International Journal of Psychophysiology 166 (2021) 127–133

Contents lists available at ScienceDirect

International Journal of Psychophysiology

journal homepage: www.elsevier.com/locate/ijpsycho

Diurnal cortisol secretion and health-related quality of life in healthy older people

Vanesa Hidalgo [a,b,*], Matias M. Pulopulos [a,b], Sara Puig-Perez [c], Teresa Montoliu [b], Alicia Salvador [b]

[a] Department of Psychology and Sociology, Area of Psychobiology, University of Zaragoza, 44003 Teruel, Spain
[b] Laboratory of Social Cognitive Neuroscience, Department of Psychobiology, IDOCAL, University of Valencia, 46010 Valencia, Spain
[c] Research Group of Psychology and Quality of Life, Valencian International University, 46002 Valencia, Spain

ARTICLE INFO

Keywords:
Quality of life
Salivary cortisol
Age
Sex
Health

ABSTRACT

Several studies have demonstrated that a dysregulated hypothalamic-pituitary-adrenal (HPA) axis is related to worse health status (e.g., depression, posttraumatic stress, or diabetes, among others). However, less is known about the association between the individual's perception of their own health status and HPA-axis functioning in healthy older people. The aim of the present study was to examine the relationship between HPA-axis functioning and health-related quality of life (HRQoL) in healthy older people. To do this, 140 healthy older people (69 men and 71 women) from 56 to 76 years old collected eight saliva samples on two consecutive weekdays to measure the diurnal cortisol cycle (i.e. awakening cortisol levels, cortisol awakening response (CAR), overall morning cortisol levels, change in the cortisol levels during the day, and bedtime cortisol levels). In addition, they completed the SF-36 questionnaire to obtain a measure of HRQoL (i.e. reflecting physical and mental functional health status). Results showed that higher awakening and bedtime cortisol levels and the CAR were associated with a better perception of both physical and mental health. In addition, the wake-to-bed cortisol slope was only positively related to physical health. No sex differences were found. These findings suggest that the awakening and bedtime cortisol levels and the CAR are the most relevant indices of diurnal cortisol secretion for understanding the relationship between HPA-axis functioning and HRQoL status in older people.

1. Introduction

There are large individual differences in people's health status during the aging process. Thus, some older people maintain good physical and mental health capacities, whereas others experience a significant decline (World Health Organization, 2018). It has been suggested that hypothalamus-pituitary-adrenal axis (HPA-axis) functioning could be a key factor in explaining part of this diversity in the effects of aging. The HPA-axis affects health through the action of cortisol, its end product. In addition to being involved in the acute and chronic stress response, the cortisol hormone participates in several bodily processes, such as glucose production, fat metabolism, inflammatory responses, and vascular responsiveness (McEwen, 1998), which have strong effects on health (Adam and Kumari, 2009).

In healthy conditions and basal or non-stress situations, cortisol follows a circadian secretion pattern. Thus, immediately after awakening, an abrupt increase in cortisol levels occurs, reaching a peak 30–45 min later, known as the Cortisol Awakening Response (CAR; Pruessner et al., 1997; Wüst et al., 2000). Afterwards, cortisol levels gradually decrease, reaching their lowest levels at the end of the day. Therefore, two discrete components are distinguished in this circadian secretion pattern: the CAR and a steeper decrease in cortisol levels during the rest of the day, known as the diurnal cortisol slope (DCS) (Adam et al., 2017; Fries et al., 2009). However, under unhealthy conditions (i.e. depression, posttraumatic stress, diabetes, among others), cortisol secretion could follow an abnormal/dysregulated pattern (i.e. excessive or deficient) with detrimental effects on mental and physical health (Adam et al., 2017; Chrousos, 2009). Therefore, the health status of the HPA-axis could help to understand age-related health differences.

Although the HPA-axis has been associated with objective health outcomes, less is known about its relationship with subjective health outcomes. In this area, health-related quality of life (HRQoL) is a

* Corresponding author at: Department of Psychology and Sociology (area of Psychobiology), University of Zaragoza, Campus Ciudad Escolar, s/n, 44003 Teruel, Spain.
E-mail address: vhidalgo@unizar.es (V. Hidalgo).

https://doi.org/10.1016/j.ijpsycho.2021.05.012
Received 10 January 2021; Received in revised form 13 May 2021; Accepted 31 May 2021
Available online 9 June 2021

measure of individuals' perceptions of their own health status, and it is an important construct that has been related to health outcomes and mortality (Idler and Benyamini, 1997). Specifically, HRQoL is a multidimensional outcome that includes the sense of well-being, physical, emotional, and social functioning, and the ability to function and perform the typical activities of daily life (Naughton et al., 1996). Among the questionnaires used to assess HRQoL, the Short Form-36 Health Survey (SF-36) stands out due to its many advantages. For example, it can be applied to both healthy people and patients with any disease, and it makes it possible to compare different health profiles. Moreover, because it includes several dimensions, it captures different health states and identifies which areas of quality of life affect a particular individual more (Ruiz and Pardo, 2005).

