

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

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1 **Ageing, physical function, and the diurnal rhythms of cortisol and**  
2 **dehydroepiandrosterone**

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24

25 **Summary**

26 The present study examined the relationship between ageing, physical function and the  
27 diurnal rhythms of cortisol and dehydroepiandrosterone (DHEA). Participants were 36  
28 community dwelling older adults aged between 65-86 years old. Salivary cortisol and  
29 DHEA were measured over the course of one day: immediately upon awakening, 30 min  
30 later, and then 3 h, 6 h, 9 h and 12 h post-awakening. Participants completed the  
31 Nottingham extended activities of daily living index, the Berg Balance Scale and their  
32 handgrip strength was assessed. Older participants had a significantly higher cortisol  
33 area under the curve (AUC), lower overall DHEA levels, lower DHEA AUC, a decreased  
34 diurnal slope of decline and increased cortisol:DHEA ratio. Lower diurnal cortisol levels  
35 were associated with poorer performance on the Berg Balance Scale and lower handgrip  
36 strength, and those with a flattened DHEA diurnal profile reported less independence in  
37 carrying out daily tasks. These associations withstood adjustment for age. In conclusion,  
38 this study suggests an association between cortisol, DHEA, ageing and physical function.

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

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## 50 **1. Introduction**

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

62

### 63 **1.1. Diurnal cortisol, DHEA and ageing**

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

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

75

76 In comparison to cortisol, little attention has been paid to the diurnal pattern of DHEA in  
77 ageing individuals or across a range of ages among older adults, with one exception,  
78 which found similar profiles in young and older individuals (Erosheva et al., 2002).

79 However, alterations in the diurnal rhythm of DHEA, as well as cortisol, and the  
80 cortisol:DHEA ratio are particularly relevant for ageing individuals where changes in  
81 endocrine function may relate to disturbances in other physiological systems, and  
82 consequently the presentation of physical frailty (Walston et al., 2006).

83

## 84 **1.2. Cortisol, DHEA and physical function**

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

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

97  
98 Neuroendocrine and immune dysregulation has also been recognised as a manifestation  
99 of frailty (Ahmed et al., 2007) and may additionally be a pathway to its onset and  
100 development (Joseph et al., 2005; Walston et al., 2006). Therefore, changes in physical  
101 function, prior to frailty onset and development, could also potentially also be associated  
102 with changes in the endocrine system. Higher cortisol levels in older adults have been  
103 associated with characteristics of frailty in several studies (Peeters et al., 2007; Varadhan  
104 et al., 2008). Further, low levels of serum DHEA sulphate (DHEA-S) have been  
105 negatively associated with a frailty phenotype (Voznesensky et al., 2009) and poorer  
106 physical function (Berkman et al., 1993). However, less is known about DHEA in its un-  
107 sulphated form and DHEA in saliva in relation to frailty. As previously mentioned,  
108 DHEA displays a diurnal variation where DHEA-S does not (Kroboth et al., 1999), and  
109 the diurnal rhythm of DHEA has been shown to be important for health and well being.  
110 For example, blunted levels of DHEA in the morning has been previously associated with  
111 depression (Goodyer et al., 1996) stress and anxiety (Luz et al., 2003), and therefore may  
112 relate to other aspects of health, such as physical function. To our knowledge, previous

113 research has not employed multiple sampling points across the day; therefore the diurnal  
114 rhythm of DHEA has not been examined in relation to physical function in older adults.  
115 Further, the advantages of employing saliva sampling, rather than serum sampling, to  
116 analyse both cortisol (Kirschbaum and Hellhammer, 1994) and DHEA (Granger et al.,  
117 1999) have been highlighted previously.

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

124

125

## 126 **2. Methods**

### 127 **2.1. Participants**

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

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

145

## 146 **2.2. Design**

147 This study was a cross sectional investigation of salivary cortisol, DHEA, age and  
148 physical function in older adults. The study comprised an initial day of saliva sampling  
149 and a follow-up frailty assessment at the University of Birmingham completed 2.7 (SD =  
150 1.93) days after saliva sampling. All participants gave written informed consent prior to  
151 the study, which had the appropriate Ethics Committee approval.

