Ageing, health behaviours and the diurnal rhythm and awakening response of salivary cortisol

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Running title: Ageing, heath behaviours and the cortisol diurnal rhythm

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Abstract

This study compared the awakening response and diurnal rhythm of cortisol in young versus older adults in relation to health behaviours. Older adults displayed significantly reduced cortisol upon awakening, a lower cortisol awakening response and a flatter diurnal profile represented by a reduced area under the curve and cortisol slope. There was also a significant interaction of age, cortisol and diet; younger adults with a higher fat and lower fruit and vegetable intake exhibited the flattened diurnal cortisol phenotype of the older adults.

Keywords

Cortisol diurnal rhythm; cortisol awakening response; ageing; diet; health behaviours
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 & Wickings, 1999). Cortisol exhibits a marked diurnal rhythm, characterised by a rapid increase in levels upon awakening peaking at around 30 minutes post awakening (Pruessner, Wolf, Hellhammer, Buske-Kirschbaum, von Auer, et al., 1997) and declining thereafter reaching a nadir in the evening (Hucklebridge, Hussain, Evans & Clow, 2005).

The diurnal rhythm of cortisol has been examined previously from the perspective of the cortisol awakening response (CAR), the secretion across the day expressed by area under the curve (AUC), and the rate of decline across the day or cortisol slope. These aspects of the circadian rhythm have been shown to relate to physiological and psychological health. For example, an elevated CAR alongside a lower AUC was observed in individuals suffering from a chronic illness compared to healthy controls (Kudielka & Kirschbaum, 2003) and a higher incidence of cardiovascular disease and type II diabetes has been associated with a reduced cortisol levels and slope of decline (Rosmond, Wallerius, Wanger, Martin, Holm, et al., 2003). A flatter diurnal profile has also been shown to relate to earlier mortality in cancer patients (Sephton, Sapolsky, Kraemer & Spiegel, 2000). Different psychosocial factors, such as job stress, general life stress and posttraumatic stress, are associated with enhanced or reduced CAR (Chida & Steptoe, 2009). Therefore, a reduction or elevated CAR, and flattened diurnal amplitude appear to be linked to adverse health outcomes. The impact of which may be particularly important for older individuals, where changes in endocrine function may be associated with immunosenescence, disturbances in physical function, and consequently independence.
Higher nadir concentrations of cortisol resulting in an overall increase in 24h mean cortisol and a flattened diurnal pattern have been observed in older participants in several studies (Deuschle, Gotthardt, Schweiger, Weber, Körner., et al., 1997; Luz, Dornelles, Preissler, Collaziol, Da Cruz., et al., 2003; VanCauter, Leproult & Kupfer, 1996; Yen & Laughlin, 1998). However, previous studies on the effects of ageing on diurnal cortisol have yielded inconsistent results and not all studies have observed a flattened diurnal cortisol profile. Ageing has also been characterised by a steeper as opposed to flatter cortisol slope (Ice, Katz-Stein, Himes & Kane, 2004) and has also been reported to show no association with cortisol secretion during the day (Edwards, Evans, Hucklebridge & Clow, 2001). Others have found a lower CAR with ageing (Kudielka & Kirschbaum, 2003) whereas some studies have failed to observe any age differences in the CAR (Pruessner et al., 1997; Wurst, Wolf, Hellhammer, Federenko, Sapolsky., et al., 2000) or the overall diurnal profile (Wolf, Convit, Thorn & de Leon, 2002). 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 employ a multiple sampling protocol and thus their analysis was restricted to the CAR (Kudielka & Kirschbaum, 2003; Pruessner et al., 1997; Wurst et al., 2000) or to the slope of decline across the day (Ice et al., 2004; Luz et al., 2003; Wolf et al., 2002). Finally, several previous studies did not control for important confounding variables such as awakening time, which has been shown to be significantly related to the diurnal cortisol cycle (Edwards et al., 2001). Given the inconsistencies in the findings of previous research, as well as variations in their methodology, it was considered important to re-address the issue of cortisol and ageing, using a multiple sampling protocol across the whole day and controlling for potential confounding factors.

