d'estrès durant els primers anys de vida contribuirien a augmentar l'estrès percebut durant l'edat adulta (Han et al., 2016; Hyman et al., 2007). Com que la investigació sobre la relació de l'ELS amb els nivells de cortisol no és concloent, i que aquest va ser el primer estudi que va explorar el paper mediador de la solitud en la relació entre l'ELS i l'estrès percebut i el funcionament basal de l'eix HPA, no vam proporcionar cap hipòtesi sobre l'existència o la direcció de

la relació entre l'ELS i el funcionament basal de l'eix HPA i de les mediacions a través de la solitud entre l'ELS i l'estrès percebut i el funcionament basal de l'eix HPA.

Estudi 2: En aquest estudi, vam investigar les relacions de la solitud amb la salut subjectiva i el funcionament basal de l'eix HPA en persones de mitjana edat i d'edat avançada. En primer lloc, vam tenir com a objectiu estudiar les associacions entre la solitud i la salut física i psicològica subjectiva i diversos indicadors del funcionament de l'eix HPA. En segon lloc, vam voler investigar el paper del sexe en aquestes relacions. Vam plantejar la hipòtesi que la solitud estaria relacionada amb una pitjor salut psicològica i física subjectiva (Beutel et al., 2017; Richard et al., 2017). Pel que fa al funcionament basal de l'eix HPA, basat en Montoliu et al. (2019), esperàvem confirmar que la solitud s'associara amb els nivells de cortisol abans d'anar a dormir, encara que no amb la CAR i la DCS. Tot i que no està clar el paper del sexe en la relació entre la solitud i la salut subjectiva i el funcionament basal de l'eix HPA, esperàvem trobar que aquestes associacions foren més fortes en hòmens que en dones (Johar et al., 2021; Zebhauser et al., 2014). Finalment, vam explorar el funcionament basal de l'eix HPA com a mecanisme subjacent a la relació entre la solitud i la salut subjectiva.

Estudi 3: En aquest estudi, vam abordar el paper de la solitud en el context de l'SCD com a símptoma neuropsiquiàtric primerenc de l'AD o CVD en persones grans no deteriorades cognitivament. El primer objectiu va ser explorar les associacions de la solitud amb la simptomatologia depressiva i els biomarcadors de la patologia de l'AD i la CVD. El segon objectiu va ser estudiar les associacions entre l'SCD i la solitud, i els biomarcadors de la patologia de l'AD i de la CVD, i vam explorar si les relacions eren independents de la simptomatologia depressiva, a causa de l'estreta relació de l'estat d'ànim depressiu i la

solitud (Erzen i Çikrikci, 2018) i la co-ocurrència de l'estat d'ànim depressiu amb l'SCD (Diaz-Galvan et al., 2021a; Jessen et al., 2014). Vam plantejar la hipòtesi que la solitud estaria associada positivament, no només a la simptomatologia depressiva (Domènech-Abella et al., 2019), sinó també als biomarcadors de la patologia de l'AD i la CVD (d'Oleire-Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017). A més, vam suposar que, independentment de la simptomatologia depressiva, l'SCD estaria relacionat amb una major solitud (Montejo et al., 2019) i amb més biomarcadors de la patologia de l'AD i la CVD (Amariglio et al., 2012; Buckley et al. 2017; Cedres et al., 2019, 2021; Diaz-Galvan et al., 2021a). Finalment, vam investigar si aquestes associacions diferirien en funció del tipus d'SCD (queixes de memòria i de no-memòria), ja que recentment s'ha suggerit que diferents SCD específics podrien reflectir diferents perfils sindròmics (Diaz-Galvan et al., 2021b).

Metodologia

Amb la finalitat de proporcionar una visió global de la metodologia utilitzada en aquesta tesi doctoral, a continuació es detalla un resum breu sobre els participants i el procediment de cada estudi i finalment les variables utilitzades en els estudis que en formen part.

