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Heaney, Jennifer; Phillips, Anna; Carroll, Douglas

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Ageing, depression, anxiety, social support and the diurnal rhythm and awakening response of salivary cortisol

Jennifer L.J. Heaney, Anna C. Phillips   , Douglas Carroll

School of Sport and Exercise Sciences, University of Birmingham, Birmingham, West Midlands, England, UK

Abstract

The present study compared the cortisol awakening response and diurnal rhythm in 24 young healthy students and 48 community-dwelling older adults. The associations with diurnal cortisol and depression, anxiety and social support were also examined in relation to age. Salivary cortisol was measured over the course of one day: immediately upon awakening, 30 min later, and then 3 h, 6 h, 9 h and 12 h post-awakening. Participants completed a questionnaire measuring symptoms of anxiety and depression and social support was assessed. Older adults exhibited a significantly reduced awakening response, overall cortisol levels, area under the curve (AUC) and diurnal slopes than younger adults, resulting in a flatter diurnal rhythm. Younger adults with higher depression scores had significantly higher overall cortisol and higher levels upon awakening and 30 min post-awakening. In the younger adults, anxiety and depression correlated positively with AUC and the cortisol awakening response (CAR). Older adults with lower social support had a reduced AUC where younger adults with lower social support displayed a larger AUC. These findings suggest that the diurnal rhythm and awakening response of salivary cortisol are significantly reduced in older adults and the associations between anxiety, depression and social support and diurnal cortisol vary with age.

1. Introduction

Cortisol, a stress hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis, is involved in a number of important functions in humans including energy metabolism, vascular activity, and inflammatory and immune responses (Schürmeyer and Wickings, 1999). Cortisol exhibits a marked diurnal rhythm, characterised by a rapid increase in levels upon awakening peaking at around 30 min post-awakening (Pruessner et al., 1997) and declining thereafter reaching a nadir in the evening (Hucklebridge et al., 2005).

The diurnal rhythm of cortisol can be examined in the context of the cortisol awakening response (CAR), and the secretion across the day expressed by area under the curve (AUC). Both these aspects of the circadian rhythm are related to physiological health. For example, individuals suffering from a chronic illness compared to healthy controls show an elevated CAR alongside a lower AUC (Kudielka and Kirschbaum, 2003). Further, a flatter cortisol diurnal pattern is related to a higher incidence of cardiovascular disease and type II diabetes (Rosmond et al., 2003), and earlier mortality in cancer patients (Sephton et al., 2000). The impact of an altered diurnal cortisol pattern may be particularly important for older individuals, for whom changes in endocrine function are associated with immunosenescence, disturbances in physical function, and independence.

1.1. Ageing and cortisol

Previous studies examining the effects of ageing on diurnal cortisol secretion have yielded contrasting results. Higher evening and nocturnal concentrations of cortisol resulting in an overall increase in 24 h mean cortisol and a flattened diurnal pattern characterised older participants in several studies ([Deuschle et al., 1997], [Luz et al., 2003], [VanCauter et al., 1996] and [Yen and Laughlin, 1998]). In contrast, older individuals have also exhibited a steeper cortisol slope (Ice et al., 2004). In addition, two studies reported no association between age and cortisol secretion during the day ([Edwards et al., 2001] and [Wolf et al., 2002]). With regard to the CAR, one study showed a lower CAR with increasing age (Kudielka and Kirschbaum, 2003) whereas others have failed to observe any age differences ([Pruessner et al., 1997] and [Wurst et al., 2000]).

Clearly there is little consensus. However, many of these studies have tested a broad age range, rather than recruit distinct young and old age cohorts. Some failed to obtain repeated saliva samples across the day and thus restricted their analysis to the CAR ([Kudielka and Kirschbaum, 2003], [Pruessner et al., 1997] and [Wurst et al., 2000]) or to the slope of decline ([Ice et al., 2004], [Luz et al., 2003] and [Wolf et al., 2002]). Finally, several previous studies did not control for important confounding variables such as awakening time, which affects the diurnal cortisol cycle (Edwards et al., 2001).

1.2. Depression, anxiety, social support and cortisol

Several studies have examined the relationship between the cortisol diurnal rhythm and psychological wellbeing. For example, depression has been associated with increased cortisol levels ([Cowen, 2009] and [Gillespie and Nemeroff, 2005]), greater diurnal variation, and a steeper diurnal decline (Bridges and Jones, 1966). However, contradictory evidence exists; other studies have found depression (Sjogren et al., 2006) and emotional distress (Miller et al., 2007) to relate to a flatter cortisol diurnal profile. In addition, one study reported no difference in the pattern of the diurnal profile between depressed patients and controls (McClure, 1966). With regard to the CAR, participants who were currently or previously depressed ([Bhagwager et al., 2005] and [Vreeburg et al., 2009a]) or had higher depressive symptoms (Pruessner et al., 2003b) exhibited a higher CAR. In contrast, one study showed a blunted CAR in depressed participants (Stetler and Miller, 2005).