152

## 153 **2.3. Measures**

### 154 **2.3.1. Physical function and activities of daily living**

155 The Nottingham extended activities of daily living (ADL) index (Nouri and Lincoln,  
156 1987) measures independence on a four point scale ranging from 0, not at all, to 3, alone



157 easily, in 21 items in the categories of mobility, kitchen, domestic tasks and leisure  
158 activity. Test retest reliabilities ranging from .62-1.00 (Nouri and Lincoln, 1987) and  
159 internal consistencies of .72- .94 (Nicholl et al., 2002) have been reported for all four  
160 categories. Internal consistency in the present sample was .96. Older adults attended the  
161 laboratory at the University of Birmingham to complete an assessment of activities of  
162 daily living (ADL) and physical function. Handgrip strength, as an index of upper body  
163 strength, was measured using a hydraulic hand dynamometer (Lafayette Instrument,  
164 70718, Lafayette, IN) and functional mobility was tested via the Berg Balance Scale.  
165 The Berg Balance Scale involves 14 tasks where the participant is mainly asked to  
166 maintain a given position for a specific time but also includes tasking involving reaching,  
167 stepping and transfers. Each task is scored on a 5 point ordinal scale ranging from 0-4  
168 where 4 is the highest level of function. Points are deducted if the time or distance  
169 requirements are not met, the participant warrants supervision or assistance is required to  
170 complete tasks. Internal consistency reliability of .83 has been reported (Berg, 1995) and  
171 the inter observer agreement of .98 when a primary researcher was compared to an  
172 independent investigator (Berg et al., 1992). The internal consistency in the present  
173 sample was .96.

174

### 175 **2.3.2. Salivary Cortisol and DHEA Measurements**

176 Saliva samples were obtained over one day to determine the diurnal pattern of free  
177 salivary cortisol and DHEA secretion. Universal tubes were centrifuged at 4000 rpm for  
178 5 min and the saliva was pipetted into eppendorfs which were stored at -20°C until assay.

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

189

#### 190 **2.4. Procedure**

191 Each participant was provided with a pack of six universal tubes labelled with the  
192 sampling times which were: immediately upon awakening, 30 min post-awakening and  
193 then 3h, 6h, 9h and 12h post awakening. They were briefed concerning the collection  
194 procedure and sampling times. Participants were asked not to eat, drink (except water),  
195 smoke or brush their teeth 30 min prior to each sample. For each sample, participants  
196 were asked to: take a sip of water, rinse their mouth, spit this water out, swallow hard,  
197 then lean forward and allow saliva to collect in their mouth while making a gentle  
198 chewing motion to stimulate saliva. After two minutes they were asked to spit the saliva  
199 that had collected in their mouth into the appropriately labelled collection tube, and store  
200 the tube in a refrigerator in a re-sealable bag which was provided. To measure

201 compliance all participants were given a diary to record the times their samples were due  
202 and the time when they actually took them. They were given a wristband on which they  
203 could write reminders of their sampling times. According to the self report diary, out of  
204 216 samples: 24% were taken up to 5 min late, 10% up to 10 min late, 1% up to 20 min  
205 late and 2% up to 45 min late. The 3% of samples that were taken more than 10 minutes  
206 late represented only 7 out of the 216 samples, and these delays only occurred in three  
207 participants. Saliva samples were collected from participants within one week. Cortisol  
208 has been found to be stable for up to 3 months when stored at 5°C (Garde and Hansen,  
209 2005) and for up to 7 days when stored at room temperature (Aardal and Holm, 1995).  
210 DHEA levels in saliva have been shown to be unaffected by storage at room temperature  
211 for up to 10 days (Whembolua et al., 2006). The first two samples of the day were  
212 excluded if taken more than 10 min late (Kunz-Ebrecht et al., 2004).

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

220

## 221 **2.5. Data analysis**

222 Analyses were conducted using the following outcome measures: the diurnal repeated  
223 measures patterns across all six samples; the cortisol awakening response (CAR); area  
224 under the curve (AUC) for both cortisol and DHEA; diurnal slopes of both hormones and  
225 the cortisol:DHEA ratio. The CAR was calculated as sample 2 minus sample 1 (Edwards  
226 et al., 2001a; Sjogren et al., 2006). AUC for cortisol and DHEA was calculated relative  
227 to zero using the trapezoid method applied to all sampling points (Pruessner et al., 2003).  
228 Diurnal slopes were calculated by regressing hormone values on the sample time for each  
229 participant separately (Cohen et al., 2006; Smyth et al., 1997; Turner-Cobb et al., 2000).  
230 This yields a slope value for each participant. The sample obtained upon awakening was  
231 used as the slope anchor (Kraemer et al., 2006). The second sample (30 minutes after  
232 waking) indicating the wakening response was excluded from the estimation of the  
233 cortisol slope across the day (Cohen et al., 2006). The cortisol: DHEA ratio was  
234 calculated by as average cortisol divided by average DHEA. Again, sample 2 was  
235 excluded from the calculating the average hormone values to exclude the awakening rise  
236 of cortisol.