Participants i procediment

Estudi 1

La *mostra* del primer estudi està composta per 187 persones (79 dones i 108 hòmens) d'edats compreses entre els 18 i els 55 anys reclutades de l'àrea metropolitana de Pensilvània. Els criteris d'exclusió van ser: persones amb antecedents de diabetis, d'un

trastorn cardiovascular o d'alguna malaltia crònica; persones que actualment estaven prenent pastilles per dormir, tranquil·litzants, esteroides, immunosupressors o altres règims de medicació habituals; persones amb diagnòstic o tractades durant l'últim any d'un trastorn psiquiàtric, o hospitalització psiquiàtrica en els últims cinc anys; i dones en període de lactància o embarassades; i no tindre totes les dades de cortisol disponibles.

Pel que fa al *procediment*, les dades van ser recollides pel Laboratori per a l'Estudi de l'Estrès, la Immunitat i la Malaltia de la Universitat Carnegie Mellon, com a part d'un estudi prospectiu de reptes virals (Pittsburgh Cold Study 3) realitzat entre 2007 i 2011 i s'hi va accedir a través del seu lloc web. El protocol tenia una duració entre 14 i 16 setmanes on els participants eren infectats amb un virus per investigar la susceptibilitat al refredat comú. Les dades d'ELS, estrès percebut actual, solitud i els nivells de cortisol diürn, es van avaluar durant les visites a l'hospital prèvies a la inoculació del virus, per tant aquesta no va afectar a les dades incloses en el present estudi. Les dades de cortisol es van recollir a través de 14 mostres (1, 2, 4, 7, 9, 11 i 14 h després del despertar) en dos dies no consecutius en l'entorn habitual i realitzant les activitats habituals dels participants.

Estudi 2

Els *participants* del segon estudi van ser 79 participants (40 dones i 39 hòmens) en un rang d'edat entre 55 i 75 anys que provenien de La Nau Gran, un programa de la Universitat de València per a persones majors de 55 anys. Els criteris d'exclusió van ser: estar fora de la franja d'edat de 55 a 75 anys; tindre un diagnòstic de diabetis, malalties psiquiàtriques o neurològiques, o altres malalties que interfereixen en el benestar diari; utilitzar medicaments com ara glucocorticoides, ansiolítics, antidepressius o altres medicaments que poden interferir directament amb el funcionament emocional,

endocrinològic o cognitiu; haver estat sota anestèsia general en els últims 12 mesos; fumar més de 10 cigarrets al dia, consumir alcohol o altres drogues; i la presència d'un esdeveniment vital estressant durant el darrer any, com ara la mort del cònjuge, l'aparició d'una malaltia important o qualsevol altre esdeveniment que el/la participant considere que li ha afectat de manera significativa.

El *procediment* d'aquest segon estudi va ser dissenyat per tal d'investigar la relació entre la solitud i la salut subjectiva i el funcionament basal de l'eix HPA. Per a aquest fi els 79 participants mencionats anteriorment van realitzar una sessió d'avaluació d'una hora aproximada de durada i es van fer a les 10, 12 i 16 h, contrapesant sexe i horari. Durant la sessió es va recollir informació sociodemogràfica i qüestionaris sobre solitud, salut subjectiva i altres variables psicològiques de control (percepció d'estrès, depressió o relacions socials). A més, els participants van realitzar diferents mostres de saliva a casa durant dos dies consecutius (immediatament després de despertar, als 15, 30, i 45 minuts després de despertar i immediatament abans d'anar a dormir). Aquesta fase experimental es va portar a terme entre el 2018 i el 2020.

Estudi 3

Els *participants* del tercer estudi són 114 dones i 101 hòmens de setanta anys que pertanyen a un estudi poblacional realitzat entre el 2014 i el 2016, a Göteborg (Suècia). El criteri d'inclusió que primer es va aplicar va ser tindre dades disponibles sobre la punció lumbar i la ressonància magnètica per poder analitzar els biomarcadors de l'AD i de la CVD. Entre els participants amb disponibilitat d'aquestes dades, els criteris posteriors d'inclusió aplicats van ser d'acord al grup de treball líder de la iniciativa internacional SCD (SCD-I): I) Cognició no deteriorada avaluada amb dos criteris. Per una banda, l'exclusió de la demència a través del diagnòstic de demència d'acord amb els