One reason for this variation in findings could be the existence of co-morbid anxiety in those who are depressed (Murphy et al., 2004). For example, depressed participants with co-morbid anxiety had a higher CAR compared to those with depression alone (Vreeburg et al., 2009a), suggesting that anxiety may exacerbate the effects of depression on diurnal cortisol. There is also evidence that anxiety is independently associated with diurnal cortisol; individuals with high levels of anxiety showed a different pattern of decline across the day compared to those with lower levels of anxiety (Vedhara et al., 2003). Conversely, others have failed to demonstrate an association between anxiety and diurnal cortisol patterns ([Adam, 2006] and [Kurina et al., 2004]). Differences in findings might relate to the intensity of depression and the environment in which the participants are studied. Clinically depressed patients may differ from individuals in the community with depressive symptomatology; the association with diurnal cortisol would appear to be less certain in the latter (Bhagwager et al., 2005). It has been proposed that mild depression may be characterised by a dysregulation of cortisol rather than specifically hypersecretion (Stetler and Miller, 2005). This lack

of consensus may also be partly explained by methodological considerations such as the age of the participants and failure to control for possible confounders ([Chida and Steptoe, 2009] and [Vreeburg et al., 2009a]).

Conflicting findings are also typical of studies of positive psychosocial factors which may buffer the effects of anxiety and depression. Individuals with higher levels of social support have shown a steeper diurnal decline (Sjogren et al., 2006) and lower mean cortisol levels (Turner-Cobb et al., 2000). Support in the form of intimacy has also been associated with smaller AUC (Ditzen et al., 2008). In contrast, studies have found no association between social support and the diurnal slope (Turner-Cobb et al., 2000) or diurnal cycle (Smyth et al., 1997). However, in comparison to anxiety and depression, fewer studies have examined the relationship between social support and diurnal cortisol.

1.3. Ageing, depression, anxiety, social support and cortisol

A high prevalence of anxiety and depression has been reported in older adults (Ritchie et al., 2004) and is associated with mortality in this population ([Blazer, 1982] and [Schulz et al., 2000]). Due to methodological differences there is variation in rates (Girling et al., 1995). However, rates of 13.5% for depression and 3.7% for anxiety have been observed in community-dwelling adults aged between 65 and 89 years old in London (Lindesay et al., 1989). The difference in prevalence between young and older adults remains unclear due to conflicting findings (Jorm, 2000). Studies which have demonstrated age differences lack longitudinal follow-up, thus it is difficult to attribute these to ageing *per se* or differences in other factors related to age group differences (Jorm, 2000).

Studies specifically examining older adults have found higher AUCs (Wrosch et al., 2007) and flattened or inconsistent diurnal patterns (Fiocco et al., 2006) in individuals with depressive symptoms. However, this occurred in the context of physical health and memory problems, and one study lacked a non-depressed comparison group (Fiocco et al., 2006). Psychosocial factors have been related to a dysregulated diurnal profile in adolescents ([Goodyer et al., 2000], [Greaves-Lord et al., 2007] and [Van den Bergh et al., 2008]). With the exception of one study, which reported greater CARs in male university students with higher depression scores (Pruessner et al., 2003b), there is limited research examining diurnal cortisol and affect in young adults. Consequently, no studies have examined the effects of depression and anxiety on both the CAR and the diurnal rhythm of cortisol in the context of ageing. Older adults' cortisol secretion over the day has been inversely related to seeking social support (O'Donnell et al., 2008); however, this study did not measure perceived social support. To our knowledge, perceived social support has not been examined in parallel with anxiety and depression in relation to diurnal cortisol and ageing.

Accordingly, the aims of the present study were to: first, compare the CAR and diurnal rhythm of cortisol in young and older community-dwelling adults; and second, examine whether any observed age differences relate to anxiety, depression and social support. Finally, these analyses were revisited while controlling for differences in health behaviours and other confounding variables. Differences have been noted in the cortisol CAR and diurnal rhythm for health behaviours including smoking (Badrick et al., 2007), alcohol consumption (Badrick et al., 2008), and sleep quality (Dahlgren et al., 2009). Other potential confounding variables including sex (Pruessner et al.,

1997; VanCauter et al., 1996), oral contraceptive use (Pruessner et al., 1997;Reinberg et al., 1996), and phase of the menstrual cycle (Bao et al., 2003) have also been shown to influence the CAR and/or diurnal rhythm of cortisol. Consequently, it is important to adjust for these potential confounders.

2. Methods

2.1. Participants

Participants were 24 (11 women) University of Birmingham students and 48 (24 women) community-dwelling older adults. Mean ages of the younger and older adults were 20.0 (SD = 1.16, range 19−22) and 75.6 (SD = 6.35, range 65−88) years, respectively. Seventy six participants were originally recruited; four were excluded, one for non compliance and three for extreme cortisol values. Older adults were recruited from Birmingham clubs and associations (for example, Age Concern, the Women's Institute), churches, and through posters displayed in businesses around the local area. Inclusion criteria were: no endocrine or immune disorder, no psychiatric illness, no periodontal disease, no eating disorder and not taking glucocorticoid medication. The majority (97%) of participants described themselves as "white", with the exception of two of the younger adults who were "mixed race". In terms of socio-economic status, 75% of the young cohort were classified as from a non-manual occupational households based on their parents' previous/current occupation, using the Registrar General's Classification of Occupations (Classification of Occupations, 1980). Sixty-five percent of older adults were from non-manual occupational households based on their previous occupation; 4% did not disclose occupational background. Among younger females, 36% reported taking oral contraceptives and four women reported being in the luteal phase of the menstrual cycle and six in the follicular phase, one participant did not provide this information.

