# AGE, BEHAVIOURAL AND PSYCHOSOCIAL FACTORS: ASSOCIATIONS WITH CORTISOL AND DEHYDROEPIANDROSTERONE

**by**

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# ABSRACT

The research in this thesis was concerned with relationships between cortisol, DHEA/S and psychosocial and behavioural factors in relation to age. First, symptoms of depression, anxiety and low social support were associated with higher diurnal cortisol and awakening response in younger adults compared to older adults. Second, younger adults who had a poorer diet were shown to have significantly lower cortisol in the morning period. Third, older adults with poorer levels of physical function were characterised by flatter diurnal profiles of cortisol and DHEA. Fourth, older adults experiencing more severe life events stress had a higher cortisol:DHEA ratio. In addition, under conditions of greater stress exposure, exercise may buffer against the effects of stress on the cortisol:DHEA ratio in older adults. Finally, long term exercise training did not attenuate age-associated hormonal changes in healthy older adults. However, it was shown that an acute bout of exercise can affect hormonal levels in older populations which are influenced by sex. Overall, a range of associations were demonstrated between behavioural, psychosocial and physical factors, which often appear to be mediated by age. These findings suggest that these hormones and their diurnal rhythms are central to various aspects of health and wellbeing.

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# LIST OF PUBLICATIONS

This thesis comprises of five papers corresponding to three empirical studies. Paper four is a shortened version of chapter five:

1) Heaney, J.L.J., Phillips, A.C. and Carroll, D. (2010) Ageing, depression, anxiety, social support and the diurnal rhythm and awakening response of salivary cortisol. **Int J Psychophysiol,** 78 (3): 201-208.

2) Heaney, J.L.J., Phillips, A.C. and Carroll, D. (2012) Ageing, health behaviours and the diurnal rhythm and awakening response of salivary cortisol. **Exp Aging Res**, (3): 38 295-313

3) Heaney, J.L.J., Phillips, A.C. and Carroll, D. (2012) Ageing, physical function, and the diurnal rhythms of cortisol and dehydroepiandrosterone. **Psychoneuroendocrinology,** 37 (3): 341-349.

4) Heaney, J.L.J., Carroll, D. and Phillips, A.C. (Under review) Stress and exercise are associated with the cortisol:DHEA ratio in older adults: Exercise may buffer against the negative effects of stress. **J Ageing Phys Act**

5) Heaney, J.L.J., Phillips, A.C. and Carroll, D. (In press) DHEA, DHEA-S and cortisol responses to acute exercise in older adults in relation to exercise training status and sex. **Age**

During the period of postgraduate study at the University of Birmingham, the following papers and presentations were also produced:

***Publications***

- Heaney, J.L.J., Ginty, A.T., Carroll, D. and Phillips, A.C. (2011) Exercise dependence and cardiovascular and cortisol reactions to acute psychological stress: Another case of blunted reactivity? **Int J Psychophysiol,** 79 (2): 323-329.

- Ginty, A.T., Phillips, A.C., Higgs, S., Heaney, J.L.J. and Carroll, C. (2012) Disordered eating behaviour is associated with blunted cortisol and cardiovascular reactions to acute psychological stress. **Psychoneuroendocrinology,** 37 (5): 715-724.

***Papers in preparation***

- Heaney, J.L.J., Carroll, D., Phillips, A.C. and Drayson, M.T. Comparison of IgA, IgG and IgM functional antibodies in saliva and serum in relation to age and vaccination history.

- Heaney, J.L.J., Carroll, D., Phillips, A.C. and Drayson, M.T. The diurnal rhythms of IgA, IgG and IgM functional antibodies in saliva.

***Poster presentations***

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Heaney, J.L.J., Phillips, A.C. & Carroll, D. (2011). Life events stress and physical activity are associated with the diurnal rhythm of dehydroepiandrosterone and the cortisol:DHEA ratio in older adults. *British Society for Research on Ageing, Brighton.* July 2011 & *Aston Research Centre for Healthy Ageing: Research Showcase*, September 2011.

Heaney, J.L.J. & Oliver. S. (2009). Endurance performance after modest hypohydration evoked by prolonged fluid restriction and exercise or diuretics. *American College of Sports Medicine Conference, Seattle.* May 2009.

# CONTENTS

# CHAPTER ONE

## 1.0. INTRODUCTION

## 1.1. HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

The hypothalamic-pituitary-adrenal (HPA) axis is an interactive neuroendocrine unit comprising of the hypothalamus, the pituitary gland and the adrenal glands. The HPA axis plays key roles in basal homeostasis and in the body's response to stress. The hypothalamus responds to basal neural input or stress, be it physiological or psychological, by increasing the secretion of corticotrophin releasing hormone (CRH). This increase in CRH acts upon the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH), which in turn circulates to the adrenal cortex to stimulate the release of the hormones cortisol from the zona fasciculata and dehydroepiandrosterone (DHEA) from the zona reticularis into the bloodstream. The HPA axis is an example of a negative feedback loop; cortisol can reduce its own secretion via feedback to the anterior pituitary to reduce ACTH and to the hypothalamus to limit the secretion of CRH. ACTH also provides negative feedback limiting its secretion to the hypothalamus. In contrast, there is no negative feedback control of DHEA secretion by the hypothalamus (Baulieu, 1996)

## 1.2. CORTISOL, DHEA, DHEA-S SYNTHESIS AND LEVELS IN HUMANS

### *1.2.1. Synthesis*

Cortisol and DHEA are synthesised from adrenal cholesterol, which is sourced from plasma lipoproteins (Tyrrell and Forsham, 1983). P450scc synthesises pregnenolone from cholesterol, and 17α-hydroxylase converts pregnenolone to 17α-hydroxy-pregnenolone. After this, the synthesis of cortisol and DHEA differs. For cortisol, 3ß-hydroxysteroid dehydrogenase converts 17α-hydroxy-pregnenolone to 17α-hydroxy-progesterone. This is then converted by 21-hydroxylase to form 11-deoxycortisol, which is then finally converted to cortisol by 11ß-

hydroxylase. DHEA requires fewer steps, as 17α-hydroxy-pregnenolone is synthesised directly to DHEA by 17, 20-desmolase (Tyrrell and Forsham, 1983). DHEA is converted to dehydroepiandrosterone sulphated (DHEA-S), its sulphated form, by hydroxysteriod sulfotransferase, and DHEA-S can be metabolised back to DHEA by steroid sulfatase (Racchi et al., 2003).

### *1.2.2. Levels in plasma/serum*

Once secreted from the adrenal cortex, only 5-10% of cortisol circulates as free unbound cortisol; this is the fraction that is biologically active. For the remaining fraction, 70-85% is bound to corticosteroid-binding globulin, and 10-15% to albumin (Kirschbaum and Hellhammer, 1994). DHEA circulates weakly bound to albumin (Tyrrell and Forsham, 1983), and is mostly present in its sulphated form in the blood, due to the longer half life and slower metabolic clearance rate of DHEA-S (Baulieu, 1996). Higher levels of both DHEA and DHEA-S are found in men compared to women (Baulieu, 1996), however, contradictory findings exist in relation to cortisol where some studies have reported higher levels in men, where others have reported no sex differences (Kirschbaum et al., 1992).

### *1.2.3. Levels in saliva*

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 et al., 1999). The advantages of saliva sampling over blood to analyse both cortisol (Kirschbaum and Hellhammer, 1994) and

DHEA (Granger et al., 1999) have been well established. For example, non-invasive measurement may be more appropriate for repeated measures, long term sampling, or for certain populations or study designs where blood sampling may not be feasible. Further, cortisol and DHEA are not affected by saliva flow rate as they enter the saliva via passive diffusion through the cells of the salivary glands, as these unconjugated steroids are small in size and lipid soluble (Kirschbaum and Hellhammer, 1994).

### 1.3. MAIN FUNCTIONS OF CORTISOL, DHEA AND DHEA-S

Cortisol is involved in a number of important functions including energy metabolism, vascular activity, and inflammatory and immune responses (Schürmeyer and Wickings, 1999). Due to these roles, cortisol plays an integral part in homeostasis under basal conditions; however, the importance of such functions is highlighted during the stress response. During stress, cortisol protects the body through enhancing vascular activity, suspending non-essential functions (e.g. reproduction and growth), inhibiting the inflammatory process, suppressing the immune system to prevent over-activation, inhibiting the actions of insulin to preserve glucose for the brain and skeletal muscle, and increasing energy availability through protein and triglyceride catabolism (Widmaier et al., 2004 ). However, if stress persists and cortisol is chronically elevated resulting in long term activation of these processes, this will clearly have negative implications for health, particularly via effects on immunity.

Aside from being a precursor to sex hormones, DHEA has been proposed to affect various systems of the body. Many of the roles in which DHEA/S has been implicated remain

contentious (Kroboth et al., 1999) although their proposed mechanisms of action are far reaching; including neuroprotection, immunomodulation, decreased apoptosis, antioxidant, anti-inflammatory, and anti-glucocorticoid effects (Maninger et al., 2009). As a result of these proposed mechanisms of action, DHEA/S has received interest from a pharmological perspective, particularly in relation to ageing (Baulieu, 1996) and psychiatric illness (Maninger et al., 2009). Although beneficial effects of supplementation are yet to be clearly demonstrated (Chahal and Drake, 2007), evidence suggests that DHEA supplementation may have the potential to improve physical or cognitive function in elderly adults who are frail or suffer from cognitive impairment (Kenny et al., 2010; Yamada et al., 2010) but have no benefit to those in an uncompromised state of health (Geol and Cappola, 2011).

In addition to the absolute concentrations of cortisol and DHEA/S, as these hormones have opposing effects on the body, the balance between these hormones, or the cortisol:DHEA/S ratio, is also significant for health. For example, higher cortisol:DHEA ratios, rather than the hormone levels independently, have been associated with immune impairments and infection risk in older adults exposed to chronic physical and psychological stress (Butcher et al., 2005).

## 1.4. DIURNAL VARIATION OF CORTISOL AND DHEA

The HPA axis has a diurnal rhythm of secretion that occurs as a result of input from the central nervous system. This neural regulation is independent of cortisol levels and negative feedback. Cortisol secretion is 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 (Pruessner et al., 1997).

As DHEA is also stimulated by ACTH it oscillates in a similar pattern, however, its diurnal rhythm does not feature an awakening response. 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 (Hucklebridge et al., 2005). Sulphated DHEA presents fairly stable 24h levels and is not subject to diurnal fluctuations on account of its low metabolic clearance rate (Baulieu, 1996).

The cortisol awakening response or CAR, has been considered separately from the rest of the diurnal cycle. It is thought to be under distinct regulatory influence (Clow et al., 2004) and serve its own specific functions in relation to memory (Wilhelm et al., 2007), the immune system (Petrovsky and Harrison, 1997) and anticipation of the demands of the day (Fries et al., 2009). The CAR and diurnal rhythm of cortisol have been shown to be affected by various intra- and inter-individual differences in a range of exposures (Smyth et al., 1997; Stone et al., 2001): time of awakening (Edwards et al., 2001b; Kudielka and Kirschbaum, 2003), oral contraceptive use (Reinberg et al., 1996), seasonal variation (Persson et al., 2008), perceived seasonality (Thorn et al., 2009), sleep (Dahlgren et al., 2009; Vreeburg et al., 2009b) and chronic stress (Steptoe et al., 2000). Factors affecting the diurnal rhythm of DHEA have received less attention than cortisol, although there is evidence to suggest that it may also be influenced by similar factors, such as time of awakening (Hucklebridge et al., 2005) and psychological factors such as depression (Heuser et al., 1998).

### *1.4.1. Cortisol, DHEA diurnal rhythms and health*

The diurnal rhythms of cortisol and DHEA influence are synchronized with other physiological parameters, highlighting their significance in relation to health. This is perhaps best illustrated by considering neuroendocrine-immune interactions. Cortisol alters the T-helper 1 (Th1, cell mediated)/ T-helper 2 (Th2, humoral) balance, via stimulating the production of Th2 cytokines and inhibition of Th1 cytokines (Marques-Deak et al., 2005). The production of pro-inflammatory cytokines peak during night-time hours, this pro-inflammatory profile then shifts towards a Th2 profile as anti-inflammatory cytokines peak during the daytime (Lange et al., 2010). The role of cortisol in orchestrating this diurnal variation of immunity has been identified through studies tracking its secretion alongside cytokine levels and the number and function of immune cells. For example, a bias towards a Th1 immune profile, demonstrated through higher IFN-γ levels and IFN-γ:interlukin-10 ratio, during the night and early morning has been shown to coincide with low plasma cortisol levels (Petrovsky and Harrison, 1997). Significant negative correlations have been shown between cortisol, absolute lymphocytes and lymphocyte subsets, whereas positive correlations have been observed between natural killer cell activity and number of neutrophils (Kronfol et al., 1997). Investigations examining lymphocytes in more detail alongside cortisol have shown circulating naive T-cells to be highest at night, compared to cytotoxic effector cells which peak during the daytime (Dimitrov et al., 2009). Although research has been predominantly focused on cortisol, the diurnal rhythm of DHEA has been put forward as a candidate that may influence cytokine diurnal profiles (Petrovsky and Harrison, 1998). Therefore, disruptions in the inherent patterns of secretion of cortisol and DHEA may disturb the harmony of other circadian rhythms.

In addition, there is a range of research that has linked both the overall levels and diurnal rhythms of cortisol and DHEA to specific aspects of health. Dysregulation of the HPA axis has been associated with physiological health outcomes. For example, a flatter cortisol diurnal pattern has been related to a higher incidence of cardiovascular disease and type II diabetes (Rosmond et al., 2003) and earlier mortality in cancer patients (Sephton et al., 2000). Further, as cortisol and DHEA represent the endpoint of the stress response, alterations in these indicate a change in the body's physiological response to stress (Kirschbaum and Hellhammer, 1994). Aspects of psychological health have also been associated with the diurnal patterns of these hormones; for example, lower levels of morning DHEA have been identified in depressed adolescents (Goodyer et al., 1996) and linked to symptoms of depression and anxiety in healthy young and old individuals (Luz et al., 2003).

### 1.5. CORTISOL, DHEA, DHEA-S AND AGE

Changes in the output of the adrenal cortex have been reported with ageing, with higher evening and nocturnal concentrations of cortisol resulting in an overall increase in 24h mean cortisol and a flattened diurnal pattern characterising older participants in several studies (Deuschle et al., 1997; Luz et al., 2003; VanCauter et al., 1996; Yen and Laughlin, 1998). However, this area of research has produced contradictory findings. A flatter diurnal profile has not always been demonstrated, with older individuals exhibiting a steeper slope of decline compared to young adults in one study (Ice et al., 2004). Further, evidence exists from longitudinal (Orentreich et al., 1992) and cohort studies (Straub et al., 2000; Wolf et al., 2002) that cortisol may decrease (Straub et al., 2000) or remain relatively unchanged with ageing (Orentreich et al., 1992; Wolf et

al., 2002). With regard to the CAR, one study showed a lower CAR with increasing age (Kudielka and Kirschbaum, 2003); although statistically significant, the authors highlighted that age accounted for only 4% of the variance. Accordingly, this finding was in line with the results of other investigations that failed to observe any impact of age in healthy individuals (Pruessner et al., 1997; Wüst et al., 2000).

A potential reason for the contrasting findings observed in studies examining cortisol and age is methodological differences. Studies have taken samples at different time points of the day; studies measuring cortisol in plasma/serum have sometimes been limited to one sampling time point, usually in the morning period (Orentreich et al., 1992; Straub et al., 2000) although some have explored cortisol over a full diurnal cycle in plasma (Deuschle et al., 1997; VanCauter et al., 1996). In saliva, some studies focused purely on the CAR (Kudielka and Kirschbaum, 2003; Pruessner et al., 1997; Wüst et al., 2000) or the slope of decline (Ice et al., 2004; Luz et al., 2003; Wolf et al., 2002), and studies employing multiple sampling protocols across the day are few. Alternatively, variability in findings could be due to differences in statistical analysis. Some studies have explored the relationship between cortisol and age using correlational analysis and continuous cortisol variables across a range of ages, where others have taken the approach of using discrete age groups. In addition, studies have varied in the extent to which they took potential confounding variables into account, with several studies failing to control for factors such as awakening time.

Regardless of the direction of change in cortisol secretion with ageing, it remains high in relation to other adrenal hormones such as DHEA (Straub et al., 2000). DHEA and DHEA-S production

peaks at age 20-30 years and then declines progressively with age in both males and females (Belanger et al., 1994; Labrie et al., 1997; Orentreich et al., 1992). The clear and progressive decline in DHEA/S with age, unparalleled by cortisol, results in an increase in the cortisol:DHEA/S ratio with ageing (Phillips et al., 2007). While it is well established that DHEA decreases with age, little attention has been paid to its diurnal rhythm, and, as previously highlighted, the pattern of secretion, not just the overall level, may have implications for health. One study found similar diurnal DHEA profiles in young and older adults (Erosheva et al., 2002). Clearly, in comparison to cortisol, investigations into the DHEA diurnal rhythm and age are scarce.

### *1.5.1. Mechanisms of age related changes in cortisol and DHEA*

Cortisol and DHEA are secreted from different zones of the adrenal cortex and independent activation of the zona fasciculata and zona reticularis has been noted previously (Velardo et al., 1991). Therefore, it is perhaps unsurprising that these hormones may respond differently to the process of ageing. The distinct decrease in DHEA/S with age has been attributed to several mechanisms. A decrease in 17, 20-desmolase activity (Labrie et al., 1997), reduced LDL receptors affecting cholesterol transport, reduced ACTH receptors, a reduction in mass of the zona reticularis (Parker, 1999) and a decrease in IGF-I and IGF-II (Yen and Laughlin, 1998), have all been implicated in the decline in DHEA/S with age.

Where increases in cortisol with ageing have been observed, this has been attributed to impairment of negative feedback of HPA activity due to neuronal loss in the hippocampal area (VanCauter et al., 1996; Yen and Laughlin, 1998) and reduced hippocampal volume has been

shown in older adults with a progressive increase in 24h cortisol over 5-6years (Lupien et al., 1998). Loss of HPA axis sensitivity has also been demonstrated via impaired dexamethasone suppression (Magri et al., 1997).

### *1.5.2. Implications of age related changes in cortisol and DHEA*

The endocrine system, along with the central nervous system, is the body's major system of communication (Widmaier et al., 2004 ) and therefore has the ability to communicate with and influence a range of other systems and functions within the body. Consequently, changes in the levels, diurnal rhythms and ratio of cortisol and DHEA/S may have significant implications for the health of ageing individuals. Neuroendocrine dysregulation and the reduction in sex hormones with ageing have been implicated in immune dysregulation, sarcopenia, osteoporosis, and ultimately the presentation of physical frailty (Fried et al., 2005; Joseph et al., 2005; Walston et al., 2006). Therefore, the impact of changes in cortisol and DHEA/S on the ageing body may be far reaching, and impinge on an individual's quality of life and ability to live independently.

Changes in the endocrine system occur as part of the biological process of ageing, although they could potentially be aggravated by other factors, such as health behaviours or psychosocial factors. Alternatively, certain behaviours or psychosocial factors may protect individuals from age-related hormone changes. As a result, the relationship between such factors, cortisol, DHEA and ageing warrants exploration.

## 1.6. PSYCHOSOCIAL FACTORS, CORTISOL, AND DHEA

The HPA axis responds to sources of psychological stress, and exposure to prolonged external stressors may lead to changes in HPA axis output. In addition, the HPA axis may also respond to psychological states and wellbeing, and potentially changes in HPA axis function may predispose individuals to psychological symptoms, such as depression or anxiety, or vice versa.

### *1.6.1. Anxiety, depression and social support*

Several studies have examined the relationship between the cortisol diurnal rhythm and psychological wellbeing. Depression has previously been associated with diurnal cortisol, however, the nature of this relationship has varied with conflicting observations of increased levels and greater diurnal amplitude (Cowen, 2009; Gillespie and Nemeroff, 2005), a flattened diurnal profile (Knight et al., 2010; Sjogren et al., 2006) and no difference between depressed and non depressed individuals (McClure, 1966). A similar pattern of mixed findings also emerges when considering specific aspects of the cortisol diurnal rhythm. Participants who were currently or previously depressed (Bhagwager et al., 2005; Vreeburg et al., 2009a) or had higher depressive symptoms (Pruessner et al., 2003) exhibited a higher CAR, where others have shown a blunted CAR in depressed participants (Stetler and Miller, 2005).

The apparent discrepancy in findings could be due to co-morbid anxiety in some depressed individuals, which may consequently produce a different cortisol output (Murphy et al., 2004). This has been illustrated previously, where depressed participants with co-morbid anxiety had a higher CAR compared to those with depression alone (Vreeburg et al., 2009a), suggesting that

anxiety may exacerbate the effects of depression on diurnal cortisol. Alternatively, this may reflect a greater severity of psychological symptoms in those with both depression and anxiety. Whereas some have failed to demonstrate independent associations between anxiety and diurnal cortisol patterns (Adam, 2006; Kurina et al., 2004), one study showed a different pattern of decline across the day in individuals with high anxiety, where lower levels where found in the afternoon period (Vedhara et al., 2003).

Another potential reason for differences in findings might be due to the severity of depression and the environment in which the participants were studied. Clinically depressed patients may differ from individuals in the community with depressive symptomatology; the association with diurnal cortisol would appear to be less certain in the latter (Bhagwager et al., 2005). It has been proposed that mild depression may be characterised by a dysregulation of cortisol rather than specifically hypersecretion (Stetler and Miller, 2005). This lack of consensus may also be partly explained by methodological considerations such as the age of the participants and consideration of potential confounders (Chida and Steptoe, 2009; Vreeburg et al., 2009a), such as another underlying health condition, time of awakening, BMI, smoking and other factors that could affect cortisol output.

Conflicting findings are also typical of studies of positive psychosocial factors which may buffer the effects of anxiety and depression. Individuals with higher levels of social support have shown a steeper diurnal decline (Sjogren et al., 2006) and lower mean cortisol levels (Turner-Cobb et al., 2000). Support in the form of intimacy has also been associated with smaller area under the curve (AUC) (Ditzen et al., 2008). In contrast, studies have found no association

between social support and the diurnal slope (Turner-Cobb et al., 2000) or diurnal cycle (Smyth et al., 1997). Fewer studies have examined the relationship between social support and diurnal cortisol in comparison to anxiety and depression. In addition, studies have failed to examine anxiety, depression and social support within the same study. Consequently, the relationships and potential interactions between both negative and positive psychosocial factors and diurnal cortisol have been overlooked.