criteris del DSM-III-R, la puntuació al test de cribatge Mini-Mental State Examination (MMSE) < 24, o la qualificació clínica de demència > 0.5. Per altra banda, l'exclusió del deteriorament cognitiu lleu segons els criteris següents suggerits per Jak et al. (2009) i Molinuevo et al. (2017) a partir d'una avaluació neuropsicològica (ajustada per edat, sexe i educació); II) Sense anomalies en ressonància magnètica a criteri del/la neuroradiòleg/a (p. ex. ictus, atac isquèmic transitori o tumors); III) Sense antecedents mèdics de trastorns neurològics o psiquiàtrics (p. ex., depressió major), malalties sistèmiques o traumatismes cranials, i IV) Sense antecedents d'abús de substàncies o d'alcohol.

A les dades d'aquest estudi s'hi va accedir mitjançant la col·laboració amb el Karolinska Institutet, gràcies a la realització d'una estada d'investigació per part de la doctoranda. El *procediment* consistia en una examinació general en la qual s'avaluava l'estat de salut física, mental i cognitiva i es recollien dades sociodemogràfiques dels participants. Es va dur a terme en un o dos dies (a elecció del participant). Tots els detalls complets de les proves i els procediments estan en Rydberg et al. (2019). Per realitzar aquest estudi vam seleccionar dades sociodemogràfiques i sobre solitud, simptomatologia depressiva, queixes subjectives de concentració i memòria i els biomarcadors relacionats amb l'AD (beta-amiloide i tau fosforilada) i amb la CVD (anomalies del senyal de la substància blanca, WMSA, per les seues sigles en anglès). Aquests biomarcadors es van utilitzar per mesurar la patologia cerebral.

Variables

Variables psicològiques

Al llarg dels estudis inclosos en la present tesi s'han estudiat diferents variables psicològiques avaluades a través de qüestionaris autoinformats. Aquestes són:

La *solitud* s'avalua en tots els estudis de la present tesi doctoral. A l'Estudi 1 s'utilitza l'escala de solitud curta que consta de 3 ítems (Hughes et al., 2004), a l'Estudi 2 utilitzem el qüestionari UCLA-R (Russell et al., 1980), que consta de 20 ítems i al tercer Estudi utilitzem un ítem directe "Se sent vosté sol/a?". Totes aquestes són mesures estandarditzades i validades.

L'estrès i adversitat durant la infància i adolescència es va avaluar a l'Estudi 1 a través de dos qüestionaris, el Qüestionari de Famílies amb Risc (RFQ, Risky Family Questionnaire; Taylor et al., 2004) que avalua la negligència de la llar i el maltractament ambiental, físic, emocional i mental. També es va avaluar amb el Qüestionari d'Estrès Percebut durant la Infància i l'Adolescència Recordat (ReCAPS, per les seues sigles en anglès) que avalua de manera general l'estrès viscut en etapes primerenques.

L'*estrès percebut* s'avalua tant a l'Estudi 1 com a l'Estudi 2, fent referència a com d'estressant, incontrolable, impredictible i sobrecarregada es percep la vida durant l'últim mes. A l'Estudi 1 es va avaluar amb l'escala d'estrès percebut de 10 ítems i a l'Estudi 2 amb la versió de 14 ítems de la mateixa escala (PSS; Cohen et al., 1983; Cohen i Janicki-Deverts, 2012).

Les mesures de *salut subjectiva* es van avaluar amb l'enquesta abreujada de Qualitat de Vida de l'Organització Mundial de la Salut (WHOQOL, Carrasco, 1998). Concretament, vam utilitzar les escales de salut psicològica i salut física per a les anàlisis principals i l'escala de relacions socials per a la descripció de la mostra.

La *simptomatologia depressiva* es va mesurar als Estudis 2 i 3 com a covariada. A l'Estudi 2 es va avaluar mitjançant l'Inventari de Depressió de Beck de 21 ítems (BDI-II; Beck et al., 1996) i a l'Estudi 3 mitjançant l'escala de valoració de la depressió de Montgomery-Åsberg de 7 ítems (MADRS; Montgomery i Åsberg, 1979).

