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Ageing, physical function, and the diurnal rhythms of cortisol and dehydroepiandrosterone

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Summary

The present study examined the relationship between ageing, physical function and the diurnal rhythms of cortisol and dehydroepiandrosterone (DHEA). Participants were 36 community dwelling older adults aged between 65-86 years old. Salivary cortisol and DHEA were 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 the Nottingham extended activities of daily living index, the Berg Balance Scale and their handgrip strength was assessed. Older participants had a significantly higher cortisol area under the curve (AUC), lower overall DHEA levels, lower DHEA AUC, a decreased diurnal slope of decline and increased cortisol:DHEA ratio. Lower diurnal cortisol levels were associated with poorer performance on the Berg Balance Scale and lower handgrip strength, and those with a flattened DHEA diurnal profile reported less independence in carrying out daily tasks. These associations withstood adjustment for age. In conclusion, this study suggests an association between cortisol, DHEA, ageing and physical function.

Keywords: Ageing, diurnal rhythm, cortisol, DHEA, physical function, saliva

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1. Introduction

Cortisol and dehydroepiandrosterone (DHEA) are stress hormones of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol is involved in a number of important functions including responses to stress, energy metabolism, vascular activity, and inflammatory and immune responses (Schürmeyer and Wickings, 1999). DHEA is a precursor to sex hormones; it has been proposed to affect various systems of the body and be anti-ageing (Chahal and Drake, 2007) and immune enhancing (Buford and Willoughby, 2005). Cortisol exhibits a marked diurnal rhythm, characterised by a rapid increase in levels upon awakening peaking at around 30 minutes post awakening and declining to reach a nadir in the evening, where DHEA has been shown to display a flat pattern of secretion after waking followed by a progressive decline to 3 hours post awakening with no significant change thereafter (Pruessner et al., 1997).

1.1. Diurnal cortisol, DHEA and ageing

Previous studies examining the effects of ageing on diurnal cortisol secretion have yielded conflicting results, with either a flattening of the diurnal pattern of secretion with increasing age (VanCauter et al., 1996; Deuschle et al., 1997; Yen and Laughlin, 1998; Luz et al., 2003), no association (Edwards et al., 2001b; Wolf et al., 2002), or decreased overall levels (Orentreich et al., 1992; Straub et al., 2000) with age. Therefore, it is

possible that cortisol *per se* may not increase with ageing, but rather that cortisol levels are high in relation to other hormones such as DHEA, which declines with age in both saliva (Ahn et al., 2007) and serum (Belanger et al., 1994; Labrie et al., 1997a). This would lead to an overrepresentation of cortisol and an increase in the cortisol:DHEA ratio (Phillips et al., 2007), which has been found to be associated with immune impairments and infection risk in older adults (Butcher et al., 2005).

In comparison to cortisol, little attention has been paid to the diurnal pattern of DHEA in ageing individuals or across a range of ages among older adults, with one exception, which found similar profiles in young and older individuals (Erosheva et al., 2002). However, alterations in the diurnal rhythm of DHEA, as well as cortisol, and the cortisol:DHEA ratio are particularly relevant for ageing individuals where changes in endocrine function may relate to disturbances in other physiological systems, and consequently the presentation of physical frailty (Walston et al., 2006).

1.2. Cortisol, DHEA and physical function

Frailty has become increasingly recognised as a key concern for older individuals (Cherniack et al., 2007). How frailty should be defined has been subject to much deliberation. However, it has been proposed that frailty is characterised by a diminished ability to carry out activities of daily living, both practically and socially (Rockwood et al., 1994; Brown et al., 1995;). Dependence on others for activities of daily living is a predictor of admission to an institution, home care use, admission to and prolonged stays

in hospital, and mortality rates Rockwood et al (1994). Alternatively, other criteria can also be used as indicators of deterioration in physical function: for example, handgrip strength, walking speed (Fried et al., 2001) and balance (Brown et al., 2000); falling due to poor balance is a key predictor of hospital admission and progression to frailty (Donaldson et al., 1990). These variables can be used separately as markers of physical function, or in combination to create a frailty index.