Previous studies carried out in both clinical and healthy samples have examined the association between HPA-axis functioning and SF-36 scores. A positive association has been reported in several studies with clinical samples, such as women with endometriosis (Petrelluzzi et al., 2008; van Aken et al., 2018) or patients with functional somatic syndrome (Mutsuura et al., 2009), but no association was found in patients with adrenal insufficiency (Andela et al., 2016). In studies with healthy people, the results are less clear. Thus, positive associations have been found between Sf-36 and blood (Peixoto et al., 2019) and urine (Ragnarsson et al., 2015) cortisol levels. In contrast, negative associations were reported when cortisol levels were obtained from saliva samples (Lindeberg et al., 2008; Mutsuura et al., 2009), and no relationships were found in saliva (Hagger-Johnsons et al., 2010), blood (Ragnarsson et al., 2015), and hair (van Aken et al., 2018) cortisol samples. The lack of homogeneity in the type of cortisol sample and, therefore, the different indices used across the studies could help to explain these mixed results. It is important to note that blood, urine, and hair cortisol samples are not used to distinguish the two dynamic components of diurnal cortisol secretion (i.e. CAR and diurnal cortisol slope) because these components can only be measured by employing salivary cortisol samples. However, in the studies mentioned above that used salivary cortisol samples, the data are quite limited because they did not employ multiple sampling points during the day, as in Mutsuura et al. (2009), who only collected cortisol levels 30 min post awakening, or in studies that only included the CAR index (Hagger-Johnsons et al., 2010; Lindeberg et al., 2008).

In addition, the age of the participants is also an important factor to consider. Studies exploring the association between HPA-axis functioning and HRQoL have been carried out in young samples (van Aken et al., 2018; Hagger-Johnsons et al., 2010), middle-aged samples (Peixoto et al., 2019), or mixed-age samples with a wide age range (Lindeberg et al., 2008: 26–63 years; Mutsuura et al., 2009: 21–64 years; Ragnarsson et al., 2015: 38–77 years). However, although a dysregulated HPA-axis pattern has been described in older people (Heaney et al., 2010), the relationship between diurnal cortisol secretion and HRQoL in healthy older people has not been specifically studied. In our opinion, analyzing this association in the older population could help to understand the mechanisms underlying age-related health differences in the aging process. In addition, drawing on Hagger-Johnsons et al. (2010), if the diurnal cortisol cycle indices are related to HRQoL in healthy older people, these findings would support the idea that HPA-axis functioning, in addition to being associated with age-related diseases, would also be a good indicator of the perception of health and functional status.

Therefore, the aim of the present study was to investigate the relationship between diurnal cortisol secretion and HRQoL in healthy older people. To do this, we jointly considered the two discrete components of diurnal HPA-axis activity, that is, the CAR and DCS, as well as other cortisol indices such as awakening and overall morning and bedtime cortisol levels. Thus, 140 healthy older men and women provided eight saliva samples on two consecutive weekdays to obtain the diurnal cortisol secretion pattern, and they completed the SF-36 to assess their HRQoL. Additionally, we aimed to explore whether there are sex differences in these relationships. We hypothesized that dysregulated HPA-axis functioning, that is, a flattened CAR and/or diurnal cortisol slope, lower awakening and overall morning cortisol, and higher bedtime cortisol levels would be related to worse HRQoL. However, no hypotheses are made about sex due to the lack of prior evidence.

2. Method

2.1. Participants

The sample was composed of 140 participants, 69 men and 71 women from 56 to 76 years old (Men: $M = 64.75$, $SD = 4.539$; Women: $M = 64.56$, $SD = 4.013$). There were no sex differences in age, subjective socioeconomic status (SES; MacArthur Scale of Subjective Social Status, Adler et al., 2000), or educational level (all $p > 0.173$). However, men had a higher mean Body Mass Index (Men: $M = 28.21$, $SD = 3.4$; Women: $M = 26.80$, $SD = 4.6$, $p = 0.041$). Table 1 shows the characteristics of the total sample.

All participants were volunteers recruited in university courses and seminars offered by the University of Valencia for people over 55 years old. Only those who met the study prerequisites were selected to participate after they had completed a general questionnaire by phone. The exclusion criteria were as follows: (i) smoking more than ten cigarettes per day, (ii) alcohol or other drug abuse, (iii) endocrine diseases that affect HPA-axis functioning, (iv) psychiatric illness, (v) taking medication that can influence hormone levels, and (vi) having been under general anesthesia in the past year. All the women were postmenopausal, with a minimum of two years since their last menstrual period and not receiving hormone replacement therapy.

All participants were informed about the study procedure verbally and in writing, and then they signed the informed consent. The protocol of the present study was approved by the Ethics Research Committee of the University of Valencia, and the study was conducted taking into account the Declaration of Helsinki.

2.2. Measurements

2.2.1. Short Form-36 Health Survey (SF-36)

To assess HRQoL, we used the Spanish version of the SF-36 (Alonso et al., 1995). The SF-36 assesses generic HRQoL, and it has been shown to be reliable and valid (Ware et al., 1993). It consists of 36 items related to different physical and mental health aspects, grouped in eight

Table 1
Characteristics of the sample.

	Mean (*SD*)
Age (years)	64.66 (27)
BMI (kg/m^2)	27.49 (4.10)
SES	5.06 (1.99)
Educational level	2.71 (1.13)
PSS	16.32 (5.63)
PCS (%)	79.56 (11.97)
MCS (%)	80.55 (10.27)
Awakening cortisol levels (nmol/L)	9.6 (5.64)
Cortisol levels 30′ after awakening (nmol/L)	14.58 (6.80)
Cortisol levels 45′ after awakening (nmol/L)	15.55 (6.46)
Bedtime cortisol levels (nmol/L)	2.68 (7.83)
CAR (nmol/L)	588.73 (260. 34)
$AUCg_{morning}$ (nmol/L)	156.69 (131.31)
Wake-to-bed cortisol levels (nmol/L)	6.92 (8.35)
Wake-up time (hh:mm)	7:23 (1:01)
Wake-to-bed time (hh:mm)	16:51 (1:01)
Go-to-bed time (hh:mm)	0:10 (1:07)