Variables biològiques

Variables hormonals

Vam mesurar el funcionament basal de l'eix HPA a través dels nivells salivals de cortisol. Per a les mostres de saliva es van utilitzar Salivettes (Sarstedt, Rommelsdorf, Alemanya), uns tubs de plàstic que contenen un cotó-en-pèl, el qual s'han de col·locar en la boca durant dos minuts seguits. L'Estudi 1 i 2 són els que inclouen la mesura de l'eix HPA, avaluant dos patrons diürns diferents. Per a l'Estudi 1 es van determinar mitjançant un immunoassaig de fluorescència resolt en el temps amb un conjugat cortisol-biotina com a traçador (Dressendörfer et al., 1992). Per a l'Estudi 2 es va utilitzar el kit ELISA de Salimetrics (Newmarket, Regne Unit) per determinar les concentracions de cortisol.

Biomarcadors de l'AD

Els principals canvis bioquímics que es produeixen al cervell des de fases preclíniques de l'AD són l'acumulació de proteïna amiloide entre les neurones formant plaques d'amiloide i posteriorment la hiperfosforilació de la proteïna tau a l'interior de les neurones, formant capdells neurofibril·lars (Jack et al., 2010) i ambdues es reflecteixen en el líquid cefaloraquidi (LCR). L'acumulació d'aquestes proteïnes pot començar fins 15-20 anys abans que apareguen els símptomes clínics de la malaltia (Lloret et al., 2019). A l'Estudi 3 utilitzem els biomarcadors ratio de beta-amiloide 42/40 (Aβ42/40) i tau fosforilada (p-tau) per reflectir patologia beta-amiloide i tau, respectivament. Aquests biomarcadors s'obtenen a través de l'assaig immunoabsorbent lligat a enzims sandvitx.

Biomarcadors de la CVD

La presència de CVD es pot observar en imatges de ressonància magnètica (Wardlaw et al., 2013) a través de WMSA. A l'Estudi 3 hem utilitzat hipointensitats WMSA com a biomarcadors de la CVD. Les hipointensitats WMSA semblen estar relacionades amb una integritat més pobra de la substància blanca i reflectir danys crònics de la substància blanca (Riphagen et al., 2018). Les hipointensitats WMSA es van segmentar automàticament amb FreeSurfer 6.0.0 i les imatges ponderades en T1 es van processar i analitzar amb la suite d'anàlisi d'imatges FreeSurfer 6.0.0.

Variables genètiques

Per a determinar les tres variacions al·lèliques del polimorfisme genètic *APOE* ε4 (ApoE-ε2, ApoE-ε3 i ApoE-ε4), es va utilitzar el sistema de genotipat KASPar PCR SNP (LGC Genomics, Hoddesdon, Herts, Regne Unit). Aquest al·lel s'inclou per a la descripció de la mostra de l'Estudi 3.

Variables cognitives

Per avaluar l'*SCD* a l'Estudi 3, vam utilitzar dues preguntes de l'entrevista semiestructurada CPRS (Åsberg et al., 1978), una per a l'SCD de queixes de concentració i altra per a les queixes de memòria. Els ítems fan referència a un empitjorament subjectiu durant l'últim mes en les dificultats autopercebudes de concentració o de memòria en comparació amb la capacitat prèvia. A més, durant el procés de selecció de la mostra de l'Estudi 3, s'utilitza una avaluació neurocognitiva per tal de poder excloure tots els participants que presenten una afectació cognitiva indicadora de deteriorament cognitiu.

Resultats principals i conclusions

L'objectiu general d'aquesta tesi doctoral era estudiar el paper de la solitud en l'estrès i la salut en persones sanes joves, de mitjana edat i d'edat avançada. Concretament, a l'Estudi 1 vam explorar si la solitud media la relació entre l'ELS i l'estrès percebut i el funcionament basal de l'eix HPA durant l'edat adulta en persones sanes joves i de mitjana edat. A l'Estudi 2, vam provar la relació entre la solitud, la salut psicològica i física subjectiva i el funcionament basal de l'eix HPA, atenent a les possibles diferències de sexe en persones sanes de mitjana edat i d'edat avançada. Finalment, a l'Estudi 3 vam investigar les associacions entre la solitud i els biomarcadors de la patologia de l'AD i la CVD i l'SCD en persones grans sanes.

