Physical activity, life events stress, cortisol, and DHEA in older adults
Heaney, Jennifer L J; Carroll, Douglas; Phillips, Anna C

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Physical activity, life events stress, and the cortisol:DHEA ratio in older adults:

Preliminary findings that physical activity may buffer against

the negative effects of stress
Abstract

The present study examined the relationship between habitual physical activity, life events stress, and the cortisol: dehydroepiandrosterone (DHEA) ratio in older adults. Thirty six participants aged 65 and over reported their habitual physical activity, and also indicated if a particular event happened to them in the past year (stress incidence) and how stressful they perceived the event to be (stress severity). Older adults with higher stress severity for life events demonstrated a significantly higher cortisol:DHEA ratio. Those with higher stress incidence scores who did not participate in aerobic exercise had a significantly higher cortisol:DHEA ratio, compared to those with high stress incidence who regularly participated in aerobic exercise. This study suggests that life events stress may have a negative impact on the cortisol:DHEA ratio in older adults, and, under conditions of high stress exposure, exercise may buffer against the effects of stress on the cortisol:DHEA ratio.

Keywords: Cortisol:DHEA ratio, physical activity, life events stress, older adults
Introduction

Cortisol and dehydroepiandrosterone (DHEA) are hormones of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol is an important component of stress response where it protects the body through its role in enhancing vascular activity, suspending non-essential functions, inhibiting the inflammatory process, immune suppression, inhibiting the actions of insulin, and increasing energy availability (Widmaier, Raff, & Strang, 2004). However, long term activation of these effects as a result of chronic cortisol elevation will clearly have negative implications for health. DHEA has been proposed to have wide reaching functions, including immunomodulation and positive effects on wellbeing (Maninger, Wolkowitz, Reues, Epel, & Mellon, 2009). As a result of these proposed actions, DHEA has received growing interest in relation to ageing (Baulieu, 1996). As these hormones have opposing effects on the body, particularly with regard to immunity, the balance and interplay between cortisol and DHEA may be as important for health as either hormone individually (Buford & Willoughby, 2005). DHEA production peaks at age 20-30 years and then declines progressively with age (Belanger et al., 1994; Labrie, Belanger, Cusan, Gomez, & Candas, 1997; Orentreich, Brind, Vogelman, Andres, & Baldwin, 1992). In contrast, cortisol has been reported to increase with age (Deuschle et al., 1997; VanCauter, Leproult, & Kupfer, 1996), although counter evidence exists (Orentreich et al., 1992). The disproportionate representation of cortisol compared to DHEA, and the consequent increase in the cortisol:DHEA ratio, with ageing (Phillips, Burns, & Lord, 2007) is associated with immune impairments and infection risk in older adults (Butcher et al., 2005) as well increasing the risk for all-cause and cause-specific mortality (Phillips et al., 2010).
Habitual physical activity and fitness levels have been previously investigated in relation to hormones in older adults. DHEA sulphate (DHEA-S) has been found to correlate positively with VO2max (Bonnefoy et al., 1998; Bonnefoy et al., 2002) and estimated energy expenditure in older women, but not men (Bonnefoy et al., 1998; Kostka, Pariante, Berthouze, Lacour, & Bonnefoy, 2002). In contrast, Abbasi et al (1998) reported an association between VO2max and DHEA-S in men but not women, although this finding did not withstand adjustment for age. With regard to cortisol, one study examined older fit and unfit females and failed to observe any significant differences in cortisol concentrations between groups at baseline prior to high intensity exercise (Traustadottir, Bosch, Cantu, & Matt, 2004). However, several of these previous investigations have failed to include both sexes and both hormones within the same study, and thus the cortisol:DHEA ratio has not been explored in both sexes simultaneously. In addition, DHEA in its un-sulphated form or, indeed, these hormones in saliva, have, to our knowledge, yet to be investigated. Cortisol and DHEA levels in saliva are linearly proportional to those in the blood. These hormones in saliva reflect approximately 5% of plasma concentrations, where only 0.2% of DHEA-S levels in serum are represented in saliva (Kroboth, Salek, Pittenger, Fabian, & Frye, 1999). The advantages of non-invasive saliva collection over blood hold for both cortisol (Kirschbaum & Hellhammer, 1994) and DHEA (Granger, Schwartz, Booth, Curran, & Zakaria, 1999) have been well established.