### *1.6.2. Stress*

Stress and the above-mentioned psychosocial factors are interrelated. For example, chronic stress is a risk factor for depression (Miller et al., 2007). Nevertheless, there is a range of literature that supports independent associations between chronic stress and altered activity of the HPA axis. Rather than just defining chronic stress as simply the presence of an enduring stressor, it has been proposed that chronic stress may best be seen as a function of the duration of the stress, perceived threat, and response (Baum et al., 1993). The authors' reasoning being that a stressor could be relatively short in duration, but it may be perceived as a chronic threat and produce a chronic response, be it psychological or physiological, from the exposed individual.

Chronic stress has typically been measured in the form of unemployment, war, through examining soldiers or refugees, widowhood, or caregiving (Miller et al., 2007) which have been shown to have different relationships with cortisol. For example, bereavement has been associated with elevated cortisol levels, whereas caregiving and domestic violence have been characterised by a reduced cortisol output (Miller et al., 2007). The effect of stress on diurnal cortisol has also been examined; for example, higher job strain (Steptoe et al., 2000) and

unemployment (Ockenfels et al., 1995) were associated with elevated morning cortisol levels. However, although trends in associations have emerged, for example for the cortisol awakening response (Chida and Steptoe, 2009), it would be an oversimplification to conclude that there are distinct patterns associated with specific types of stressors, given the inconsistent findings.

Chronic stress has also been associated with DHEA-S, where it appears reduced levels are generally found in the presence of stress. For example, healthy caregivers of Alzheimer's disease patients were shown to have reduced salivary DHEA-S levels and an increased cortisol:DHEA-S ratio compared to non stressed controls (Jeckel et al., 2010). Similarly, perceived stress was inversely related to DHEA-S levels in male army officers (Labbate et al., 1995). However, little is known about the associations between chronic stress and DHEA in its non-sulphated form. It is important to also explore DHEA as only unsulphated DHEA exhibits a diurnal rhythm of secretion and, as previously mentioned, this rhythm may have important associations with health, rather than just stable concentrations on their own. Further, DHEA may have different mechanisms of action (Maninger et al., 2009).

One form of chronic stress that has received less attention in relation to these hormones is life events stress. One study examining life events stress in older adults, demonstrated 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). However, the diurnal rhythm of DHEA has yet to be examined in relation to life events stress, and there is a general lack of data on life events stress within the past year on cortisol and DHEA. Despite this,

life events stress is important as it has been shown to be a risk factor for future adverse health outcomes, for example depression (Blazer et al., 1987) and the metabolic syndrome (Raikkonen et al., 2007). This form of stress encompasses traumatic events but also less serious exposures and daily hassles, which if they occur in abundance, or are perceived as stressful, may be a significant source of stress.

## 1.7. PSYCHOSOCIAL FACTORS, CORTISOL, DHEA AND AGE

There is a high prevalence of anxiety and depression in older adults (Ritchie et al., 2004) and depression has been associated with mortality in this population (Blazer et al., 1987; Schulz et al., 2000). Studies have specifically examined older adults and cortisol, revealing both higher AUCs (Wrosch et al., 2007) and flattened or inconsistent diurnal patterns (Fiocco et al., 2006) in individuals with depressive symptoms. However, these studies were in the context of physical health and memory problems and either lacked non-depressed comparison groups (Fiocco et al., 2006) or included crude measurements of depressive mood. Wrosch et al (2007) measured this using a basic questionnaire which included only two items, sad and unhappy, measured on a 0-4 scale.

Psychosocial factors have been related to a dysregulated diurnal profile in adolescents in several studies. For example, higher cortisol awakening responses have been observed with persistent anxiety problems (Greaves-Lord et al., 2007), and higher evening cortisol has been associated with trait anxiety (Goodyer et al., 2000; Greaves-Lord et al., 2007; Van den Bergh et al., 2008). One study reported greater CARs in male university students with higher depression scores

(Pruessner et al., 2003). However, there is limited research which has focused on anxiety and depression in young adults, rather than adolescents. Older adults' cortisol output over the day has been inversely related to seeking social support (O'Donnell et al., 2008); however, this study did not measure perceived social support. Consequently, perceived social support has not been examined in parallel with anxiety and depression in relation to diurnal cortisol in both younger and older adults, although the relationships between these psychosocial variables may differ depending on age.

Aside from these psychosocial variables, chronic stress has received its own particular attention in relation to ageing. Chronic stress is particularly relevant for elderly individuals, as it is a risk factor for adverse health outcomes, in terms of both acute problems, such as upper respiratory tract infection, and chronic disease, such as cardiovascular disease (Miller et al., 2007). Further, it has been theorised that chronic psychosocial stress, and its impact on various physiological systems, accelerates ageing (Juster et al., 2010). The implications of stress on the ageing immune system, and consequently health, have been previously discussed (Graham et al., 2006; Kiecolt-Glaser et al., 2002). Neuroendocrine-immune networks and their relationship in ageing are well established (Bauer et al., 2009; Graham et al., 2006) Therefore, chronic stress, through its interaction with the neuroendocrine system, may also exacerbate the effects of ageing. Another theory is that stress may not only accelerate ageing, but that older adults may be more at risk from the effects of chronic stress. The reduction in DHEA, increase in the cortisol:DHEA ratio, and changes in the immune system that occur with ageing, have been proposed to increase vulnerability to stress exposures in older adults (Phillips et al., 2007).

Studies have previously investigated relationships between stress and cortisol or DHEA-S, in the form of both caregiving and DHEA-S (Jeckel et al., 2010) and life events and cortisol (Gerritsen et al., 2010) in older populations. In 8-16 year olds, the cortisol:DHEA ratio has been associated with disappointing life events (Goodyer et al., 2000). However, studies have yet to compare the impact of chronic stress on both younger and older adults, and indeed middle-aged adults, within the same study. It may be that age influences how an individual perceives stressful events and their potential threat, and therefore age may affect the physiological reaction to stress. It is possible that older adults show an amplified response to stress, manifested by alterations in the neuroendocrine system, as a consequence of increased stress vulnerability as a result of age related physiological changes, or heightened stress sensitivity.

## 1.8. HEALTH BEHAVIOURS AND CORTISOL

A range of health behaviours have been associated with cortisol levels and aspects of its diurnal rhythm of secretion. Smoking was associated with an increased CAR and higher levels of cortisol across the day (Badrick et al., 2007), and women who are heavy drinkers have been characterised by a larger CAR whereas male heavy drinkers showed a flatter diurnal slope (Badrick et al., 2008). Individuals who rated a high level of sleepiness upon awakening had lower levels of cortisol upon awakening (Dahlgren et al., 2009) and short sleep duration and high sleep disturbance have been associated with a flatter diurnal slope, as a result of higher evening cortisol levels (Kumari et al., 2009). Flatter cortisol profiles have also been observed in individuals with a higher intake of saturated fat and central body fat distribution (Garcia-Prieto et al., 2007). However, counter evidence exists and often for each of these associations a study has

been published on the same health behaviour and has failed to find an association. For example, diurnal cortisol activity has also been shown to not be influenced by smoking status (Edwards et al., 2001a) and alcohol consumption (Kunz-Ebrecht et al., 2004).

Most studies have focused on one or two health behaviours in isolation, such as smoking (Badrick et al., 2007; Steptoe and Ussher, 2006) or alcohol intake (Badrick et al., 2008). An exception here is the study by Vreeburg et al (2009), which examined a range of socio-demographic and health characteristics, and multiple health behaviours and the CAR and cortisol. This study found various associations between sex, age, awakening time, smoking, physical activity and cardiovascular disease, with different aspects of the cortisol diurnal rhythm. Some facets of health behaviour are still yet to be investigated in relation to diurnal cortisol. For example, research has largely focused on obesity (Mårin et al., 1992; Strain et al., 1980) rather than aspects of diet and food intake.

It is possible that the relationship between health behaviours and cortisol varies as a function of age and, as already discussed, the impact of changes in diurnal cortisol may be particularly relevant for ageing individuals. Although some researchers have examined diurnal cortisol and a health behaviour among older adults (Badrick et al., 2008; Kumari et al., 2010) the majority of studies have not included adults aged over 65 years, nor included multiple behaviours, or young and old participants within the same study. As a result, the relationship between multiple health behaviours and diurnal cortisol in relation to age has yet to be tested.

## 1.9. CHRONIC STRESS AND EXERCISE

Exercise has been proposed as an intervention to protect against the damaging effects of stress on the neuroendocrine system in older adults (Phillips et al., 2007). It was found that physical activity defended individuals experiencing high stress against age-related telomere shortening (Puterman et al., 2010). Exercise also protected against functional decline in a longitudinal study of ageing, and buffered the effects of stress in the form of widowhood on functional decline (Unger et al., 1997). These studies in ageing individuals confirm earlier findings in younger adults and adolescents, that the effects of stress, namely life events stress, on health can be buffered by physical activity and fitness (Brown, 1991). However, the potential ameliorating effects of exercise on life events stress on cortisol and DHEA, or their diurnal rhythms, have yet to be explored, nor has the impact of exercise been compared between different age groups.

Independently of stress, the relationship between the diurnal rhythms of cortisol and DHEA and exercise has received little attention. One study found the diurnal rhythm of cortisol to be absent in elite gymnasts (Georgopoulos et al., 2011). However, the relationship between elite sport performance and diurnal cortisol is likely to differ from that of more mundane physical activity and cortisol, nor has this been explored outside of young individuals. Therefore, it would be of interest to examine if habitual exercise is associated with cortisol and DHEA among participants of different ages.

## 1.10. PHYSICAL FUNCTION, CORTISOL AND DHEA IN OLDER ADULTS

The population of older adults is rapidly increasing, and it has been projected that by 2050 numbers of individuals aged 60 years and over will account for 35% of the total population in Europe. Further, those aged 80 years and over are the fastest growing division of this ageing population, with projections of 26% of the total population in Europe being of this age or above by 2050 (UN, 1999). However, there is a large discrepancy between increase in life expectancy and increase in healthy life expectancy, which has failed to grow at the same rate (HoL, 2005). Older adults are more likely to suffer from a chronic illness, have a reduced functional capacity, and be more dependent (Tinker, 2002). However, this is not the case for all older adults, and many maintain physical function and independence.

Neuroendocrine and immune dysregulation has been recognised as a manifestation of frailty (Ahmed et al., 2007) and may additionally be a pathway to its presentation and development (Joseph et al., 2005; Walston et al., 2006). Therefore, changes in physical function, prior to frailty onset, could also potentially be associated with changes in the endocrine system. Although changes in the neuroendocrine system occur naturally as part of the ageing process, it may be that these changes are more pronounced in certain individuals, and as a result they may be more at risk of deteriorations in physical functioning and consequently becoming frail in older age.

Higher cortisol levels and blunted diurnal variation have been found in older women presenting with frailty (Varadhan et al., 2008). The decrease in amplitude in diurnal variation in this case

was a result of higher cortisol during the day and at bedtime. Frailty in this study was defined using the criteria of Fried et al. (2001) which include weight loss of more than 4.5kg in the previous year, grip strength, walking speed and self reported exhaustion and physical activity. Higher cortisol levels have also been associated with poorer physical performance in older men and women (Peeters et al., 2007; Varadhan et al., 2008) as measured using chair stands, tandem stands, and a walk test.

Lower levels of serum DHEA-S have been associated with poorer physical function in community dwelling older adults measured using assessments of balance, gait and upper body strength, such as tandem stands, leg stands, a timed walk and handgrip strength (Berkman et al., 1993). Lower DHEA-S has also been associated with a frailty phenotype, as defined by the criteria of Fried et al (2001), in community dwelling older adults and individuals living in an assisted living facility (Voznesensky et al., 2009). However, less is known about DHEA in its non-sulphated form in relation to physical functioning in older adults, nor has its diurnal rhythm been explored. Further, there is a lack of studies exploring both cortisol and DHEA simultaneously in relation to physical function. As previously mentioned, it may not just be the levels of these hormones independently that is relevant, rather their diurnal rhythms of secretion and the balance between them that may be important for health outcomes in older individuals, such as physical function.

## 1.11. EXERCISE TRAINING STATUS , CORTISOL, DHEA, AND DHEA-S IN OLDER ADULTS

Not all older adults become frail or experience poor physical function, and some older adults participate in regular exercise and maintain a good or even high level of aerobic fitness for their age. It is possible that exercise may protect against the age associated changes in adrenal hormones and that older adults who are physically active might present with a more favourable endocrine profile.

DHEA-S has been found to be significantly higher in endurance trained older men who had training experience of more than 15 years (Tissandier et al., 2001). Higher levels of DHEA-S have also been shown in older men who for the past 10 years regularly participated in moderate intensity cycling (Ravaglia et al., 2001). In contrast, in a similar study comparing older recreational male runners, who had been running between 21-25 years, to age-matched sedentary controls no significant differences were found in DHEA-S (Arai et al., 2006); suggesting that long term training may not have the potential to attenuate age related endocrine change. These studies included men only aged between 50-80 years and did not include women. However, other studies investigating $VO_{2max}$ and energy expenditure rather than exercise training status have tested women. DHEA-S correlated positively with $VO_{2max}$ (Bonnefoy et al., 1998; Bonnefoy et al., 2002) and physical activity energy expenditure, measured via questionnaire, in older women, but not men (Bonnefoy et al., 1998; Kostka et al., 2002). In contrast, an association between $VO_{2max}$ and DHEA-S has been reported in men but not women (Abbasi et al., 1998), however, it should be noted that this finding did not withstand adjustment for age.

Where studies have included women, participant's fitness was of an average level for their age and did not include individuals who were endurance trained. Studies in this area of research typically have included only two groups of interest; therefore, individuals who take part in moderate activities have not been compared to endurance trained individuals, alongside sedentary older adults, within the same study. In addition, the attention has been focused on DHEA-S, and DHEA and cortisol have not been examined in parallel. Thus, it seems important to establish if long term exercise behaviour, be it endurance training or engaging in moderate intensity exercise, can protect against the age-associated decline in DHEA/S levels and increase in the cortisol:DHEA ratio in both men and women.

## 1.12. ACUTE EXERCISE, CORTISOL, DHEA, AND DHEA-S IN OLDER ADULTS

The HPA axis responds to physiological stress, which may present in the form of exercise. Acute exercise can stimulate increases in cortisol which is proportional to exercise intensity and duration (Kjaer and Dela, 1996; Pedersen and Hoffman-Goetz, 2000). Therefore, intense or prolonged exercise induces the release of cortisol, and has been implicated as a mediator of post-exercise immune suppression. DHEA (Aldred et al., 2009; Cumming et al., 1986) and DHEA-S levels (Tremblay et al., 2004) have also been shown to increase in response to acute exercise in younger adults. This has been observed in response to both low-moderate and moderate-high intensity aerobic exercise (Aldred et al., 2009; Cumming et al., 1986) and endurance and resistance exercise (Tremblay et al., 2004).

Less is known regarding hormone responses to acute exercise in older adults. Studies that have investigated adrenal hormones and exercise have mainly been restricted to females in the post-menopausal age range, and less is known about males and individuals of an older age. For example, DHEA significantly increased to response to resistance exercise in females aged up to 69 years (Copeland et al., 2002). Early post-menopausal females demonstrated an increase in DHEA-S immediately after and 2 h post a combined endurance and strength training session (Kemmler et al., 2003). Another study in postmenopausal females found an increase in DHEA, but not DHEA-S, in response to submaximal exercise (Giannopoulou et al., 2003). In contrast, DHEA and DHEA-S have also be shown to remain unchanged in older adults following an acute bout of submaximal exercise (Aldred et al., 2009). Although this study included both males and females, only seven participants were tested and only one sample was taken post-exercise and consequently did not include the recovery period. Differences in the existing literature may be due to the different exercise protocols employed and participants studied.

As older adults of different training status and fitness may vary in hormone levels at rest, they may also display different hormonal responses to acute exercise. One study in older men concluded that chronic endurance training can enhance the hormonal response to both maximal and submaximal exercise (Silverman and Mazzeo, 1996). On the other hand, one study examined older fit and unfit female's baseline levels of cortisol, response and recovery to acute submaximal exercise, and failed to observe any significant differences between groups (Traustadottir et al., 2004). DHEA/S were not measured in these studies, therefore, responses of DHEA, DHEA-S alongside cortisol in relation to exercise training status and gender in older adults have yet to be investigated. If exercise is to be used as a possible intervention to buffer against age induced

changes in the neuroendocrine system, such as the reduction in DHEA/DHEA-S and increase in cortisol:DHEA/S ratio, it is important to ascertain: first, whether long term exercise training has a beneficial effect on these hormones in older age; second if acute exercise is able to stimulate changes in adrenal hormones and if any changes are sustained post-exercise during recovery; and finally, if sex or training status affect hormonal responses to exercise in older adults.

## 1.13. OVERVIEW

The present thesis comprises three cross-sectional studies that have produced five research papers, presented in chapters two to six, examining the relationships between cortisol, DHEA/S and psychosocial factors and physiological factors in the context of ageing. The first study yielded two papers. The initial paper focused on the diurnal rhythm of cortisol in saliva and its association with depression, anxiety and social support, comparing a sample of students with community dwelling older adults. The second paper again examined the cortisol diurnal rhythm in students and older adults, this time in relation to a range of health behaviours. It was hypothesised that the diurnal profile of cortisol would be associated with psychosocial factors and health behaviours and that these associations may differ between age groups, potentially having more of an impact on older adults. The third paper investigated the diurnal rhythms of cortisol and DHEA in old and very old community dwelling adults, and the relationship between these hormones and physical function. It was hypothesised that individuals indicating lower levels of physical function would exhibit flatter diurnal profiles of cortisol and DHEA, based on previous associations of flat diurnal rhythms with negative health outcomes. The fourth paper examined whether or not stressful life events and exercise behaviour were associated with the diurnal

rhythms of cortisol and DHEA, and the cortisol:DHEA ratio in young, middle aged and older adults. It was hypothesised that the impact of stress on cortisol and DHEA would be more pronounced in older adults, and secondly that exercise may buffer against the effects of stress on these hormones. The older participants were the same in papers three and four. Finally, the last paper included sedentary, moderately active and endurance trained older adults. The aim of this paper was to examine if long term exercise training influenced levels of cortisol, DHEA and DHEA-S in serum at rest, and also in response to acute exercise. In addition to training status, a further objective was to explore whether sex influenced the responses of these hormones to acute exercise. It was hypothesised that those who engaged in exercise would be characterised by higher levels of DHEA/S and a lower cortisol:DHEA/S ratio, and that they may also differ in their response to acute exercise.

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# CHAPTER TWO

## 2.0. AGE, DEPRESSION, ANXIETY, SOCIAL SUPPORT, AND THE DIURNAL RHYTHM AND AWAKENING RESPONSE OF SALIVARY CORTISOL

## 2.1. ABSTRACT

The present study compared the cortisol awakening response and diurnal rhythm in 24 young healthy students and 48 community dwelling older adults. The associations with diurnal cortisol and depression, anxiety and social support were also examined in relation to age. Salivary cortisol was measured over the course of one day: immediately upon awakening, 30 min later, and then 3h, 6h, 9h and 12h post-awakening. Participants completed a questionnaire measuring symptoms of anxiety and depression and social support was assessed. Older adults exhibited a significantly reduced awakening response, lower overall cortisol levels, AUCs and diurnal slopes than younger adults, resulting in a flatter diurnal rhythm. Younger adults with higher depression scores had significantly higher overall cortisol and higher levels upon awakening and 30 minutes post awakening. In the younger adults, anxiety and depression correlated positively with AUC and the CAR. Older adults with lower social support had a reduced AUC where younger adults with lower social support displayed a larger AUC. These findings suggest that the diurnal rhythm and awakening response of salivary cortisol is significantly reduced in older adults and the associations between anxiety, depression and social support and diurnal cortisol vary with age.

## 2.2. INTRODUCTION

Cortisol, a stress hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis, is involved in a number of important functions in humans including energy metabolism, vascular activity, and inflammatory and immune responses (Schürmeyer and Wickings, 1999). Cortisol exhibits a marked diurnal rhythm, characterised by a rapid increase in levels upon awakening peaking at around 30 minutes post awakening (Pruessner et al., 1997) and declining thereafter reaching a nadir in the evening (Hucklebridge et al., 2005).

The diurnal rhythm of cortisol has been previously examined using measures such as the cortisol awakening response (CAR), the secretion across the day expressed by area under the curve (AUC), and also the rate of decline across the day or cortisol slope. These aspects of the circadian rhythm are related to physiological health. For example, individuals suffering from a chronic illness compared to healthy controls show an elevated CAR alongside a lower AUC (Kudielka and Kirschbaum, 2003). Further, a flatter cortisol diurnal pattern related to a higher incidence of cardiovascular disease and type II diabetes (Rosmond et al., 2003), and earlier mortality in cancer patients (Sephton et al., 2000). The impact of an altered diurnal cortisol pattern may be particularly important for older individuals, where neuroendocrine dysregulation is associated with immunosenescence and disturbances in physical functioning, which may consequently impact upon independence and quality of life.

### *2.2.1. Ageing and cortisol*

Previous studies examining the effects of ageing on diurnal cortisol secretion have yielded contrasting results. Higher evening and nocturnal concentrations of cortisol resulting in an overall increase in 24h mean cortisol and a flattened diurnal pattern characterised older participants in several studies (Deuschle et al., 1997; Luz et al., 2003; VanCauter et al., 1996; Yen and Laughlin, 1998). In contrast, older individuals have also exhibited a steeper cortisol slope (Ice et al., 2004). In addition, two studies reported no association between age and cortisol secretion during the day (Edwards et al., 2001; Wolf et al., 2002). With regard to the CAR, one study showed a lower CAR with increasing age (Kudielka and Kirschbaum, 2003) whereas others have failed to observe any age differences (Pruessner et al., 1997; Wüst et al., 2000).

Clearly there is little consensus. Many of these studies have tested a broad age range, rather than recruit distinct age groups. Some failed to obtain repeated saliva samples across the day and thus restricted their analysis to the CAR (Kudielka and Kirschbaum, 2003; Pruessner et al., 1997; Wüst et al., 2000) or to the slope of decline (Ice et al., 2004; Luz et al., 2003; Wolf et al., 2002). Finally, several previous studies did not control for important confounding variables such as awakening time, which affects the diurnal cortisol cycle (Edwards et al., 2001).

### *2.2.2. Depression, anxiety, social support and cortisol*

Several studies have examined the relationship between the cortisol diurnal rhythm and psychological wellbeing. For example, depression has been associated with increased cortisol levels (Cowen, 2009; Gillespie and Nemeroff, 2005), greater diurnal variation, and a steeper diurnal decline (Bridges and Jones, 1966). However, contradictory evidence exists; other studies

have found depression (Sjogren et al., 2006) and emotional distress (Miller et al., 2007) to relate to a flatter cortisol diurnal profile. In addition, one study reported no difference in the pattern of the diurnal profile between depressed patients and controls (McClure, 1966). With regard to the CAR, participants who were currently or previously depressed (Bhagwager et al., 2005; Vreeburg et al., 2009a) or had higher depressive symptoms (Pruessner et al., 2003b) exhibited a higher CAR. In contrast, one studied showed a blunted CAR in depressed participants (Stetler and Miller, 2005).