A l'*Estudi 1*, vam analitzar la solitud, l'ELS, l'estrès percebut i els nivells de cortisol en 2 dies de 187 persones sanes d'entre 18 i 55 anys de l'àrea metropolitana de Pennsilvània (EUA). El funcionament basal de l'eix HPA es va mesurar amb 7 mostres de cortisol de saliva al dia i es van obtenir nivells de cortisol diürn general (AUCg), DCS i cortisol abans d'anar a dormir. Els resultats de les anàlisis de regressió lineal i de mediació van mostrar que l'ELS es va associar amb un estrès percebut més alt durant l'edat adulta, mediant la solitud aquesta relació. En aquest sentit, les persones que havien experimentat un ELS més alt, van sentir una major solitud i, al seu torn, van informar d'un estrès percebut més alt a l'edat adulta. Tanmateix, no vam trobar una associació significativa d'ELS o solitud amb el funcionament basal de l'eix HPA a l'edat adulta. Els resultats coincideixen amb estudis previs que observen una pitjor gestió i un augment de la percepció de l'estrès en persones que han viscut ELS (Betz et al., 2021; Bossé et al., 2018; Han et al., 2016; Hyman et al., 2007). A més, estan en línia amb estudis que van

trobar que les persones amb experiència d'ELS tenen menys satisfacció en els contactes socials en l'edat adulta (Beutel et al., 2017; Germine et al., 2015; Repetti et al., 2002), la qual cosa al seu torn, s'ha relacionat amb una major percepció de l'estrès (Matthews et al., 2019).

Els resultats podrien estar relacionats amb el paper de les figures d'aferrament en les primeres etapes, a través de qui aprenem a gestionar les emocions i l'estrès, i a entendre com funciona el món de les relacions (Bowlby, 1982). En experiències adverses com la negligència o l'abús per part dels pares, la figura d'aferrament podria no estar disponible per a aquest aprenentatge relacional, per tant, s'interioritzen esquemes inadequats per afrontar les emocions, l'estrès i els vincles amb els altres (Fonagy & Luyten, 2018). L'anterior influiria en les relacions interpersonals formades al llarg de la vida (Crowell et al., 2009; Pilkington et al., 2021; Repetti et al., 2002), experimentant una major solitud i percepció d'estrès. Finalment, la manca de relacions entre l'ELS i la solitud amb el funcionament basal de l'eix HPA es podria explicar per aspectes metodològics. Les mesures de cortisol utilitzades podrien no ser prou sensibles per captar aquestes associacions. A més, els baixos nivells d'ELS dels nostres participants es podrien expressar psicològicament però no biològicament mitjançant el funcionament basal de l'eix HPA. En resum, els nostres resultats suggereixen que les experiències estressants primerenques dificulten la relació amb els altres, augmentant la solitud i accentuant la percepció de l'estrès durant l'edat adulta. En aquest sentit, els resultats d'aquest estudi posen de manifest la importància de les intervencions socials o psicoterapèutiques en solitud en persones que han experimentat ELS amb l'objectiu de promoure vincles saludables significatius amb els altres i reduir la solitud i els seus efectes negatius associats.