Although counter evidence exists (Vreeburg, Kruijtzner, van Pelt, van Dyck, DeRijk., et al., 2009), health behaviours, such as diet, sleep and alcohol consumption, have been associated with the cortisol
diurnal rhythm. Flatter cortisol profiles have been observed in individuals with a higher intake of saturated fat and central body fat distribution (Garcia-Prieto, Tebar, Nicolas, Larque, Zamora, et al., 2007). Smoking (Badrick, Kirschbaum & Kumari, 2007), alcohol consumption (Badrick, Bobak, Britton, Kirschbaum, Marmot, et al., 2008), and sleep quality (Dahlgren, Kecklund, Theorell & Akerstedt, 2009) have also been shown to affect the CAR and/or the diurnal rhythm of cortisol. However, the effects of multiple health behaviours on the CAR and cortisol diurnal rhythm are rarely investigated within the same study. With the exception of Vreeburg et al (2009), who examined a range of socio-demographic and health characteristics, most have focused on one or two health behaviours in isolation, for example smoking (Badrick et al., 2007; Steptoe & Ussher, 2006) or alcohol (Badrick et al., 2008). In addition, although one study has examined cortisol and food intake (Garcia-Prieto et al., 2007), research has largely focussed on obesity (Márin, Darin, Amemiya, Andersson, Jern, et al., 1992; Strain, Zumoff, Strain, Levin & Fukushima, 1980), rather than diet specifically. Although some have examined diurnal cortisol and a health behaviour in relation to age (Badrick et al., 2008), the majority of studies have not included adults aged over 65 or included multiple behaviours and older participants. As a result, the relationship between multiple health behaviours and diurnal cortisol in the context of ageing has yet to be identified. It is possible that the impact of health behaviours on cortisol varies as a function of age. Accordingly, the aims of the present study were to compare the CAR and diurnal rhythm of cortisol in younger and older adults, and secondly, examine whether any observed age differences relate to customary health behaviours.

Methods

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) and 75.6 (SD = 6.35) years, respectively. 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.

**Study design**

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

**Questionnaires**

Participants completed a socio-demographic questionnaire at entry to the study. Data gathered included: date of birth, height (m), weight (kg), ethnicity, if suffering from chronic illness or taking ongoing medication, if taking oral contraception, and previous or parental occupation. Body mass
index (BMI) was computed as kg/m². Health behaviours over the past year were assessed using a questionnaire adapted from the Whitehall study (Marmot, Smith, Stansfeld, Patel, North., et al., 1991). Participants indicated how much time (0, 1-2, 3-5, 6-8, 9-10, 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 hours 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+, 40+ per day), how many units of alcohol they consumed per week (0, 1-5, 6-10, 11-20, 20-40, 40+), how often they consumed alcohol (never, special occasions only, 1-2 per month, 1-2 per week, almost daily, 2 or more per day), and on average, how many hours they slept per night (0-3, 4-5, 6-7, 8-9, 10-11, 12+). A binary variable was created for smoking (current smoke, non smoker) and sleep (≥ 6 h per night), alcohol units (≥ 6 units per week) and alcohol frequency (≥ 1-2 times per week) were collapsed into binary variables based on median splits. The questionnaire contained a dietary section from which two measures were derived (Burns, Carroll, Ring, Harrison & Drayson, 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, 4 or more times a day.

Salivary Cortisol Measurements
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%.

**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; 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 (Sarstedt Ltd, Leicester, UK) labelled with the sampling times which were: immediately upon awakening, 30 min post-awakening and then 3h, 6h, 9h and 12h 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 excessive alcohol consumption and 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 until collection.
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, Kirschbaum, Marmot & Steptoe, 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 five min, 10% were up to 20 min late, 4% were up to 30 min late, 5% over 30 min up to 1hr late and 2% were over 1hr late. In conjunction with the saliva sampling pack participants received the questionnaire pack to complete at home which was collected with the saliva samples.

Data analysis

Age cohort differences in health behaviours, BMI, 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, consuming alcohol ≥ 1-2 times per week, sleeping ≥ 6 h a night and chronic illness).