One reason for this variation in findings could be the existence of co-morbid anxiety in those who are depressed (Murphy et al., 2004). For example, depressed participants with co-morbid anxiety had a higher CAR compared to those with depression alone (Vreeburg et al., 2009a), suggesting that anxiety may exacerbate the effects of depression on diurnal cortisol. There is also evidence that anxiety is independently associated with diurnal cortisol; individuals with high levels of anxiety showed a different pattern of decline across the day compared to those with lower levels of anxiety (Vedhara et al., 2003). Conversely, others have failed to demonstrate an association between anxiety and diurnal cortisol patterns (Adam, 2006; Kurina et al., 2004). Differences in findings may also relate to the intensity of depression and the environment in which the participants are studied. Clinically depressed patients may differ from individuals in the community with depressive symptomatology; the association with diurnal cortisol would appear to be less certain in the latter (Bhagwager et al. 2005). It has been proposed that mild depression may be characterised by a dysregulation of cortisol rather than specifically hypersecretion (Stetler and Miller, 2005). This lack of consensus may also be partly explained by methodological

considerations such as the age of the participants and failure to control for possible confounders (Chida and Steptoe, 2009; Vreeburg et al., 2009a).

Conflicting findings are also typical of studies of positive psychosocial factors which may buffer the effects of anxiety and depression. Individuals with higher levels of social support have shown a steeper diurnal decline (Sjogren et al., 2006) and lower mean cortisol levels (Turner-Cobb et al., 2000). Support in the form of intimacy has also been associated with smaller AUC (Ditzen et al., 2008). In contrast, studies have found no association between social support and the diurnal slope (Turner-Cobb et al., 2000) or diurnal cycle (Smyth et al., 1997). However, in comparison to anxiety and depression, fewer studies have examined the relationship into social support and diurnal cortisol, nor have these three psychosocial factors between examined simultaneously.

### *2.2.3. Ageing, depression, anxiety, social support and cortisol*

There is a high prevalence of anxiety and depression in older adults (Ritchie et al., 2004) and depression has been associated with mortality in this population (Blazer, 1982; Schulz et al., 2000). Due to methodological differences, variations in reported rates exist (Girling et al., 1995). However, rates of 13.5% for depression and 3.7% for anxiety have been observed in community-dwelling adults aged between 65 and 89 years old in London (Lindsay et al., 1989). The difference in prevalence between young and older adults remains unclear due to conflicting findings (Jorm, 2000). Studies which have demonstrated age differences lack longitudinal follow up, thus it is difficult to attribute these to ages *per se* or differences in other factors related to age differences (Jorm, 2000).

Studies specifically examining older adults have found higher AUCs (Wrosch et al., 2007) and flattened or inconsistent diurnal patterns (Fiocco et al., 2006) in individuals with depressive symptoms. However, in one case this occurred in the context of physical health and memory problems and absence of a non-depressed comparison group (Fiocco et al., 2006). Psychosocial factors have been related to a dysregulated diurnal profile in adolescents (Goodyer et al., 2000; Greaves-Lord et al., 2007; Van den Bergh et al., 2008). With the exception of one study, which reported greater CARs in male university students with higher depression scores (Pruessner et al., 2003b), there is limited research examining diurnal cortisol and affect in young adults. Consequently, no studies have examined the effects of depression and anxiety on the both the CAR and the diurnal rhythm of cortisol in the context of ageing. Older adults' cortisol outputs over the day has been inversely related to seeking social support (O'Donnell et al., 2008); however, this study did not measure perceived social support. To our knowledge, perceived social support has not been examined in parallel with anxiety and depression in relation to diurnal cortisol among individuals of different ages.

Accordingly, the aims of the present study were to: first, compare the CAR and diurnal rhythm of cortisol in young and older community dwelling adults; and second, examine whether any observed age differences related to anxiety, depression, or social support. Finally, these analyses were revisited while controlling for differences in health behaviours and other potential confounding variables. Differences have been noted in the CAR and cortisol diurnal rhythm for health behaviours including smoking (Badrick et al., 2007), alcohol consumption (Badrick et al., 2008), and sleep quality (Dahlgren et al., 2009). Other potential confounding variables including

sex (Pruessner et al., 2007; VanCauter et al., 1996), oral contraceptive use (Pruessner et al., 1997; Reinberg et al., 1996), and phase of the menstrual cycle (Bao et al., 2003) have also been shown to influence the CAR and/or diurnal rhythm of cortisol. Consequently, it is important to adjust for these potential confounders.

## 2.3. METHODS

### *2.3.1. Participants*

Participants were 24 (11 women) University of Birmingham students and 48 (24 women) community dwelling older adults. Mean ages of the younger and older adults were 20.0 (SD = 1.16, range 19-22) and 75.6 (SD = 6.35, range 65-88) years, respectively. Seventy six participants were originally recruited; four were excluded, one for non compliance and three for extreme cortisol values ($\geq \pm 3$ SD above the mean). Older adults were recruited from Birmingham clubs and associations (for example, Age Concern, the Women's Institute), churches, and through posters displayed in businesses around the local area. Inclusion criteria were: no endocrine or immune disorder, no psychiatric illness, no periodontal disease, no eating disorder and not taking glucocorticoid medication. The majority (97%) of participants described themselves as "white", with the exception of two of the younger adults who were "mixed race". In terms of socio-economic status, 75% of the young cohort were classified as from a non-manual occupational households based on their parent's previous/current occupation, using the Registrar General's Classification of Occupations (Classification of Occupations, 1980). Sixty-five percent of older adults were from non-manual occupational households based on their previous occupation; 4% did not disclose occupational background. Among younger females, 36%

reported taking oral contraceptives; this information was gathered as cortisol can differ between women taking oral contraceptives and those who are not (Pruessner et al., 2003a). Four women reported being in the luteal phase of the menstrual cycle and six in the follicular phase, one participant did not provide this information.

### *2.3.2. Study design*

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

### *2.3.3. Questionnaires*

Participants completed all questionnaires at home on the same day as the saliva sampling protocol.

*Depression and anxiety*

The Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983) was used to measure depression and anxiety. It contains seven items measuring anhedonic rather than somatic aspects of depression and seven measuring anxiety. Items are scored from 0-3, with higher scores indicating greater depression and anxiety. The HADS has good concurrent validity (Bramley et al., 1988; Herrmann, 1997), an internal consistency of .90 and .93 for anxiety and depression respectively (Zigmond and Snaith, 1983) and test retest reliability coefficients of .85 for depression and .84 for anxiety (Herrmann, 1997). In the present study, internal consistency was .84 for depression and .81 for anxiety.

*Social Support*

Social support was measured using the Medical Outcomes Study Social Support Survey (MOSSSS) (Sherbourne and Stewart, 1991). This questionnaire assesses structural social support (i.e. the number of close friends and relatives from whom social support can be sought) and functional support (i.e. the availability of different types of support) via 19 items in four domains: emotional/informational, tangible, affectionate and positive social interaction. The questionnaire is scored on a five-point scale ranging from none of the time to all of the time. Internal consistency is high at .91, and one year test reliability values ranging from 0.72-0.78 have been reported (Sherbourne and Stewart, 1991). For the present study, internal consistency averaged .75 for the four categories of functional support.

*Socio-demographic and health behaviours*

Participants also completed a socio-demographic questionnaire. Data gathered included: date of birth, height (m), weight (kg), ethnicity, if suffering from chronic illness or taking ongoing medication, if taking oral contraception, the day of their menstrual cycle and previous or parental occupation. Body mass index (BMI) was computed as $kg/m^2$. Health behaviours over the past year were assessed using a questionnaire adapted from the Whitehall study (Marmot et al., 1991). Participants indicated how much time (0, 1-2, 3-5, 6-8, 9-10, 11+ hours a week) they spent participating in light (i.e. walking), moderately energetic (swimming, golf) and vigorous (i.e. running, squash) per week. A 0-5 categorical scoring system was applied to all behaviours, e.g. if they spent 1-2 hours performing an activity they were awarded a score of 1. A combined exercise score was calculated by multiplying the category score by a weighting of 1, 2, 3 for light, moderate, and vigorous activity respectively. Participants reported how many cigarettes (0,

1-5, 6-10, 11-20, 21+, 40+ per day), how many units of alcohol they consumed per week (0, 1-5, 6-10, 11-20, 20-40, 40+) and, on average, how many hours they slept per night (0-3, 4-5, 6-7, 8-9, 10-11, 12+). A binary variable (current smoker, non-smoker) was created and sleep and alcohol data were also collapsed into binary variables, denoting whether participants slept ≥ 6 hours per night and if they consumed ≥ 6 units of alcohol per week. The questionnaire contained a dietary section from which two measures were derived (Burns et al., 2002): scores for fresh fruit, fruit juice and cooked vegetables were summed to obtain a measure of fruit and vegetable intake; scores for chips/fried food, crisps/similar, sweets/chocolate, biscuits/cakes/puddings, full fat dairy products and processed meat were summed to provide an index of fat intake. Participants reported how frequently they consumed these types of food in a typical week during the past year on a 0-7 categorical scale defined as: never, less than once a week, 1 or 2 a week, most days (3-6), once a day, 2-3 times a day, 4 or more times a day.

### *2.3.4. Salivary Cortisol Measurements*

Stimulated saliva samples were obtained over one day to determine the diurnal pattern of free salivary cortisol secretion. Salivettes were centrifuged at 4000 rpm for 5 min and the saliva was pipetted into eppendorfs which were stored at -20°C until assay. Salivary cortisol samples were analysed all in the same day in duplicate by ELISA (DRG Diagnostics, Germany). This assay is based on the competition principle and microplate separation. An unknown amount of cortisol present in the sample and a fixed amount of cortisol conjugated with horseradish peroxidise compete for the binding sites of mouse monoclonal cortisol antiserum. After an hour the microplate is washed to stop the competition reaction. After addition of a substrate solution the

concentration of cortisol is inversely proportional to the optical density measured at 450 nm. Intra-assay coefficients were always < 10%.

### *2.3.4. Procedure*

A one day saliva sampling protocol was chosen as the diurnal rhythm of cortisol had been shown to display intra-individual stability between days (Edwards et al., 2001; Hucklebridge et al., 2005). Participants were instructed to complete the sampling protocol on a weekday. Each participant was provided with a pack of six salivette tubes (Sarstdedt Ltd, Leicester, UK) labelled with the sampling times which were: immediately upon awakening, 30 min post-awakening, and then 3h, 6h, 9h and 12h post awakening. They were briefed concerning the collection procedure and sampling times. Participants were asked to refrain from excessive alcohol consumption on the day prior to sampling, and to avoid consuming alcohol and undertaking vigorous exercise on the sampling day. Participants were asked not to eat, drink (except water), smoke or brush their teeth 30 min prior to each sample. Participants placed the salivette dental swab into their mouths and gently chewed for 1 min to collect saliva. The swab was returned to the salivette and stored in the participant's refrigerator within 24 hours until collection.

To assess compliance all participants were given a diary to record the times their samples were due and the time when they actually took them. Younger participants were asked to set alarms on their mobile phones to prompt them when to take a sample and older adults were given a wristband where they could write reminders of their sampling times. The first two samples of the day (used to calculate the CAR) were included only if taken on time or within 10 minutes, as recommended (Kunz-Ebrecht et al., 2004). For the remainder of the samples, one participant was

excluded from the study as they had repeatedly taken samples over an hour late, resulting in our final overall sample size of 72. Overall, according to the self report diary, out of 432 samples: 79% were taken on time or within five minutes, 10% were up to 20 min late, 4% were up to 30 min late, 5% over 30 min up to 1hr late and 2% were over 1hr late.

### *2.3.5. Data analysis*

Age cohort differences in psychosocial variables, health behaviours, BMI, chronic illness and time of awakening were examined using ANOVA and chi-square, in the case of categorical variables such as current smoker, consuming ≥6 alcohol units a week, and sleeping ≥ 6 hours a night.

Analyses were conducted using four cortisol outcome measures: the diurnal repeated measures pattern across all six samples; the cortisol awakening response (CAR); AUC; and diurnal slope. The CAR was calculated as sample 2 minus sample 1 (Edwards et al., 2001; Sjogren et al., 2006). AUC was calculated relative to zero using the trapezoid method applied to all sampling points (Pruessner et al., 2003a). Diurnal cortisol slopes were calculated by regressing cortisol values on the sample time for each participant separately (Cohen et al., 2006; Smyth et al., 1997; Turner-Cobb et al., 2000). This yields a slope value for each participant. The sample obtained upon awakening was used as the slope anchor (Kraemer et al., 2006) and the second sample (30 minutes after waking) was excluded from the estimation of the slopes (Cohen et al., 2006).

Repeated measures ANOVA was used to examine the diurnal cortisol rhythm, first in relation to age cohort, and second, in relation to age cohort and each separate psychosocial variable, in order

to test main effects of age and psychosocial variables, and any interaction effects. For all continuous variables, binary variables were created using median splits to form high and low groups within each age cohort. Anxiety and depression were split at a score of 11, indicating a high probability of clinical depression (Zigmond and Snaith, 1983), to form groups indicating high and low depressive symptoms. Greenhouse-Geisser corrected statistics and partial $\eta^2$ as a measure of effect size are reported.

Univariate ANOVA was applied to analyse age cohort, then age cohort × psychosocial differences in the CAR, AUC, and diurnal slopes, respectively. The relationships between CAR, AUC, and diurnal slope with psychosocial variables were then analysed in their continuous form using correlations. In all of the above analyses, where significant effects emerged, subsequent ANCOVA was performed to adjust for potential confounding variables: diet, alcohol consumption, exercise, BMI, time of awakening, smoking, sleep and chronic illness (Table 2). Slight variations in degrees of freedom reflect occasional missing data or insufficient saliva for analysis.

## 2.4. RESULTS

No sex differences were found for any other analysis; accordingly genders were grouped together and results presented below are for both males and females collectively.

### *2.4.1. Diurnal cortisol and age*

The repeated measures ANOVA yielded a significant main effect of time on cortisol concentration, $F(5,320) = 50.11$, $p < .001$, $\eta^2 = .439$. The pattern was characterised by significant

linear and cubic components, $p < .001$. The quadratic component was not statistically significant, $p = .110$. There was also a main effect of age cohort, $F(1,64) = 14.23$, $p < .001$, $\eta^2 = .184$, such that younger adults had higher cortisol levels overall. The time × age interaction effect was also significant, $F(5,320) = 7.29$, $p < .001$, $\eta^2 = .102$; as can be seen in Figure 1, with younger adults exhibiting higher cortisol concentrations than the older adults up to 3h post waking. Analysis of AUC revealed a significant difference, $F(1,64) = 11.73$, $p = .001$, $\eta^2 = .155$, between the older (65.1, SD = 34.99 ng/ml) and the younger (95.7, SD = 34.70 ng/ml) cohort. There was also a significant difference in cortisol slope between the younger (-.60, SD = .41) and older (-.32, SD = .51) adults, $F(1,66) = 5.52$, $p = .022$, $\eta^2 = .07$. The younger cohort exhibited a steeper decline across the day.

### *2.4.2. Cortisol awakening response and age*

For the CAR, there was a significant cohort effect, $F(1,66) = 6.96$, $p = .010$, $\eta^2 = .095$, with younger adults showing a greater awakening response than older adults, 8.82, SD = 10.54 and 2.99, SD =7.54 ng/ml, respectively.

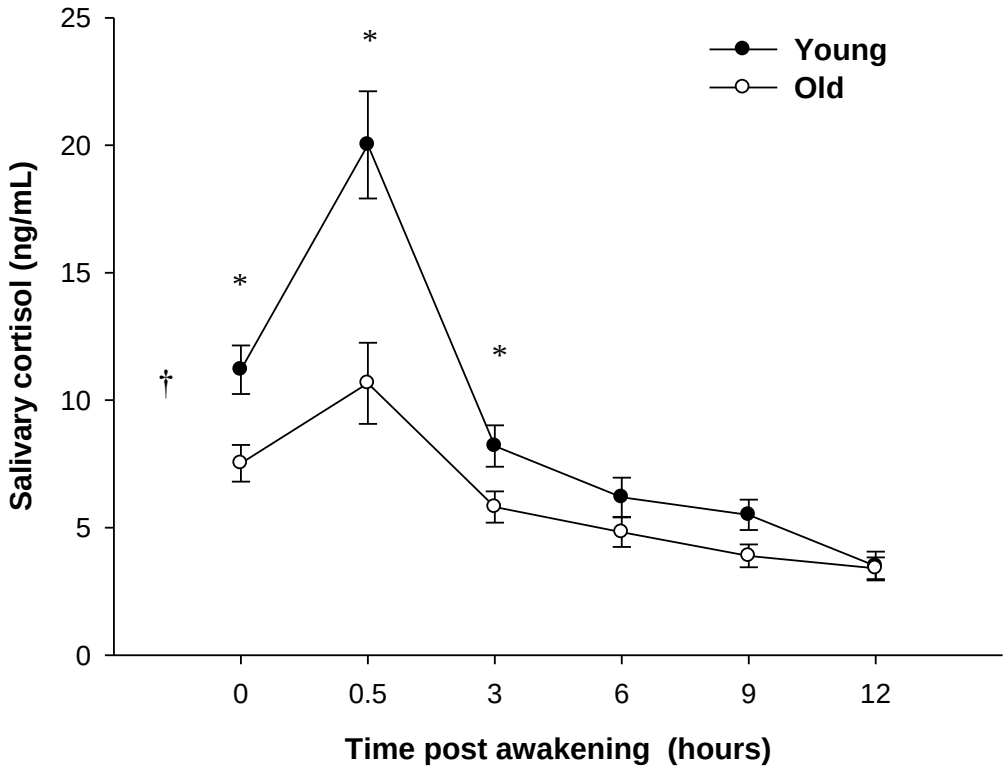


**Figure 1.** Mean (SEM) diurnal cortisol pattern by age cohort. Significant main effect of age group indicated by † $p$ <.001, and group × time interaction by * $p$ <.001

### *2.4.3. Age, anxiety, depression and social support*

There were no significant differences between age cohorts for anxiety, depressive symptoms or social support variables with the exception of positive social interaction; where older adults had significantly lower social interaction scores than the younger cohort.

**Table 1**. Mean (SD) anxiety, depression and social support scores in younger and older adults.

| Variable | Older Adults | Younger | F(df) | *p* | $\eta^2$ |
|---|---|---|---|---|---|
| HADS total | 14.7 (9.22) | 13.6 (9.01) | 0.44 (1,70) | .835 | .001 |
| HADS anxiety score | 7.1 (4.52) | 7.0 (4.08) | 0.01 (1,70) | .924 | .000 |
| HADS depression score | 7.0 (5.39) | 6.6 (5.75) | 0.07 (1,70) | .786 | .001 |
| MOS friends & relatives | 6.6 (4.88) | 8.0 (2.38) | 1.68 (1,54) | .199 | .030 |
| MOS total | 75.7 (19.53) | 83.2 (15.98) | 2.68 (1,69) | .106 | .037 |
| MOS tangible | 16.1 (4.78) | 18.4 (11.32) | 1.46 (1,68) | .231 | . 021 |
| MOS emotional | 31.2 (8.54) | 34.8 (4.69) | 3.60 (1,69) | .062 | .050 |
| MOS affectionate | 12.6 (3.08) | 12.3 (2.70) | 0.15 (1,69) | .704 | .002 |
| MOS positive social | 16.0 (4.29) | 17.8 (2.13) | 3.88 (1,68) | .050 | .054 |
| Categorical variables | | | $\chi^2$ (df) | *p* | |
| HADS depression score ≥ 11 (%) | 22 | 38 | .818 | .366 | |
| HADS anxiety score ≥ 11 (%) | 25 | 17 | .643 | .423 | |
| Low depressive symptoms average score | 2.7 (3.10) | 4.3 (3.18) | | | |
| High depressive symptoms average score | 13.0 (2.00) | 14.15 (2.85) | | | |
| Low anxiety symptoms average score | 5.7 (2.67) | 5.13 (2.96) | | | |
| High anxiety symptoms average score | 13.5 (3.78) | 13.0 (2.92) | | | |

HADS, Hospital Anxiety and Depression Scale; MOS, Medical Outcomes Study Social Support Survey.

### *2.4.4. Diurnal cortisol, age, anxiety, depression and social support*

Repeated measures ANOVA revealed a significant main effect of depression on diurnal cortisol, $F(5,310) = 5.38$, $p = .004$, $\eta^2 = .080$, where higher depressive symptoms was associated with higher cortisol levels overall. There was also a significant two-way interaction between cohort and depression on cortisol, $F(1,62) = 7.65$, $p = .007$, $\eta^2 = .110$, such that young adults with higher depressive symptoms had higher overall cortisol levels. Finally, there was a significant age cohort × depressive symptoms × time interaction effect for diurnal cortisol, $F(5,310) = 10.29$, $p < .001$, $\eta^2 = .142$. As seen in Figure 2, the younger adults with depressive symptoms had significantly higher cortisol levels upon awakening and 30 minutes later than the other three groups, $F(1,66) = 14.68$, $p < .001$, $\eta^2 = .182$ and $F(1,67) = 36.68$, $p < .001$, $\eta^2 = .354$, respectively. The age cohort × depressive symptoms interaction effect for AUC did not quite reach statistical significance, $F(1,62) = 3.71$, $p = .059$, $\eta^2 = .056$, nor was there an interaction effect for diurnal slope.

No significant interaction effects occurred for diurnal cortisol, age and anxiety or social support or for diurnal slope and these variables. However, there was a significant interaction effect for AUC of cohort and total social support, $F(1,61) = 3.94$, $p = .05$, $\eta^2 = .061$, such that older adults with lower total social support had a reduced AUC. In addition, younger adults with lower affectionate support had a significantly higher AUC, $F(1,61) = 16.65$, $p = <.001$, $\eta^2 = .214$.

### *2.4.5. CAR, age and depression*

There was a significant age cohort × depression interaction effect for the CAR, $F(1,64) = 10.09$, $p = .002$, $\eta^2 = .136$; younger participants with depressive symptoms showed a higher awakening

response. No significant effects emerged for CAR, age and anxiety or social support, although there was a trend for younger adults with lower affectionate support to display a higher CAR, $F(1,63) = 3.78$, $p = .056$, $\eta^2 = .057$.