BMI: Body Mass Index; SES: subjective socioeconomic status; educational level (range: 0 = no studies, 1 = primary school, 2 = secondary education, 3 = university and higher education, 4 = postgraduate); PSS: Perceived Stress Scale; PCS: physical component score; MCS: mental component score; CAR: cortisol awakening response; $AUCg_{morning}$: cortisol area under the curve with awakening and 30 min and 45 min post-awakening saliva samples.

subscales: (i) physical functioning, (ii) role-physical, (iii) bodily pain, (iv) general health, (v) vitality, (vi) social functioning, (vii) role-emotional, and (viii) mental health. These eight subscales are grouped, in turn, into two components: the physical component summary (PCS; physical functioning, role-physical, bodily pain, general health, and vitality) and the mental component summary (MCS; general health, vitality, social functioning, role-emotional, and mental health). The raw scores were transformed into a 0–100 scale, so that higher scores indicate better HRQL. In the present study, Cronbach's α was 0.88, indicating high internal scale reliability.

2.2.2. Salivary cortisol

To measure the diurnal cortisol cycle, participants provided four saliva samples per day on two consecutive weekdays, using salivettes (Sarstedt, Nümbrecht, Germany) at home. The saliva samples were provided immediately after awakening, 30 min and 45 min post-awakening, and immediately before bedtime. In order to check whether the second and third salivary samples were collected 30 min and 45 min post-awakening, the salivettes were stored in MEMS TrackCap containers (MEM 6 TrackCap Monitor, Aardex Ltd., Switzerland). Moreover, participants completed a log with their awakening time, bedtime, and time of each saliva collection.

Participants received instructions about how to provide the saliva samples in a demonstration by the experimenter in the lab. Moreover, they received written instructions and were advised to drink only water and not eat, smoke, or brush their teeth at least 1 h before providing each saliva sample. After providing the saliva samples, participants stored them in their refrigerator and took them to the university as soon as possible within three days of completion. These storage conditions ensure the stability of saliva cortisol concentrations (Garde and Hansen, 2005; Nalla et al., 2015), and they follow the consensus guidelines for the assessment of the CAR (Stalder et al., 2016).

Once in the lab, the samples were centrifuged at 4000 rpm for 15 min to obtain a clear supernatant, and they were frozen at −80 °C until the biochemical analyses were carried out in the central Research Unit of the Faculty of Medicine at the University of Valencia (Spain). Cortisol concentrations were determined by radioimmunoassay with the commercial kit Spectria Cortisol RIA from Orion Diagnostica (Espoo, Finland). All samples were analyzed in duplicate, and each participant's samples were analyzed in the same trial. Assay sensitivity was 0.8 nmol/L, and the within- and inter-assay variation coefficients were all below 8%.

2.2.3. Demographic, health-related, and stress measurements

Participants were asked to complete a questionnaire on demographic and health-related characteristics (e.g., age, smoking status, physical activity), and they filled out the Spanish version (Remor, 2006) of the Perceived Stress Scale (PSS; Cohen et al., 1983). The PSS is a 14-item questionnaire that assesses the degree to which life situations that occurred in the previous month are appraised as stressful. Participants answered using a 5-point Likert scale ranging from 1 (“never”) to 5 (“very often”). Thus, higher scores indicate more perceived stress. The internal consistency was adequate (α = 0.78).

2.3. Statistical analyses and data management

To investigate sex or group differences in the demographic variables, student's *t*-tests or one-way ANOVA, respectively, were used, except for educational level, which was investigated using X^2.

From the saliva samples collected, five different components of the diurnal cortisol cycle were used in the analyses: (i) Awakening, the first saliva sample collected immediately post-awakening; (ii) CAR, calculated by the cortisol area under the curve with respect to the increase (AUCi; Pruessner et al., 2003); (iii) $AUCg_{morning}$, the cortisol area under the curve with respect to the awakening and 30 min and 45 min post-awakening saliva samples, an index that reflects overall cortisol secretion during the first 45 min; (iv) the Wake-to-bed cortisol slope, calculated as the difference between the awakening and bedtime saliva samples (Adam et al., 2017); and (v) Bedtime cortisol, the last saliva sample of the day.

To investigate differences in the diurnal cortisol cycle across days and between sexes, an ANOVA for repeated measures was performed, with Time (Awakening, 30 min, 45 min and Bed) as within-subject factor and Sex (men and women) as a between-subject factor. When the requirement of sphericity in the ANOVA was violated, the Greenhouse-Geisser adjustment was used. *Post-hoc* planned comparisons were performed using Bonferroni adjustments for the *p* values. Because the residual values were not normal (all $p < 0.008$), the cortisol values used in the ANOVA for repeated measures were log transformed.

Pearson's correlation was used to investigate the unadjusted association between the covariates, the cortisol indices, and the two HRQL summary components. Regression analyses were performed to investigate whether the different indices of the diurnal cortisol cycle were related to HRQL, controlling for possible confounders. Separate analyses were conducted for each summary component. In Step 1, the following variables were included: age, BMI, SES, PSS, and the wake-up time (when the cortisol awakening, CAR or $AUCg_{morning}$ indices were investigated), the wake-to-bed time (when wake-to-bed was included), or the go-to-bed time (when the cortisol bedtime was investigated), and the cortisol awakening, CAR, $AUCg_{morning}$, Wake-to-bed, or bedtime cortisol. Moreover, in Step 2, the product of Sex and the cortisol index included in Step 1 was added in order to analyze whether sex moderated these relationships, according to the procedure described by Aiken and West (1991). Because the residual values followed a normal distribution (all $p > 0.07$), the cortisol values were not transformed for these analyses. The variable time, in each case, was introduced as covariate as a decimal numeral, using the following formula: Time (hours:minutes:seconds) × 24.