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; Sjogren, Leanderson & Kristenson, 2006). AUC was calculated relative to zero using the trapezoid method applied to all sampling points (Pruessner, Kirschbaum, Meinlschmid & Hellhammer, 2003). Diurnal cortisol slopes were calculated by regressing cortisol values on the sample time for each participant separately (Cohen, Schwartz,
Epel, Kirschbaum, Sidney., et al., 2006; Smyth, Ockenfels, Gorin, Catley, Porter., et al., 1997; Turner-Cobb, Sephton, Koopman, Blake-Mortimer & Spiegel, 2000). This yields a slope value for each participant. The sample obtained upon awakening was used as the slope anchor (Kraemer, Giese-Davis, Yutsis, Neri, Gallagher-Thompson., et al., 2006) and the second sample (30 min after waking) was excluded from the estimation of the slopes (Cohen et al., 2006) to eliminate the initial amplitude of the CAR.

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 health behaviour variable, in order to test main effects of age and health behaviours, and any interaction effects. For continuous health behaviours, binary variables were created using median splits to form high and low groups within each age cohort, for example, high exercise score versus low exercise score groups. Greenhouse-Geisser corrected statistics and partial $\eta^2$ as a measure of effect size are reported.

Univariate ANOVA was applied to analyse age cohort, then age cohort × health behaviour differences in the CAR, AUC, and diurnal slopes, respectively. In all of the above analyses, where significant effects emerged, subsequent ANCOVA was performed to adjust for potential confounding variables: BMI, time of awakening and chronic illness. To ensure the variance of independent variables and covariates were not significantly different, homogeneity of covariance was assessed and confirmed. Any significant effects from the ANOVAs were also explored using correlational analysis. Slight variations in degrees of freedom reflect occasional missing data or insufficient saliva for analysis.

Results

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, $p = .11$. 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 × age interaction effect was also significant, $F(5,320) = 7.29, p < .001, \eta^2 = .102$; as can be seen in Figure 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) cohort. The younger cohort also exhibited a significantly steeper slope of decline across the day (-.60, SD = .41 versus -.32, SD = .51), $F(1,66) = 5.52, p = .022, \eta^2 = .07$.

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).

Health behaviours and age
Health behaviours differed between the age cohorts with the exception of fat intake score and smoking status. Descriptive statistics are shown in Table 1.

[Insert Table 1 about here]

**Diurnal cortisol, age, and health behaviours**

There was a significant two-way interaction between cohort and fat intake on cortisol, $F(1,59) = 6.90, p = .011, \eta^2 = .105$, such that young adults with low fat intake had higher overall cortisol levels. There was also significant age cohort $\times$ fat intake $\times$ time interaction effect for diurnal cortisol, $F(5,295) = 4.28, p = .016, \eta^2 = .068$. As shown in Figure 2, younger adults with a lower fat intake had a higher cortisol immediately after waking and 30 min post waking; they also tended to have higher cortisol levels later in the afternoon. Younger adults with a lower fat intake had a significantly higher cortisol AUC, $F(1, 59) = 4.93, p = .030, \eta^2 = .077$, however, there was no significant age $\times$ fat intake interaction for slope. There was also a significant age cohort $\times$ fruit and vegetable intake interaction effect, $F(5, 305) = 3.74, p = .026, \eta^2 = .058$. Figure 3 indicates that participants in the younger cohort who had a higher intake of fruit and vegetables had higher early cortisol concentrations. There were no significant age cohort $\times$ fruit and vegetable intake interactions for AUC or slope. There were no significant main or interaction effects for any of the other health behaviours on diurnal cortisol.

[Insert Figures 2 and 3 about here]
Diurnal cortisol, age and health behaviours adjusting for potential confounding variables

The two age cohorts significantly differed in BMI, time of awakening and chronic illness (Table 1). The most commonly reported illnesses by older adults were: hypertension (44%) and arthritis (25%), the other illnesses reported were angina, gastrointestinal disorders and Parkinson’s disease (2% in each case). Younger adults reported asthma only. The significant repeated measures ANOVA findings for age × diet for diurnal cortisol were adjusted for each potential confounding variable separately. The age cohort × fat intake interaction for diurnal cortisol remained significant following adjustment for BMI, F(5,290) = 4.24, p = .017, η² = .068, time of awakening, F(5,290) = 3.95, p = .022, η² = .064, and chronic illness, F(5,290) = 3.74, p = .027, η² = .061. The age cohort× fruit and vegetable intake interaction for diurnal cortisol also withstood adjustment for BMI, F(5,300) = 3.84, p = .024, η² = .060, time of awakening, F(5,300) = 3.70, p = .027, η² = .058, and chronic illness, F(5,300) = 3.62, p = .029, η² = .057.