En *l'Estudi 2*, hem realitzat una avaluació de 39 hòmens i 40 dones d'entre 55 i 75 anys de la Nau Gran, un programa educatiu de la Universitat de València (Espanya). Hem mesurat com a variables principals la solitud, la salut psicològica i física subjectiva i la simptomatologia depressiva. A més, es va avaluar el funcionament basal de l'eix HPA mitjançant 5 mostres de saliva en 2 dies, que ens van permetre obtenir els següents índexs de cortisol: CAR, DCS i nivells de cortisol abans d'anar a dormir. Es van realitzar anàlisis de regressió lineal, moderació i mediació. Els resultats van indicar que la solitud estava relacionada amb la salut psicològica subjectiva en els hòmens, però no en les dones. La solitud no estava relacionada amb la salut física subjectiva. A més, el funcionament basal de l'eix HPA no estava relacionat amb la solitud i no va aparèixer com un mecanisme subjacent a l'associació entre la solitud i la salut subjectiva. Aquests resultats suggereixen que el sexe és un factor crític en la relació entre la solitud i la salut psicològica subjectiva en persones de mitjana edat i d'edat avançada. Açò està en línia amb un estudi anterior que va observar una afecció més pronunciada de la solitud en la salut psicològica en hòmens que en dones (Zebhauser et al., 2014).

Aquestes diferències de sexe es podrien explicar per la variabilitat en les experiències sobre les relacions interpersonals en hòmens i dones. D'una banda, les dones tendeixen a moure's en relacions íntimes i diàdiques (Baumeister i Sommer, 1997; Gardner i Gabriel, 2004), la qual cosa facilita trobar un espai segur per expressar-se, enfortint la seua salut psicològica. D'altra banda, els hòmens tendeixen a moure's en grups més grans, on és més complicat compartir les seues emocions i preocupacions (Hoza et al., 2000). A més, aquestes diferències solen estar vinculades a valors sobre la masculinitat com l'autosuficiència o la dificultat per demanar suport psicosocial (Ogrodniczuk et al., 2016; Roy et al., 2017). Aquests motius podrien explicar perquè la solitud està relacionada amb una pitjor salut psicològica en els hòmens i no en les dones.

En general, la manca de relació entre la solitud i la salut física subjectiva i el funcionament basal de l'eix HPA suggereix l'important paper dels factors protectors de la solitud. Suposem que les característiques que representen l'envelliment òptim dels nostres participants (sense malalties cròniques, assistir a classes a la universitat, alt nivell socioeconòmic, baix estrès i símptomes depressius) podrien compensar factors relacionats amb una major solitud en l'envelliment (pèrdues naturals o jubilació) o almenys retardar els seus efectes nocius, actuant com a amortidor de l'estressor solitud (Hawkley et al., 2008; Teater et al., 2021). En resum, sembla que els hòmens tenen més vulnerabilitat a la solitud que les dones, mostrant una pitjor salut psicològica. Per tant, aquestes diferències de sexe s'han de tenir en compte en les intervencions de solitud.

L'*Estudi 3* estava compost per 215 participants de setanta anys sense alteracions cognitives, de la cohort de naixement de Gothenburg H70 1944. Es van analitzar mesures de solitud, d'SCD i de simptomatologia depressiva. A més, es van mesurar els biomarcadors de la patologia de l'AD (ratio Aβ42/40 i p-tau) mitjançant els nivells al líquid cefaloraquidi i es va avaluar el biomarcador de la CVD (WMSA) en imatges de ressonància magnètica. Es van realitzar anàlisis de classificació de boscs aleatòris per investigar la contribució dels predictors sobre la solitud i l'SCD i es van realitzar anàlisis de regressió logística per investigar l'efecte parcial de cada predictor sobre la solitud i l'SCD. Els resultats van mostrar que els biomarcadors de la patologia de l'AD i la CVD i, especialment, la simptomatologia depressiva, van contribuir a discriminar els individus que reportaven sentiments de solitud. La solitud es va associar amb l'SCD, tot i que va mostrar una baixa capacitat per discriminar el grup d'SCD. La simptomatologia depressiva i la beta-amiloide van ser els principals factors que van contribuir a discriminar els individus que reportaven SCD de queixes de concentració. Els biomarcadors de la

patologia de l'AD i la CVD van ser els principals factors que van contribuir a discriminar les persones que reportaven SCD de queixes de memòria.