Three participants were excluded from the analyses due to: (i) being a multivariate outlier for cortisol levels, (i) having missing data for the cortisol sample time recollection, and (iii) having missing data for two items on the SF-36 questionnaire. In addition, two bedtime cortisol values were not detected by the kit. Therefore, for these two participants, we only considered the cortisol values of the day, without including the undetectable samples.

All *p*-values reported are two-tailed, and the level of significance was set at $p = 0.05$. The results shown are means ± standard error of mean (SEM). To aid the interpretation of the figures, the values represented are raw values and not log-transformed values. Statistical analyses were performed with SPSS 22.0.

3. Results

3.1. Preliminary analyses

As indicated in previous studies, a delay in the collection of the first sample could compromise the reliability of the CAR measurement (Thorn et al., 2006; Stalder et al., 2016). To control this issue, based on previous studies carried out by our team (Almela et al., 2012: Hidalgo et al., 2016; Puig-Perez et al., 2016; Pulopulos et al., 2016a, 2016b) and other research groups (O'Connor et al., 2009; Thorn et al., 2006; Walker et al., 2011), the participants were divided into three groups: (i) 2-Day CAR group: those who had a positive CAR (i.e. AUCi >0) on both days, (ii) 1-Day CAR group: those who had a positive CAR on only one day, and (iii) 0-Day CAR group: those participants who had a negative CAR on both days. A total of 83 participants (33 men and 50 women) showed a positive CAR on both days, 47 participants (28 men and 19 women) showed a positive CAR on only one day, and 10 participants (8 men and 2 women) did not show a positive CAR on any day.

There were no significant differences between the three groups in: age, BMI, SES, educational level, PSS, PCS, MCS, or bedtime (all $p > 0.247$). However, there were significant group differences in wake-up

time ($p = 0.008$) and trending differences in the time between waking-up and going to bed ($p = 0.073$). In the 0-Day CAR group, the wake-up time was later than in the other two groups (both $p < 0.009$), and the time between waking-up and bedtime was trending lower than in the 2-Day CAR group ($p = 0.069$), but not lower than in the 1-Day CAR group ($p = 0.196$). No differences in these two variables were found between the 2-Day CAR group and the 1-Day-CAR group (both $p > 0.99$). Therefore, we decided to exclude the 0-Day CAR group from the statistical analysis because they did not present the expected CAR on any day. In addition, based on previous research on the 2-Day CAR, the data for the two days were averaged to compute the cortisol indices and the time outcomes, and in the 1-Day CAR group, the data for the day that showed a positive CAR were selected (Pulopulos et al., 2016a, 2016b).

3.2. Diurnal cortisol secretion

The repeated-measures ANOVAs showed a main effect of Time ($F(1.95, 249.85) = 1099.41$, $p < 0.001$). Participants showed an increase from the awakening to +30 min saliva samples ($p < 0.001$), reaching the maximum peak 45 min after awakening ($p = 0.001$). Then, cortisol levels decreased, reaching the lowest levels in the bedtime sample (all $p < 0.001$). The Sex factor ($F(1, 128) = 0.12$, $p = 0.726$) and the interaction between Time and Sex ($F(1.95, 249.85) = 2.41$, $p = 0.093$) were not significant (Fig. 1).

3.3. Diurnal cortisol secretion and the HRQoL: correlation analyses

Table 2 shows the results of the unadjusted correlation analyses. Among the most important results, PCS was positively related to CAR ($r = 0.222$, $p = 0.013$), bedtime cortisol levels ($r = 0.189$, $p = 0.034$), and bedtime ($r = 0.276$, $p = 0.002$). In addition, PCS was also negatively related to BMI ($r = -0.193$, $p = 0.031$) and PSS ($r = -0.450$, $p < 0.001$). However, MCS was only positively related to bedtime ($r = 0.186$, $p = 0.037$) and negatively to PSS ($r = -0.619$, $p < 0.001$).

3.4. Diurnal cortisol secretion and the HRQoL: regression analyses

Table 3 shows the results of the regression analyses, controlling for possible confounders (i.e., age, BMI, SES, PSS and the wake-up, wake-to-bed, and bedtime).

Regression analyses showed positive relationships between the PCS and all the cortisol indexes assessed, awakening ($p = 0.006$), CAR ($p = 0.001$), wake-to-bed ($p = 0.027$) and bedtime ($p = 0.003$), except $AUCg_{morning}$ ($p = 0.109$). Sex did not moderate any of the relationships studied (all $p > 0.191$).

Similarly to PCS, MCS was positively associated with cortisol levels on awakening ($p = 0.032$), CAR ($p = 0.005$) and at bedtime ($p = 0.004$).

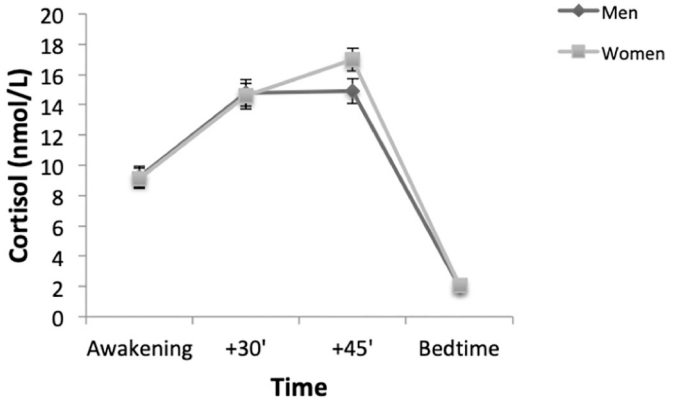

Fig. 1. Diurnal cortisol pattern for men and women. Depicted values are means, and error bars represent the SEM.