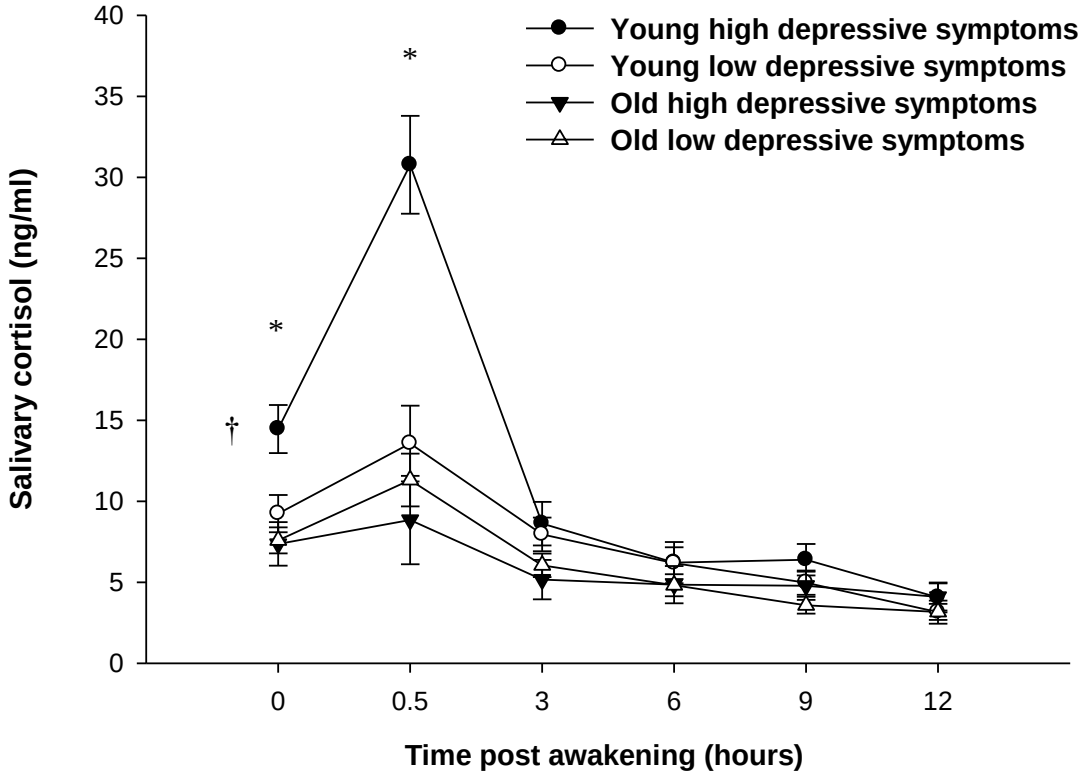


**Figure 2**. Mean (SEM) diurnal cortisol pattern by age cohort and HADS depression score. Significant main effect of age × depressive symptoms indicated by † $p = .007$, and age × depression × time interaction by * $p < .001$.

### *2.4.6. Diurnal cortisol, oral contraceptives and the menstrual cycle*

There was no significant difference in diurnal cortisol or CAR between women taking oral contraceptives and those who were not. The diurnal rhythm was significantly different between the two phases of the menstrual cycle, $F(5,40) = 6.52$, $p = .012$, $\eta^2 = .449$. Females in the luteal phase had significantly higher cortisol after waking and 30 min after waking compared to those in the follicular phase. However, the interaction between depression and diurnal cortisol in the younger female cohort was not attenuated by adjustment for menstrual cycle phase.

### *2.4.7. Diurnal cortisol, CAR, age and HADS depression adjusting for potential confounding variables*

The interactions of diurnal cortisol and CAR for age cohort × depressive symptoms were adjusted individually for each of the variables displayed in Table 2. For these analyses, the continuous versions of these variables were entered as covariates with the exception of alcohol consumption, sleep duration and smoking status. The interactions for both diurnal cortisol and CAR withstood adjustment for these potential confounders. Further, there were no differences in sex and socio-economic status distribution between the cohorts, nor any interaction effects for these variables on diurnal cortisol. There was also no significant difference in diurnal cortisol or CAR between women taking oral contraceptives and those who were not.

### *2.4.7. Correlations between AUC, CAR and diurnal slope and the psychosocial variables*

Significant positive correlations were found for younger adults between the AUC and depressive symptoms, $r(22) = .52$, $p < .01$ and anxiety, $r(22) = .41$, $p = .049$. For older adults, the AUC

positively correlated with emotional/informational support, r(39) =.32, $p$ = .042. No significant correlations were found for the CAR and psychosocial variables for older adults. However, there was a significant positive correlation for the younger cohort for depressive symptoms, r(22) =.59, $p$ < .01, as would be expected given the previous diurnal cortisol interactions, and for anxiety, r(22) =.46, $p$ = .026. There were no significant correlations between the cortisol slope and the psychosocial variables for either cohort.

**Table 2**. Mean (SD) health behaviours, BMI, chronic illness and time of awakening for the old and young cohorts.

| Variable | Older Adults | Younger Adults | F(df) | *p* | $\eta^2$ |
|---|---|---|---|---|---|
| Exercise score | 4.8 (4.51) | 13.7 (6.85) | 41.47 (1,66) | < .001 | .386 |
| Cooked meals (per day) | 1.2 (0.80) | 1.8 (0.51) | 39.13 (1,70) | < .001 | .359 |
| Caffeine (drinks per day) | 4.6 (2.62) | 1.4 (1.20) | 29.93 (1,69) | < .001 | .303 |
| Fat score | 9.6 (2.66) | 10.9 (3.87) | 2.00 (1,67) | .162 | .029 |
| Fruit and vegetable score | 4.8 (4.51) | 11.1 (2.15) | 5.79 (1,69) | .019 | .077 |
| BMI ($kg/m^2$) | 28.2 (4.17) | 21.7 (2.79) | 47.29 (1,70) | < .001 | .403 |
| Time of awakening (am/hr) | 7.10 (1.01) | 8.45 (1.01) | 28.79 (1,70) | < .001 | .291 |
| Categorical variables | | | $\chi^2$ (df) | *p* | |
| Alcohol ≥ 6 units per week (%) | 27 | 63 | 8.45 (1) | .004 | |
| Current smokers (%) | 6 | 4 | 0.13 (1) | .593 | |
| Sleep ≥ 6 hours (%) | 29 | 71 | 9.06 (1) | .003 | |
| Chronic illness (%) | 67 | 17 | 17.38 (1) | < .001 | |

## 2.5. DISCUSSION

### *2.5.1. Age and cortisol*

Older adults displayed a blunted CAR and a flatter diurnal profile which is consistent with previous findings for the CAR (Kudielka and Kirschbaum, 2003) and diurnal profile (Deuschle et al., 1997; VanCauter et al., 1996). Although the exact function of the CAR is unknown, it has been proposed to be linked to memory systems (Wilhelm et al., 2007), play an important role in regulating the immune system (Petrovsky and Harrison, 1997) and in anticipating the demands of the day (Fries et al., 2009). The reduced CAR observed in the older adults may therefore have implications for such functions.

In contrast to some previous findings, the flatter profile observed in the present study was due to lower awakening levels and a reduced CAR in older adults, as opposed to higher evening levels (Deuschle et al., 1997). However, in this previous study, cortisol, although sampled over a 24 hour period, was not measured over the awakening period. Whereas older adults in the present study had lower overall cortisol levels, others have reported higher cortisol levels in the elderly (Luz et al., 2003; VanCauter et al., 1996). Nevertheless, the present pattern of results is in line with previous observations of lower morning (Maes et al., 1994) and overall cortisol levels as a function of age (Ahn et al., 2007). Although these studies analysed cortisol in plasma, salivary cortisol accurately reflects plasma free cortisol (Kirschbaum and Hellhammer, 1989). Thus, different media of measurement would not appear to explain contrasting results. It is possible that variations in findings reflect differences in sampling and the recruitment of distinct age cohorts rather than continuous age sampling. Alternatively, lack of consistency may reflect the

considerable individual variability in changes in diurnal cortisol with ageing. A longitudinal study of cortisol in healthy older adults revealed that although the majority showed increasing diurnal cortisol levels over the years some individuals registered a decrease (Lupien et al., 1996); the aggregate pattern of secretion did not change significantly over time in this study. Marked individual differences in cortisol secretion have also been noted in younger adults (Smyth et al., 1997). Accordingly, marked individual variability may partly explain the lack of consensus regarding cortisol and ageing. Although there may be a general shift towards a flatter diurnal profile, this may be characterised through either increased nadir levels or blunted secretion in the morning.

Increases in cortisol previously observed with ageing have been attributed to impairment of feedback inhibition of HPA activity due to neuronal loss in hippocampal area (VanCauter et al., 1996; Yen and Laughlin, 1998) and reduced hippocampal volume has been shown in older adults with a progressive increase in 24h cortisol over 5-6 years (Lupien et al., 1998). Loss of HPA axis sensitivity has also been demonstrated via impaired dexamethasone suppression (Magri et al., 1997). The attenuated diurnal levels of cortisol observed in our older participants imply dysregulation at another stage of the HPA axis. Variations in the diurnal profile with age in the present study appear to be largely driven by an attenuated CAR in the older cohort. Although speculative, it is possible that the decrease in morning cortisol is possibly a consequence of fatigue of the adrenal cortex and thus a reduced ability to respond dynamically to the stress of awakening. However, it should be conceded that the blunted CAR may also reflect changes in hippocampal function with age, given that the awakening response is abolished in patients with hippocampal damage (Buchanan et al., 2004).

### 2.5.2. *Age, depression, anxiety, social support and cortisol*

The present finding that a higher CAR related to higher depressive symptoms among male university students has been reported previously (Pruessner et al., 2003b). However, the present study extended this finding to both male and female students. An elevated CAR in individuals with diagnosed depression has been found in community samples (Bhagwager et al., 2005; Vreeburg et al., 2009a). Also in line with previous findings, cortisol returned to similar levels irrespective of depression status after awakening (Bhagwager et al., 2005). In contrast, a blunted CAR (Stetler and Miller, 2005) and a flatter diurnal profile (Sjogren et al., 2006) has also been reported in those in community settings, implying that the effects of depression in the community may be best viewed from the perspective of dysregulation and not in terms of a specific increase/decrease in usual levels (Stetler and Miller, 2005). However, interestingly, there was no significant difference in any of the cortisol parameters between the high and low depressive symptom groups in the older adults where altered diurnal patterns have been previously observed with depressive symptoms (Fiocco et al., 2006; Wrosch et al., 2007). This could be attributable to the positive health status of the older participants recruited to the present study. Previous studies on cortisol and depression in older adults have included participants with health problems and/or cognitive dysfunction (Fiocco et al., 2006; Wrosch et al., 2007).

In the present study no gender or oral contraceptive use differences were found in relation to cortisol, in contrast to previous studies (Pruessner et al., 1997; Vreeburg et al., 2009b), but in line with others (Würst et al, 2000). There was a significant difference between young females in different phases of the menstrual cycle, as observed previously (Bao et al., 2003). However, our main findings with regard to age and depression withstood adjustment for this difference.

Further, changes in cortisol over the menstrual cycle have not been shown to vary between depressed and non-depressed females (Bao et al., 2004).

Anxiety was positively associated with a higher CAR and AUC in the younger cohort but again there were no significant relationships in the older cohort. Others have failed to show an association between anxiety with diurnal cortisol in adolescents (Adam, 2006), however, we are not aware of any studies specifically among those aged over 65 year for comparison. Within the higher depressive symptoms group, there was no difference in the CAR between those with high and low anxiety symptoms, where co-morbid anxiety has been previously found to increase the CAR (Vreeburg et al., 2009a). Social support was related to the CAR in the younger adults and AUC in both cohorts. However, the direction of these associations differed whereby low social support related to reduced cortisol in older adults but had the opposite association in students. It could be speculated that social support has differing relationships with physiological variables depending on age. For example, social support has been associated with vaccination response in students (Gallagher et al., 2008) but not an older adult sample (Phillips et al., 2006). Social support may be interpreted differently by these age cohorts and the relevance of different domains of support may differ. Other aspects of social support not measured in this study, such as marriage, may be more important for older adults, as suggested previously (Phillips et al., 2006).

It has been speculated that an increased CAR in depression could be a stress response to disturbed mood upon awakening (Bridges and Jones, 1966). However, a causal association between depression and HPA activity is yet to be established. It has been suggested that cortisol

dysregulation may represent an endophenotype (Hasler et al., 2004) or vulnerability marker of depression (Bhagwager et al., 2005; Cowen, 2009; Harris et al., 2000); further, corticotrophin-releasing hormone has been argued to be a key aetiological factor in depression and anxiety (Alldredge, 2010). Alternatively, changes in the diurnal cortisol rhythm could be a residue from previous depression implying a causal pathway from depression to altered HPA activity (Bhagwager et al., 2005). In contrast, elevated cortisol has been proposed to be a compensatory mechanism to overcome glucocorticoid resistance as a result of impaired/reduced glucocorticoid receptors in depression (Pariante, 2009). Thus, the association between depression and cortisol would appear to have multiple determinants and may be bi-directional.

Cortisol deregulation could be influenced by genetics, stress exposure, and personality (Heim et al., 2000) and these could play role a role in the individual variability observed in diurnal cortisol with both ageing and psychosocial factors. Further, it may not be the relative magnitude of cortisol in comparison to others that is most important in terms of health consequences, but rather how much cortisol changes in relation to an individual's usual level and its balance with other parameters. Future research might investigate how changes in diurnal cortisol with ageing and psychosocial factors relate to the diurnal patterns of other endocrine variables, as it may not be changes in cortisol *per se,* but rather the balance of cortisol with other hormones, such as the cortisol:DHEA ratio, that is of greater relevance to psychological and physiological health in ageing individuals (Straub et al., 2001).

### *2.5.3. Limitations*

The present study suffers from a number of limitations. Only younger and older adults were tested, whereas flattened cortisol profiles have been observed in participants with a mean age of 46 years (Lasikiewicz et al., 2008). The inclusion of a third middle-aged cohort in this study may have captured more precisely the age at which changes in cortisol rhythm become apparent. Nevertheless, the study was still novel in using two distinct age cohorts. Although studies have shown that the CAR is influenced by state factors (Hellhammer et al., 2007; Stalder et al., 2009; Thorn et al., 2009), there is also evidence from studies sampling on more than one day that the diurnal cortisol profile (Edwards et al., 2001; Hucklebridge et al., 2005) and the CAR (Clow et al., 2004; Pruessner et al., 1997; Würst et al., 2000) shows reasonable temporal stability. Previous depression was not measured in this study and self report was employed to measure depressive symptoms as opposed to an interview. However, the HADS has been validated as a reliable method for evaluating anxiety and depression. Participants were asked to refrain from eating and drinking 30 min prior to samples. Compliance was measured subjectively, in terms of sampling diaries and oral self report. However, cortisol peaks across the day as a result of meal consumption were not observed. Although it is possible that some participants did not comply, this is unlikely to have occurred only in the young depressed group, and unlikely to account for observed differences. Finally, although confounding by unmeasured or poorly measured variables remains possible, we did adjust for a range of potential confounders, including awakening time and health behaviours. Further, the present associations were not explained by perceived or stressful life events (data not reported) which has been proposed to influence the relationship between depression and cortisol (Pruessner et al., 2003b).

### *2.4.4. Conclusion*

Compared to the young cohort, older adults displayed significantly reduced cortisol upon awakening, a lower CAR and a flatter diurnal profile reflected in a reduced AUC. The associations between anxiety, depression, and social support and diurnal cortisol varied by age cohort.

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# CHAPTER THREE

## 3.0. AGE, HEALTH BEHAVIOURS, AND THE DIURNAL RHYTHM AND AWAKENING RESPONSE OF SALIVARY CORTISOL

## 3.1. ABSTRACT

The cortisol diurnal rhythm has previously been examined in relation to age and health behaviours. However, less is known about the relationship between multiple health behaviours and diurnal cortisol in the context of ageing, where it is possible that the impact of health behaviours on cortisol varies as a function of age. This study compared the awakening response and diurnal rhythm of cortisol in young versus older adults in relation to health behaviours. Twenty four young students (aged 18-22) and 48 community dwelling older adults (aged 65-88) completed an assessment of health behaviours (exercise, smoking, sleep, diet, alcohol) over the past year. Salivary cortisol was measured over the course of one day: immediately upon awakening, 30 min later, and then 3h, 6h, 9h and 12h post-awakening. Older adults displayed significantly reduced cortisol upon awakening, a lower cortisol awakening response and a flatter diurnal profile represented by a reduced area under the curve and cortisol slope. There was also a significant interaction of age, cortisol and diet; younger adults with a higher fat and lower fruit and vegetable intake exhibited the flattened diurnal cortisol phenotype of the older adults. These findings suggest that the diurnal rhythm and awakening response of salivary cortisol is significantly reduced in older adults and that variations in the cortisol diurnal rhythm of younger adults are associated with dietary factors. Younger adults with a poor quality of food intake may be vulnerable to a reduction in the amplitude of the cortisol diurnal profile and this may have implications for other aspects of health.

## 3.2. INRODUCTION

Cortisol, a stress hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis, is involved in a number of important functions in humans including energy metabolism, vascular activity, and inflammatory and immune responses (Schürmeyer and Wickings, 1999). Cortisol exhibits a marked diurnal rhythm, characterised by a rapid increase in levels upon awakening peaking at around 30 minutes post awakening (Pruessner et al., 1997) and declining thereafter reaching a nadir in the evening (Hucklebridge et al., 2005).

The diurnal rhythm of cortisol has been examined previously from the perspective of the cortisol awakening response (CAR), the secretion across the day expressed by area under the curve (AUC), and the rate of decline across the day or cortisol slope. These aspects of the circadian rhythm have been shown to relate to physiological and psychological health. For example, an elevated CAR alongside a lower AUC was observed in individuals suffering from a chronic illness compared to healthy controls (Kudielka and Kirschbaum, 2003) and a higher incidence of cardiovascular disease and type II diabetes has been associated with a reduced cortisol levels and slope of decline (Rosmond et al., 2003). A flatter diurnal profile has also been shown to relate to earlier mortality in cancer patients (Sephton et al., 2000). Different psychosocial factors, such as job stress, general life stress and posttraumatic stress, are associated with enhanced or reduced CAR (Chida and Steptoe, 2009). Therefore, a reduction or elevated CAR, and flattened diurnal amplitude appear to be linked to adverse health outcomes. The impact of which may be particularly important for older individuals, where changes in endocrine function may be

associated with immunosenescence, disturbances in physical function, and consequently independence.

Higher nadir concentrations of cortisol resulting in an overall increase in 24h mean cortisol and a flattened diurnal pattern have been observed in older participants in several studies (Deuschle et al., 1997; Luz et al., 2003; VanCauter et al., 1996; Yen and Laughlin, 1998). However, previous studies on the effects of ageing on diurnal cortisol have yielded inconsistent results and not all studies have observed a flattened diurnal cortisol profile. Ageing has also been characterised by a steeper as opposed to flatter cortisol slope (Ice et al., 2004) and has also been reported to show no association with cortisol secretion during the day (Edwards et al., 2001). Others have found a lower CAR with ageing (Kudielka and Kirschbaum, 2003) whereas some studies have failed to observe any age differences in the CAR (Pruessner et al., 1997; Wüst et al., 2000) or the overall diurnal profile (Wolf et al., 2002). Clearly there is little consensus. However, many of these studies have tested a broad age range, rather than compare distinct young and old age cohorts in order to characterise potential differences. Some failed to employ a multiple sampling protocol and thus their analysis was restricted to the CAR (Kudielka and Kirschbaum, 2003; Pruessner et al., 1997; Wüst et al., 2000) or to the slope of decline across the day (Ice et al., 2004; Luz et al., 2003; Wolf et al., 2002). Finally, several previous studies did not control for important confounding variables such as awakening time, which has been shown to be significantly related to the diurnal cortisol cycle (Edwards et al., 2001). Given the inconsistencies in the findings of previous research, as well as variations in their methodology, it was considered important to re-address the issue of cortisol and age, using a multiple sampling protocol across the whole day and controlling for potential confounding factors.

Although counter evidence exists (Vreeburg et al., 2009), health behaviours, such as diet, sleep and alcohol consumption, have been associated with the cortisol diurnal rhythm. Flatter cortisol profiles have been observed in individuals with a higher intake of saturated fat and central body fat distribution (Garcia-Prieto et al., 2007). Smoking was associated with an increased CAR and higher levels of cortisol across the day (Badrick et al., 2007), and women who are heavy drinkers have been characterised by a larger CAR whereas male heavy drinkers showed a flatter diurnal slope (Badrick et al., 2008). Individuals who rated a high level of sleepiness upon awakening had lower levels of cortisol upon awakening (Dahlgren et al., 2009). However, the effects of multiple health behaviours on the CAR and cortisol diurnal rhythm are rarely investigated within the same study. With the exception of Vreeburg et al (2009), who examined a range of socio-demographic and health characteristics, most have focused on one or two health behaviours in isolation, for example smoking (Badrick et al., 2007; Steptoe and Ussher, 2006) or alcohol (Badrick et al., 2008). In addition, although one study has examined cortisol and food intake (Garcia-Prieto et al., 2007), research has largely focussed on obesity (Mårin et al., 1992; Strain et al., 1980), rather than diet specifically. Although some have examined diurnal cortisol and a health behaviour in relation to age (Badrick et al., 2008), the majority of studies have not included adults aged over 65 or included multiple behaviours and older participants. As a result, the relationship between multiple health behaviours and diurnal cortisol in the context of ageing has yet to be identified. It is possible that the impact of health behaviours on cortisol varies as a function of age. Accordingly, the aims of the present study were to compare the CAR and diurnal rhythm of cortisol in younger and older adults, and secondly, examine whether any observed age differences relate to customary health behaviours.

## 3.3. METHODS

### *3.3.1. Participants*

Participants were 24 (11 women) University of Birmingham students and 48 (24 women) community dwelling older adults. Mean ages of the younger and older adults were 20.0 (SD = 1.16) and 75.6 (SD = 6.35) years, respectively. Older adults were recruited from Birmingham clubs and associations (for example, Age Concern, the Women's Institute), churches, and through posters displayed in businesses around the local area. Inclusion criteria were: no endocrine or immune disorder, no psychiatric illness, no periodontal disease, no eating disorder and not taking glucocorticoid medication. The majority (97%) of participants described themselves as "white", with the exception of two of the younger adults who were "mixed race". In terms of socio-economic status, 75% of the young cohort were classified as from a non-manual occupational households based on their parents' previous/current occupation, using the Registrar General's Classification of Occupations (Classification of Occupations, 1980). Sixty-five percent of older adults were from non-manual occupational households based on their previous occupation; 4% did not disclose occupational background. Among younger females, 36% reported taking oral contraceptives.