In order to eliminate the possibility that other health behaviours were confounding these diet findings, differences between groups within the younger cohort were explored (Tables 2 and 3). Participants in the high fat intake group were more likely to consume over ≥ 6 units of alcohol per week, however, the age × fat intake × time interaction withstood adjustment for this, F(5,290) = 4.03, p = .021, η² = .065.

There were no significant differences in sex, smoking and socio-economic status distribution between the cohorts, nor any main or interaction effects for these variables on diurnal cortisol. There was also no significant difference in diurnal cortisol or CAR between women taking oral contraceptives and those who were not.

[Insert Table 2 and Table 3 about here]
When these relationships with age and health behaviours were examined for CAR alone, there was again a significant age cohort × fat intake interaction effect, F(1,61) = 3.87, p = .05, η² = .060; younger participants who reported consuming lower levels of fat showed a higher awakening response. However, this became a non-significant trend following adjustment for BMI, awakening time, and chronic illness (p = .06 - .07). There was no significant interaction effect with age cohort for fruit and vegetable intake.

**Sensitivity analyses**

In order to confirm the robustness of the interaction effects for age group and diet, age and diet interaction variables were created as products of: age group and fat intake, and age group and fruit and vegetable intake, and correlational analyses performed. There were significant negative correlations between the age group x fat interaction variable and cortisol mean, r(61) = -.30, p = .02, and AUC, r(61) = -.28, p = .02; the higher the fat intake, the lower the mean cortisol and AUC. The association for the CAR did not quite reach significance, r(61) = -.23, p = .07. For the age group x fruit and vegetable intake interaction variable, there were significant positive correlations for cortisol mean, r(63) = .31, p = .01, AUC, r(63) = -.28, p = .03, and CAR, r(65) = .31, p = .01. The lower the fruit and vegetable intake the lower the mean cortisol, the CAR and the AUC. Accordingly, the correlational analyses largely confirmed the outcomes from the ANOVAs.
Discussion

Older adults displayed a blunted CAR and a flatter diurnal profile which is consistent with previous findings for the CAR (Kudielka & Kirschbaum, 2003) and diurnal profile (Deuschle et al., 1997; VanCauter et al., 1996). Young adults with high fat and low fruit and vegetable diets also showed an attenuated diurnal profile, and those with high fat intake a reduced CAR. The correlational analyses also indicated an association between a low fruit and vegetable diet and a lower CAR. Although the exact function of the CAR is unknown, it has been proposed to be linked to memory systems (Wilhelm, Born, Kudielka, Schlotz & Wüst, 2007), play an important role in regulating the immune system (Petrovsky & Harrison, 1997) and in anticipating the demands of the day (Fries, Dettenborn & Kirschbaum, 2009). The reduced CAR observed in the older adults and younger adults with high fat intake in this study may therefore have implications for such functions, although this latter finding should be interpreted with caution, given that the interaction effect with fat intake for the CAR was attenuated with adjustment for potential confounding variables. This could, however, reflect low statistical power.