Neuroendocrine and immune dysregulation has also been recognised as a manifestation of frailty (Ahmed et al., 2007) and may additionally be a pathway to its onset and development (Joseph et al., 2005; Walston et al., 2006). Therefore, changes in physical function, prior to frailty onset and development, could also potentially also be associated with changes in the endocrine system. Higher cortisol levels in older adults have been associated with characteristics of frailty in several studies (Peeters et al., 2007; Varadhan et al., 2008). Further, low levels of serum DHEA sulphate (DHEA-S) have been negatively associated with a frailty phenotype (Voznesensky et al., 2009) and poorer physical function (Berkman et al., 1993). However, less is known about DHEA in its un-sulphated form and DHEA in saliva in relation to frailty. As previously mentioned, DHEA displays a diurnal variation where DHEA-S does not (Kroboth et al., 1999), and the diurnal rhythm of DHEA has been shown to be important for health and well being. For example, blunted levels of DHEA in the morning has been previously associated with depression (Goodyer et al., 1996) stress and anxiety (Luz et al., 2003), and therefore may relate to other aspects of health, such as physical function. To our knowledge, previous

research has not employed multiple sampling points across the day; therefore the diurnal rhythm of DHEA has not been examined in relation to physical function in older adults. Further, the advantages of employing saliva sampling, rather than serum sampling, to analyse both cortisol (Kirschbaum and Hellhammer, 1994) and DHEA (Granger et al., 1999) have been highlighted previously.

Given the scant research on cortisol and DHEA and particularly their rhythms in relation to physical function in older adults, the present study investigated the diurnal rhythms of cortisol and DHEA and the cortisol:DHEA ratio in relation to age among older adults. It also examined how these endocrine parameters related to physical function among older adults. It was hypothesised that those indicating lower levels of physical function would exhibit flatter diurnal profiles of cortisol and DHEA.

2. Methods

2.1. Participants

Participants were 36 (18 women) community dwelling older adults aged between 65-86 years (mean = 72.5, SD = 6.47), with mean BMI of 26.7 (SD = 4.73). Forty one participants were originally recruited, five were excluded for non compliance and/or extreme ($\geq \pm 3$ SD from the mean) hormone values. Older adults were recruited from clubs and associations in Birmingham, UK, and through posters displayed in businesses around the local area. The majority (94%) of participants described themselves as "white", and the remaining participants described themselves as "Asian". In terms of

socio-economic status, 69% were classified as from a non-manual occupational households based on their previous/current occupation, using the Registrar General's Classification of Occupations (Occupations., 1980). Inclusion criteria were: no endocrine or immune disorder, no psychiatric illness, no periodontal disease, no eating disorder and not taking glucocorticoid medication. Forty seven percent of participants reported suffering from a chronic illness, the most commonly reported were: hypertension (35%), arthritis (29%), osteoarthritis (18%), renal disease (12%) and glaucoma (12%). Fifty percent of participants reported taking chronic medication, most frequently reported were: diuretics (33%), antihypertensive (22%), gastrointestinal (22%) and pain medication (22%).

2.2. Design

This study was a cross sectional investigation of salivary cortisol, DHEA, age and physical function in older adults. The study comprised an initial day of saliva sampling and a follow-up frailty assessment at the University of Birmingham completed 2.7 (SD = 1.93) days after saliva sampling. All participants gave written informed consent prior to the study, which had the appropriate Ethics Committee approval.

2.3. Measures

2.3.1. Physical function and activities of daily living

The Nottingham extended activities of daily living (ADL) index (Nouri and Lincoln, 1987) measures independence on a four point scale ranging from 0, not at all, to 3, alone

easily, in 21 items in the categories of mobility, kitchen, domestic tasks and leisure activity. Test retest reliabilities ranging from .62-1.00 (Nouri and Lincoln, 1987) and internal consistencies of .72- .94 (Nicholl et al., 2002) have been reported for all four categories. Internal consistency in the present sample was .96. Older adults attended the laboratory at the University of Birmingham to complete an assessment of activities of daily living (ADL) and physical function. Handgrip strength, as an index of upper body strength, was measured using a hydraulic hand dynamometer (Lafayette Instrument, 70718, Lafayette, IN) and functional mobility was tested via the Berg Balance Scale. The Berg Balance Scale involves 14 tasks where the participant is mainly asked to maintain a given position for a specific time but also includes tasking involving reaching, stepping and transfers. Each task is scored on a 5 point ordinal scale ranging from 0-4 where 4 is the highest level of function. Points are deducted if the time or distance requirements are not met, the participant warrants supervision or assistance is required to complete tasks. Internal consistency reliability of .83 has been reported (Berg, 1995) and the inter observer agreement of .98 when a primary researcher was compared to an independent investigator (Berg et al., 1992). The internal consistency in the present sample was .96.