2.2. Study design

This study was a cross-sectional cohort investigation of ageing, diurnal salivary cortisol, and psychological factors. All participants gave written informed consent prior to the study, and the study had the appropriate ethics committee approval.

2.3. Questionnaires

Participants completed all questionnaires at home on the same day as the saliva sampling protocol.

2.3.1. Depression and anxiety

The Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983) was used to measure depression and anxiety. It contains seven items measuring anhedonic rather than somatic aspects of depression and seven measuring anxiety. Items are scored from 0−3, with higher scores indicating greater depression and anxiety. The HADS has good concurrent validity ([Bramley et al., 1988] and [Herrmann, 1997]), an internal consistency of .90 and .93 for anxiety and depression respectively (Zigmond and Snaith, 1983) and test retest reliability coefficients of .85 for depression and .84 for anxiety (Herrmann, 1997). In the present study, internal consistency was .84 for depression and .81 for anxiety.

2.3.2. Social support

Social support was measured using the Medical Outcomes Study Social Support Survey (MOSSSS) (Sherbourne and Stewart, 1991). This questionnaire assesses structural social support (i.e. the number of close friends and relatives from whom social support can be sought) and functional support (i.e. the availability of different types of support) via 19 items in four domains: emotional/informational, tangible, affectionate and positive social interaction. The questionnaire is scored on a five-point scale ranging from none of the time to all of the time. Internal consistency is high at .91, and one year test reliability values ranging from .72 to .78 have been reported (Sherbourne and Stewart, 1991). For the present study, internal consistency averaged .75 for the four categories of functional support.

2.3.3. Socio-demographic and health behaviours

Participants also completed a socio-demographic questionnaire. Data gathered included: date of birth, height (m), weight (kg), ethnicity, if suffering from chronic illness or taking ongoing medication, if taking oral contraception, the day of their menstrual cycle, and previous or parental occupation. Body mass index (BMI) was computed as kg/m^2. Health behaviours over the past year were assessed using a questionnaire adapted from the Whitehall study (Marmot et al., 1991). Participants indicated how much time (0, 1−2, 3−5, 6−8, 9−10, or 11+ hours a week) they spent participating in light (i.e. walking), moderately energetic (swimming, golf) and vigorous (i.e. running, squash) per week. A 0−5 categorical scoring system was applied to all behaviours, e.g. if they spent 1−2 h performing an activity they were awarded a score of 1. A combined exercise score was calculated by multiplying the category score by a weighting of 1, 2, 3 for light, moderate, and vigorous activity respectively. Participants reported how many cigarettes (0, 1−5, 6−10, 11−20, 21+, or 40+ per day), how many units of alcohol they consumed per week (0, 1−5, 6−10, 11−20, 20−40, or 40+) and, on average, how many hours they slept per night (0−3, 4−5, 6−7, 8−9, 10−11, or 12+). A binary variable (current smoker or non-smoker) was created and sleep data was also collapsed into a binary variable, denoting whether participants slept ≥ 6 h per night. The questionnaire contained a dietary section from which two measures were derived (Burns et al., 2002): scores for fresh fruit, fruit juice and cooked vegetables were summed to obtain a measure of fruit and vegetable intake; scores for chips/fried food, crisps/similar, sweets/chocolate, biscuits/cakes/puddings, full fat dairy products and processed meat were summed to provide an index of fat intake. Participants reported how frequently they consumed these types of food in a typical week during the past year on a 0−7 categorical scale defined as: never, less than once a week, 1 or 2 a week, most days (3−6), once a day, 2−3 times a day, or 4 or more times a day.

2.4. Salivary cortisol measurements

Stimulated saliva samples were obtained over one day to determine the diurnal pattern of free salivary cortisol secretion. Salivettes were centrifuged at 4000 rpm for 5 min and the saliva was pipetted into Eppendorfs which were stored at − 20 ° C until assay. Salivary cortisol samples were analysed all in the same day in duplicate by ELISA (DRG Diagnostics, Germany). This assay is based on the competition principle and microplate separation. An unknown amount of cortisol present in the

sample and a fixed amount of cortisol conjugated with horseradish peroxidise compete for the binding sites of mouse monoclonal cortisol antiserum. After an hour the microplate is washed to stop the competition reaction. After addition of a substrate solution the concentration of cortisol is inversely proportional to the optical density measured at 450 nm. Intra-assay coefficients were always < 10%.