Table 2
Unadjusted correlation analyses.

	PCS	MCS	Awak	CAR	$AUCg_{morning}$	Wake-to-bed	Bedtime	Age	BMI	SES	PSS	Wake-up time	Go-to-bed time
PCS													
MCS	0.842**												
Awak.	0.173#	0.076											
CAR	0.222*	0.114	0.903**										
$AUCg_{morning}$	0.142	0.103	−0.051	0.384**									
Wake-to-bed	0.130	0.035	0.965**	0.863**	−0.068								
Bedtime	0.189*	0.159#	0.315**	0.313**	0.050	0.053							
Age	−0.144	−0.070	0.074	0.007	−0.142	0.041	0.131						
BMI	−0.193*	0.088	−0.103	−0.150	−0.128	−0.107	−0.003	0.186*					
SES	0.085	0.122	0.097	0.138	0.111	0.074	0.099	−0.120	−0.054				
PSS	−0.450**	−0.619**	0.134	0.136	0.030	0.129	0.043	0.002	0.145	−0.040			
Wake-up time	0.007	−0.059	0.240**	0.262**	0.094	0.222*	0.108	0.049	−0.069	−0.129	0.077		
Go-to-bed time	0.276**	0.186*	0.090	0.108	0.057	0.083	0.044	0.057	−0.104	−0.008	−0.110	0.170	
Wake-to-bed time	0.039	0.060	−0.194*	−0.223*	−0.101	−0.183*	−0.076	−0.069	0.140	0.070	−0.022	−0.753**	0.258**

PCS: physical component score; MCS: mental component score; CAR: cortisol awakening response; $AUCg_{morning}$: cortisol area under the curve with awakening, and 30 min and 45 min post-awakening saliva samples; BMI: Body Mass Index; SES: subjective socioeconomic status; PSS: Perceived Stress Scale.

** $p < 0.01$.

* $p < 0.05$.

# $p < 0.10$.

However, no relationships were found between this component and the $AUCg_{morning}$ ($p = 0.101$) and wake-to-bed ($p = 0.122$) indexes. No sex differences were found in any relationship (all $p > 0.394$).

4. Discussion

The goal of the present study was to examine the relationship between HPA-axis functioning and health-related quality of life (HRQoL) in healthy older men and women. To do so, participants provided eight saliva samples on two consecutive weekdays that were used to calculate different components of the diurnal cortisol secretion (i.e. awakening, CAR, $AUCg_{morning}$, wake-to-bed, and bedtime). In addition, they completed the SF-36 questionnaire to assess their individual perceptions of their own physical (i.e., PCS) and mental (i.e., MCS) health status. We found that the awakening, CAR, and bedtime cortisol levels were positively associated with the perception of both physical and mental health. In addition, the wake-to-bed cortisol slope was only positively related to physical health. Finally, sex did not moderate these associations.

Our results showed that both awakening and CAR were related to a higher perception of physical and mental health-related quality of life. If we consider higher morning cortisol and CAR to be indices of regulated HPA-axis functioning, taking into account the circadian rhythm of cortisol secretion in basal situations and healthy conditions (Stone et al., 2001), then these results confirm our hypothesis. These findings agree with previous studies showing a positive relationship between the magnitude of CAR and physical and mental health outcomes in older people (Fries et al., 2009; Almela et al., 2012; Evans et al., 2012; Pulopulos et al., 2016a, 2016b). In line with our results, the magnitude of CAR has previously been related to physical health outcomes, such as cardiovascular activity and sleep (Fries et al., 2009) and walking speed (Pulopulos et al., 2016b), an objective measure of physical performance related to executive function (Cooper et al., 2011; Sustakoski et al., 2015). Regarding mental health, our results contribute to confirming its relationship with the magnitude of CAR, as in other studies showing its relationship with working memory (Almela et al., 2012) or executive functions (Evans et al., 2012; Pulopulos et al., 2016a). However, it is important to note that Pulopulos et al. (2016b) failed to find an association between awakening cortisol levels and walking speed. Hence, more research is needed to clarify the relationship between awakening cortisol levels and physical and mental outcomes in older people.

Regarding studies investigating HRQoL outcomes, our results contrast with those found by Mutsuura et al. (2009) in healthy people. These authors showed a negative correlation between the cortisol levels 30 min after awakening and the mental component score on the SF-8. It is possible that these contradictory findings can be explained by methodological differences, such as the number of saliva samples collected, the age of the participants studied, and the sample size. Thus, Mutsuura et al. (2009) only collected one saliva sample 30 min after awakening in 29 people from 21 to 64 years old. In contrast, in the current study, we collected three saliva samples (awakening and 30 and 45 min after awakening) on two consecutive weekdays to measure the cortisol morning levels in 140 older people from 56 to 76 years old. Future studies should investigate the involvement of these factors in the relationship between morning cortisol levels and HRQoL.

In contrast to awakening and CAR, the $AUCg_{morning}$ index was not related to the two health components. This result may seem contradictory because $AUCg_{morning}$ is calculated from morning cortisol levels (i.e. awakening and 30 and 45 min after awakening samples), as is CAR. However, the regulatory mechanism underlying the CAR is independent from cortisol output the rest of the day (Chida and Steptoe, 2009; Edwards et al., 2001), and so the CAR is considered an independent component of HPA-axis functioning (Fries et al., 2009; Clow et al., 2010a, 2010b). Therefore, its relationship with these two subjective health outcomes may be different from that of the overall morning cortisol levels. It is important to note that, despite the evidence indicating that the magnitude of the CAR may be affected by the health status (for a review see: Fries et al., 2009), to the best of our knowledge, this is the first study to investigate the relationship between the CAR and HRQoL, measured with the SF-36 questionnaire, in a sample only consisting of older people. More research including different morning cortisol indices is warranted.