2.3.2. Salivary Cortisol and DHEA Measurements

Saliva samples were obtained over one day to determine the diurnal pattern of free salivary cortisol and DHEA secretion. Universal tubes were centrifuged at 4000 rpm for 5 min and the saliva was pipetted into eppendorfs which were stored at -20°C until assay.

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Salivary cortisol and DHEA samples were analysed in duplicate using separate assays by ELISA (IBL international, Hamburg, Germany). These cortisol and DHEA assays are based on the competition principle and microplate separation. An unknown amount of cortisol/DHEA present in the sample and a fixed amount of cortisol/DHEA conjugated with horseradish peroxidase compete for the binding sites of antibody directed towards cortisol/DHEA which are coated to the wells. After 1h (DHEA) or 2h (cortisol), the microplate is washed to stop the competition reaction. After addition of a substrate solution and further incubation, the enzymatic reaction is stopped and the concentration of these hormones is inversely proportional to the optical density measured at 450 nm. Intra assay coefficients were < 10%.

2.4. Procedure

Each participant was provided with a pack of six universal tubes 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 not to eat, drink (except water), smoke or brush their teeth 30 min prior to each sample. For each sample, participants were asked to: take a sip of water, rinse their mouth, spit this water out, swallow hard, then lean forward and allow saliva to collect in their mouth while making a gentle chewing motion to stimulate saliva. After two minutes they were asked to spit the saliva that had collected in their mouth into the appropriately labelled collection tube, and store the tube in a refrigerator in a re-sealable bag which was provided. To measure

compliance all participants were given a diary to record the times their samples were due and the time when they actually took them. They were given a wristband on which they could write reminders of their sampling times. According to the self report diary, out of 216 samples: 24% were taken up to 5 min late, 10% up to 10 min late, 1% up to 20 min late and 2% up to 45 min late. The 3% of samples that were taken more than 10 minutes late represented only 7 out of the 216 samples, and these delays only occurred in three participants. Saliva samples were collected from participants within one week. Cortisol has been found to be stable for up to 3 months when stored at 5°C (Garde and Hansen, 2005) and for up to 7 days when stored at room temperature (Aardal and Holm, 1995). DHEA levels in saliva have been shown to be unaffected by storage at room temperature for up to 10 days (Whembolua et al., 2006). The first two samples of the day were excluded if taken more than 10 min late (Kunz-Ebrecht et al., 2004).

At the laboratory, handgrip strength was measured in the standing position by asking participants to hold the dynamometer out at 90 degrees from their body then grip as strongly as they could, pulling the dynamometer down towards themselves. A practice grip was followed by three assessments with 30 seconds rest in between. The mean of the three measures was used to calculate average handgrip strength. Following this, participants completed the Berg Balance Scale activities as described above. Participants then completed the ADL scale, were thanked, and given a form to claim travel expenses.

2.5. Data analysis

Analyses were conducted using the following outcome measures: the diurnal repeated measures patterns across all six samples; the cortisol awakening response (CAR); area under the curve (AUC) for both cortisol and DHEA; diurnal slopes of both hormones and the cortisol:DHEA ratio. The CAR was calculated as sample 2 minus sample 1 (Edwards et al., 2001a; Sjogren et al., 2006). AUC for cortisol and DHEA was calculated relative to zero using the trapezoid method applied to all sampling points (Pruessner et al., 2003). Diurnal slopes were calculated by regressing hormone values on the sample time for each participant separately (Cohen et al., 2006; Smyth et al., 1997; 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). The second sample (30 minutes after waking) indicating the wakening response was excluded from the estimation of the cortisol slope across the day (Cohen et al., 2006). The cortisol: DHEA ratio was calculated by as average cortisol divided by average DHEA. Again, sample 2 was excluded from the calculating the average hormone values to exclude the awakening rise of cortisol.