In contrast to prior studies, the flatter profile observed in this investigation was due to lower awakening levels and a reduced CAR in older adults, as opposed to higher evening levels (Deuschle et al., 1997). However, whilst Deuschle et al (1997) took samples over a 24 h period, the CAR was not measured and thus age differences may have also occurred in this parameter. Older adults in the present study had lower overall cortisol levels, whereas higher cortisol has been observed previously (Luz et al., 2003; VanCauter et al., 1996). However, others have found a lower morning (Maes, Calabrese, Lee & Meltzer, 1994) and overall cortisol levels with ageing (Ahn, Lee, Choi, Kwon & Chun, 2007). Although these studies analysed plasma, salivary cortisol accurately reflects plasma free cortisol (Kirschbaum & 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 of individuals showed increasing diurnal cortisol levels over the years some individuals also showed a decrease (Lupien, Lecours, Schwartz, Sharma, Hauger., et al., 1996); the pattern of secretion did not differ significantly with 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; Yen & Laughlin, 1998) and reduced hippocampal volume has been shown in older adults with a progressive increase in 24h cortisol over 5-6 years (Lupien, de Leon, de Santi, Convit, Tarshish., et al., 1998). Loss of HPA axis sensitivity of has also been demonstrated via impaired dexamethasone suppression (Magri, Locatelli, Balza, Molla, Cuzzoni., 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 possibly 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, Kern, Allen, Tranel & Kirschbaum, 2004).

A bi-directional relationship exists between cortisol and metabolism (Lasikiewicz, Hendrickx, Talbot & Dye, 2008) and the purpose of the CAR has been suggested to provide energy in the transition from sleep (Pruessner et al., 1997) via cortisol induced gluconeogenesis. Given the relationship between cortisol with metabolism, the association between diet and cortisol observed is perhaps unsurprising. However, there are no previous reports of interactions between age, diurnal cortisol and diet. It is worth noting that there is evidence of a flatter cortisol rhythm in individuals with a higher intake of saturated fat (Garcia-Prieto et al., 2007) and a decreased CAR in individuals with greater body mass indices and central body fat deposition (Lasikiewicz et al., 2008). In the present study, however, the flattened diurnal profile observed in the younger participants with a high fat intake was independent of body mass index, although adjustment for BMI did attenuate the age × fat intake interaction effect for the CAR. However, all of our younger participants had body mass indices within the normal range. Nevertheless, it is possible that a flattened diurnal profile is a marker of susceptibility to obesity or metabolic disturbance later in life. However, in the absence of longitudinal data, this must remain highly speculative.

The present study suffers from a number of limitations. First, it is possible that the findings relate to more specific aspects of diet which were not investigated, such as dietary composition or energy balance. For example, the micronutrient content of diet has been shown to effect cortisol metabolism (Stimson, Johnstone, Homer, Wake, Morton., et al., 2007). A diet diary could have been used to ascertain food intake in more detail. However, food frequency questionnaires have been demonstrated to closely agree with diet diaries (Brunner, Stallone, Juneja, Bingham & Marmot, 2001) and the present aim was to assess habitual rather than current intake whilst avoiding response burden for participants,
particularly older adults. In addition it is possible that the effects for fat intake and fruit and vegetable intake were not independent of one another. However, fat and fruit and vegetable intake were not correlated in either cohort. Second, 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, Fries, Schweisthal, Schlotz, Stone, et al., 2007; Stalder, Hucklebridge, Evans & Clow, 2009; Thorn, Hucklebridge, Evans & Clow, 2009), there is also evidence from studies sampling on more than one day that the diurnal cortisol profile (Edwards et al., 2001; Hucklebridge et al., 2005) and the CAR (Clow, Thorn, Evans & Hucklebridge, 2004; Pruessner et al., 1997; Wurst et al., 2000) show reasonable temporal stability. Finally, although confounding by unmeasured or poorly measured variables remains possible, we did adjust for a range of potential confounders, including awakening time. Further, the present associations were not explained by perceived or life events stress (data not reported here).

Future research might investigate how changes in diurnal cortisol with ageing relate to the diurnal patterns of other endocrine and immune variables. As it may not be changes in cortisol per se, but rather the balance of cortisol with other hormones, such as dehydroepiandrosterone, that is of greater importance to physiological and psychological health in ageing individuals (Straub, Cutolo, Zietz & Schölmerich, 2001). Further, the CAR and diurnal profile could be additionally examined in relation to physical function and independence to better determine the practical implications of an altered diurnal cortisol rhythm in older adults.
In conclusion, compared to the young cohort older adults displayed significantly reduced cortisol upon awakening, a lower CAR and a flatter diurnal profile represented by a reduced AUC and diurnal slope. This phenotype was, to an extent, imitated by younger adults with higher fat and lower fruit and vegetable intake; the significant interactions between age and diet on diurnal cortisol were further supported by correlational analysis and withstood adjustment for potential confounding variables. The findings of the present study suggest that age and diet interact in their relationship with diurnal cortisol, and this should be taken into consideration when examining health behaviours and cortisol or age and cortisol. Younger adults with a poor quality of food intake may be vulnerable to a reduction in the amplitude of the cortisol diurnal profile and this may have implications for other aspects of health.
References


cortisol indicators in a large sample without psychopathology. *Psychoneuroendocrinology*, 34, 1109-1120.