237

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

244 sample, and therefore do not represent a clinical cut off. Based on the cut off criteria  
245 used to indicate frailty from (Ahmed et al., 2007), high and low handgrip strength groups  
246 were formed. This handgrip strength criteria is based on sex and BMI, see Ahmed et al.  
247 (2007) for ranges and cut offs.

248 Repeated measures ANOVA was used to examine the diurnal cortisol rhythm, first in  
249 relation to age group, and second, in relation to each separate physical function variable,  
250 in order to test main effects of age and physical function and any interaction effects of  
251 age group  $\times$  time or physical function group  $\times$  time, on these hormones. Greenhouse-  
252 Geisser corrections were applied in repeated measures analyses and partial  $\eta^2$  is reported  
253 throughout as a measure of effect size. In order to examine the patterns over time  
254 between groups, using SPSS version 17, orthogonal polynomial contrasts were fitted  
255 within each repeated measures model. Statistical significance for linear, quadratic, and  
256 cubic components are reported below, where appropriate. Univariate ANOVA was  
257 applied to analyse effects of age group, then frailty on the CAR, AUCs, diurnal slopes  
258 and the cortisol:DHEA ratio. Where significant effects emerged for the function  
259 measures, subsequent ANCOVA was performed to adjust for potential confounding  
260 variables: time of awakening, age and delay in sampling time. These covariates were  
261 entered separately. Age was significantly correlated with chronic illness,  $r(34) = .43$ ,  $p =$   
262  $.009$ , and medication use,  $r(34) = .335$ ,  $p = .046$ ; accordingly, because of issues of co-  
263 linearity, we did not additionally adjust for these variables in models controlling for age.  
264 To control for delays in sampling times, average sampling time delay was computed for  
265 each participant and used as a covariate. In addition, for significant group  $\times$  time interactions,

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

271

### 272 **3. Results**

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

275

276 [Insert Table 1 about here]

277

278

#### 279 **3.1. Age, cortisol and DHEA**

280 There was a significant quadratic effect for diurnal cortisol,  $F(1,26) = 7.54, p = .01, \eta^2 =$   
281  $.225$ , such that the older old adults had higher cortisol levels at 3 h and 6 h post waking.

282 This pattern is shown in Figure 1a. They also had a significantly higher AUC (62.8, SD

283  $= 20.53$  versus 49.6, SD = 12.45),  $F(1,26) = 4.26, p = .05, \eta^2 = .141$ . Females in the

284 younger old adult group had a significantly higher CAR compared to the male younger

285 old adults,  $F(1,17) = 6.37, p = .02, \eta^2 = .273$ .

286

287 There was a significant main effect of age for DHEA levels overall where the older  
288 participants exhibited lower DHEA levels (.49, SD = .35 nmol/l) compared to the  
289 younger old adults (.23, SD = .12 nmol/l),  $F(1,31) = 7.35, p = .01, \eta^2 = .192$ . This effect is  
290 displayed in Figure 1b. Older participants also demonstrated a significantly lower,  
291  $F(1,31) = 7.88, p = .009, \eta^2 = .203$ , DHEA AUC (917.9, SD = 447.39 versus 1795.6, SD =  
292 1137.09) which decreased progressively with age,  $r(31) = -.49, p = .004$ . With  
293 increasing age, the DHEA slope became significantly less steep,  $r(31) = .42, p = .01$ .

294

295 [Insert Figure 1 about here]

296

297 Finally, older adults had a significantly higher cortisol:DHEA ratio (20.5, SD = 9.56  
298 nmol/l versus 11.8, SD = 9.64 nmol/l),  $F(1,26) = 5.64, p = .02, \eta^2 = .178$ , which increased  
299 linearly with age,  $r(26) = .40, p = .03$ . There was no significant differences between  
300 time of awakening between age groups ( $p = .17$ ) and significant findings in relation to  
301 age withstood adjustment for sampling delays. There were no sex differences for any of  
302 the above cortisol or DHEA variables, nor any sex  $\times$  age interaction effects, with the  
303 exception of the CAR  $\times$  sex finding for the younger old adults.

304

### 305 **3.2. Cortisol and physical function**

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

308  $=.04$ ,  $\eta^2 = .105$ , such that those with a lower score indicating worse balance exhibited  
309 lower cortisol immediately after and 30 minutes post-waking, as reflected by a significant  
310 quadratic trend,  $F(1,26) = 4.45$ ,  $p = .04$ ,  $\eta^2 = .146$ . This is shown in Figure 2. There was  
311 also a significant main effect of the Berg Balance Scale on cortisol,  $F(1,26) = 6.50$ ,  $p$   
312  $=.02$ ,  $\eta^2 = .200$ , such that those with poorer balance had lower overall cortisol levels (4.7,  
313  $SD = 1.47$  nmol/l) than those with relatively good balance (6.2,  $SD = 1.48$  nmol/l).