Participants were split into two age groups using the median, an old group (mean = 67.6 SD = 2.36) and an older group (mean = 78.1, SD = 4.87), for the analysis of diurnal cortisol and DHEA in relation to ageing. Secondly, for the separate analysis of physical function in relation to these hormones, binary variables were created for the Berg Balance Scale and Nottingham ADL index using median splits to form high and low groups. It should be noted that these high and low groups are based on the median of the present

sample, and therefore do not represent a clinical cut off. Based on the cut off criteria used to indicate frailty from (Ahmed et al., 2007), high and low handgrip strength groups were formed. This handgrip strength criteria is based on sex and BMI, see Ahmed et al. (2007) for ranges and cut offs.

Repeated measures ANOVA was used to examine the diurnal cortisol rhythm, first in relation to age group, and second, in relation to each separate physical function variable, in order to test main effects of age and physical function and any interaction effects of age group × time or physical function group × time, on these hormones. Greenhouse-Geisser corrections were applied in repeated measures analyses and partial η^2 is reported throughout as a measure of effect size. 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 effects of age group, then frailty on the CAR, AUCs, diurnal slopes and the cortisol:DHEA ratio. Where significant effects emerged for the function measures, subsequent ANCOVA was performed to adjust for potential confounding variables: time of awakening, age and delay in sampling time. These covariates were entered separately. Age was significantly correlated with chronic illness, $r(34) = .43$, $p = .009$, and medication use, $r(34) = .335$, $p = .046$; accordingly, because of issues of co-linearity, we did not additionally adjust for these variables in models controlling for age. To control for delays in sampling times, average sampling time delay was computed for each participant and used as a covariate. In addition, for significant group × time interactions,

specific time delays for the samples where significant differences were found were also used as a covariate. For example, if the groups significantly differed upon waking and 30 minutes post waking, sample time delays for these two samples were entered separately as covariates for that finding. Slight variations in degrees of freedom reflect occasional missing data or insufficient saliva for analysis.

3. Results

Participants mean cortisol and DHEA levels overall and at each time point are shown in Table 1, along with their mean handgrip strength, Berg Balance Scale and ADL scores.

[Insert Table 1 about here]

3.1. Age, cortisol and DHEA

There was a significant quadratic effect for diurnal cortisol, $F(1,26) = 7.54$, $p = .01$, $\eta^2 = .225$, such that the older old adults had higher cortisol levels at 3 h and 6 h post waking. This pattern is shown in Figure 1a. They also had a significantly higher AUC (62.8, SD = 20.53 versus 49.6, SD = 12.45), $F(1,26) = 4.26$, $p = .05$, $\eta^2 = .141$. Females in the younger old adult group had a significantly higher CAR compared to the male younger old adults, $F(1,17) = 6.37$, $p = .02$, $\eta^2 = .273$.

There was a significant main effect of age for DHEA levels overall where the older participants exhibited lower DHEA levels (.49, SD = .35 nmol/l) compared to the younger old adults (.23, SD =.12 nmol/l), $F(1,31) = 7.35$, $p = .01$, $\eta^2 = .192$. This effect is displayed in Figure 1b. Older participants also demonstrated a significantly lower, $F(1,31) = 7.88$, $p = .009$, $\eta^2 = .203$, DHEA AUC (917.9, SD = 447.39 versus 1795.6, SD = 1137.09) which decreased progressively with age , $r(31) = -.49$, $p = .004$. With increasing age, the DHEA slope became significantly less steep, $r(31) = .42$, $p = .01$.

[Insert Figure 1 about here]

Finally, older adults had a significantly higher cortisol:DHEA ratio (20.5, SD = 9.56 nmol/l versus 11.8, SD = 9.64 nmol/l), $F(1,26) = 5.64$, $p = .0$, $\eta^2 = .178$, which increased linearly with age, $r(26) = .40$, $p = .03$. There was no significant differences between time of awakening between age groups ($p = .17$) and significant findings in relation to age withstood adjustment for sampling delays. There were no sex differences for any of the above cortisol or DHEA variables, nor any sex × age interaction effects, with the exception of the CAR × sex finding for the younger old adults.

3.2. Cortisol and physical function

Regarding associations between cortisol and physical function, there was a significant interaction effect of diurnal cortisol × Berg Balance Scale score, $F(5,130) = 3.04$, p

=.04, η^2 = .105, such that those with a lower score indicating worse balance exhibited lower cortisol immediately after and 30 minutes post-waking, as reflected by a significant quadratic trend, F(1,26) = 4.45, *p* =.04, η^2 = .146. This is shown in Figure 2. There was also a significant main effect of the Berg Balance Scale on cortisol, F(1,26) = 6.50, *p* =.02, η^2 = .200, such that those with poorer balance had lower overall cortisol levels (4.7, SD = 1.47 nmol/l) than those with relatively good balance (6.2, SD = 1.48 nmol/l).