While physical activity may have a positive effect on the endocrine system of older adults, other exposures, such as stress, could have negative effects and consequently accelerate ageing (Juster, McEwen, & Lupien, 2010).

There is certainly evidence that older adults who experience stress are at risk of accelerated age-related hormonal changes. Older adults who had experienced bereavement, and its associated emotional distress, within two months had a significantly higher
Previous studies therefore have mainly looked at stress using the framework of caregiving and bereavement. Not all older adults may be subject to specific stress such as caregiving, although most are exposed to more general life events, which has received scant attention in relation to cortisol and DHEA. The one study to date examining life events stress in older adults found that temporally distal life events were associated with attenuated morning cortisol and reduced variability between morning and evening cortisol, whereas more proximal events were associated with higher morning cortisol and increased diurnal variability (Gerritsen et al., 2010).

Exercise has been proposed as an intervention to mitigate the damaging effects of stress on the neuroendocrine system in older adults (Phillips et al., 2007). Physical activity appears to protect those experiencing high stress against age-related telomere shortening (Puterman et al., 2010). Exercise also buffered against the effects of stress, in the form of widowhood, on functional decline in a longitudinal study of ageing (Unger, Johnson, & Marks, 1997). The stress buffering potential of exercise has also been demonstrated in younger adults; a prospective study of adolescent health found that exercise buffered against the negative health effects of life events stress (Brown & Siegel, 1988). However, the
potential ameliorating effects of exercise on life events stress in relation to the cortisol:DHEA ratio in older adults has yet to be investigated.

The aim of the present study was to examine the relationship between habitual physical activity and life events stress on the cortisol:DHEA ratio in older adults. Specifically, we were concerned to determine whether participating in regular exercise buffered any negative effects of stress on these hormones. It was hypothesised that that older adults who participated in regular exercise would have a lower cortisol:DHEA ratio. It was also hypothesised that older adults experiencing higher levels of life events stress would have a higher cortisol:DHEA ratio, but this would be less pronounced in physically active individuals.

Methods

Participants

Participants were 36 (18 women) community dwelling older adults recruited from around the local area of Birmingham, UK, who were aged between 65-88. Participants were recruited from Birmingham social clubs and associations, churches, and through information stands in local supermarkets and posters displayed in businesses in the local area. Forty one participants were originally recruited, 5 were excluded for non compliance and/or extreme (≥ ± 3 SD from the mean) hormone values, resulting in the final sample size of 36. Inclusion criteria were no endocrine or immune disorder, no psychiatric illness, no periodontal disease, no eating disorder, and not taking glucocorticoid medication. The majority (94%) of participants described themselves as “white”, 4% described themselves as “Asian” and 2% as “black”. Socio-economic status was defined using previous occupation, as classified by the Registrar General’s Classification of Occupations (Occupations, 1980). Participant
characteristics are displayed in Table 1. 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%).

Study design

This study was a cross sectional investigation of salivary cortisol, DHEA, life events stress, and exercise in older adults. The study comprised a day of saliva sampling and the assessment of life events stress and habitual exercise behaviour via questionnaires. All participants gave written informed consent prior to the study, which was approved by the University Research Ethics Committee.

Questionnaires

Habitual physical activity. Participants completed the physical activity scale from the West of Scotland Twenty-07 study (Ford, Ecob, Hunt, Macintyre and West, 1994). Participants are asked to choose one activity category that describes their usual pattern of exercise. There were five categories to choose from ranging from level 1 “inactive or little activity other than usual daily activities” to level 5 “participate in aerobic activities such as brisk walking, jogging at a comfortable pace or other activities requiring similar exertion for over 3h a week”. Only aerobic exercise was measured in this scale, however, participants were asked if they engaged in any other sort of exercise not included in this scale, such as resistance exercise, to which all participants responded no.
Stressful life events over the past year. Stressful life events over the past year were measured using questionnaires designed to capture both the occurrence of events and the perception of their severity. This has been proposed to be the most effective way to measure psychosocial stress (Holmes & Rache, 1967). Stressful life events exposure over the past year was assessed by a Life Events Survey (LES) from the West of Scotland Twenty-07 study (Ford et al., 1994). It contains 61 items examining several areas of life: health, marriage/co-habitation, relationships, death, work, housing, finances and general. Participants were asked to rate the seriousness of events which had occurred on a 10-point scale, where 1 was very small and unimportant and 10 was the worst thing that could happen to you. Total scores for stress incidence and seriousness were created. In addition, participants completed a stressful events questionnaire specific to older adults. This questionnaire was designed by older adults and consists of 70 items with a 4-point severity scale ranging from not at all stressful to very stressful. Events are in the following categories: health, family, home and neighbourhood, marriage and relationships, care, bereavements, finance, work, ageing, and outside agencies. Participants received a score for total number of events and a total stress severity score. Initial piloting of this scale produced an acceptable test-retest reliability rating of .73 (Phillips et al, unpublished observations).

