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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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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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).

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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

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.

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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 × time or physical function group × time, on these hormones.   Greenhouse-

252   Geisser corrections were applied in repeated measures analyses and partial η^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 × 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), $F(1,26) = 5.64$, $p =.0$, $\eta^2 = .178$, 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

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

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

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.

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454   **References**

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