Aquests resultats afegeixen nova evidència a l'estudi de l'associació entre biomarcadors de la patologia de l'AD i la CVD i la solitud (d'Oleire-Uquillas et al., 2018; Donovan et al., 2016; Duan et al., 2017) i suggereixen que aquesta relació no és independent dels nivells de depressió. A més, aquests resultats reforcen la idea de considerar la solitud com un símptoma neuropsiquiàtric primerenc de l'AD i la CVD. És important aprofundir en com es relacionen la solitud i els biomarcadors, la direccionalitat i el mecanisme subjacent entre ells. D'una banda, les persones que se senten soles semblen tenir habilitats socials més pobres (Cacioppo et al., 2006) i també un menor volum de substància grisa a àrees cerebrals relacionades amb el processament social (Kanai et al., 2012). Per tant, la solitud es podria reflectir en sistemes neuronals implicats en un funcionament social menys elaborat, amb una reserva cognitiva i neuronal reduïda. Açò implicaria menys capacitat per compensar el dany neuronal en altres sistemes neuronals (a causa de factors relacionats amb l'edat o de patologia cerebral). D'altra banda, la patologia cerebral podria estar afectant les xarxes neuronals relacionades amb el processament socioemocional i, per tant, amb la solitud. Pel que fa a la relació entre la solitud i l'SCD, els resultats van mostrar que la solitud, la simptomatologia depressiva i els biomarcadors de la patologia de l'AD i la CVD contribueixen als dos subtipus d'SCD. Tanmateix, la solitud no juga un paper important explicant l'SCD quan la simptomatologia depressiva i els biomarcadors s'inclouen en un model competitiu. En conclusió, aquest estudi mostra que la solitud té el potencial de detectar risc de deteriorament cognitiu a causa de patologies cerebrals. L'associació entre la solitud i la simptomatologia depressiva s'ha de dilucidar encara més, especialment en el context de l'AD i la CVD i en etapes primerenques d'aquestes malalties, com ara l'SCD.

En general, aquesta tesi doctoral afegeix evidències rellevants a la literatura centrada en l'estudi de la solitud en relació amb diferents estressors i factors de salut en persones sanes joves, de mitjana edat i d'edat avançada. S'ha vist que la solitud és un sentiment que es pot relacionar amb experiències adverses en etapes molt primerenques de la vida i que pot provocar una major percepció d'estrès en l'edat adulta. En persones de mitjana edat i majors, podrien ser diferències de sexe en la relació de la solitud amb una pitjor salut psicològica subjectiva, ja que aquesta associació apareix només en els hòmens. La solitud sembla no estar relacionada ni amb una pitjor salut física subjectiva ni amb una desregulació del funcionament basal de l'eix HPA tal com s'ha avaluat ací, la qual cosa suggereix possibles factors resilients que actuen com a amortidor. Finalment, cal destacar el potencial de la solitud per ser entesa com un símptoma neuropsiquiàtric precoç de l'AD i la CVD preclíniques, encara que calen més investigacions per entendre amb més profunditat la seua relació amb la simptomatologia depressiva, la SCD i les patologies cerebrals relacionades amb l'AD i les CVD.

Limitacions i fortaleses

En cadascun dels estudis s'han detallat i discutit les limitacions específiques i ací es presenten alguns dels aspectes generals a tenir en compte. En primer lloc, el disseny transversal dels estudis fa impossible concloure sobre les relacions causals. En segon lloc, en els tres estudis hem avaluat els principals potencials factors de confusió descrits a la literatura, però podria ser que altres variables estranyes tinguen un paper important i que hi hagen explicacions alternatives als resultats. La mostra dels estudis van ser persones sanes, ja que vam excloure molts potencials participants aplicant criteris d'exclusió rigorosos basats en la presa de medicaments i la salut general. Açò ens va permetre obtenir una mostra homogènia en la qual les malalties i factors relacionats amb l'estat de salut no

eren una variable de confusió, tot i que, alhora, açò dificulta la generalització dels resultats.