Table 3
Regression analyses with the indexes of diurnal cortisol secretion as predictors and the summary component outcomes of the SF-36 as dependent variable.

	PCS			MCS		
	Adj R^2	β	*p*	Adj R^2	β	*p*
Awakening	0.329	0.216	0.006	0.460	0.152	0.032
CAR	0.349	0.265	0.001	0.474	0.198	0.005
$AUCg_{morning}$	0.300	0.124	0.109	0.451	0.112	0.101
Wake-to-bed	0.311	0.173	0.027	0.450	0.107	0.122
Bedtime	0.379	0.220	0.003	0.488	0.190	0.004

PCS: physical component score; MCS: mental component score; CAR: cortisol awakening response; $AUCg_{morning}$: cortisol area under the curve with awakening and 30 min and 45 min post-awakening saliva samples.

We expected that a flatter wake-to-bed cortisol slope and higher cortisol at bedtime, as indicators of dysregulated HPA-axis functioning, would be related to a worse HRQoL status or vice versa. In this regard, we partially confirmed this hypothesis because we found a positive relationship between the wake-to-bed cortisol slope and the physical health component. This result is in line with a previous study that reported a negative correlation between exhaustion (measured with the inverted SF-36 vitality score) and diurnal cortisol variability (i.e. the difference between the maximum morning cortisol concentration, between the awakening and 30 min after awakening cortisol levels, and the evening cortisol levels) in healthy people (Lindeberg et al., 2008). Accordingly, a recent review and meta-analysis indicated that there was a significant association between flatter diurnal cortisol slopes and poorer health across all the studies included (Adam et al., 2017). Our results confirm the findings of Pulopulos et al. (2016b), who failed to show an association between the awakening cortisol slope and physical status (walking speed) in older people. However, they do not agree with Hagger-Johnsons et al. (2010), who found a significant relationship between the DCS and the mental component of HRQoL in healthy young men and women, although this relationship did not remain significant when they controlled for age, sex, and waking time.

In addition, it is important to note that the wake-to-bed cortisol slope was only related to the physical component, but not to the mental health component. It is possible that this cortisol index is more sensitive to physical health outcomes than to mental health outcomes, at least when these health indicators are assessed subjectively with the SF-36 questionnaire. In fact, in a previous study carried out in a sample of healthy older people, we did not find any association between the diurnal cortisol slope and different memory domains such as verbal, visual, and working memory (Hidalgo et al., 2016), thus supporting the previously reported lack of association between the diurnal cortisol slope and memory performance (Beluche et al., 2010; Fiocco et al., 2006; Singh-Manoux et al., 2014; Stawski et al., 2011). Therefore, more research is needed to understand the possible predictor role of this cortisol secretion index and the HRQoL status in healthy older people.

Unexpectedly, the relationship between bedtime cortisol levels and the physical and mental health components was positive. That is, higher cortisol levels at night (a clear sign of deregulated HPA-axis functioning) were associated with higher scores on both health outcomes. In our opinion, given that our sample consisted of healthy older people, they may have had a better perception of their health. Physically they feel fine, are very active people, and take on new challenges, which could lead them to have higher cortisol levels at night. In this regard, we previously reported a positive relationship between hair cortisol levels and cognitive performance (e.g., working memory, learning, short-term and long-term verbal memory) in healthy older people (Pulopulos et al.,

2014). In any case, these results must be interpreted with caution because they seem counterintuitive. Some variables that were not considered in the current study may explain this relationship. Hence, further research should consider this HPA-axis functioning index in order to clarify this issue.

This study has important implications. First, our results show that some HPA-axis indices, such as awakening and bedtime cortisol levels and the CAR, are good biomarkers for the perception of physical and mental status in older people. Our findings also help to shed light on the functionality of indices such as the CAR. Second, our results indicate that the SF-36 is a subjective instrument that is able to capture the health status of older people associated with the HPA-axis functioning. This suggests that the SF-36 may offer important information as a screening measure to obtain an initial impression of people's health before other objective health measures that can only be evaluated by clinicians, thus simplifying the initial assessment protocols. Finally, understanding the associations between the HPA-axis and SF-36 in healthy older people could provide further knowledge about specific illnesses or other populations in which this association is studied.

One limitation of this study is that the correlational nature of the design does not allow us to draw causal conclusions. In addition, several strategies were used to control the participants' adherence to the sampling times. Thus, participants completed a diary log, and the times of the saliva sample collections were registered by the electronic monitoring containers. We only included participants with a positive CAR on one or two days. However, we did not use an objective measure of awakening to exclude sampling days with a delay in the collection of the first salivary sample. Finally, it is important to mention that quality of life was only obtained from a self-report questionnaire, as we intended, but further research would benefit from also including specific physical or mental objective measures, such as examinations by clinicians.

In conclusion, this study suggests that, in healthy older people, higher awakening, CAR, and bedtime cortisol levels are related to better physical and mental HRQoL, whereas a higher wake-to-bed cortisol slope is only associated with better physical HRQoL. These findings support the idea that the HPA-axis functioning indices could be good predictors of the perception of quality of life in healthy older people, and they highlight the need to continue to study the role of some of these indices.

Funding

This research study was supported by the Spanish Education and Science Ministry with grants no. PSI2016-78763-P and PSI2017-90806-REDT and by the Generalitat Valenciana no. PROMETEOII2015/20. Moreover, the contribution of V. Hidalgo has been supported by the Government of Aragón (Department of Innovation, Research and University) and FEDER “Construyendo desde Aragón” for the research group S31_20D.