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. 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%.

**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 ask to refrain from exercise (other than walking and 
activities of daily living) on the day prior to and the day of saliva sampling. 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 and rinse their mouth, then 
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 and not swallow. 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 were actually taken. Participants 
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 & Hansen, 2005) and for up to 7 days when stored at room temperature (Aardal & Holm, 1995). DHEA levels in saliva have been shown to be unaffected by storage at room temperature for up to 10 days (Whembolua, Granger, Singer, Kivlighan, & Marguin, 2006). The first two samples of the day were excluded if taken more than 10 min late (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004).

In conjunction with the saliva sampling pack participants received a questionnaire pack to complete at home on the same day as saliva sampling. Participants were orally briefed on each questionnaire prior to completing them on their own. The investigator went through all written instructions, providing examples and clarification where needed, and encouraged participants to ask any questions they may have relating to the questionnaires. It was emphasised that all questionnaires were anonymous and participants should be honest in their responses. Participants were instructed to contact the investigator via telephone if they had any queries during completing the questionnaires. The questionnaires were collected at the same time as the saliva samples. Upon collection of the samples and questionnaire pack, participants were asked if they had adhered to the protocol (including refraining from exercise the day prior to, and on the day of sampling and if they missed any samples or took any late).

Data analysis

Analyses were conducted using the outcome measures of the overall average level of each hormone across the day, and the cortisol:DHEA ratio. The cortisol:DHEA ratio was calculated as average cortisol across the day divided by average DHEA. For the ratio, sample
2 was excluded from the calculating the average hormone values to exclude the awakening rise of cortisol.

Binary variables of stressful life events, both the number of events and seriousness or severity, were created using median splits to form high and low groups: for example, high life events severity score versus low score. Median splits were primarily used as exposure to life events was not normally distributed with scores clustering at the lower end of the scale, and also to follow the analytic strategy of previous studies examining life events (Pedersen et al., 2009) who have examined those with little or no exposure compared to those who experienced a number of events. Median splits was applied to physical activity level as this was a categorical rather than continuous variable, with participants having a tendency to engage in little or no exercise or exercise on a frequent basis. This resulted in participants being split into those who participated in aerobic exercise for 1 h a week or more versus those who did not.

Univariate ANOVA was applied to analyse physical activity and stressful events differences in average cortisol across the day, average DHEA across the day and the cortisol:DHEA ratio, respectively. In all of the above analyses, where significant effects emerged, subsequent ANCOVA was performed to adjust for potential confounding variables known to be associated with one or both of these hormones: age, time of awakening, and BMI. 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 colinearity, we did not additionally adjust for these variables in models controlling for age. Further, where significant group effects emerged, variables were examined in their continuous form as a sensitivity analysis variables were then analysed in their continuous form using correlations. Slight variations in degrees of freedom reflect occasional missing data or insufficient saliva for analysis.
Results

Descriptive statistics for age, BMI, time of awakening, chronic illness, smoking, and socioeconomic status are displayed in Table 1. Descriptive statistics for hormone and stress variables for male and female participants are also displayed in Table 1. No significant sex differences were found for variables displayed in Table 1. No sex differences were found for any analysis; subsequently, genders were grouped together in the following results.

[Insert Table 1 about here]

Physical activity, cortisol and DHEA

Those with higher levels of physical activity, who spent at least an hour a week engaging in aerobic exercise, demonstrated significantly higher average DHEA levels, $F(1, 32) = 7.64, p = .009, \eta^2 = .193$, resulting in a lower cortisol:DHEA ratio, $F(1, 32) = 4.05, p = .05, \eta^2 = .111$. However, these findings did not withstand adjustment for the confounding variable of age.