314

315 [Insert Figure 2 about here]

316

317 There was a significant main effect of handgrip strength on cortisol,  $F(1,26) = 4.83$ ,  $p$   
318  $=.04$ ,  $\eta^2 = .157$ , such that those with lower handgrip strength, who met the cut off criteria  
319 for frailty risk according to Ahmed et al. (2007), had lower overall cortisol levels ( 4.7,  
320  $SD = 1.51$  nmol/l) than those with greater handgrip strength (6.0,  $SD = 1.51$  nmol/l).

321

322 [Insert Figure 3 about here]

323

324 The main effect of Berg score on cortisol withstood adjustment for age,  $F(1,25) = 8.59$ ,  $p$   
325  $=.007$ ,  $\eta^2 = .256$ . However, the interaction effect was attenuated following adjustment  
326 for age,  $F(5,125) = 1.77$ ,  $p = .17$ ,  $\eta^2 = .066$ . The main effect of handgrip strength on  
327 cortisol also withstood adjustment for age,  $F(1,25) = 4.67$ ,  $p = .04$ ,  $\eta^2 = .157$ . There was  
328 no significant difference in time of waking between those with high and low Berg scores  
329 ( $p = .91$ ) or high and low handgrip strength scores ( $p = .78$ ). The above findings



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

332

### 333 **3.3. DHEA and physical function**

334 Those with lower independence in carrying out activities of daily living displayed a  
335 significantly different diurnal DHEA pattern over the day,  $F(5,155) = 3.80, p = .03, \eta^2 =$   
336  $.109$ . The pattern was characterised by significant linear,  $F(1,31) = 5.56, p = .03, \eta^2 =$   
337  $.109$ , and quadratic effects,  $F(1,31) = 4.45, p = .04, \eta^2 = .126$ , such that those with lower  
338 DHEA in the morning period, and consequently a flatter diurnal profile, were less  
339 independent. This effect is displayed in Figure 4. Those with lower independence scores  
340 were also characterised by a lower DHEA slope ( $-4.51, SD = 6.46$ ) compared to those  
341 with higher independence ( $-17.15, SD = 15.90$ ),  $F(1,31) = 5.82, p = .02, \eta^2 = .158$ .

342

343 [Insert Figure 4 about here]

344

345 The interaction of diurnal DHEA  $\times$  ADL independence remained significant when  
346 controlling for age,  $F(5,150) = 3.03, p = .05, \eta^2 = .092$ , although the effect for diurnal  
347 slope did not,  $F(1,30) = 2.21, p = .15, \eta^2 = .069$ . There was no significant difference in  
348 time of waking between those with high and low independence on the ADL scale ( $p =$   
349  $.52$ ) and sampling time delays did not attenuate the interaction finding. No significant  
350 findings emerged in relation to handgrip strength or the Berg Balance Scale for DHEA.  
351 There were no significant findings for the cortisol:DHEA ratio in relation to any of the

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

354

#### 355 **4. Discussion**

##### 356 **4.1. Diurnal cortisol, DHEA and ageing**

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

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

381 The observed reduction in DHEA levels coincident with no overall change in cortisol was  
382 reflected in a significantly higher cortisol:DHEA ratio with increasing age: a finding not  
383 without precedent (Butcher et al., 2005). Several mechanisms have been proposed for the  
384 age related decline in DHEA alongside no overall change in cortisol. A decrease in 17,  
385 20-desmolase activity (Labrie et al., 1997), reduced LDL receptors affecting cholesterol  
386 transport, reduced ACTH receptors, a reduction in mass of the zona reticularis (Parker,  
387 1999) and a decrease in IGF-I and IGF-II, (Yen and Laughlin, 1998), have all been  
388 implicated in the reduction of DHEA with age. Due to the diurnal rhythms of cortisol and  
389 DHEA, the elevated cortisol:DHEA ratio is most pronounced in the morning period, and  
390 it could be speculated that this may represent a more vulnerable endocrine profile of our  
391 oldest participants, at this time of day.

392

#### 393 **4.2. Cortisol, DHEA and physical function**

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

402

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

409

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

416

417 **4.3. Limitations and conclusions**

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

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

445

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

452

453

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455

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