Acknowledgements

The authors wish to thank Ms. Cindy DePoy for the revision of the English text.

References

Adam, E.K., Kumari, M., 2009. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 34, 1423–1436.
Adam, E.K., Quinn, M.E., Tavernier, R., McQuillan, M.T., Dahlke, K.A., Gilbert, K.E., 2017. Diurnal cortisol slopes and mental and physical health outcomes: a systematic review and meta-analysis. Psychoneuroendocrinology 83, 25–41.
Adler, N.E., Epel, E.S., Castellazzo, G., Ickovics, J.R., 2000. Relationship of subjective and objective social status with psychological and physiological functioning: preliminary data in healthy, white women. Health Psychol. 19, 586–592.
Aiken, L.S., West, S.G., 1991. Multiple Regression: Testing and Interpreting Interactions. Sage Publications, Inc., Thousand Oaks, CA, US.
Almela, M., van der Meij, L., Hidalgo, V., Villada, C., Salvador, A., 2012. The cortisol awakening response and memory performance in older men and women. Psychoneuroendocrinology 37, 1929–1940.
Alonso, J., Prieto, L., Anto, J.M., 1995. La versión española del SF-36 Health Survey (Cuestionario de Salud SF-36): un instrumento para la medida de los resultados clínicos. Med. Clin. (Barc.) 104, 771–776.
Andela, C.D., Staufenbiel, S.M., Joustra, S.D., Pereira, A.M., van Rossum, E.F.C., Biermasz, N.R., 2016. Quality of life in patients with adrenal insufficiency correlates stronger with hydrocortisone dosage, than with long-term systemic cortisol levels. Psychoneuroendocrinology 72, 80–86.
Beluche, I., Carrière, I., Ritchie, K., Ancelin, M.L., 2010. A prospective study of diurnal cortisol and cognitive function in community-dwelling elderly people. Psychol. Med. 40, 1039–1049.
Chida, Y., Steptoe, A., 2009. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. Biol. Psychol. 80 (3), 265–278.
Chrousos, G.P., 2009. Stress and disorders of the stress system. Nat. Rev. Endocrinol. 5 (7), 374–381.
Clow, A., Hucklebridge, F., Stalder, T., Evans, P., Thorn, L., 2010a. The cortisol awakening response: more than a measure of HPA axis function. Neurosci. Biobehav. Rev. 35 (1), 97–103.
Clow, A., Hucklebridge, F., Thorn, L., 2010b. The cortisol awakening response in context. Int. Rev. Neurobiol. 93, 153–175.
Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24 (4), 385–396.
Cooper, R., Kuh, D., Cooper, C., Gale, C.R., Lawlor, D.A., Matthews, F., Hardy, R., FALCon and HALCon Study Teams, 2011. Objective measures of physical capability and subsequent health: a systematic review. Age Ageing 40, 14–23.
Edwards, S., Evans, P., Hucklebridge, F., Clow, A., 2001. Association between time of awakening and diurnal cortisol secretory activity. Psychoneuroendocrinology 26 (6), 613–622.
Evans, P., Clow, A., Hucklebridge, F., Loveday, C., 2012. The cortisol awakening response is related to executive function in older age. Int. J. Psychophysiol. 84 (2), 201–204.
Fiocco, A.J., Wan, N., Weekes, N., Pim, H., Lupien, S.J., 2006. Diurnal cycle of salivarycortisol in older adult men and women with subjective complaints of memorydeficits and/or depressive symptoms: relation to cognitive functioning. Stress 9 (3), 143–152.
Fries, E., Dettenborn, L., Kirschbauem, C., 2009. The cortisol awakening response (CAR): facts and future directions. Int. J. Psychophysiol. 72, 67–73.
Garde, A.H., Hansen, A.M., 2005. Long-term stability of salivary cortisol. Scand. J. Clin. Lab. Invest. 65, 433–436.
Hagger-Johnsons, G.E., Whiteman, M.C., Wawrzyniak, A.J., Holroyd, W.G., 2010. The SF-36 component summary scales and the daytime diurnal cortisol profile. Qual. Life Res. 19, 643–651.
Heaney, J.L., Phillips, A.C., Carroll, D., 2010. Ageing, depression, anxiety, social support and the diurnal rhythm and awakening response of salivary cortisol. Int. J. Psychophysiol. 78, 201–208.
Hidalgo, V., Almela, M., Pulopulos, M.M., Salvador, A., 2016. Memory performance is related to the cortisol awakening response in older people, but not to the diurnal cortisol slope. Psychoneuroendocrinology 71, 136–146.
Idler, E.L., Benyamini, Y., 1997. Self-rated health and mortality: a review of twenty-seven community studies. J. Health Soc. Behav. 38, 21–37.
Lindeberg, S.I., Eek, F., Lindbladh, E., östergren, P.O., Hansen, A.M., Karlson, B., 2008. Exhaustion measured by the SF-36 vitality scale is associated with a flattened diurnal cortisol profile. Psychoneuroendocrinology 33, 471–477.
McEwen, B.S., 1998. Stress, adaptation, and disease: allostasis and allostatic load. Ann. N. Y. Acad. Sci. 840 (1), 33–44.
Mutsuura, H., Kanbara, K., Fukunaga, M., Yamamoto, K., Ban, I., Kitamura, K., Nakai, Y., 2009. Depression and anxiety correlate differently with salivary free cortisol in the morning in patients with functional somatic syndrome. Appl. Psychophysiol. Biofeedback 34, 291–298.
Nalla, A.A., Thomsen, G., Knudsen, G.M., Frokjaer, G., 2015. The effect of storage conditions on salivary cortisol concentrations using and enzyme immunoassay. Scand. J. Clin. Lab. Invest. 75, 92–95.
Naughton, M.J., Shumaker, S.A., Anderson, R.T., Czajkowski, S.M., 1996. In: Spilker, B. (Ed.), Quality of Life and Pharmacoeconomics in Clinical Trials, 2nd ed. Raven Press, New York, pp. 118–120.
O'Connor, D.B., Hendrickx, H., Dadd, T., Elliman, T.D., Willis, T.A., Talbot, D., Mayes, A. E., Thethi, K., Powell, J., Dye, L., 2009. Cortisol awakening rise in middle-aged women in relation to psychological stress. Psychoneuroendocrinology 34 (10), 1486–1494.
Peixoto, C., Carrilho, C.G., Ribeiro, T.T.S.B., da Silva, L.M., Gonçalves, E.A., Fernandes, L., Nardi, A.E., Cardoso, A., Veras, A.B., 2019. Relationship between sexual hormones, quality of life and postmenopausal sexual function. Trends Psychiatry Psychother. 41 (2), 136–143.
Petrelluzzi, K.F.S., Garcia, M.C., Petta, C.A., Grassi-Kassisse, D.M., Sapadari-Bratfisch, R. C., 2008. Salivary cortisol concentrations, stress and quality of life in women with endometriosis and chronic pelvic pain. Stress 11 (5), 390–397.
Pruessner, J.C., Wolf, O.T., Hellhammer, D.H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., Kaspers, F., Kirschbaum, C., 1997. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 61, 2539–2549.
Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28 (7), 916–931.