Entre els punts forts d'aquesta tesi doctoral, cal destacar la rellevància d'estudiar la solitud i les seues relacions amb factors estressants i de salut específics, ja que és un tema de salut mental a la nostra societat que està rebent més atenció en els darrers anys. Hem estudiat la solitud en persones amb una franja d'edat àmplia, la qual cosa ens permet tenir una informació més àmplia de com ens afecta aquest sentiment en les diferents etapes de la nostra vida. A més, en aquesta tesi doctoral s'han inclòs persones de diferents nacionalitats, fet que facilita una major comprensió de la influència de la solitud en les diferents cultures. Pel que fa a la metodologia, vam ser estrictes en la recollida i/o selecció de mostres de saliva per obtenir nivells de cortisol principalment no esbiaixats (Adam et al., 2017; Stalder et al., 2016). Així mateix, hem utilitzat anàlisis estadístiques complexes i sofisticades, com ara anàlisis de regressió de mediació o moderació mitjançant tècniques de bootstrapping o l'ús d'anàlisis de classificació de boscs aleatòris, que ens permeten aconseguir una major profunditat d'anàlisi i abordar els nostres objectius de manera més concisa.

Orientacions futures

Els resultats d'aquesta tesi doctoral afegeixen evidència a l'estat actual de la literatura, i obren la porta a nous estudis en aquest camp emergent de recerca.

Es necessiten estudis longitudinals que comencen en etapes molt primerenques per abordar més profundament l'associació entre les experiències adverses i els sentiments de solitud, incloent l'efecte i l'evolució dels estils d'aferrament. Hem vist que la solitud no estava relacionada amb la salut física subjectiva i el funcionament basal de l'eix HPA,

contrari al que s'esperava. Suggerim ampliar l'estudi d'aquestes relacions per tal d'identificar factors resilients que influeixen en aquesta no associació. A més, es podrien estudiar diferents formes de socialització en hòmens i dones, així com els valors relacionats amb la masculinitat per tal d'explicar com poden contribuir a la relació entre la solitud i la salut psicològica. Pel que fa a les mostres de cortisol, donada la naturalesa relativament estable de la solitud, valdria la pena estudiar la solitud amb una mesura estable de cortisol acumulat a llarg termini. Les mostres de cortisol del cabell serien una opció vàlida, ja que poden reflectir la secreció integrada de cortisol durant diverses setmanes o mesos (Iob i Steptoe, 2019; Stalder i Kirschbaum, 2012). Finalment, calen més investigacions i estudis longitudinals per entendre completament la naturalesa de la relació entre la solitud, la simptomatologia depressiva, l'SCD, els biomarcadors d'AD i CVD, considerant diferents tipus de mesurar de la patologia cerebral i la solitud. Finalment, calen més investigacions i estudis longitudinals per entendre completament el paper de la solitud en l'SCD i en la patologia de l'AD i la CVD, considerant la seua relació amb la simptomatologia depressiva.

Conclusions principals

1) L'ELS està relacionat amb l'estrès percebut, però no amb el funcionament basal de l'eix HPA (cortisol diürn general, DCS i cortisol abans d'anar a dormir) durant l'edat adulta en persones joves i de mitjana edat.

2) La solitud media la relació entre l'ELS i l'estrès percebut durant l'edat adulta en persones joves i de mitjana edat.

3) La solitud no media la relació entre l'ELS i el funcionament basal de l'eix HPA (cortisol diürn general, DCS i cortisol abans d'anar a dormir) en persones joves i de mitjana edat.

4) La solitud s'associa amb pitjor salut psicològica subjectiva, però no s'associa amb la salut física subjectiva en persones de mitjana edat i gent gran. Aquesta associació només es produeix en hòmens.

5) La solitud no està relacionada amb el funcionament basal de l'eix HPA (CAR, DCS i cortisol abans d'anar a dormir) en persones de mitjana edat i edat avançada.

6) El funcionament basal de l'eix HPA (CAR, DCS i cortisol abans d'anar a dormir) no juga un paper rellevant com a mecanisme subjacent a la relació entre la solitud i la salut psicològica i física subjectiva en persones de mitjana edat i edat avançada.

7) La solitud reflexa patologia de l'AD i la CVD i, especialment, simptomatologia depressiva en persones sanes de setanta anys sense alteracions cognitives.

8) La solitud contribueix a l'SCD, encara que no és independent de la simptomatologia depressiva i dels nivells de beta amiloide en persones de setanta anys sense alteracions cognitives.

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