Table 1. Mean (SD) health Behaviours, BMI, Chronic Illness and Time of Awakening for Old and Young cohorts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Older Adults (n = 48)</th>
<th>Younger Adults (n = 24)</th>
<th>F(df)</th>
<th>p</th>
<th>η²</th>
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<tr>
<td>Exercise score</td>
<td>4.8 (4.51)</td>
<td>13.7 (6.85)</td>
<td>41.47 (1,66)</td>
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<td>.386</td>
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<td>Cooked meals (per day)</td>
<td>1.2 (0.80)</td>
<td>1.8 (0.51)</td>
<td>39.13 (1,70)</td>
<td>&lt; .001</td>
<td>.359</td>
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<tr>
<td>Caffeine (drinks per day)</td>
<td>4.6 (2.62)</td>
<td>1.4 (1.20)</td>
<td>29.93 (1,69)</td>
<td>&lt; .001</td>
<td>.303</td>
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<td>Fat score</td>
<td>9.6 (2.66)</td>
<td>10.9 (3.87)</td>
<td>2.00 (1,67)</td>
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<td>.029</td>
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<td>Fruit and vegetable score</td>
<td>4.8 (4.51)</td>
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<td>5.79 (1,69)</td>
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<td>.077</td>
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<td>BMI (kg/m²)</td>
<td>28.2 (4.17)</td>
<td>21.7 (2.79)</td>
<td>47.29 (1,70)</td>
<td>&lt; .001</td>
<td>.403</td>
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<tr>
<td>Time of awakening (am/h)</td>
<td>7.10 (1.01)</td>
<td>8.45 (1.01)</td>
<td>28.79 (1,70)</td>
<td>&lt; .001</td>
<td>.291</td>
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Categorical variables

<table>
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<th>Older Adults</th>
<th>Younger Adults</th>
<th>χ² (df)</th>
<th>p</th>
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<td>Alcohol ≥ 6 units per week (%)</td>
<td>27</td>
<td>63</td>
<td>8.45 (1)</td>
<td>.004</td>
</tr>
<tr>
<td>Alcohol ≥ 1-2 times per week (%)</td>
<td>48</td>
<td>75</td>
<td>4.79 (1)</td>
<td>.029</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>6</td>
<td>4</td>
<td>.132 (1)</td>
<td>.593</td>
</tr>
<tr>
<td>Sleep ≥ 6 h (%)</td>
<td>29</td>
<td>71</td>
<td>9.06 (1)</td>
<td>.003</td>
</tr>
<tr>
<td>Chronic illness (%)</td>
<td>67</td>
<td>17</td>
<td>17.38 (1)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>
Table 2. Mean (SD) Health Behaviours, BMI and Time of Awakening for Diet Groups for the Younger Cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>High Fat Intake</th>
<th>Low Fat Intake</th>
<th>Low Fruit &amp; Vegetable Intake</th>
<th>High Fruit &amp; Vegetable Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise score</td>
<td>13.2 (7.01)</td>
<td>14.2 (6.95)</td>
<td>14.0 (6.92)</td>
<td>13.5 (7.03)</td>
</tr>
<tr>
<td>Cooked meals (per day)</td>
<td>1.8 (0.45)</td>
<td>1.8 (0.58)</td>
<td>1.7 (0.49)</td>
<td>1.8 (0.53)</td>
</tr>
<tr>
<td>Caffeine (drinks per day)</td>
<td>1.8 (1.22)</td>
<td>1.1 (1.14)</td>
<td>1.5 (.54)</td>
<td>1.4 (1.37)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 (3.38)</td>
<td>21.6 (2.20)</td>
<td>23.1 (3.20)</td>
<td>21.1 (2.50)</td>
</tr>
<tr>
<td>Time of awakening (am/h)</td>
<td>8.51 (0.92)</td>
<td>8.38 (1.14)</td>
<td>8.54 (0.82)</td>
<td>8.4 (1.10)</td>
</tr>
<tr>
<td>Fruit and vegetable score</td>
<td>10.7 (2.27)</td>
<td>11.5 (2.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat score</td>
<td></td>
<td></td>
<td>10.3 (3.20)</td>
<td>11.1 (4.18)</td>
</tr>
</tbody>
</table>