Cortisol, DHEA, life events stress

Descriptive statistics in relation to stress and the overall sample are displayed in Table 1. Descriptive statistics for high and low stress groups for each of the life events variables are displayed in Table 2. There was a significant difference between older adults with high and low severity ratings for average DHEA across the day, where those in the high stress severity group had significantly lower DHEA, $F(1, 33) = 3.92, p = .05, \eta^2 = .106$. Consequently, those with higher stress severity had a significantly higher cortisol:DHEA
ratio, $F(1, 33) = 13.23, \ p = .001, \eta^2 = .286$, compared to those with low ratings. The ratio finding was supported by correlational analysis, $r(32) = .365, \ p = .035$, and is illustrated in Figure 1. The ratio findings withstood adjustment for all potential confounding variables; time of awakening, chronic illness and BMI, however, the average DHEA finding was attenuated to non significance. There were no other significant findings in relation to stressful life events for cortisol alone.

There was a significant interaction between stress $\times$ physical activity for the cortisol:DHEA ratio for the older adults. Older individuals who reported a higher number of stressful events on the LES, and who reported less than 1h a week of aerobic exercise had a significantly higher cortisol:DHEA ratio compared to those with high stress exposure who exercise for 1h a week or more, and both low stress groups, $F(1, 30) = 7.67, \ p = .010, \eta^2 = .204$. This interaction effect is illustrated in Figure 2. There was no significant difference between the low stress groups and the high stress group who exercised. Out of the participants who exercised for at least 1h a week, half reported 1 to 3h per week, and half reported > 3h per week. This interaction finding remained significant when controlling for confounding variables.
Discussion

Older adults reporting with more severe life events stress had a significantly higher cortisol:DHEA ratio than those reporting lower stress severity. This finding was supported by a significant positive correlation, where the cortisol:DHEA ratio increased with stress severity ratings. This is consistent with previous studies that found an increased cortisol:DHEA-S ratio in older adults experiencing stress in the form of bereavement (Khanfer et al., 2011) and caregiving (Jeckel et al., 2010). The present study not only extends such results to DHEA in its non-sulphated form, but suggests that life events stress, in addition to more extreme or unrelenting stress such as caregiving, can also have a significant impact on the endocrine system of older adults and produce a more advanced age-related hormonal profile. The observed association between stress severity and the cortisol:DHEA was driven by lower DHEA values in those reporting more severe stress, rather than higher levels of cortisol. Cortisol and DHEA are secreted from separate areas of the adrenal cortex, the zona fasciculate and the zona reticularis, respectively. Independent activation of these two areas has been noted previously (Velardo et al., 1991). It may be that the relationship between life events stress is specific to the synthesis of DHEA, although the mechanisms underlying the association observed in the present study can only be speculated upon.

Despite the relationship between life events stress and DHEA, it is important to remember only the ratio finding remained significant after adjustment for confounding variables. Significance in relation to the ratio rather than these hormones independently mirrors prior results in this area. Where previous research has found the ratio to be associated with immune function and susceptibility to infection (Arlt et al., 2006; Butcher et al., 2005; Khanfer, Lord & Phillips, 2011), no significant effects were found for these
hormones separately, thus again illustrating the importance of the balance between these hormones.

Previously, DHEA-S has been found to correlate positively with VO$_{2\text{max}}$ (Bonnefoy et al., 1998; Bonnefoy et al., 2002) and estimated energy expenditure in older women, but not men (Bonnefoy et al., 1998; Kostka et al., 2002). Interestingly, it was only stress that showed an independent association with the hormone measures, where physical activity was not related to any hormone variables. While some significant findings emerged initially, they did not withstand adjustment for age, a similar story to a prior investigation which examined DHEA-S and VO$_{2\text{max}}$ (Abbasi et al., 1998). This may be due to the slightly larger sample sizes acquired in previous investigations (Bonnefoy et al., 2008; Bonnefoy et al., 2002; Kostka et al., 2002). Alternatively, differences may be due to variation in the participants studied, in terms of cardio-respiratory fitness, volume and frequency of exercise, and the number of years they had been participating in physical activity. Although half of the individuals in the high physical activity group reported exercising for > 3h per week, the other half reported only 1 to 3h per week. It may be that participants were not exercising at a sufficient level to have an independent impact. However, this level of physical activity was enough to buffer against the effects of stress on the cortisol:DHEA ratio.