[Insert Figure 2 about here]

There was a significant main effect of handgrip strength on cortisol, F(1,26) = 4.83, *p* =.04, η^2 = .157, such that those with lower handgrip strength, who met the cut off criteria for frailty risk according to Ahmed et al. (2007), had lower overall cortisol levels (4.7, SD = 1.51 nmol/l) than those with greater handgrip strength (6.0, SD = 1.51 nmol/l).

[Insert Figure 3 about here]

The main effect of Berg score on cortisol withstood adjustment for age, F(1,25) = 8.59, *p* =.007, η^2 = .256. However, the interaction effect was attenuated following adjustment for age, F(5,125) = 1.77, *p* =.17, η^2 = .066. The main effect of handgrip strength on cortisol also withstood adjustment for age, F(1,25) = 4.67, *p* =.04, η^2 = .157. There was no significant difference in time of waking between those with high and low Berg scores (*p* = .91) or high and low handgrip strength scores (*p* = .78). The above findings

withstood adjustment for sampling delays. No significant findings emerged in relation to the Nottingham ADL index for cortisol.

3.3. DHEA and physical function

Those with lower independence in carrying out activities of daily living displayed a significantly different diurnal DHEA pattern over the day, $F(5,155) = 3.80$, $p = .03$, $\eta^2 = .109$. The pattern was characterised by significant linear, $F(1,31) = 5.56$, $p = .03$, $\eta^2 = .109$, and quadratic effects, $F(1,31) = 4.45$, $p = .04$, $\eta^2 = .126$, such that those with lower DHEA in the morning period, and consequently a flatter diurnal profile, were less independent. This effect is displayed in Figure 4. Those with lower independence scores were also characterised by a lower DHEA slope (-4.51, SD = 6.46) compared to those with higher independence (-17.15, SD = 15.90), $F(1,31) = 5.82$, $p = .02$, $\eta^2 = .158$.

[Insert Figure 4 about here]

The interaction of diurnal DHEA × ADL independence remained significant when controlling for age, $F(5,150) = 3.03$, $p = .05$, $\eta^2 = .092$, although the effect for diurnal slope did not, $F(1,30) = 2.21$, $p = .15$, $\eta^2 = .069$. There was no significant difference in time of waking between those with high and low independence on the ADL scale ($p = .52$) and sampling time delays did not attenuate the interaction finding. No significant findings emerged in relation to handgrip strength or the Berg Balance Scale for DHEA. There were no significant findings for the cortisol:DHEA ratio in relation to any of the

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physical function variables. Finally, there were no interactions between function scores and sex for either cortisol or DHEA.

4. Discussion

4.1. Diurnal cortisol, DHEA and ageing

Older old adults showed higher diurnal cortisol levels and a higher AUC. This elevation in diurnal cortisol with ageing is consistent with previous findings; however, it has mainly been observed as a result of higher evening and nocturnal concentrations (VanCauter et al., 1996;Deuschle et al., 1997), as opposed to the higher daytime levels in the present study. Van Cauter et al. (1996) and Deuschle et al. (2007) measured cortisol in plasma, thus the different specimen of measurement may account for contrasting results. However, salivary cortisol has been shown to accurately reflect plasma free cortisol (Kirschbaum and Hellhammer, 1989). Increases in cortisol 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 and Laughlin, 1998). Despite being evident at different times of the day, it is possible that the increase in cortisol among the older adults, wherever manifest in the diurnal cycle, is due to the same mechanisms. Further, as evening and nocturnal samples were not collected in the present study, it remains possible that our two age groups differed at these times. It is important to note that a change in the diurnal pattern did not translate into a significant increase in overall cortisol.

Older participants exhibited lower DHEA levels overall, and with increasing age, the DHEA AUC was attenuated and the slope of decline became less steep. The observed decrease in DHEA levels is in line with previous research (Belanger et al., 1994; Labrie et al., 1997; Ahn et al., 2007), however, to our knowledge, the diurnal rhythm of DHEA has not been examined previously in older individuals. Rather than maintaining its normal pattern of secretion and a lower overall level with increasing age, DHEA secretion appears to be most reduced in the morning period resulting in a flatter diurnal rhythm among the oldest old.