### *3.3.2. Study design*

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

### *3.3.3. Questionnaires*

Participants completed a socio-demographic questionnaire at entry to the study. Data gathered included: date of birth, height (m), weight (kg), ethnicity, if suffering from chronic illness or taking ongoing medication, if taking oral contraception, and previous or parental occupation. Body mass index (BMI) was computed as $kg/m^2$. Health behaviours over the past year were assessed using a questionnaire adapted from the Whitehall study (Marmot et al., 1991). Participants indicated how much time (0, 1-2, 3-5, 6-8, 9-10, 11+ hours a week) they spent participating in light (i.e. walking), moderately energetic (swimming, golf) and vigorous (i.e. running, squash) per week. A 0-5 categorical scoring system was applied to all behaviours, e.g. if they spent 1-2 hours performing an activity they were awarded a score of 1. A combined exercise score was calculated by multiplying the category score by a weighting of 1, 2, 3 for light, moderate and vigorous activity respectively. Participants reported how many cigarettes (0, 1-5, 6-10, 11-20, 21+, 40+ per day), how many units of alcohol they consumed per week (0, 1-5, 6-10, 11-20, 20-40, 40+), how often they consumed alcohol (never, special occasions only, 1-2 per month, 1-2 per week, almost daily, 2 or more per day), and on average, how many hours they slept per night (0-3, 4-5, 6-7, 8-9, 10-11, 12+). Binary variables were created for smoking (current smoke, non smoker) and sleep (≥ 6 h per night), alcohol units (≥ 6 units per week) and alcohol frequency (≥ 1-2 times per week) based on median splits. The questionnaire contained a dietary section from which two measures were derived (Burns et al., 2002): scores for fresh fruit, fruit juice and cooked vegetables were summed to obtain a measure of fruit and vegetable intake; scores for chips/fried food, crisps/similar, sweets/chocolate, biscuits/cakes/puddings, full fat dairy products and processed meat were summed to provide an index of fat intake. Participants reported how frequently they consumed these types of food in a typical week during the past year

on a 0-7 categorical scale defined as: never, less than once a week, 1 or 2 a week, most days (3-6), once a day, 2-3 times a day, 4 or more times a day.

### *3.3.4. Salivary Cortisol Measurements*

Saliva samples were obtained over one day to determine the diurnal pattern of free salivary cortisol secretion. Salivettes were centrifuged at 4000 rpm for 5 min and the saliva was pipetted into eppendorfs which were stored at -20°C until assay. Salivary cortisol samples were analysed all in the same day in duplicate by ELISA (DRG Diagnostics, Germany). This assay is based on the competition principle and microplate separation. An unknown amount of cortisol present in the sample and a fixed amount of cortisol conjugated with horseradish peroxidise compete for the binding sites of mouse monoclonal cortisol antiserum. After an hour the microplate is washed to stop the competition reaction. After addition of a substrate solution the concentration of cortisol is inversely proportional to the optical density measured at 450 nm. Intra-assay coefficients were always < 10%.

### *3.3.5. Procedure*

A one day saliva sampling protocol was chosen as the diurnal rhythm of cortisol had been shown to display intra-individual stability between days (Edwards et al., 2001; Hucklebridge et al., 2005). Participants were instructed to complete the sampling protocol on a weekday. Each participant was provided with a pack of six salivette tubes (Sarstdedt Ltd, Leicester, UK) labelled with the sampling times which were: immediately upon awakening, 30 min post-awakening and then 3h, 6h, 9h and 12h post awakening. They were briefed concerning the collection procedure and sampling times. Participants were asked to refrain from excessive alcohol consumption on

the day prior to sampling, and to avoid excessive alcohol consumption and vigorous exercise on the sampling day. Participants were asked not to eat, drink (except water), smoke or brush their teeth 30 min prior to each sample. Participants placed the salivette dental swab into their mouths and gently chewed for 1 min to collect saliva. The swab was returned to the salivette and stored in the participant's refrigerator until collection.

To assess compliance all participants were given a diary to record the times their samples were due and the time when they actually took them. Younger participants were asked to set alarms on their mobile phones to prompt them when to take a sample and older adults were given a wristband where they could write reminders of their sampling times. The first two samples of the day (used to calculate the CAR) were included only if taken on time or within 10 min, as recommended (Kunz-Ebrecht et al., 2004). For the remainder of the samples, one participant was excluded from the study as she had repeatedly taken samples over an hour late, resulting in our final overall sample size of 72. Overall, according to the self report diary, out of 432 samples: 79% were taken on time or within five min, 10% were up to 20 min late, 4% were up to 30 min late, 5% over 30 min up to 1hr late and 2% were over 1hr late. In conjunction with the saliva sampling pack participants received the questionnaire pack to complete at home which was collected with the saliva samples.

### *3.3.6. Data analysis*

Age cohort differences in health behaviours, BMI, and time of awakening were examined first using ANOVA. Chi-square was used in the case of categorical variables (such as current smoker,

consuming ≥6 alcohol units a week, consuming alcohol ≥ 1-2 times per week, sleeping ≥ 6 h a night and chronic illness).

Analyses were conducted using four cortisol outcome measures: the diurnal repeated measures pattern across all six samples; the cortisol awakening response (CAR); AUC; and diurnal slope. The CAR was calculated as sample 2 minus sample 1 (Edwards et al., 2001; Sjogren et al., 2006). AUC was calculated relative to zero using the trapezoid method applied to all sampling points (Pruessner et al., 2003). Diurnal cortisol slopes were calculated by regressing cortisol values on the sample time for each participant separately (Cohen et al., 2006; Smyth et al., 1997; Turner-Cobb et al., 2000). This yields a slope value for each participant. The sample obtained upon awakening was used as the slope anchor (Kraemer et al., 2006) and the second sample (30 min after waking) was excluded from the estimation of the slopes (Cohen et al., 2006) to eliminate the initial amplitude of the CAR.

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

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

## 3.4. RESULTS

### *3.4.1. Diurnal cortisol and age*

The repeated measures ANOVA yielded a significant main effect of time on cortisol concentration, $F(5,320) = 50.11$, $p < .001$, $\eta^2 = .439$. The pattern was characterised by significant linear and cubic components, $p < .001$. The quadratic component was not statistically significant, $p = .11$. There was also a main effect of age cohort, $F(1,64) = 14.23$, $p < .001$, $\eta^2 = .184$, such that younger adults had higher cortisol levels overall. The time × age interaction effect was also significant, $F(5,320) = 7.29$, $p < .001$, $\eta^2 = .102$; as can be seen in Figure 1, with younger adults exhibiting higher cortisol concentrations than the older adults in the morning. Analysis of AUC revealed a significant difference, $F(1,64) = 11.73$, $p = .001$, $\eta^2 = .155$, between the older (65.1, SD = 34.99 ng/ml) and the younger (95.7, SD = 34.70 ng/ml) cohort. The younger cohort also exhibited a significantly steeper slope of decline across the day (-.60, SD = .41 versus -.32, SD = .51), $F(1,66) = 5.52$, $p = .022$, $\eta^2 = .07$.

### *3.4.2. Cortisol awakening response and age*

For the CAR, there was a significant cohort effect, F(1,66) = 6.96, $p$ = .010, $\eta^2$ = .095, with younger adults showing a greater awakening response than older adults (8.82, SD = 10.54 and 2.99, SD =7.54 ng/ml, respectively).

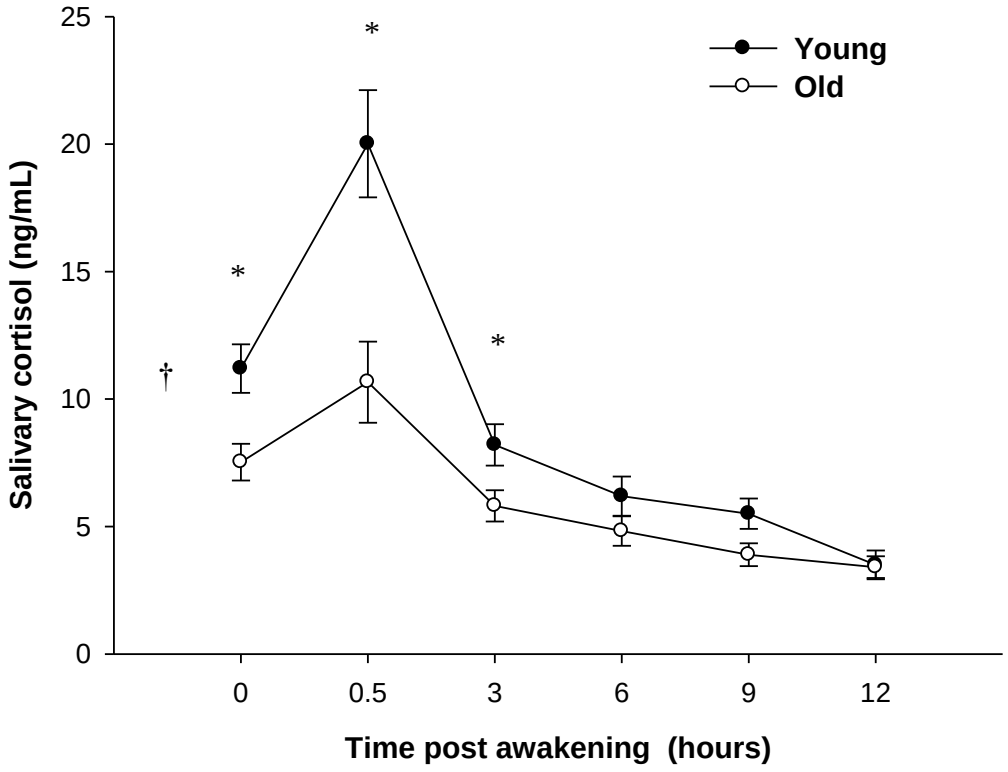


**Figure 1.** Mean (SEM) diurnal cortisol pattern by age cohort. Significant main effect of age group indicated by † $p$ <.001, and group × time interaction by * $p$ <.001.

### *3.4.3. Health behaviours and age*

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

**Table 1.** Mean (SD) health Behaviours, BMI, chronic illness and time of awakening for old and younger cohorts.

| Variable | Older Adults | Younger Adults | F(df) | *p* | $\eta^2$ |
|---|---|---|---|---|---|
| Exercise score | 4.8 (4.51) | 13.7 (6.85) | 41.47 (1,66) | < .001 | .386 |
| Cooked meals (per day) | 1.2 (0.80) | 1.8 (0.51) | 39.13 (1,70) | < .001 | .359 |
| Caffeine (drinks per day) | 4.6 (2.62) | 1.4 (1.20) | 29.93 (1,69) | < .001 | .303 |
| Fat score | 9.6 (2.66) | 10.9 (3.87) | 2.00 (1,67) | .162 | .029 |
| Fruit and vegetable score | 4.8 (4.51) | 11.1 (2.15) | 5.79 (1,69) | .019 | .077 |
| BMI ($kg/m^2$) | 28.2 (4.17) | 21.7 (2.79) | 47.29 (1,70) | < .001 | .403 |
| Time of awakening (am/h) | 7.10 (1.01) | 8.45 (1.01) | 28.79 (1,70) | < .001 | 291 |
| Categorical variables | | | $\chi^2$ (df) | *p* | |
| Alcohol ≥ 6 units per week (%) | 27 | 63 | 8.45 (1) | .004 | |
| Alcohol ≥ 1-2 times per week (%) | 48 | 75 | 4.79 (1) | .029 | |
| Current smokers (%) | 6 | 4 | .132 (1) | .593 | |
| Sleep ≥ 6 h (%) | 29 | 71 | 9.06 (1) | .003 | |
| Chronic illness (%) | 67 | 17 | 17.38 (1) | <.001 | |

### *3.4.4. Diurnal cortisol, age, and health behaviours*

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

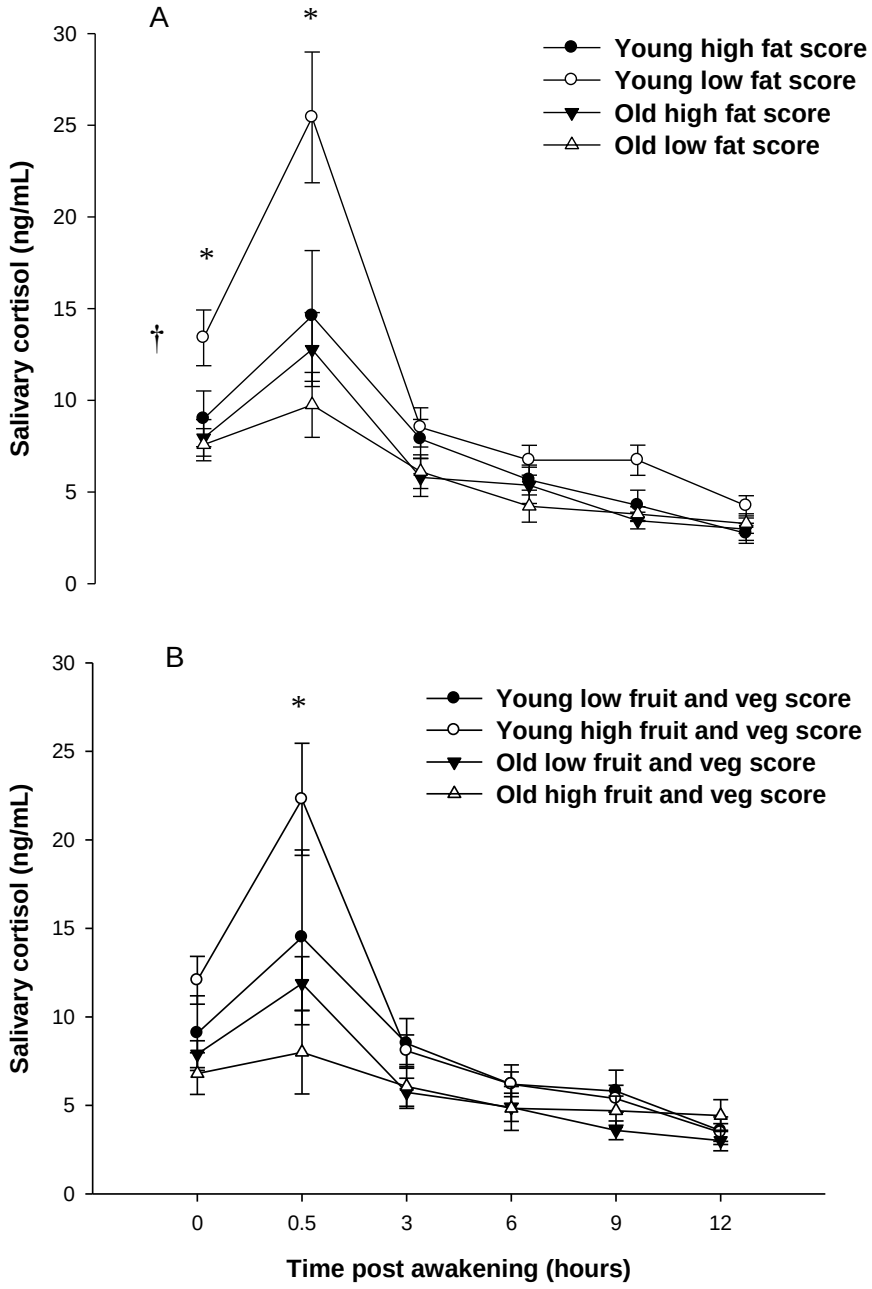


**Figure 2.** Mean (SEM) diurnal cortisol by age cohort and fat intake score (2A) and mean (SEM) diurnal cortisol by age cohort and fruit and vegetable intake score (2B). Significant main effect of age × fat indicated by † $p$ = .011, age × fat × time interaction by * $p$ =.016 and age × fruit and veg × time interaction by *, $p$ = .026.

### *3.4.5. Diurnal cortisol, age and health behaviours adjusting for potential confounding variables*

The two age cohorts significantly differed in BMI, time of awakening and chronic illness (Table 1). The most commonly reported illnesses by older adults were: hypertension (44%) and arthritis (25%), the other illnesses reported were angina, gastrointestinal disorders and Parkinson's disease (2% in each case). Younger adults reported asthma only. The significant repeated measures ANOVA findings for age × diet for diurnal cortisol were adjusted for each potential confounding variable separately. The age cohort × fat intake interaction for diurnal cortisol remained significant following adjustment for BMI, $F(5,290) = 4.24$, $p = .017$, $\eta^2 = .068$, time of awakening, $F(5,290) = 3.95$, $p = .022$, $\eta^2 = .064$, chronic illness, $F(5,290) = 3.74$, $p = .027$, $\eta^2 = .061$, depressive symptoms, $F(5,290) = 3.54$, $p = .031$, $\eta^2 = .058$, symptoms of anxiety, $F(5,290) = 4.09$, $p = .019$, $\eta^2 = .066$, and fruit and vegetable intake, $F(5,285) = 4.12$, $p = .019$, $\eta^2 = .067$. The age cohort × fruit and vegetable intake interaction for diurnal cortisol also withstood adjustment for BMI, $F(5,300) = 3.84$, $p = .024$, $\eta^2 = .060$, time of awakening, $F(5,300) = 3.70$, $p = .027$, $\eta^2 = .058$, chronic illness, $F(5,300) = 3.62$, $p = .029$, $\eta^2 = .057$, depressive symptoms, $F(5,300) = 4.43$, $p = .013$, $\eta^2 = .069$, symptoms of anxiety, $F(5,300) = 3.55$, $p = .031$, $\eta^2 = .056$, and fat intake, $F(5,285) = 3.55$, $p = .032$, $\eta^2 = .059$.

In order to eliminate the possibility that other health behaviours were confounding these diet findings, differences between groups within the younger cohort were explored. Descriptives for diet groups for both the young and older chorts are displayed in Tables 2 and 3. Participants in the high fat intake group were more likely to consume over ≥ 6 units of alcohol per week, $\chi^2 (1) = 4.44$, $p = .035$. However, the age × fat intake × time interaction withstood adjustment for this,

$F(5,290) = 4.03$, $p = .021$, $\eta^2 = .065$. There were no significant differences in sex, smoking and socio-economic status distribution between the cohorts, nor any main or interaction effects for these variables on diurnal cortisol. There was also no significant difference in diurnal cortisol or CAR between women taking oral contraceptives and those who were not.

### *3.4.6. CAR, age, and health behaviour*

When these relationships with age and health behaviours were examined for CAR alone, there was again a significant age cohort × fat intake interaction effect, $F(1,61) = 3.87$, $p = .05$, $\eta^2 = .060$; younger participants who reported consuming lower levels of fat showed a higher awakening response. However, this became a non-significant trend following adjustment for BMI, awakening time, chronic illness, and symptoms of depression and anxiety ($p = .06$ - $.09$). There was no significant interaction effect with age cohort for fruit and vegetable intake.

### **3.4.7.** ***Sensitivity analyses***

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

the AUC. Accordingly, the correlational analyses largely confirmed the outcomes from the ANOVAs.

**Table 2.** Mean (SD) health behaviours, BMI and time of awakening for diet groups for the younger cohort. * indicates significance between high and low intake groups, $p < .05$

| Variable | High Fat Intake | Low Fat Intake | Low Fruit & Vegetable Intake | High Fruit & Vegetable Intake |
|---|---|---|---|---|
| Exercise score | 13.2 (7.01) | 14.2 (6.95) | 14.0 (6.92) | 13.5 (7.03) |
| Cooked meals (per day) | 1.8 (0.45) | 1.8 (0.58) | 1.7 (0.49) | 1.8 (0.53) |
| Caffeine (drinks per day) | 1.8 (1.22) | 1.1 (1.14) | 1.5 (.54) | 1.4 (1.37) |
| BMI ($kg/m^2$) | 21.8 (3.38) | 21.6 (2.20) | 23.1 (3.20) | 21.1 (2.50) |
| Time of awakening (am/h) | 8.51 (0.92) | 8.38 (1.14) | 8.54 (0.82) | 8.4 (1.10) |
| Fruit and vegetable score | 10.7 (2.27) | 11.5 (2.02) | 8.2 (1.49) | 12.2 (.97) |
| Fat score | 13.9 (2.42) | 7.8 (2.28) | 10.3 (3.20) | 11.1 (4.18) |
| Categorical variables | | | | |
| Sleep ≥ 6 h (%) | 75 | 67 | 71 | 71 |
| Alcohol ≥ 6 units per week (%) | 83* | 42 | 86 | 53 |
| Alcohol ≥ 1-2 times per week (%) | 83 | 67 | 86 | 71 |

**Table 3.** Mean (SD) health behaviours, BMI and time of awakening for diet groups for the older cohort

| Variable | High Fat Intake | Low Fat Intake | Low Fruit & Vegetable Intake | High Fruit & Vegetable Intake |
|---|---|---|---|---|
| Exercise score | 4.8 (5.27) | 4.5 (4.14) | 5.0 (4.79) | 3.9 (3.61) |
| Cooked meals (per day) | 1.2 (0.42) | 1.1 (0.33) | 1.1 (0.33) | 1.2 (0.43) |
| Caffeine (drinks per day) | 4.8 (2.75) | 4.4 (2.56) | 5.0 (2.65) | 3.9 (2.40) |
| BMI ($kg/m^2$) | 28.1 (4.32) | 27.9 (4.16) | 28.6 (4.20) | 26.9 (4.10) |
| Time of awakening (am/h) | 7.0 (1.19) | 7.2 (0.85) | 7.1 (1.05) | 7.1 (0.95) |
| Fruit and vegetable score | 9.3 (2.45) | 9.9 (2.79) | 8.1 (1.70) | 12.9 (1.17) |
| Fat score | 12.5 (1.86) | 7.5 (2.12) | 8.2 (1.70) | 9.6 (2.63) |
| Categorical variables | | | | |
| Sleep ≥ 6 h (%) | 21 | 38 | 39 | 21 |
| Alcohol ≥ 6 units per week (%) | 31 | 21 | 27 | 29 |
| Alcohol ≥ 1-2 times per week (%) | 54 | 42 | 58 | 29 |

## 3.5. DISCUSSION

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

### *3.5.1. Diurnal cortisol and age*

In contrast to prior studies, the flatter profile observed in this investigation was due to lower awakening levels and a reduced CAR in older adults, as opposed to higher evening levels (Deuschle et al., 1997). However, whilst Deuschle et al (1997) took samples over a 24 h period, the CAR was not measured and thus age differences may have also occurred in this parameter. Older adults in the present study had lower overall cortisol levels, whereas higher cortisol has been observed previously (Luz et al., 2003; VanCauter et al., 1996). However, others have found

a lower morning (Maes et al., 1994) and overall cortisol levels with ageing (Ahn et al., 2007). Although these studies analysed plasma, salivary cortisol accurately reflects plasma free cortisol (Kirschbaum and Hellhammer, 1989). Thus, different media of measurement would not appear to explain contrasting results. It is possible that variations in findings reflect differences in sampling and the recruitment of distinct age cohorts rather than continuous age sampling. Alternatively, the lack of consistency may reflect the considerable individual variability in changes in diurnal cortisol with ageing. A longitudinal study of cortisol in healthy older adults revealed that although the majority of individuals showed increasing diurnal cortisol levels over the years some individuals also showed a decrease (Lupien et al., 1996); the pattern of secretion did not differ significantly with time in this study. Marked individual differences in cortisol secretion have also been noted in younger adults (Smyth et al., 1997). Accordingly, marked individual variability may partly explain the lack of consensus regarding cortisol and ageing. Although there may be a general shift towards a flatter diurnal profile, this may be characterised through either increased nadir levels or blunted secretion in the morning.