2.5. Procedure

A one day saliva sampling protocol was chosen as the diurnal rhythm of cortisol had been shown to display intra-individual stability between days ([Edwards et al., 2001] and [Hucklebridge et al., 2005]). Participants were instructed to complete the sampling protocol on a weekday. Each participant was provided with a pack of six salivette tubes (Sarstdedt Ltd, Leicester, UK) labelled with the sampling times which were: immediately upon awakening, 30 min post-awakening, and then 3 h, 6 h, 9 h and 12 h post-awakening. They were briefed concerning the collection procedure and sampling times. Participants were asked to refrain from excessive alcohol consumption on the day prior to sampling, and to avoid consuming alcohol and undertaking vigorous exercise on the sampling day. Participants were asked not to eat, drink (except water), smoke or brush their teeth 30 min prior to each sample. Participants placed the salivette dental swab into their mouths and gently chewed for 1 min to collect saliva. The swab was returned to the salivette and stored in the participant's refrigerator within 24 h until collection. The study coordinators had ascertained that all participants had access to a refrigerator.

To assess compliance all participants were given a diary to record the times their samples were due and the time when they actually took them. Younger participants were asked to set alarms on their mobile phones to prompt them when to take a sample and older adults were given a wristband where they could write reminders of their sampling times. The first two samples of the day (used to calculate the CAR) were included only if taken on time or within 10 min, as recommended (Kunz-Ebrecht et al., 2004). For the remainder of the samples, one participant was excluded from the study as she had repeatedly taken samples over an hour late, resulting in our final overall sample size of 72. Overall, according to the self-report diary, out of 432 samples: 79% were taken on time or within 5 min, 10% were up to 20 min late, 4% were up to 30 min late, 5% over 30 min up to 1 h late and 2% were over 1 h late.

2.6. Data analysis

Age cohort differences in psychosocial variables, health behaviours, BMI, chronic illness and time of awakening were examined first using ANOVA. Chi-square was used in the case of categorical variables such as current smoker, consuming ≥ 6 alcohol units a week, and sleeping ≥ 6 h a night.

Analyses were conducted using four cortisol outcome measures: the diurnal repeated measures pattern across all six samples; the cortisol awakening response (CAR); AUC; and diurnal slope. The CAR was calculated as sample 2 minus sample 1 ([Edwards et al., 2001] and [Sjogren et al., 2006]). AUC was calculated relative to zero using the trapezoid method applied to all sampling points (Pruessner et al., 2003a). Diurnal cortisol slopes were calculated by regressing cortisol values on the sample time for each participant separately ([Cohen et al., 2006], [Smyth et al., 1997] and [Turner-

Cobb et al., 2000]). This yields a slope value for each participant. The sample obtained upon awakening was used as the slope anchor (Kraemer et al., 2006) and the second sample (30 min after waking) was excluded from the estimation of the slopes (Cohen et al., 2006).

Repeated measures ANOVA was used to examine the diurnal cortisol rhythm, first in relation to age cohort, and second, in relation to age cohort and each separate psychosocial variable, in order to test main effects of age and psychosocial variables, and any interaction effects. For all continuous psychosocial variables, binary variables were created using median splits to form high and low groups within each age cohort. Anxiety and depression were split at a score of 11, indicating a high probability of depression (Zigmond and Snaith, 1983) to form high and low groups. Greenhouse−Geisser corrected statistics and partial η^2 as a measure of effect size are reported. In order to examine the patterns over time between groups, using SPSS version 17, orthogonal polynomial contrasts were fitted within each repeated measures model. Statistical significance for linear, quadratic, and cubic components are reported below, where appropriate.

Univariate ANOVA was applied to analyse age cohort, then age cohort x psychosocial differences in the CAR, AUC, and diurnal slopes, respectively. The relationships between CAR, AUC, and diurnal slope with psychosocial variables were then analysed in their continuous form using correlations. In all of the above analyses, where significant effects emerged, subsequent ANCOVA was performed to adjust for potential confounding variables: diet, alcohol consumption, exercise, BMI, time of awakening, smoking, sleep and chronic illness. Slight variations in degrees of freedom reflect occasional missing data or insufficient saliva for analysis.

3. Results

No sex differences were found for any analysis; accordingly, genders were grouped together and the results presented below are for both males and females collectively.