Older individuals who reported more stressful life events exposure, and who did not participate in regular aerobic exercise had a significantly higher cortisol:DHEA ratio than all other stress × exercise groups. Therefore, not only did these individuals exhibit a higher ratio compared to low stress groups, but also compared to individuals with high stress exposure who reported participating in aerobic exercise for a minimum of 1h a week or more. This suggests, that under conditions of high stress exposure, exercise can guard against elevations in the cortisol:DHEA ratio. Once again, significant findings emerged in relation to the ratio only rather than the hormones independently. Interestingly, there was no difference between
those who did and did not exercise in the low stress group. Consequently exercise only had a protective effect when individuals had relatively high stress exposure: this is parallel to previous research that has shown potential buffers may act only under conditions of stress. For example, stressful life events have been associated with high mortality in middle-aged men; however, this relationship was not evident in those with high levels of social support (Rosengren, Orth-Gomer, Wedel, & Wilhelsen, 1993).

Exercise has previously been shown to buffer against the effects of life events stress on health (Brown, 1991) and illness (Kobasa, Maddi, & Puccetti, 1982). Further, aerobic exercise training has been found to reduce reported stress (Norris, Carroll, & Cochrane, 1990). However, the present finding that exercise may potentially buffer against the negative influence of stressful life events on the cortisol:DHEA ratio in older adults has not been described previously. Overall, our results offer support to the proposal that exercise could be used as a possible intervention to protect the neuroendocrine system from stress in older adults (Phillips et al., 2007). The older adults in the present study were healthy with no endocrine or immune disorders, and of a relatively high socio-economic status, as is characteristic of study volunteers and is associated with better health (Anderson & Armstead, 1995). It may be that exercise may be most beneficial to more vulnerable older adults, for example, those who are frail or suffer from an illness.

The present study is not without limitations; first, the sample size was modest. This may have limited the ability to obtain more significant findings. A greater number of participants may have produced a broader distribution of life events stress and physical activity scores. As a result, larger studies are required to confirm and build upon the current findings. Although, recruiting large numbers of older adults in the community can be challenging. Second, this study measured only self reported exercise behaviour as opposed to cardiovascular fitness. However, both self reported fitness and objective fitness levels have
previously been shown to relate to stress and health (Brown, 1991). The scale employed was not able to distinguish between moderate and vigorous intensity exercise, only the time engaging in activities that were at least moderate, although it was suitable to discriminate between older adults who exercise on a regular basis and those who do not. Further, it could be speculated that older adults would generally be exercising at a moderate intensity rather than high intensity exercise. Assisting participants with their responses to questionnaires may have increased the accuracy to the self-report measures employed. However, the presence of an investigator may have also lead to response bias and participants may have felt more comfortable answering personal questions, such as those in the life events questionnaires, independently. Third, stressful life events exposure was only measured over the past year. It is possible that stressful life events earlier on in life may leave a permanent mark on the neuroendocrine system, or predispose an individual to a maladaptive response to stress later on in life (Graham et al., 2006). Further, early and more recent life events may yield different associations with the HPA axis (Gerritsen et al., 2010). Although confounding by unmeasured variables remains possible, we did adjust for the main potential confounders. Further, the present associations were not explained by depression or anxiety (data not presented). As this study was not longitudinal, the nature of the relationship between life events stress and the cortisol:DHEA ratio in older adults cannot be unquestionably determined. Longitudinal studies are required to untangle directional relationships between stress and the endocrine system, and intervention studies are necessary to establish if exercise can guard against age and stress related changes in the neuroendocrine system. Finally, it may be that physical activity protected against stress through a direct physiological pathway or indirectly through psychosocial mechanisms. For example, it may be that physical activity exerted a positive benefit through social interaction, although the type of exercise and the exercise environment were not assessed in the present study. Future
research should consider the psychosocial mechanisms through which exercise may have a potential positive effect, such as social support or self-efficacy.

In conclusion, life events stress was associated with an increased cortisol:DHEA ratio in older adults, adding further support to the theory that stress may accelerate age-related changes in this hormone parameter, which may have negative implications for immunity and other aspects of health. Physical activity was not associated with cortisol, DHEA or the balance between these hormones; however, under conditions of high stress, older adults who exercised regularly were protected from an increased cortisol:DHEA, suggesting exercise may defend against stress in older individuals.

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