Categorical variables

<table>
<thead>
<tr>
<th></th>
<th>High Fat Intake</th>
<th>Low Fat Intake</th>
<th>Low Fruit &amp; Vegetable Intake</th>
<th>High Fruit &amp; Vegetable Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep ≥ 6 h (%)</td>
<td>75</td>
<td>67</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Alcohol ≥ 6 units per week (%)</td>
<td>83*</td>
<td>42</td>
<td>86</td>
<td>53</td>
</tr>
<tr>
<td>Alcohol ≥ 1-2 times per week (%)</td>
<td>83</td>
<td>67</td>
<td>86</td>
<td>71</td>
</tr>
</tbody>
</table>

* Significantly different from low fat intake group, $\chi^2 (1) = 4.44, p = .035.$
### Table 3. Mean (SD) Health Behaviours, BMI and Time of Awakening for Diet Groups for the Older Cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>High Fat Intake</th>
<th>Low Fat Intake</th>
<th>Low Fruit &amp; Vegetable Intake</th>
<th>High Fruit &amp; Vegetable Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise score</td>
<td>4.8 (5.27)</td>
<td>4.5 (4.14)</td>
<td>5.0 (4.79)</td>
<td>3.9 (3.61)</td>
</tr>
<tr>
<td>Cooked meals (per day)</td>
<td>1.2 (0.42)</td>
<td>1.1 (0.33)</td>
<td>1.1 (0.33)</td>
<td>1.2 (0.43)</td>
</tr>
<tr>
<td>Caffeine (drinks per day)</td>
<td>4.8 (2.75)</td>
<td>4.4 (2.56)</td>
<td>5.0 (2.65)</td>
<td>3.9 (2.40)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.1 (4.32)</td>
<td>27.9 (4.16)</td>
<td>28.6 (4.20)</td>
<td>26.9 (4.10)</td>
</tr>
<tr>
<td>Time of awakening (am/h)</td>
<td>7.0 (1.19)</td>
<td>7.2 (0.85)</td>
<td>7.1 (1.05)</td>
<td>7.1 (0.95)</td>
</tr>
<tr>
<td>Fruit and vegetable score</td>
<td>9.3 (2.45)</td>
<td>9.9 (2.79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat score</td>
<td></td>
<td></td>
<td>8.2 (1.70)</td>
<td>9.6 (2.63)</td>
</tr>
</tbody>
</table>

**Categorical variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>High Fat Intake</th>
<th>Low Fat Intake</th>
<th>Low Fruit &amp; Vegetable Intake</th>
<th>High Fruit &amp; Vegetable Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep ≥ 6 h (%)</td>
<td>21</td>
<td>38</td>
<td>39</td>
<td>21</td>
</tr>
<tr>
<td>Alcohol ≥ 6 units per week (%)</td>
<td>31</td>
<td>21</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td>Alcohol ≥ 1-2 times per week (%)</td>
<td>54</td>
<td>42</td>
<td>58</td>
<td>29</td>
</tr>
</tbody>
</table>
Figure 1. Mean (SEM) diurnal cortisol pattern by age cohort.

Figure 2. Mean (SEM) diurnal cortisol by age cohort and fat intake score.

Figure 3. Mean (SEM) diurnal cortisol by age cohort and fruit and vegetable intake score
Time post awakening (hours)

Salivary cortisol (ng/ml)

- Young low fruit and veg score
- Young high fruit and veg score
- Old low fruit and veg score
- Old high fruit and veg score