3.1. Diurnal cortisol and age

The repeated measures ANOVA yielded a significant main effect of time on cortisol concentration, $F(5,320) = 50.11$, $p < .001$, $\eta^2 = .439$. The pattern was characterised by significant linear and cubic components, $p < .001$. The quadratic component was not statistically significant, $p = .110$. There was also a main effect of age cohort, $F(1,64) = 14.23$, $p < .001$, $\eta^2 = .184$, such that younger adults had higher cortisol levels overall. The time $\times$ age interaction effect was also significant, $F(5,320) = 7.29$, $p < .001$, $\eta^2 = .102$; as can be seen in Fig. 1, with younger adults exhibiting higher cortisol concentrations than the older adults in the morning. Analysis of AUC revealed a significant difference, $F(1,64) = 11.73$, $p = .001$, $\eta^2 = .155$, between the older (65.1, SD = 34.99 ng/ml) and the younger (95.7, SD = 34.70 ng/ml) cohorts. There was also a significant difference in cortisol slope between the younger (−.60, SD = .41) and older (−.32, SD = .51) adults, $F(1,66) = 5.52$, $p = .022$, $\eta^2 = .07$. The younger cohort exhibited a steeper decline across the day.
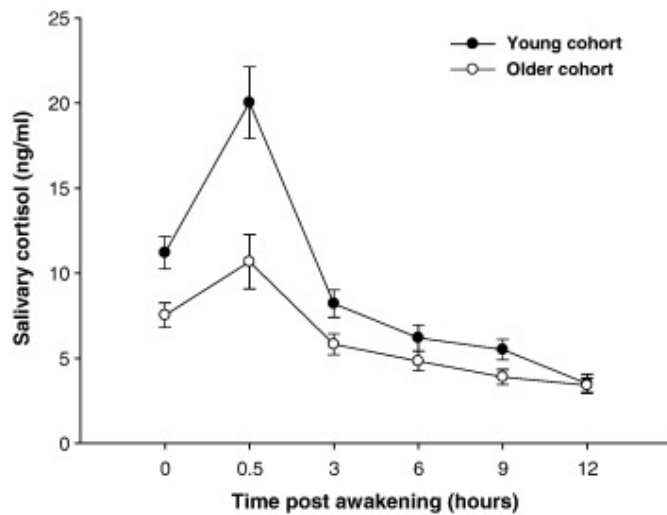
Fig. 1. Mean (standard error) diurnal cortisol pattern by age cohort.

3.2. Cortisol awakening response and age

For the CAR, there was a significant cohort effect, $F(1,66) = 6.96$, $p = .010$, $\eta^2 = .095$, with younger adults showing a greater awakening response than older adults, 8.82, SD = 10.54 and 2.99, SD = 7.54 ng/ml, respectively.

3.3. Age, anxiety, depression and social support

There were no significant differences between age cohorts for anxiety, depression or social support variables with the exception of positive social interaction; where older adults had significantly lower social interaction scores than the younger cohort. Descriptive statistics are displayed in Table 1.

Table 1. Mean (SD) anxiety, depression and social support scores in younger and older adults.

Variable	Older adults (n = 48)	Younger adults (n = 24)	F(df)	p	η^2
HADS total	14.7 (9.22)	13.6 (9.01)	.44 (1,70)	.835	.001
HADS anxiety score	7.1 (4.52)	7.0 (4.08)	.01 (1,70)	.924	.000
HADS depression score	7.0 (5.39)	6.6 (5.75)	.07 (1,70)	.786	.001
MOS friends & relatives	6.6 (4.88)	8.0 (2.38)	1.68 (1,54)	.199	.030

Variable	Older adults (n = 48)	Younger adults (n = 24)	F(df)	p	η^2
MOS total	75.7 (19.53)	83.2 (15.98)	2.68 (1,69)	.106	.037
MOS tangible	16.1 (4.78)	18.4 (11.32)	1.46 (1,68)	.231	.021
MOS emotional	31.2 (8.54)	34.8 (4.69)	3.60 (1,69)	.062	.050
MOS affectionate	12.6 (3.08)	12.3 (2.70)	.15 (1,69)	.704	.002
MOS positive social	16.0 (4.29)	17.8 (2.13)	3.88 (1,68)	.050	.054
Categorical variables			χ^2 (df)	p	
HADS depression score ≥ 11 (%)	22	38	.818	.366	
HADS anxiety score ≥ 11 (%)	25	17	.643	.423	

HADS, Hospital Anxiety and Depression Scale; MOS, Medical Outcomes Study Social Support Survey. Scores ≥ 11 on HADS indicate a high probability of depression or anxiety.