Puig-Perez, S., Almela, M., Pulopulos, M.M., Hidalgo, V., Salvador, A., 2016. Are neuroticism and extraversion related to morning cortisol release in healthy older people? Int. J. Psychophysiol. 110, 243–248.
Pulopulos, M.M., Hidalgo, V., Almela, M., Puig-Perez, S., Villada, C., Salvador, A., 2014. Hair cortisol and cognitive performance in healthy older people. Psychoneuroendocrinology 44, 100–111.
Pulopulos, M.M., Hidalgo, V., Puig-Perez, S., Salvador, A., 2016a. Cortisol awakening response and cognitive performance in hypertensive and normotensive older people. Horm. Behav. 83, 75–82.
Pulopulos, M.M., Puig-Perez, S., Hidalgo, V., Villada, C., Salvador, A., 2016b. Cortisol awakening response and walking speed in older people. PLoS ONE 11 (5), e0152071.
Ragnarsson, O., Trimpou, P., Oleröd, G., Landin-Wilhelmsen, K., 2015. The association between urinary cortisol excretion and cardiovascular risk factors, bone status and quality of life in the population. Steroids 101, 71–77.
Remor, E., 2006. Psychometric properties of a European Spanish version of the Perceived Stress Scale (PSS). Span. J. Psychol. 9 (1), 86–93.
Ruiz, M., Pardo, A., 2005. Calidad de vida relacionada con la salud: definición y utilización en la práctica médica. PharmacoEconomics-Spanish Research Articles 2 (1), 31–43.
Singh-Manoux, A., Dugravot, A., Elbaz, A., Shipley, M., Kivimaki, M., Kumari, M., 2014. No evidence of a longitudinal association between diurnal cortisolpatterns and cognition. Neurobiol. Aging 35 (10), 2239–2245.
Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D.H., Miller, R., Wetherell, M.A., Lupien, S.J., Clow, A., 2016. Assessment of the cortisol awakening response: expert consensus guidelines. Psychoneuroendocrinology 63, 414–432.
Stawski, R.S., Almeida, D.M., Lachman, M.E., Tun, P.A., Rosnick, C.B., Seeman, T., 2011. Associations between cognitive function and naturally occurring daily cortisol during middle adulthood: timing is everything. J. Gerontol. B Psychol. Sci. Soc. Sci. 66B (suppl.1), i71–i81.
Stone, A.A., Schwartz, J.E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., Grossman, S., 2001. Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. Psychoneuroendocrinology 26, 295–306.
Sustakoski, A., Perera, S., VanSwearingen, J.M., Studenski, S.A., Brach, J.S., 2015. The impact of testing protocol on recorded gait speed. Gait Posture 41, 329–331.
Thorn, L., Hucklebridge, F., Evans, P., Clow, A., 2006. Suspected non-adherence and weekend versus week day differences in the awakening cortisol response. Psychoneuroendocrinology 31 (8), 1009–1018.
van Aken, M., oosterman, J., van Rijn, T., Ferdek, M., Ruigt, G., Kozicz, T., Braat, D., Peeters, A., Nap, A., 2018. Hair cortisol and the relationship with chronic pain and quality of life in endometriosis patients. Psychoneuroendocrinology 89, 216–222.
Walker, S., O'Connor, D.B., Schaefer, A., Talbot, D., Hendrickx, H., 2011. The cortisol awakening response: associations with trait anxiety and stress reactivity. Personal. Individ. Differ. 51 (2), 123–127.
Ware, J.E., Snow, K.K., Kosisnki, M., Gandek, B., 1993. SF-36 Health Survey Manual and Interpretation Guide. The Health Institute, Boston, MA.
WHO, 2018. Ageing and health. Retrieved from. https://www.who.int/news-room/fact-sheets/detail/ageing-and-health.
Wüst, S., Federenko, I., Hellhammer, D.H., Kirschbaum, C., 2000. Genetic factors, perceived chronic stress, and the free cortisol response to awakening. Psychoneuroendocrinology 25, 707–720.