The observed reduction in DHEA levels coincident with no overall change in cortisol was reflected in a significantly higher cortisol:DHEA ratio with increasing age: a finding not without precedent (Butcher et al., 2005). Several mechanisms have been proposed for the age related decline in DHEA alongside no overall change in cortisol. A decrease in 17, 20-desmolase activity (Labrie et al., 1997), reduced LDL receptors affecting cholesterol transport, reduced ACTH receptors, a reduction in mass of the zona reticularis (Parker, 1999) and a decrease in IGF-I and IGF-II, (Yen and Laughlin, 1998), have all been implicated in the reduction of DHEA with age. Due to the diurnal rhythms of cortisol and DHEA, the elevated cortisol:DHEA ratio is most pronounced in the morning period, and it could be speculated that this may represent a more vulnerable endocrine profile of our oldest participants, at this time of day.

4.2. Cortisol, DHEA and physical function

Those with poorer performance on the Berg Balance Scale and lower handgrip strength exhibited significantly lower overall cortisol levels. Although attenuated cortisol concentrations upon awakening has been shown to predict higher levels of fatigue later that day (Adam, 2006), the present result it was higher levels of cortisol that were associated with frailty, assessed by chair stands, a tandem stand and walk test (Peeters et al., 2007). One reason for the discrepancy could be the different assessments of physical function used in the two studies; it is possible that the relationship between cortisol and physical function may vary depending on the assessment and/or criteria employed.

Those with less independence in carrying out activities of daily living displayed lower levels of DHEA in the morning period generating a flat diurnal rhythm. The negative association between DHEA and physical function is consistent with previous findings in relation to DHEA-S (Berkman et al., 1993; Voznesensky et al., 2009). The present study extends this association with physical function to salivary DHEA and illustrates that the diurnal rhythm may also be altered among individuals with lower levels of function.

Both cortisol and DHEA affect metabolism, and the balance between these two hormones has been considered as a marker of catabolic/anabolic status; sarcopenia has been proposed as one pathway through which neuroendocrine dysregulation relates to frailty (Walston, 2004). Interestingly, in the present study lower levels of both DHEA and cortisol related to physical function and consequently there was no significant associations between our measures of function and the cortisol:DHEA ratio.

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4.3. Limitations and conclusions

The present study is not without limitations. First, cross-sectional designs cannot establish the direction of causation. However, it is reasonable to speculate that neuroendocrine function contributes to the deterioration of physical function through interaction with several other systems, such as the immune and musculoskeletal systems. Second, the relatively small sample size may have limited the power to find further significant associations The original aim of the present study was to recruit equal numbers of frail and non frail participants. However, it proved difficult to recruit frail individuals from the community and thus became a study focused on physical function. Future research should consider recruiting in residential settings. Third, half of the present participants reported suffering from a chronic illness or taking continuous medication and it is possible that either their condition or medication could have influenced HPA axis function. However, although age was highly correlated with illness and medication usage and we did adjust significant findings for age. Further, due to the age group investigated a high prevalence of chronic medical conditions and medication use is somewhat expected and difficult to avoid. Additional measures of function could have been included. However, it is important in testing older adults to strike a balance between a broad assessment and what is feasible in terms of the demands of testing. In addition, the present assessments are commonly used and well regarded within frailty research. Fourth, although the findings could be confounded by other variables, we did adjust for the likely confounders of awakening time and age. It is also possible that the

observed associations between these hormones and physical function may reflect changes in psychological health. However, the present associations were not influenced by symptoms of depression or anxiety, perceived stress, or life events stress (data not reported here). Finally, we would like to have sample across more than one day, but costs precluded this. However, there is evidence that the diurnal profile of cortisol and DHEA are stable across days (Edwards, et al., 2001; Hucklebridge, et al., 2005), and all participants were retired, thus unlikely to differ vastly in terms of daily activities.

In conclusion, we found an association between cortisol, DHEA, ageing and physical function. The diurnal rhythms of cortisol and DHEA and their ratio differed between old adults and older old adults. Poorer performance on the Berg Balance Scale and lower handgrip strength was associated with lower diurnal cortisol levels, and those who reported less independence in carrying out daily tasks showed a flatter DHEA diurnal profile.

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