3.4. Diurnal cortisol, age, anxiety, depression and social support

Repeated measures ANOVA revealed a significant main effect of depression on diurnal cortisol, $F(5,310) = 5.38$, $p = .004$, $\eta^2 = .080$, where higher depression was associated with higher cortisol levels overall. There was also a significant two-way interaction between cohort and depression on cortisol, $F(1,62) = 7.65$, $p = .007$, $\eta^2 = .110$, such that young adults with high depression had higher overall cortisol levels. Finally, there was a significant age cohort $\times$ depression $\times$ time interaction effect for diurnal cortisol, $F(5,310) = 10.29$, $p < .001$, $\eta^2 = .142$. As seen in Fig. 2, the younger adults with higher depression scores had significantly higher cortisol levels upon awakening and 30 min later than the other three groups, $F(1,66) = 14.68$, $p < .001$, $\eta^2 = .182$ and $F(1,67) = 36.68$, $p < .001$, $\eta^2 = .354$, respectively. The age cohort $\times$ depression interaction effect for AUC did not quite reach statistical significance, $F(1,62) = 3.71$, $p = .059$, $\eta^2 = .056$, nor was there an interaction effect for diurnal slope.
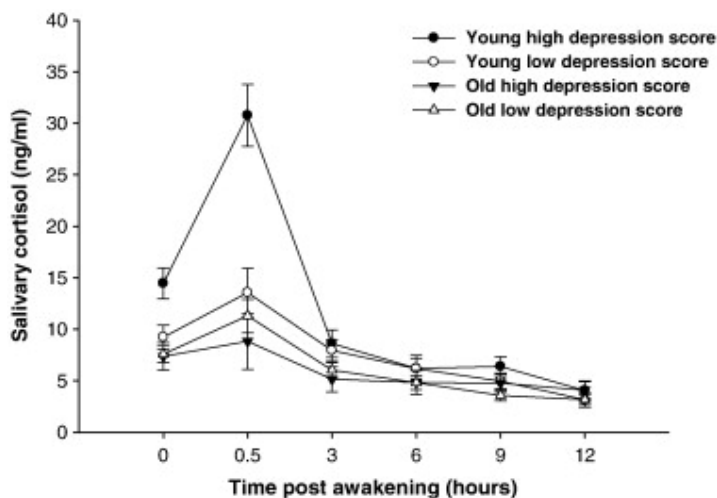
Fig. 2. Mean (standard error) diurnal cortisol pattern by age cohort and HADS depression score.

No significant interaction effects occurred for diurnal cortisol, age and anxiety or social support or for diurnal slope and these variables. However, there was a significant interaction effect for AUC of cohort and total social support, $F(1,61) = 3.94$, $p = .05$, $\eta^2 = .061$, such that older adults with lower total social support had a reduced AUC. In addition, younger adults with lower affectionate support had a significantly higher AUC, $F(1,61) = 16.65$, $p = <.001$, $\eta^2 = .214$.

3.5. CAR, age and depression

There was a significant age cohort $\times$ depression interaction effect for the CAR, $F(1,64) = 10.09$, $p = .002$, $\eta^2 = .136$; younger participants with higher depression scores showed a higher awakening response. No significant effects emerged for CAR, age and anxiety or social support, although there was a trend for younger adults with lower affectionate support to display a higher CAR, $F(1,63) = 3.78$, $p = .056$, $\eta^2 = .057$.

3.6. Diurnal cortisol, oral contraceptives and the menstrual cycle

There was no significant difference in diurnal cortisol or CAR between women taking oral contraceptives and those who were not. The diurnal rhythm was significantly different between the two phases of the menstrual cycle, $F(5,40) = 6.52$, $p = .012$, $\eta^2 = .449$. Females in the luteal phase had significantly higher cortisol after waking and 30 min after waking compared to those in the follicular phase. However, the interaction between HADS depression score and diurnal cortisol in the younger female cohort was not attenuated by adjustment for menstrual cycle phase.

3.7. Diurnal cortisol, CAR, age and HADS depression adjusting for potential confounding variables

The interactions of diurnal cortisol and CAR for age cohort $\times$ depression were adjusted individually for each of the variables displayed in Table 2. For these analyses, the continuous versions of these variables were entered as covariates with the exception of alcohol consumption, sleep duration, and smoking status. The interactions for both diurnal cortisol and CAR withstood adjustment for these

potential confounders. In addition, there were no significant effects of these variables independently of cohort on diurnal cortisol. Further, there were no differences in socio-economic status distribution between the cohorts or any interaction effects for this variable on diurnal cortisol.

Table 2. Mean (SD) health behaviours, BMI, chronic illness and time of awakening for older and younger cohorts.

Variable	Older adults (n = 48)	Younger adults (n = 24)	F(df)	p	η^2
Exercise score	4.8 (4.51)	13.7 (6.85)	41.47 (1,66)	<.001	.386
Cooked meals (per day)	1.2 (.80)	1.8 (.51)	39.13 (1,70)	<.001	.359
Caffeine (drinks per day)	4.6 (2.62)	1.4 (1.20)	29.93 (1,69)	<.001	.303
Fat score	9.6 (2.66)	10.9 (3.87)	2.00 (1,67)	.162	.029
Fruit and vegetable score	4.8 (4.51)	11.1 (2.15)	5.79 (1,69)	.019	.077
BMI (kg/m^2)	28.2 (4.17)	21.7 (2.79)	47.29 (1,70)	<.001	.403
Time of awakening (am/h)	7.10 (1.01)	8.45 (1.01)	28.79 (1,70)	<.001	.291
Categorical variables			χ^2 (df)	p	
Alcohol ≥ 6 units per week (%)	27	63	8.45 (1)	.004	
Current smokers (%)	6	4	.13 (1)	.593	
Sleep ≥ 6 h (%)	29	71	9.06 (1)	.003	
Chronic illness (%)	67	17	17.38 (1)	<.001	

3.8. Correlations between AUC, CAR and diurnal slope and the psychosocial variables

Significant positive correlations were found for younger adults between the AUC and depression, r(22) = .52,*p* < .01 and anxiety, r(22) = .41, *p* = .049. For older adults, the AUC positively correlated with emotional/informational support, r(39) = .32, *p* = .042. No significant correlations were found for the CAR and psychosocial variables for older adults. However, there was a significant positive correlation for the younger cohort for depression, r(22) = .59, *p* < .01, as would be expected given the previous diurnal cortisol interactions, and for anxiety, r(22) = .46, *p* = .026. There were no significant correlations between the cortisol slope and the psychosocial variables for either cohort.