Increases in cortisol previously observed with ageing have been attributed to impairment of feedback inhibition of HPA activity due to neuronal loss in hippocampal area (VanCauter et al., 1996; Yen and Laughlin, 1998) and reduced hippocampal volume has been shown in older adults with a progressive increase in 24h cortisol over 5-6years (Lupien et al., 1998). Loss of HPA axis sensitivity of has also been demonstrated via impaired dexamethasone suppression (Magri et al., 1997). The attenuated diurnal levels of cortisol observed in our older participants imply dysregulation at another stage of the HPA axis. Variations in the diurnal profile with age in the present study appear to be largely driven by an attenuated CAR in the older cohort. Although

speculative, it is possible that the decrease in morning cortisol is possibly a consequence of fatigue of the adrenal cortex and thus a reduced ability to respond dynamically to the stress of awakening. However, it should be conceded that the blunted CAR may also reflect changes in hippocampal function with age, given that the awakening response is abolished in patients with hippocampal damage (Buchanan et al., 2004).

### *3.5.2. Diurnal cortisol and diet*

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

### *3.5.3. Limitations and conclusions*

The present study suffers from a number of limitations. First, it is possible that the findings relate to more specific aspects of diet which were not investigated, such as dietary composition or energy balance. For example, the micronutrient content of diet has been shown to effect cortisol metabolism (Stimson et al., 2007). A diet diary could have been used to ascertain food intake in more detail. However, food frequency questionnaires have been demonstrated to closely agree with diet diaries (Brunner et al., 2001) and the present aim was to assess habitual rather than current intake whilst avoiding response burden for participants, particularly older adults. In addition, it is possible that the effects for fat intake and fruit and vegetable intake were not independent of one another. However, fat and fruit and vegetable intake were not correlated in either cohort. Second, only younger and older adults were tested, whereas flattened cortisol profiles have been observed in participants with a mean age of 46 years (Lasikiewicz et al., 2008). The inclusion of a third middle-aged cohort in this study may have captured more precisely the age at which changes in cortisol rhythm become apparent. Nevertheless, the study was still novel in using two distinct age cohorts. Although studies have shown that the CAR is influenced by state factors (Hellhammer et al., 2007; Stalder et al., 2009; Thorn et al., 2009), there is also evidence from studies sampling on more than one day that the diurnal cortisol profile (Edwards et al., 2001; Hucklebridge et al., 2005) and the CAR (Clow et al., 2004; Pruessner et al., 1997; Wüst et al., 2000) show reasonable temporal stability. Finally, although confounding by unmeasured or poorly measured variables remains possible, we did adjust for a range of potential confounders, including awakening time. Further, the present associations were not explained by perceived or life events stress (data not reported here).

Future research might investigate how changes in diurnal cortisol with ageing relate to the diurnal patterns of other endocrine and immune variables. As it may not be changes in cortisol *per se*, but rather the balance of cortisol with other hormones, such as dehydroepiandrosternone, that is of greater importance to physiological and psychological health in ageing individuals (Straub et al., 2001). Further, the CAR and diurnal profile could be additionally examined in relation to other health parameters and physical and cognitive functioning to better determine the practical implications of an altered diurnal cortisol rhythm in older adults.

In conclusion, compared to the young cohort older adults displayed significantly reduced cortisol upon awakening, a lower CAR and a flatter diurnal profile represented by a reduced AUC and diurnal slope. This phenotype was, to an extent, imitated by younger adults with higher fat and lower fruit and vegetable intake; the significant interactions between age and diet on diurnal cortisol were further supported by correlational analysis and withstood adjustment for potential confounding variables. The findings of the present study suggest that age and diet interact in their relationship with diurnal cortisol, and this should be taken into consideration when examining health behaviours and cortisol or age and cortisol. Younger adults with a poor quality of food intake may be vulnerable to a reduction in the amplitude of the cortisol diurnal profile and this may have implications for other aspects of health.

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# CHAPTER FOUR

## 4.0. AGE, PHYSICAL FUNCTION AND THE DIURNAL RHYTHMS OF SALIVARY CORTISOL AND DEHYDROEPIANDROSTERONE IN OLDER ADULTS

## 4.1. ABSTRACT

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

## 4.2. 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 (Hucklebridge et al., 2005; Pruessner et al., 1997).

### *4.2.1. 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 (Deuschle et al., 1997; Luz et al., 2003; VanCauter et al., 1996; Yen and Laughlin, 1998), no association (Edwards et al., 2001b; Wolf et al., 2002), or decreased overall levels (Orentreich et al., 1992; Straub et al., 2000) with age. Therefore, it is possible that cortisol *per se* may not increase with ageing, but rather that cortisol levels are high in relation to other hormones such as DHEA, which declines with age in both saliva (Ahn et al., 2007) and serum (Belanger et al., 1994; Labrie et al., 1997). This would lead to an over-representation of cortisol and an increase

in the cortisol:DHEA ratio (Phillips et al., 2007), which has been found to be associated with immune impairments and infection risk in older adults (Butcher et al., 2005).

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

### *4.2.2. Cortisol, DHEA and physical function*

Frailty has become increasingly recognised as a key concern for older individuals (Cherniack et al., 2007). How frailty should be defined has been subject to much deliberation. However, it has been proposed that frailty is characterised by a diminished ability to carry out activities of daily living, both practically and socially (Brown et al., 1995; Rockwood et al., 1994). Dependence on others for activities of daily living is a predictor of admission to an institution, home care use, admission to and prolonged stays in hospital, and mortality rates Rockwood et al (1994). Alternatively, other criteria can also be used as indicators of deterioration in physical function: for example, handgrip strength, walking speed (Fried et al., 2001) and balance (Brown et al., 2000); falling due to poor balance is a key predictor of hospital admission and progression to frailty (Donaldson et al., 1990). These variables can be used separately as markers of physical function, or in combination to create a frailty index.

Neuroendocrine and immune dysregulation has also been recognised as a manifestation of frailty (Ahmed et al., 2007) and may additionally be a pathway to its onset and development (Joseph et al., 2005; Walston et al., 2006). Therefore, changes in physical function, prior to frailty onset and development, could also potentially be associated with changes in the endocrine system. Higher cortisol levels in older adults have been associated with characteristics of frailty in several studies (Peeters et al., 2007; Varadhan et al., 2008). Further, low levels of serum DHEA sulphate (DHEA-S) have been negatively associated with a frailty phenotype (Voznesensky et al., 2009) and poorer physical function (Berkman et al., 1993). However, less is known about DHEA in its un-sulphated form and DHEA in saliva in relation to frailty. As previously mentioned, DHEA displays a diurnal variation where DHEA-S does not (Kroboth et al., 1999), and the diurnal rhythm of DHEA has been shown to be important for health and well being. For example, blunted levels of DHEA in the morning has been previously associated with depression (Goodyer et al., 1996) stress and anxiety (Luz et al., 2003), and therefore may relate to other aspects of health, such as physical function. To our knowledge, previous research has not employed multiple sampling points across the day; therefore the diurnal rhythm of DHEA has not been examined in relation to physical function in older adults. Further, the advantages of employing saliva sampling, rather than serum sampling, to analyse both cortisol (Kirschbaum and Hellhammer, 1994) and DHEA (Granger et al., 1999) have been highlighted previously.

Given the scant research on cortisol and DHEA and particularly their rhythms in relation to physical function in older adults, the present study investigated the diurnal rhythms of cortisol and DHEA and the cortisol:DHEA ratio in relation to age among older adults, and examined how these endocrine parameters related to physical function among older adults. It was hypothesised

that those indicating lower levels of physical function would exhibit flatter diurnal profiles of cortisol and DHEA.

## 4.3. METHODS

### *4.3.1. Participants*

Participants were 36 (18 women) community dwelling older adults aged between 65-86 years (mean = 72.5, SD = 6.47), with a mean BMI of 26.7 (SD = 4.73). Forty one participants were originally recruited, five were excluded for non compliance and/or extreme ($\geq \pm 3$ SD from the mean) hormone values. Older adults were recruited from clubs and associations in Birmingham, UK, and through posters displayed in businesses around the local area. The majority (94%) of participants described themselves as "white", and the remaining participants described themselves as "Asian". In terms of socio-economic status, 69% were classified as from a non-manual occupational households based on their previous/current occupation, using the Registrar General's Classification of Occupations (Occupations., 1980). Inclusion criteria were: no endocrine or immune disorder, no psychiatric illness, no periodontal disease, no eating disorder and not taking glucocorticoid medication. Forty seven percent of participants reported suffering from a chronic illness, the most commonly reported were: hypertension (35%), arthritis (29%), osteoarthritis (18%), renal disease (12%) and glaucoma (12%). Fifty percent of participants reported taking chronic medication, most frequently reported were: diuretics (33%), antihypertensive (22%), gastrointestinal (22%) and pain medication (22%).

### *4.3.2. Design*

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

### *4.3.3. Measures*

*Physical function and activities of daily living*

Older adults attended the laboratory at the University of Birmingham to complete an assessment of activities of daily living (ADL) and physical function. The Nottingham extended activities of daily living (ADL) index (Nouri and Lincoln, 1987) measures independence on a four point scale ranging from 0, not at all, to 3, alone easily, in 21 items in the categories of mobility, kitchen, domestic tasks and leisure activity. Test retest reliabilities ranging from .62-1.00 (Nouri and Lincoln, 1987) and internal consistencies of .72- .94 (Nicholl et al., 2002) have been reported for all four categories. Internal consistency in the present sample was .96. Handgrip strength, an index of upper body strength, was measured using a hydraulic hand dynamometer (Lafayette Instrument, 70718, Lafayette, IN) and functional mobility was tested via the Berg Balance Scale. The Berg Balance Scale involves 14 tasks where the participant is mainly asked to maintain a given position for a specific time but also includes tasks involving reaching, stepping and transfers. Each task is scored on a 5 point ordinal scale ranging from 0-4 where 4 is the highest level of function. Points are deducted if the time or distance requirements are not met, the

participant warrants supervision or assistance is required to complete tasks. Internal consistency reliability of .83 has been reported (Berg, 1995) and the inter observer agreement of .98 when a primary researcher was compared to an independent investigator (Berg et al., 1992). The internal consistency in the present sample was .96.

*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%.

### *4.3.4. Procedure*

Each participant was provided with a pack of six universal tubes labelled with the sampling times which were: immediately upon awakening, 30 min post-awakening and then 3h, 6h, 9h and 12h

post awakening. They were verbally briefed concerning the collection procedure and sampling times, and also given written instructions regarding the saliva sampling protocol. Participants were asked not to eat, drink (except water), smoke or brush their teeth 30 min prior to each sample. For each sample, participants were asked to: take a sip of water, rinse their mouth, spit this water out, swallow hard, then lean forward and allow saliva to collect in their mouth while making a gentle chewing motion to stimulate saliva. After two minutes they were asked to spit the saliva that had collected in their mouth into the appropriately labelled collection tube, and store the tube in a refrigerator in a re-sealable bag which was provided. To measure compliance all participants were given a diary to record the times their samples were due and the time when they actually took them. They were given a wristband on which they could write reminders of their sampling times. According to the self report diary, out of 216 samples: 24% were taken up to 5 min late, 10% up to 10 min late, 1% up to 20 min late and 2% up to 45 min late. The 3% of samples that were taken more than 10 minutes late represented only 7 out of the 216 samples, and these delays only occurred in three participants. Saliva samples were collected from participants within one week. Cortisol has been found to be stable for up to 3 months when stored at 5°C (Garde and Hansen, 2005) and for up to 7 days when stored at room temperature (Aardal and Holm, 1995). DHEA levels in saliva have been shown to be unaffected by storage at room temperature for up to 10 days (Whembolua et al., 2006). The first two samples of the day were excluded if taken more than 10 min late (Kunz-Ebrecht et al., 2004).

At the laboratory, handgrip strength was measured in the standing position by asking participants to hold the dynamometer out at 90 degrees from their body then grip as strongly as they could, pulling the dynamometer down towards themselves. A practice grip was followed by three

assessments with 30 seconds rest in between. The mean of the three measures was used to calculate average handgrip strength. Following this, participants completed the Berg Balance Scale activities as described above. Participants then completed the ADL scale, were thanked, and given a form to claim travel expenses.

### *4.3.5. Data analysis*

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

Participants were split into two age groups using the median, an old group (mean = 67.6 SD = 2.36, range 65-71 years) and an older group (mean = 78.1, SD = 4.87, range 72-86 years), for the analysis of diurnal cortisol and DHEA in relation to age. Secondly, for the separate analysis of

physical function in relation to these hormones, binary variables were created for the Berg Balance Scale and Nottingham ADL index using median splits to form high and low groups. It should be noted that these high and low groups are based on the median of the present sample, and therefore do not represent a clinical cut off. Based on the cut off criteria used to indicate frailty from Ahmed et al. (2007), high and low handgrip strength groups were formed. This handgrip strength criteria is based on sex and BMI, see Ahmed et al. (2007) for ranges and cut offs.

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

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

## 4.4. RESULTS

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

**Table 1**. Descriptive statistics for cortisol, DHEA, and physical function measures. Data is presented as mean (SD). Higher handgrip, activities of daily living (ADL) and Berg balance scale scores indicate higher levels of physical function. * indicates a significant sex difference, $p < .0$

| | Old (aged 65-71) | | Older (aged 72-86) | |
|---|---|---|---|---|
| Variable | Males | Females | Males | Females |
| Cortisol overall (nmol/l) | 3.47 (.85) | 3.37 (.86) | 4.16 (1.58) | 3.90 (1.20) |
| After waking | 10.18 (2.86) | 7.26 (3.89) | 6.48 (3.95) | 7.47 (6.47) |
| 30 min post waking | 10.40 (4.62) | 12.98 (4.45) | 10.56 (4.07) | 10.51 (6.66) |
| 3 h after waking | 3.63 (1.13) | 4.39 (1.83) | 6.80 (4.39) | 6.94 (2.89) |
| 6 h after waking | 3.25 (3.29) | 3.99 (5.35) | 4.61 (2.61) | 4.47 (3.16) |
| 9 h after waking | 2.48 (1.59) | 3.54 (1.65) | 1.70 (2.09) | 2.12 (1.82) |
| 12 h after waking | 1.92 (2.16) | 3.49 (3.39) | 1.68 (1.18) | 1.54 (1.06) |
| DHEA overall (nmol/l) | .51 (.24) | .47 (.50) | .27 (.14) | .20 (.10) |
| After waking | 1.24 (.90) | 1.15 (1.46) | .62 (.48) | .41 (.31) |
| 30 min post waking | 1.00 (.61) | .74 (.63) | .59 (.30) | .24 (.16) |
| 3 h after waking | .48 (.22) | .74 (.63) | .39 (.19) | .24 (.16) |
| 6 h after waking | .45 (.23) | .46 (.37) | .21 (.10) | .23 (.11) |
| 9 h after waking | .47 (.36) | .27 (.26) | .20 (.11) | .17 (.11) |
| 12 h after waking | .40 (.42) | .31 (.36) | .20 (.08) | .21 (.11) |
| Handgrip (kg) | 36.24 (8.31)* | 19.70 (6.91) | 32.80 (10.91)* | 18.40 (2.38) |
| ADL score | 68.63 (9.87) | 56.37 (10.62) | 63.00 (19.72) | 58.30 |
| Berg Balance Scale | 42.63 (22.41) | 46.75 (15.35) | 49.57 (10.03) | 43.70 |

### *4.4.1. Age, cortisol and DHEA*

There was a significant quadratic effect for diurnal cortisol, $F(1,26) = 7.54$, $p = .01$, $\eta^2 = .225$, such that the older old adults had higher cortisol levels at 3 h and 6 h post waking. This pattern is shown in Figure 1A. They also had a significantly higher AUC (62.8, SD = 20.53 versus 49.6, SD = 12.45), $F(1,26) = 4.26$, $p = .05$, $\eta^2 = .141$. Females aged 65-71 had a significantly higher CAR compared to the males aged 65-71, $F(1,17) = 6.37$, $p = .02$, $\eta^2 = .273$. No significant correlations emerged between age and cortisol parameters.

There was a significant main effect of age for DHEA levels overall where the older participants exhibited lower overall DHEA levels across the day (.49, SD = .35 nmol/l) compared to the participants aged 65-71 (.23, SD =.12 nmol/l), $F(1,31) = 7.35$, $p = .01$, $\eta^2 = .192$. This effect is displayed in Figure 1B. Older participants also demonstrated a significantly lower, $F(1,31) = 7.88$, $p = .009$, $\eta^2 = .203$, DHEA AUC which decreased progressively with age , $r(31) = -.49$, $p = .004$. With increasing age, the DHEA slope became significantly less steep, $r(31) = .42$, $p = .01$.

Finally, older adults had a significantly higher cortisol:DHEA ratio (20.5, SD = 9.56 nmol/l versus 11.8, SD = 9.64 nmol/l), $F(1,26) = 5.64$, $p = .01$, $\eta^2 = .178$, which increased linearly with age, $r(26) = .40$, $p = .03$. There was no significant difference between time of awakening between age groups ($p = .17$) and significant findings in relation to age withstood adjustment for sampling delays. There were no sex differences for any of the above cortisol or DHEA variables, nor any sex × age interaction effects, with the exception of the CAR × sex finding for participants aged 65-71.

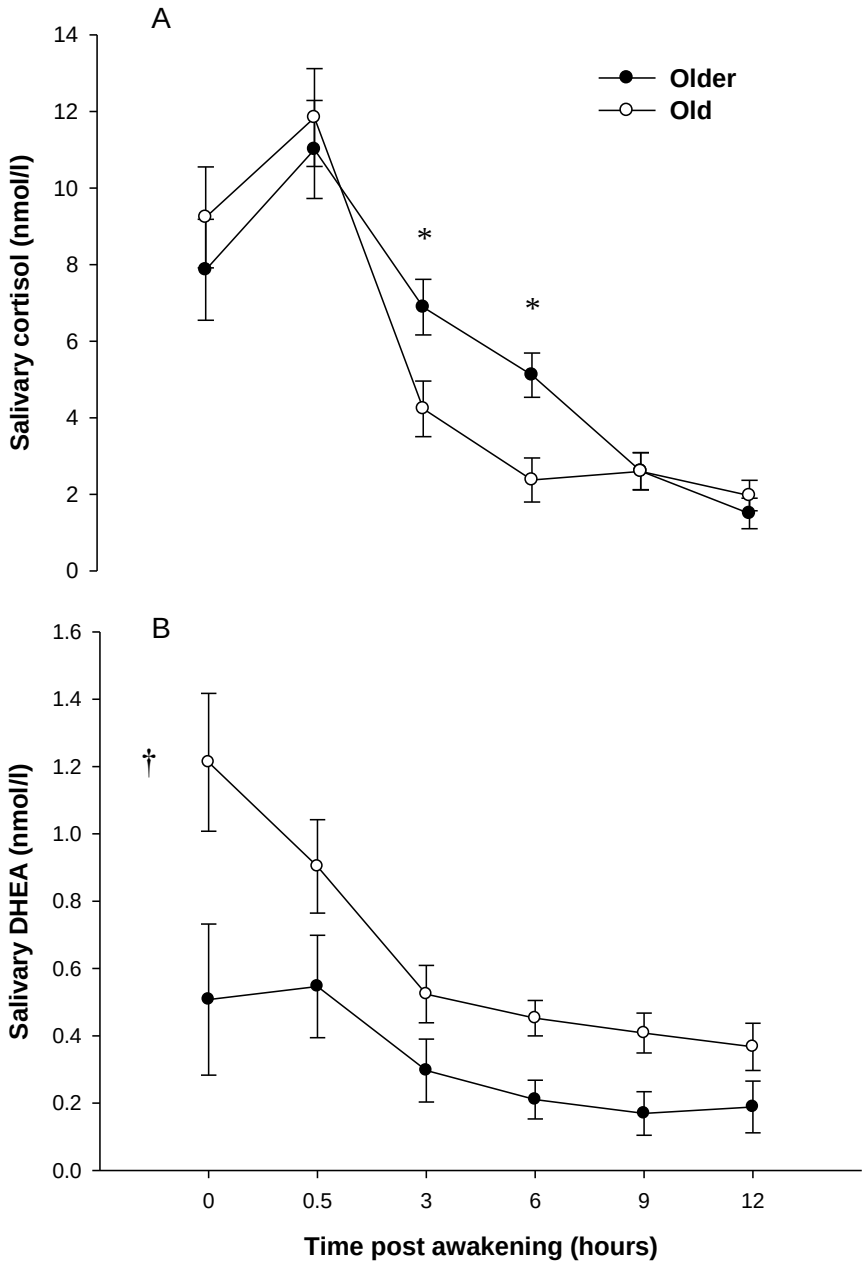


**Figure 1**. Mean (SEM) diurnal cortisol (A) and DHEA (B) patterns by age. Significant group × time interaction is indicated by * and significant main effect of group by †, $p = .01$.

### *4.4.2 Cortisol and physical function*

Regarding associations between cortisol and physical function, there was a significant interaction effect of diurnal cortisol × Berg Balance Scale score, $F(5,130) = 3.04$, $p = .04$, $\eta^2 = .105$, such that those with a lower score indicating worse balance exhibited lower cortisol immediately after and 30 minutes post-waking, as reflected by a significant quadratic trend, $F(1,26) = 4.45$, $p = .04$, $\eta^2 = .146$. This is shown in Figure 2A. There was also a significant main effect of the Berg Balance Scale on cortisol, $F(1,26) = 6.50$, $p = .02$, $\eta^2 = .200$, such that those with poorer balance had lower overall cortisol levels (4.7, SD = 1.47 nmol/l) than those with relatively good balance (6.2, SD = 1.48 nmol/l).