4. Discussion

4.1. Age and cortisol

Older adults displayed a blunted CAR and a flatter diurnal profile which is consistent with previous findings for the CAR (Kudielka and Kirschbaum, 2003) and diurnal profile ([Deuschle et al., 1997] and [VanCauter et al., 1996]). Although the exact function of the CAR is unknown, it has been proposed to be linked to memory systems (Wilhelm et al., 2007), play an important role in regulating the immune system (Petrovsky and Harrison, 1997) and in anticipating the demands of the day (Fries et al., 2009). The reduced CAR observed in the older adults may therefore have implications for such functions.

In contrast to some previous findings, the flatter profile observed in the present study was due to lower awakening levels and a reduced CAR in older adults, as opposed to higher evening levels (Deuschle et al., 1997). However, in this previous study, cortisol, although sampled over a 24 hour period, was not measured over the awakening period. Whereas older adults in the present study had lower overall cortisol levels, others have reported higher cortisol levels in the elderly ([Luz et al., 2003] and [VanCauter et al., 1996]). Nevertheless, the present pattern of results is in line with previous observations of lower morning (Maes et al., 1994) and overall cortisol levels as a function of age (Ahn et al., 2007). Although these studies analysed cortisol in plasma, salivary cortisol accurately reflects plasma free cortisol (Kirschbaum and Hellhammer, 1989). Thus, different media of measurement would not appear to explain contrasting results. It is possible that variations in findings reflect differences in sampling and the recruitment of distinct age cohorts rather than continuous age sampling. Alternatively, the lack of consistency may reflect the considerable individual variability in changes in diurnal cortisol with ageing. A longitudinal study of cortisol in healthy older adults revealed that although the majority showed increasing diurnal cortisol levels over the years some individuals registered a decrease (Lupien et al., 1996); the aggregate pattern of secretion did not change significantly over time in this study. Marked individual differences in cortisol secretion have also been noted in younger adults (Smyth et al., 1997). Accordingly, marked individual variability may partly explain the lack of consensus regarding cortisol and ageing. Although there may be a general shift towards a flatter diurnal profile, this may be characterised through either increased nadir levels or blunted secretion in the morning.

Increases in cortisol previously observed with ageing have been attributed to impairment of feedback inhibition of HPA activity due to neuronal loss in hippocampal area ([VanCauter et al., 1996] and [Yen and Laughlin, 1998]) and reduced hippocampal volume has been shown in older adults with a progressive increase in 24 h cortisol over 5−6 years (Lupien et al., 1998). Loss of HPA

axis sensitivity has also been demonstrated via impaired dexamethasone suppression (Magri et al., 1997). The attenuated diurnal levels of cortisol observed in our older participants imply dysregulation at another stage of the HPA axis. Variations in the diurnal profile with age in the present study appear to be largely driven by an attenuated CAR in the older cohort. Although speculative, it is possible that the decrease in morning cortisol is a consequence of fatigue of the adrenal cortex and thus a reduced ability to respond dynamically to the stress of awakening. However, it should be conceded that the blunted CAR may also reflect changes in hippocampal function with age, given that the awakening response is abolished in patients with hippocampal damage (Buchanan et al., 2004).

4.2. Age, depression, anxiety, social support and cortisol

The present finding that a higher CAR related to higher depression scores among male university students has been reported previously (Pruessner et al., 2003b). However, the present study extended this finding to both male and female students. An elevated CAR in individuals with diagnosed depression has been found in community samples ([Bhagwager et al., 2005] and [Vreeburg et al., 2009a]). Also in line with previous findings, cortisol returned to similar levels irrespective of depression status after awakening (Bhagwager et al., 2005). In contrast, a blunted CAR (Stetler and Miller, 2005) and a flatter diurnal profile (Sjogren et al., 2006) have also been reported in those in community settings, implying that the effects of depression in the community may be best viewed from the perspective of dysregulation and not in terms of a specific increase/decrease in usual levels (Stetler and Miller, 2005). However, interestingly, there was no significant difference in any of the cortisol parameters between the high and low depressive symptom groups in the older adults where altered diurnal patterns have been previously observed with depressive symptoms ([Fiocco et al., 2006] and [Wrosch et al., 2007]). This could be attributable to the positive health status of the older participants recruited to the present study. Previous studies on cortisol and depression in older adults have included participants with health problems and/or cognitive dysfunction ([Fiocco et al., 2006] and [Wrosch et al., 2007]).

In the present study no gender or oral contraceptive use differences were found in relation to cortisol in either cohort in contrast to previous studies ([Pruessner et al., 1997] and [Vreeburg et al., 2009b]), but in line with others (Wurst et al., 2000). There was a significant difference in diurnal cortisol rhythm between young females in different phases of the menstrual cycle, as observed previously (Bao et al., 2003). However, our main findings with regard to age and depression withstood adjustment for this difference. Further, changes in cortisol over the menstrual cycle have not been shown to vary between depressed and non-depressed females (Bao et al., 2004).

Anxiety was positively associated with a higher CAR and AUC in the younger cohort but again there were no significant relationships in the older cohort. Others have failed to show an association between anxiety and diurnal cortisol in adolescents (Adam, 2006), however, we are not aware of any studies specifically among those aged over 65 years for comparison. Within the higher depressive symptoms group, there was no difference in the CAR between those with high and low anxiety symptoms, where co-morbid anxiety has been previously found to increase the CAR (Vreeburg et al., 2009a). Social support was related to the CAR in the younger adults and AUC in both cohorts.

However, the direction of these associations differed whereby low social support related to reduced cortisol in older adults but had the opposite association in students. It could be speculated that social support has differing relationships with physiological variables depending on age. For example social support has been associated with vaccination response in students (Gallagher et al., 2008) but not an older adult sample (Phillips et al., 2006). Social support may be interpreted differently by these age cohorts and the relevance of different domains of support may differ. Other aspects of social support not measured in this study, such as marriage, may be more important for older adults, as suggested previously (Phillips et al., 2006).

It has been speculated that an increased CAR in depression could be a stress response to disturbed mood upon awakening (Bridges and Jones, 1966). However, a causal association between depression and HPA activity is yet to be established. It has been suggested that cortisol dysregulation may represent an endophenotype (Hasler et al., 2004) or vulnerability marker of depression ([Bhagwager et al., 2005], [Cowen, 2009] and [Harris et al., 2000]); further, corticotrophin-releasing hormone has been argued to be a key aetiological factor in depression and anxiety (Alldredge, 2010). Alternatively, changes in the diurnal cortisol rhythm could be a residue from previous depression implying a causal pathway from depression to altered HPA activity (Bhagwager et al., 2005). In contrast, elevated cortisol has been proposed to be a compensatory mechanism to overcome glucocorticoid resistance as a result of impaired/reduced glucocorticoid receptors in depression (Pariante, 2009). Thus, the association between depression and cortisol would appear to have multiple determinants and may be bi-directional.

Cortisol dysregulation could be influenced by genetics, stress exposure, and personality (Heim et al., 2000) and these could play a role in the individual variability observed in diurnal cortisol with both ageing and psychosocial factors. Further, it may not be the relative magnitude of cortisol in comparison to others that is most important in terms of health consequences, but rather how much cortisol changes in relation to an individual's usual level and its balance with other parameters. For example, increased DHEA levels in depressed younger and older adults parallel to cortisol without changes in diurnal pattern have been observed (Heuser et al., 1998). Future research might investigate how changes in diurnal cortisol with ageing and psychosocial factors relate to the diurnal patterns of other endocrine variables, as it may not be changes in cortisol *per se*, but rather the balance of cortisol with other hormones, such as the cortisol:DHEA ratio, that is of greater relevance to psychological and physiological health in ageing individuals (Straub et al., 2001).

4.3. Limitations

The present study suffers from a number of limitations. Only younger and older adults were tested, whereas flattened cortisol profiles have been observed in participants with a mean age of 46 years (Lasikiewicz et al., 2008). The inclusion of a third middle-aged cohort in this study may have captured more precisely the age at which changes in cortisol rhythm become apparent. Nevertheless, the study was still novel in using two distinct age cohorts. Although studies have shown that the CAR is influenced by state factors ([Hellhammer et al., 2007], [Stalder et al., 2009] and [Thorn et al., 2009]), there is also evidence from studies sampling on more than one day that the diurnal cortisol profile ([Edwards et al., 2001] and [Hucklebridge et al., 2005]) and the CAR ([Clow et al., 2004], [Pruessner

et al., 1997] and [Wurst et al., 2000]) show reasonable temporal stability. Previous depression was not measured in this study and self-report was employed to measure depressive symptoms as opposed to an interview. However, the HADS has been validated as a reliable method for evaluating anxiety and depression. Participants were asked to refrain from eating and drinking 30 min prior to samples. Compliance was measured subjectively, in terms of participant oral self-report. However, cortisol peaks as a result of meal consumption were not observed across the day. Although it is possible that some participants did eat and drink in time between waking and the 30 minute sample, this is unlikely to have occurred only in the young depressed group, and therefore unlikely to account for the observed CAR differences. Finally, although confounding by unmeasured or poorly measured variables remains possible, we did adjust for a range of potential confounders, including awakening time and health behaviours. Further, the present associations were not explained by perceived or life event stress (data not reported here) which has been proposed to influence the relationship between depression and cortisol (Pruessner et al., 2003b).

4.4. Conclusion

Compared to the young cohort, older adults displayed significantly reduced cortisol upon awakening, a lower CAR and a flatter diurnal profile reflected in a reduced AUC. The associations between anxiety, depression, social support and diurnal cortisol varied by age cohort.

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