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

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

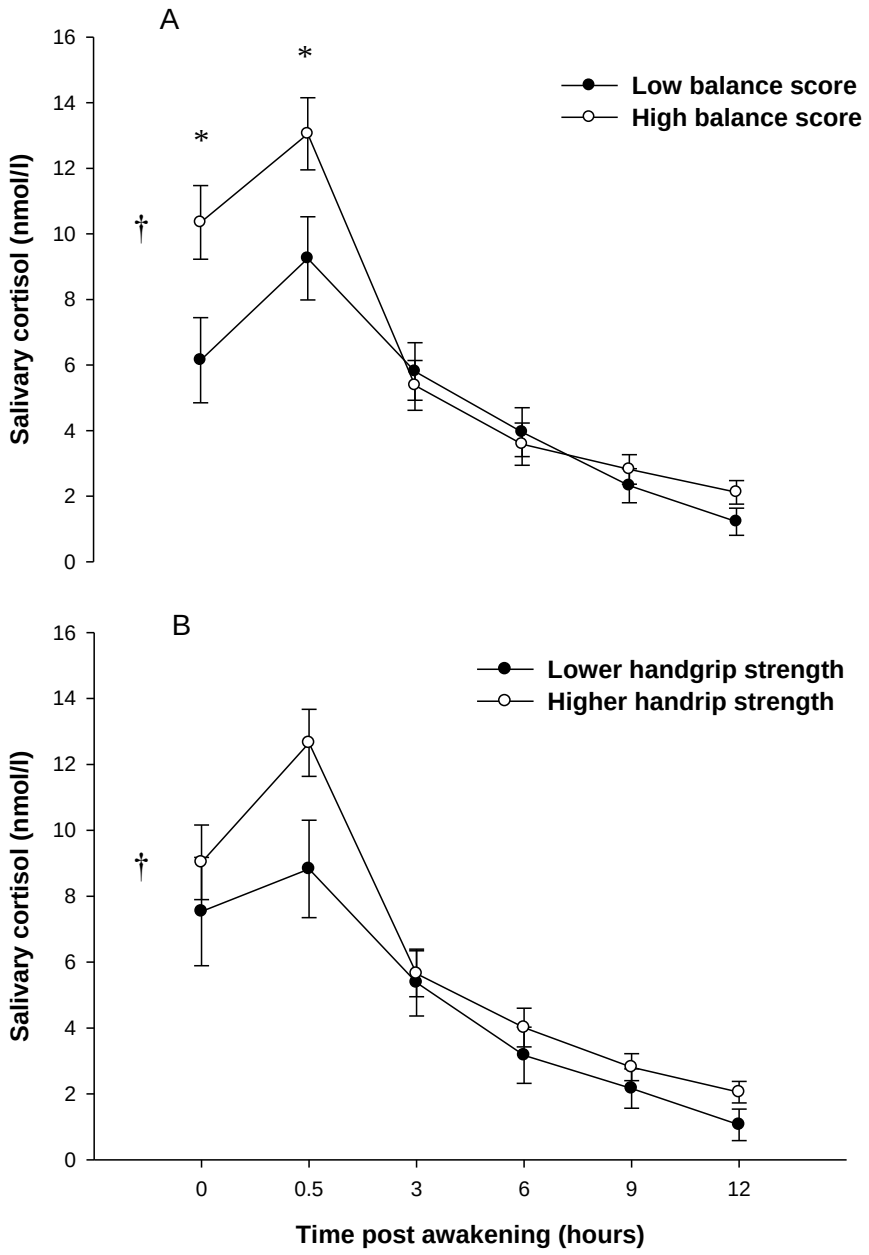


**Figure 2.** **A** Mean (SEM) diurnal cortisol pattern by Berg Balance Scale scores. Significant group × time interaction is indicated by * $p$ =.04, and significant main effect of group by †, $p$ = .02. **B** Mean (SEM) diurnal cortisol pattern by handgrip strength. Significant main effect of group is indicated by †, $p$ = .04

### *4.4.4 DHEA and physical function*

Those with lower independence in carrying out activities of daily living displayed a significantly different diurnal DHEA pattern over the day, $F(5,155) = 3.80$, $p = .03$, $\eta^2 = .109$. The pattern was characterised by significant linear, $F(1,31) = 5.56$, $p = .03$, $\eta^2 = .109$, and quadratic effects, $F(1,31) = 4.45$, $p = .04$, $\eta^2 = .126$, such that those with lower DHEA in the morning period, and consequently a flatter diurnal profile, were less independent. This effect is displayed in Figure 3. Those with lower independence scores were also characterised by a lower DHEA slope (-4.51, SD = 6.46) compared to those with higher independence (-17.15, SD = 15.90), $F(1,31) = 5.82$, $p = .02$, $\eta^2 = .158$.

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

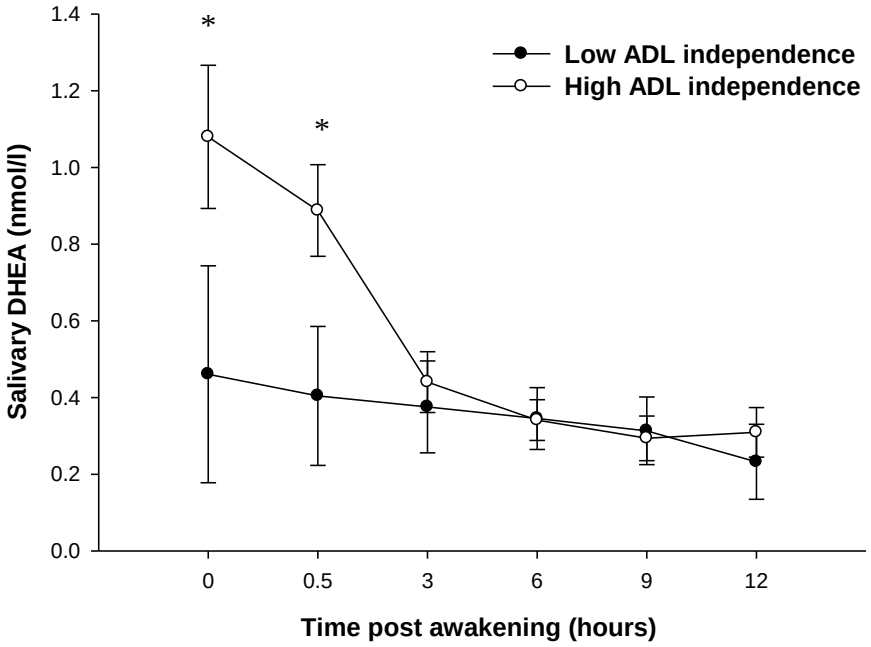


**Figure 3.** Mean (SEM) diurnal DHEA pattern by independence in activities of daily living (ADL). Significant group × time interaction is indicated by * $p$ =.03.

## 4.5. DISCUSSION

### *4.5.1. Diurnal cortisol, DHEA and age*

Participants aged 72-86 showed higher diurnal cortisol levels and a higher AUC. This elevation in diurnal cortisol with ageing is consistent with previous findings; however, it has mainly been observed as a result of higher evening and nocturnal concentrations (Deuschle et al., 1997; VanCauter et al., 1996), as opposed to the higher daytime levels in the present study. Van Cauter et al. (1996) and Deuschle et al. (1997) measured cortisol in plasma, thus the different specimen of measurement may account for contrasting results. However, salivary cortisol has been shown to accurately reflect plasma free cortisol (Kirschbaum and Hellhammer, 1989). Increases in cortisol observed with ageing have been attributed to impairment of feedback inhibition of HPA activity due to neuronal loss in hippocampal area (VanCauter et al., 1996; Yen and Laughlin, 1998). Despite being evident at different times of the day, it is possible that the increase in cortisol among the older adults, wherever manifest in the diurnal cycle, is due to the same mechanisms. Further, as evening and nocturnal samples were not collected in the present study, it remains possible that our two age groups differed at these times. It is important to note that a change in the diurnal pattern did not translate into a significant increase in overall cortisol. Older participants exhibited lower DHEA levels overall, and with increasing age, the DHEA AUC was attenuated and the slope of decline became less steep. The observed decrease in DHEA levels is in line with previous research (Ahn et al., 2007; Belanger et al., 1994; Labrie et al., 1997), however, to our knowledge, the diurnal rhythm of DHEA has not been examined previously in older individuals. Rather than maintaining its normal pattern of secretion and a

lower overall level with increasing age, DHEA secretion appears to be most reduced in the morning period resulting in a flatter diurnal rhythm among the oldest old.

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

### *4.5.2. Cortisol, DHEA and physical function*

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

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

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

### *4.5.3. 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, and thus potential risk of future frailty. 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, age was highly correlated with illness and medication usage and we did adjust significant findings for age. Further, due to the age group investigated a high prevalence of chronic medical conditions and medication use is somewhat expected and difficult to avoid. Additional measures of function could have been included. However, it is important in testing older adults to strike a balance between a broad assessment and what is feasible in terms of the demands of testing. In addition, the present assessments are commonly used and well regarded within frailty research. Fourth, although the findings could be confounded by other variables, we did adjust for the likely confounders of awakening time and age. It is also possible that the observed associations between these hormones and physical function may reflect changes in psychological health. However, the present associations were not influenced by symptoms of depression or anxiety, perceived stress, or life events stress (data not reported here). Finally, we would like to have sampled across more than one day and also use further samples to measure the CAR rather than two time points only, but costs precluded this. However, there is evidence that the diurnal profile of cortisol and DHEA are stable across days (Edwards, et al., 2001a; Edwards et al., 2001b; Hucklebridge et al., 2005), and all participants were retired, thus unlikely to differ vastly in terms of daily activities. Further, the two time points we used to measure the CAR have been previously shown to capture the peak point post awakening (Hucklebridge et al., 2005).

In conclusion, we found an association between cortisol, DHEA, age and physical function. The diurnal rhythms of cortisol and DHEA and their ratio differed between old adults and older old

adults. Poorer performance on the Berg Balance Scale and lower handgrip strength was associated with lower diurnal cortisol levels, and those who reported less independence in carrying out daily tasks showed a flatter DHEA diurnal profile.

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# CHAPTER FIVE

## LIFE EVENTS STRESS, EXERCISE AND AGE: THE DIURNAL RHYTHMS OF CORTISOL AND DEHYDROEPIADROSTERONE, AND THE CORTISOL:DHEA RATIO IN SALIVA

## 5.1. ABSTRACT

The present study examined relationships between life events stress, exercise and the diurnal rhythms of cortisol and dehydroepiandrosterone (DHEA) in the context of ageing. Seventy one participants 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. The cortisol:DHEA ratio increased linearly with increasing stress severity. Older adults 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. Young adults who participated in less exercise had lower levels of DHEA and a flatter diurnal pattern. This study suggests that stress in the form of life events may augment the increase in the cortisol:DHEA ratio observed with ageing, and older adults may be more vulnerable to stress of this nature. Further, under conditions of stress exposure, exercise may buffer against the effects of stress on the cortisol:DHEA ratio in older adults.

## 5.2. INTRODUCTION

Cortisol and dehydroepiandrosterone (DHEA) are hormones of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol plays an integral part in homeostasis under basal conditions, however, its importance is highlighted during the stress response where cortisol protects the body through: enhancing vascular activity, suspending non-essential functions, inhibiting the inflammatory process, immune suppression, inhibiting the actions of insulin to preserve glucose, and increasing energy availability via catabolism (Widmaier et al., 2004 ). However, if stress persists and cortisol is chronically elevated resulting in long term activation of these processes, this will clearly have negative implications for health. DHEA has been proposed to have far reaching mechanisms of action, including neuroprotection, immunomodulation, decreased apoptosis, antioxidant, anti-inflammatory, and anti-glucocorticoid effects (Maninger et al., 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, the balance and interplay between cortisol and DHEA is important for health, in addition to the absolute concentrations of these hormones (Buford and Willoughby, 2005).

DHEA production peaks at age 20-30 and then declines progressively with age, a phenomenon termed adrenopause (Belanger et al., 1994; Labrie et al., 1997; Orentreich et al., 1992). In contrast, cortisol has been reported to increase with age (Deuschle et al., 1997; VanCauter et al., 1996), although counter evidence exists (Orentreich et al., 1992). The over-representation of cortisol compared to DHEA, and the consequent increase in the cortisol:DHEA ratio with ageing

(Phillips et al., 2007) is associated with immune impairments and infection risk in older adults (Butcher et al., 2005).

In addition to the ratio, the diurnal rhythms of these hormones are significant for health. Both cortisol and DHEA exhibit marked diurnal rhythms, with higher levels in the morning which decline throughout the day (Hucklebridge et al., 2005; Pruessner et al., 1997). The diurnal rhythms of cortisol and DHEA have been shown to relate to both physiological health (Rosmond et al., 2003), psychological wellbeing (Luz et al., 2003), and mortality (Sephton et al., 2000), where a flattened diurnal amplitude appears to be linked to adverse health outcomes. Consequently, changes in the diurnal rhythms of these hormones may have significant implications for the health of ageing individuals. Given the rapidly increasing population of older adults, and growing discrepancy between life expectancy and healthy life expectancy, factors that may influence physiological homeostasis and impact upon health of older adults, such as stress, are ever more applicable.

### *5.2.1. Cortisol, DHEA and chronic stress*

Changes in the endocrine system occur as part of the biological process of ageing, although they could potentially be aggravated by other factors, such as stress. It has been theorised that chronic psychosocial stress, and its impact on various physiological systems, accelerates ageing (Juster et al., 2010). The implications of stress on the ageing immune system, and consequently health, have been previously discussed (Graham et al., 2006; Kiecolt-Glaser et al., 2002). Neuroendocrine-immune networks and their relationship in ageing are well established, and the effects of psychological stress on the immune regulation have been proposed to be mediated by

the neuroendocrine system (Bauer et al., 2000; Graham et al., 2006). Therefore, chronic stress, through its interaction with the neuroendocrine system, may exacerbate the effects of ageing. It has also been hypothesised that older adults may be more vulnerable to stress exposures (Phillips et al., 2007). Psychosocial stress has been shown to have a negative impact on hormonal levels and immune function in individuals of different ages (Glaser and Kiecolt-Glaser, 2005; Goodyer et al., 2000; Kiecolt-Glaser et al., 2002). However, the reduction in DHEA, increase in the cortisol:DHEA ratio, and changes in the immune system that occur with ageing, have been proposed to place older adults at greater risk of the negative effects of stress.

There is evidence to support both these theories of stress and ageing. Older adults who had experienced bereavement, and its associated emotional distress, within two months had a significantly higher cortisol:DHEA-S ratio compared to age matched non-bereaved controls (Khanfer et al., 2011). Older adults who were spousal caregivers of dementia patients, Alzheimer's disease and multi-infarct dementia, were observed to have an elevated salivary cortisol in the morning period and AUC over the day, compared to elderly non-caregivers (Bauer et al., 2000). Healthy caregivers of Alzheimer's disease patients have also been shown to have reduced salivary DHEA-S levels and an increased cortisol:DHEA-S ratio compared to non stressed controls (Jeckel et al., 2010), although this study included middle aged as well as older adults. In these studies, these changes in endocrine parameters occurred parallel to impaired innate (Khanfer et al., 2011) and adaptive immunity (Bauer et al., 2000; Jeckel et al., 2010). These previous investigations included DHEA-S rather than DHEA, which may differ in its mechanisms of action (Maninger et al., 2009). Further, only DHEA exhibits a diurnal rhythm of

secretion and, as previously mentioned, this rhythm may have important associations with health, rather than just stable concentrations on their own.

The notion that older adults are more vulnerable to stress has received initial substantiation (Kiecolt-Glaser and Glaser, 2001; Phillips et al., 2007); however, this is based on comparisons between studies of older and young adults, as studies in this area have generally failed to compare across ages within the same study. One study supports this hypothesis in relation to physical trauma and immunity; older adults who had experienced hip fracture had significantly reduced immune function and increased infection, which was not observed in younger adults who had experienced a similar fracture (Butcher et al., 2005). Consequently, the relationships between prolonged stress and the diurnal rhythms of cortisol and DHEA may vary as a function of age, although this has yet to be examined.

As previous studies have mainly looked at stress using the framework of caregiving and bereavement, less is known about life events stress and these hormones. One study examining life events stress in older adults demonstrated 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). However, DHEA, and consequently its diurnal rhythm, was not measured in this study and thus the cortisol:DHEA ratio, which may be the key factor influencing immunity, was not obtained. Life events stress not only recognises traumatic events, but also encompasses less serious exposures and daily hassles, which if they occur in accumulation, or are perceived as stressful, may be a significant source of stress. Further, life

events stress has been shown to be a risk factor for future adverse health outcomes, for example depression (Blazer et al., 1987) and the metabolic syndrome (Raikkonen et al., 2007). Therefore, the impact of life events stress may be particularly relevant for ageing individuals.

### *5.2.2. Cortisol, DHEA and stress and exercise*

Although factors such as stress may advance ageing, certain factors could protect against age-related changes in hormones. 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 et al., 1997). There is also evidence that the effects of stress on health can be buffered by physical activity within younger adults (Brown, 1991). A prospective study of adolescent health found that exercise buffered against the negative health effects of life events stress (Brown and Siegel, 1988). However, the potential ameliorating effects of exercise on life events stress in relation to diurnal DHEA and cortisol and the ratio between these hormones is, to our knowledge, yet to be investigated or compared among adults of different ages.

Independently of stress, the relationship between the diurnal rhythms of cortisol and DHEA and exercise has received little attention. One study found the diurnal rhythm of cortisol to be absent in elite gymnasts (Georgopoulos et al., 2011); however, the relationship between elite sport and diurnal cortisol is likely to differ from that between more mundane physical activity and cortisol. In older individuals, physical activity has been found to be associated with higher levels of

DHEA-S in women (Bonnefoy et al., 1998). However, the diurnal rhythms of DHEA and cortisol have yet to be examined.

The aim of the present study was to examine, independently, if stressful life events and habitual exercise behaviour were associated with the diurnal rhythms of cortisol and DHEA, and the cortisol:DHEA ratio. Second, if participating in regular exercise buffered against any negative effects of stress on these hormones. Lastly, if any observed relationships varied on the basis of age. It was hypothesised that life events stress would have the greatest impact on the older cohort, demonstrated by an increased ratio and flatter diurnal profiles, and any negative associations with stress would be less pronounced in exercising older adults.

## 5.3. METHODS

### *5.3.1. Participants*

Participants were 18 (9 women) University of Birmingham students, 17 middle-aged adults (10 women) employed by the University or from the local area and aged between 40-60 years, and 36 (18 women) community dwelling older adults aged between aged 65-88. Eighty one participants were originally recruited, ten (4 young, 2 middle-aged and 4 older adults) were excluded for non compliance and/or extreme ($\geq \pm 3$ SD from the mean) hormone values, resulting in the final sample size of 71. 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 (Table 1) was defined using previous (older

cohort), current (middle-aged cohort) and parental previous/current occupation (young cohort), as classified by the Registrar General's Classification of Occupations (Occupations., 1980).

### *5.3.2. Study design*

This study was a cross sectional investigation of salivary cortisol, DHEA, life events stress, and physical activity in relation to age. 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.

### *5.3.3. Questionnaires*

*Stressful life events over the past year*

Age specific stress scales were used to capture the different types of stress most likely to be experienced by each age group. It is likely that young students, middle-aged adults and older adults differ in terms of the specific events experienced, which could be acknowledged by using specific measures appropriate for each age group. Stressful life events over the past year were measured using questionnaires designed to assess 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 and Rache, 1967). The young cohort completed the Life Events Scale for Students (LESS) (Linden, 1984) (Linden, 1984) . Participants selected from a 36 item student-specific inventory of events which they have experienced in the past year. Participants

rated how stressful they found each event they experienced on a 4-point scale of severity. A total score for number of events experience or stress incidence, and a total severity score were generated.

Stressful life events exposure over the past year in middle-aged and older adults was assessed by a Life Events Survey (LES) from the West of Scotland Twenty-07 study (Ford et al., 1994). It contains 58 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, older 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).

*Habitual exercise behaviour*

Health behaviours over the past year were assessed using a questionnaire adapted from the Whitehall study (Marmot et al., 1991). Participants indicated how much time (0, 1-2, 3-5, 6-8, 9-10, 11+ hours a week) they spent participating in light (i.e. walking), moderately energetic

(swimming, golf) and vigorous (i.e. running, squash) per week. A 0-5 categorical scoring system was applied to all behaviours, e.g. if they spent 1-2 hours performing an activity they were awarded a score of 1. A combined exercise score was calculated by multiplying the category score by a weighting of 1, 2, 3 for light, moderate, and vigorous activity, respectively. In addition, participants completed the physical activity scale from the West of Scotland Twenty-07 study (Ford et al., 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". This was included as older adults may not take part in vigorous activity and, accordingly, this scale may be more sensitive to the types of physical activity that older adults are more likely to engage in.

### *5.3.4. 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%.

### *5.3.5. Procedure*

Each participant was provided with a pack of six universal tubes labelled with the sampling times which were: immediately upon awakening, 30 min post-awakening and then 3h, 6h, 9h and 12h post awakening. They were briefed concerning the collection procedure and sampling times. Participants were asked not to eat, drink (except water), smoke or brush their teeth 30 min prior to each sample. For each sample, participants were asked to: take a sip of water 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. Older adults were given a wristband on which they could write reminders of their sampling times and participants were encouraged to set reminders on their mobile phones. The first two samples of the day (used to calculate the CAR) were included only if taken on time or within 10 minutes, as recommended (Kunz-Ebrecht et al., 2004). According to the self report diary, out of 426 samples: 59% were taken on time, 22% were taken up to 5 min late, 11% up to 10 min late, 3% up to 20 min late, and 5% up to 45 min late. The 8% of samples that were taken more than 10 minutes late represented only 35 out of the 426 samples. In conjunction with the saliva sampling pack, participants

received a questionnaire pack to complete at home which was collected with the saliva samples within one week of completion. Cortisol has been found to be stable for up to 3 months when stored at 5°C (Garde and Hansen, 2005) and for up to 7 days when stored at room temperature (Aardal and Holm, 1995). DHEA levels in saliva have been shown to be unaffected by storage at room temperature for up to 10 days (Whembolua et al., 2006). The first two samples of the day were excluded if taken more than 10 min late (Kunz-Ebrecht et al., 2004). Participants were asked to complete the stressful life events and exercise measures on the saliva sampling day.

### *5.3.6. Data analysis*

Analyses were conducted using the following outcome measures: the overall average level of each hormone across the day, the diurnal repeated measures patterns across all six samples; area under the curve (AUC); diurnal slopes, the cortisol awakening response (CAR), and the cortisol:DHEA ratio. AUC for cortisol and DHEA was calculated relative to zero using the trapezoid method applied to all sampling points (Pruessner et al., 2003). Diurnal cortisol slopes were calculated by regressing hormone values on the sample time for each participant separately (Cohen et al., 2006; Smyth et al., 1997; Turner-Cobb et al., 2000). This yields a slope value for each participant. The sample obtained upon awakening was used as the slope anchor (Kraemer et al., 2006) and the second sample (30 minutes after waking) was excluded from the estimation of the cortisol slope (Cohen et al., 2006) to exclude the CAR, which was calculated as sample 2 minus sample 1 (Edwards et al., 2001; Sjogren et al., 2006). The cortisol:DHEA ratio was calculated as average cortisol divided by average DHEA. For the ratio, sample 2 for both hormones were 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. Where significant group differences were found, variables were examined in their continuous form as a sensitivity analysis. Median splits were applied to exercise score and physical activity as these were categorical rather than continuous variables, with participants having a tendency to engage in little or no exercise or exercise on a frequent basis. Median splits into high and low categories were based on the median for each specific age group, to ensure individuals were deemed as high and low exercisers compared to other individuals in their age group, and not the sample as a whole.

Repeated measures ANOVA was used to examine the diurnal cortisol rhythm in relation to each stress variable, exercise score and physical activity level. Greenhouse-Geisser corrected statistics and partial $\eta^2$, a measure of effect size, are reported. Univariate ANOVA was applied to analyse stressful events and exercise behaviour differences in the AUC, CAR, diurnal slopes and cortisol:DHEA ratio, respectively. In all of the above analyses, where significant effects emerged, subsequent ANCOVA was performed to adjust for potential confounding variables: time of awakening, chronic illness, and BMI. Slight variations in degrees of freedom reflect occasional missing data or insufficient saliva for analysis.

## 5.4. RESULTS

Descriptive statistics for age, BMI, time of awakening, chronic illness, smoking and socioeconomic status for the young, middle-aged and older cohorts are displayed in Table 1. Reported chronic illness, BMI and time of awakening were significantly different between age cohorts. No sex differences were found for any analysis; subsequently, genders were grouped together and the results presented below are for both males and females collectively.

**Table 1.** Descriptive statistics for age, BMI, time of awakening, chronic illness status and smoking status for the young, middle-aged and older cohorts. * indicates significant difference to the other cohorts

| Variable | Young Adults | Middle-aged Adults | Older Adults | F(df) | *p* | $\eta^2$ |
|---|---|---|---|---|---|---|
| Age | 19.22* (1.11) | 47.23* (5.94) | 72.52* (6.47) | 577.34 (2,68) | <.001 | .944 |
| BMI ($kg/m^2$) | 22.92 (3.29) | 24.00 (3.01) | 26.65* (4.73) | 5.93 (2,68) | .004 | .148 |
| Time of awakening(am/hr) | 8.24* (1.40) | 6.49 (.97) | 7.25 (.96) | 8.14 (2,68) | .001 | .193 |
| Categorical variables | | | | $\chi^2$ (df) | *p* | |
| Current smokers (%) | 11 | 6 | 6 | 0.61 (2) | .736 | |
| Chronic illness (%) | 6 | 18 | 47* | 11.52 (2) | .003 | |
| Non manual socio-economic status (%) | 83 | 94 | 69 | 4.50 (2) | .105 | |

### *5.4.1. Cortisol, DHEA and Age*

Descriptive statistics for cortisol and DHEA for the three age cohorts are displayed in Table 2. There was a significant main effect for DHEA, where the younger adults had significantly higher DHEA across the day compared to middle-aged and older adults, $F(2,67) = 6.97$, $p = .002$, $\eta^2 = .172$. There was also a significant difference for the cortisol:DHEA ratio, $F(2,66) = 6.59$, $p = .002$, $\eta^2 = .167$; the older adults exhibited a higher cortisol:DHEA ratio compared to the middle-aged and younger adults. There was no significant difference in cortisol across the day between age groups. The age differences in DHEA and cortisol:DHEA withstood adjustment for potential confounders of chronic illness, BMI, time of awakening. There were no significant age × time interactions for cortisol or DHEA, nor any differences in AUC, CAR or diurnal slopes and age groups that withstood adjustment for potential confounders, illustrating no differences in diurnal patterns based on age.

**Table 2.** Mean (SD) hormone parameters for the three age cohorts. * indicates a significant difference from the other two age cohorts, $p < .05$.

| Hormone variable | Young Adults | Middle-aged Adults | Older Adults |
|---|---|---|---|
| Average cortisol across the day (nmol/l) | 3.99 (1.13) | 4.35 (1.23) | 4.08 (1.65) |
| Average DHEA across the day (nmol/l) | 1.34 (.87)* | .80 (.44) | .51 (.69) |
| Cortisol:DHEA ratio | 7.31 (6.69) | 8.56 (4.01) | 14.95 (9.93)* |

### *5.4.2. Cortisol, DHEA, life events stress*

Descriptive statistics in relation to stress for the three age cohorts are displayed in Table 3.

**Table 3.** Median and interquartile range for stress questionnaires across age groups. Variable scale refers to the possible range of scores for each questionnaire.

| | Young Adults | | Middle-aged Adults | | Older Adults | |
|---|---|---|---|---|---|---|
| Variable (scale) | Median | Interquartile Range | Median | Interquartile Range | Median | Interquartile Range |
| LESS incidence (0-36) | 8.5 | 6.3 | | | | |
| LESS ratings (0-144) | 18.5 | 20 | | | | |
| LES incidence (0-58) | | | 7 | 7 | 5.0 | 7.5 |
| LES ratings (0-580) | | | 24 | 50 | 17.5 | 30.5 |
| Older stress incidence (0-70) | | | | | 14 | 10 |
| Older stress ratings (0-280) | | | | | 29 | 30 |

LESS, Life Events Scale for Students; LES, Life Events Survey

Significant findings in relation to stress and hormones emerged for the older adults only; therefore, hormone parameters for high and low stress groups for the older adults are displayed in Table 4. There was a significant difference between older adults with high and low severity ratings on the older adult questionnaire for average DHEA across the day, where those in the high stress severity group had significantly lower DHEA, $F(1,33) = 3.92$, $p = .05$, $\eta^2 = .106$. There was no significant difference in cortisol between these groups, 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 withstood adjustment for potential confounding variables; time of awakening, chronic illness and BMI, however, the average DHEA finding was attenuated to non significance. The ratio finding was supported by correlational analysis, $r(32) = .365$, $p = .035$, and is illustrated in Figure 1.

There were no other significant findings for in relation to stressful life events for any other continuous hormone parameters, CAR, AUC and slope, nor any stress × time interactions.

No significant findings emerged in relation to stress in the younger and middle-aged adults. For younger adults, correlational analysis revealed that LESS incidence was associated with lower levels of DHEA; however, this did not reach significance ($p = .085$). Other correlations failed to approach significance or suggest trends. Similarly, in the middle-aged adults, there were no significant associations or trends from correlational analysis between stress variables and hormones parameters.

**Table 4.** Mean (SD) cortisol, DHEA and cortisol:DHEA ratio values for high and low stress groups for the older adults for each of the stressful life events measures. * indicates significance between low and high groups, $p < .05$

| | Stressful life events measures | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Older stress incidence | | Older stress severity | | LES incidence | | LES ratings | |
| Hormone variable | Low | High | Low | High | Low | High | Low | High |
| Average cortisol over the day (nmol) | 4.31 (1.31) | 5.06 (1.99) | 4.23 (1.33) | 5.12 (1.91) | 4.30 (1.37) | 5.08 (1.93) | 4.29 (1.35) | 5.10 (1.91) |
| Average DHEA over the day(nmol) | .44 (.39) | .43 (.29) | .54* (.41) | .32 (.21) | .40 (.25) | .51 (.41) | .47 (.39) | .43 (.29) |
| Average cortisol:DHEA ratio across the day | 13.33 (8.44) | 17.11 (17.58) | 10.14* (5.84) | 20.65 (10.88) | 13.51 (7.96) | 16.86 (12.11) | 13.86 (8.96) | 16.40 (11.24) |

LES, Life Events Survey

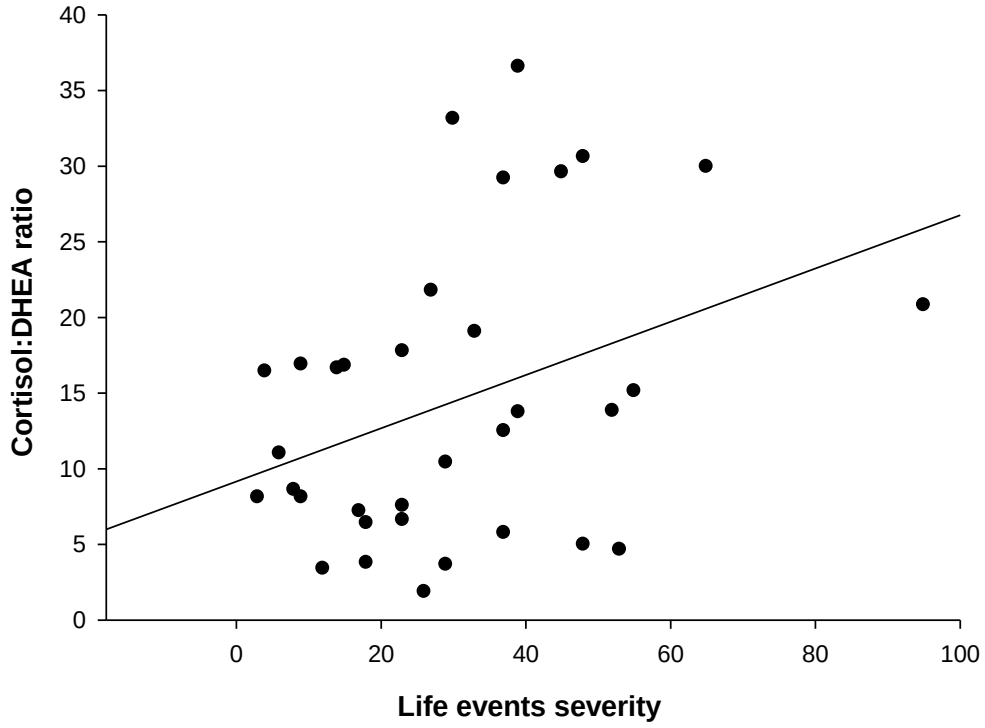


**Figure 1**. Scatterplot of stressful life events severity and cortisol:DHEA ratio for the older adults, $p = .03$

### *5.4.3. Cortisol, DHEA and exercise*

When analysing high and low exercise scores, based on the median for each age group, significant findings were observed for the young adults, but not the middle-aged or older adults. For younger adults, there was a significant main effect of exercise on diurnal DHEA such that those who undertook more exercise had higher overall diurnal DHEA, $F(1,14) = 5.62$, $p = .033$, $\eta^2 = .287$. They also had a significantly higher DHEA AUC compared to those who undertook less exercise, $F(1,14) = 5.45$, $p = .035$, $\eta^2 = .280$, 15.2 (SD = 7.20), compared to 7.85 (SD = 4.60). A non-significant trend emerged for slope steepness ($p = .06$, $\eta^2 = .224$), where those who undertook more exercise had a steeper slope of decline, -38.57 (SD = 31.25) and -12.15 (SD =16.98), respectively. These findings in relation to DHEA can be seen by referring to Figure 2.

There were no significant differences in cortisol between the exercise groups, but, as might be expected from the outcomes above, there was a significantly higher cortisol:DHEA ratio among those with lower exercise scores, $F(1,15) = 4.59$, $p = .05$, $\eta^2 = .239$. No significant associations were found between continuous cortisol and DHEA parameters and exercise score.

The main effect of exercise on diurnal DHEA and DHEA AUC withstood adjustment for potential confounders. However, the effect of exercise on the cortisol:DHEA ratio was attenuated when controlling for BMI ($p = .06$, $\eta^2 = .226$). There were no significant differences between exercise groups for the number of stressful events or seriousness/severity scores, nor did exercise score correlate with continuous stress variables for any of the age cohorts.

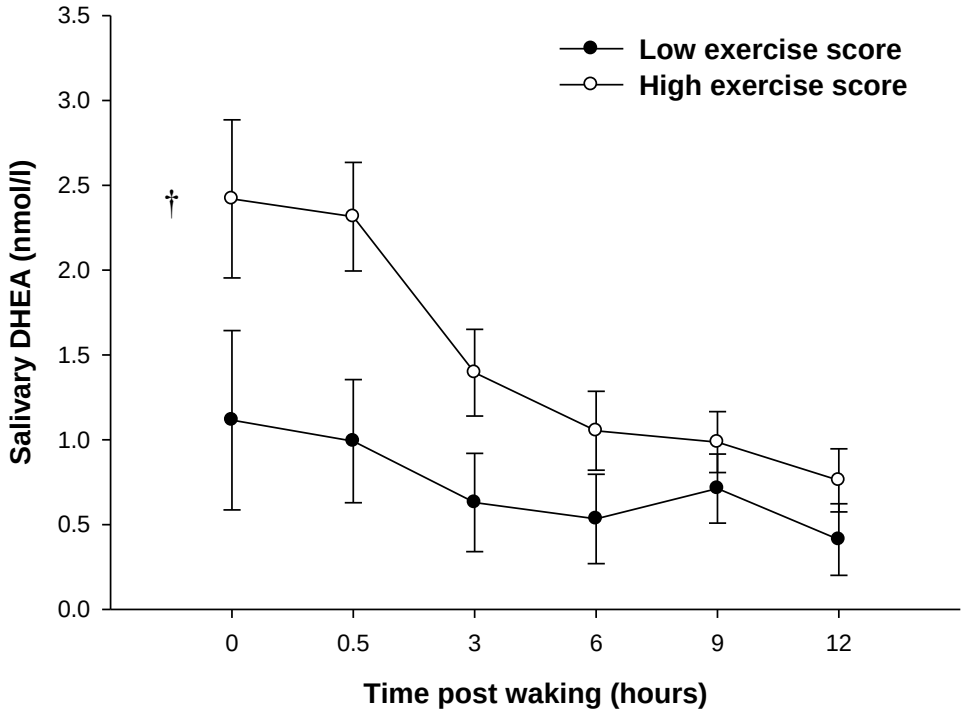


**Figure 2.** Mean (SEM) diurnal DHEA pattern by exercise score for younger adults. Significant main effect of group is indicated by †, $p = .03$.

### *5.4.4. Cortisol, DHEA, life events stress and exercise*

There was a significant interaction between stress × exercise for the cortisol:DHEA ratio for the older adults. This was significant when habitual exercise was measured using the physical activity scale from the West of Scotland study; as opposed to the exercise score generated using the Whitehall study questionnaire. 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 3. There was no significant difference between the low stress groups and the high stress group who exercised. The majority of individuals who reported less than 1h a week of aerobic exercise performed very little activity other than those of daily living or performed only physical activities requiring low levels of exertion for 10-20 min at a time. 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.

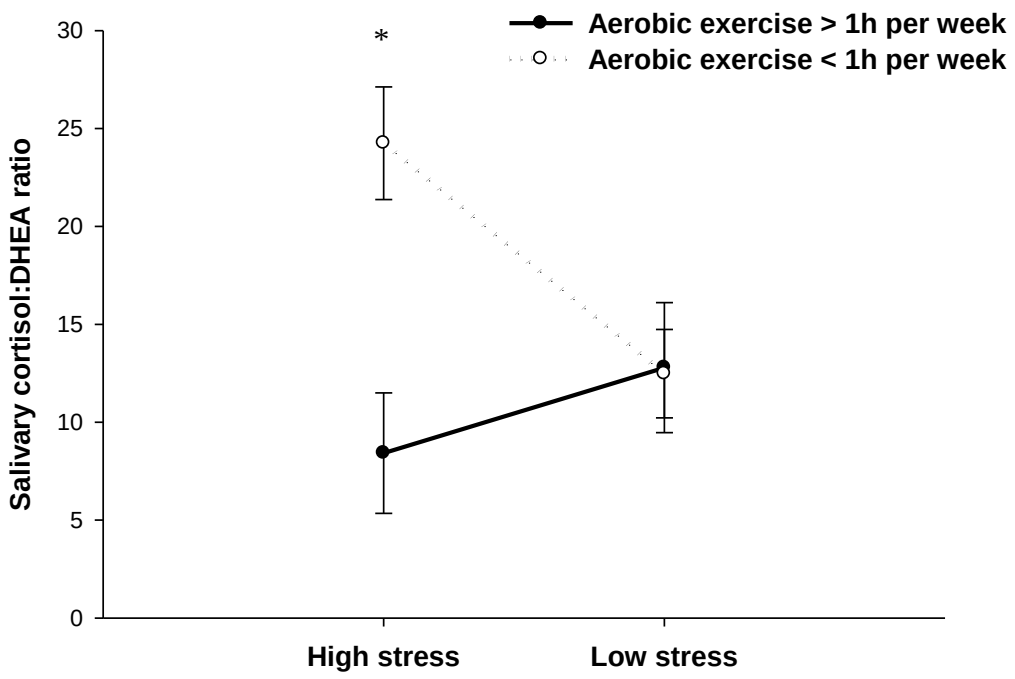


**Figure 2**. Mean (SE) salivary cortisol:DHEA ratio for aerobic exercise groups × high and low incidence of stressful life events for older adults, * significant interaction, high stress exercise > 1h per week, versus high stress exercise < 1 h per week, and also versus both low stress groups, $p = .010$.

## 5.5. DISCUSSION

### *5.5.1. Cortisol, DHEA and life events stress*

The cortisol:DHEA ratio was found to be higher in older adults compared to middle aged and younger adults, over-representation of cortisol in relation to DHEA with ageing has been well established (Phillips et al., 2007). However, older adults reporting with more severe life events stress had a significantly higher cortisol:DHEA ratio than those reporting less severe stress. 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. However, 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. Previous research has found the ratio to be associated with immune impairments and infection risk in older adults (Khanfer et al., 2011; Butcher et al., 2005); no significant effects were found for these hormones separately, thus again illustrating the importance of the balance between these hormones. Interestingly, despite other associations with aspects of health and wellbeing, significant findings did not emerge in relation to life events stress and the diurnal rhythms of these hormones. This suggests that the overall levels of cortisol and DHEA and the balance between them, rather than the patterns of

secretion and features of these diurnal rhythms, such as the CAR and AUC, may be susceptible to this form of stress. Although studies with a larger sample size examining stress and the diurnal rhythms of these hormones are required for confirmation. Notably, it was the severity ratings that impacted on the cortisol:DHEA ratio, implying that it is the product of the number of events and the perception of how stressful such events are that is important among these older adults, rather than simply number of events that occur.

In addition to providing evidence to support the hypothesis that older adults who experience stress are at risk of accelerated age-related hormonal change, as life events did not relate to the cortisol:DHEA ratio in the younger or middle-aged cohorts, this provides initial support to the view that older adults may be more vulnerable to stress as a result of changes in the neuroendocrine system (Phillips et al., 2007). Older adults already have an increased cortisol:DHEA ratio and chronic stress in the form of life events may exacerbate this age related change. However, as this study was not longitudinal, the nature of the relationship between life events stress and the cortisol:DHEA ratio in older adults can only be speculated upon.

The implications of an increased cortisol:DHEA ratio may not only impact upon the immune system of older individuals, but may be a risk factor for other adverse health outcomes. For example, this ratio has been considered a marker of catabolic/anabolic status and therefore an increased ratio may lead to sarcopenia, and potentially future frailty onset (Walston, 2004). Further, a higher ratio has also been associated with all-cause, and cause-specific mortality (Phillips et al., 2010).

In contrast to others, we observed no significant associations between cortisol *per se* and chronic stress in older individuals (Gerritsen et al., 2010). How cortisol responds to chronic stress has been suggested to be influenced by factors such as: time since stress onset, nature of the threat, controllability and individual differences (Miller et al., 2007), such as personality (Van Eck et al., 1996). Accordingly, the cortisol response to stress could be viewed as individualised and contextual dependent on the characteristics of the stress. This perspective certainly limits the likelihood of finding meaningful patterns of association in studies with a modest sample size.

### *5.5.2. DHEA and exercise in young adults*

The younger adults with a higher exercise scores had significantly higher overall DHEA, a higher DHEA AUC and lower cortisol:DHEA ratio (although the ratio finding did not withstand adjustment for potential confounders), where those with lower exercise scores had a flatter DHEA diurnal rhythm. It has been shown previously that DHEA increases in response to an acute exercise bout (Aldred et al., 2009). However, the present study is the first we are aware of to suggest that younger adults who engage in more regular exercise have higher levels of DHEA and a more dynamic diurnal rhythm. Given the small sample of younger adults in the present study, larger studies are required to confirm the association between habitual exercise, DHEA and cortisol.

All younger adults in the present sample participated in at least mild and moderate exercise, although differed in the volume and intensity of training. It would be interesting to analyse the hormone profiles of young adults who are sedentary; it may be that those in the lower exercise score do not have low DHEA as such, rather that those with higher scores have

managed to boost their DHEA levels above normal values. Although it could be speculated that while exercise may have a positive effect on DHEA, if those in the high exercise group increased their training above a certain threshold this may no longer be the case. This assumption is based on prior evidence that intense training and competition has been shown to have a negative impact on cortisol and DHEA. For example, the diurnal rhythm of cortisol has been shown to be blunted in gymnasts (Georgopoulos et al., 2011), and increases in training load have been associated with an increase in the cortisol:DHEA-S ratio in cyclists (Bouget et al., 2006).

Interestingly, the association observed in the present study between physical activity and DHEA was evident only in the youngest cohort adults and not in the middle-aged or older cohorts. One possible reason for this could be that those who exercised more in the young cohort were participating in more vigorous activities compared to those who exercised more in the middle-aged and older cohorts. Vigorous activity has been shown to promote greater cardio-protective benefits than moderate activity (Swain and Franklin, 2006) and although highly speculative, this may also be the case for the endocrine system. Therefore, despite having higher exercise scores, individuals in the middle-aged and older adult high exercise groups may not have been participating in exercise that was sufficiently vigorous to have beneficial effects on DHEA levels and its rhythm.

### *5.5.3. DHEA, stress, and exercise in older adults*

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. Notably, there was no difference between those who did and did not exercise in the low stress group. As a result, 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 et al., 1993). It was only when exercise was measured using the West of Scotland scale and not the Whitehall score that interacted with stress and DHEA. It is possible that the physical activity level is a more sensitive and appropriate measure in older populations.

Exercise has previously been shown to buffer against the effects of life events stress on health (Brown, 1991) and illness (Kobasa et al., 1982). Further, aerobic exercise training has been found to reduce reported stress (Norris et al., 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 and 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.

### *5.5.4. Limitations and conclusions*

The present study is not without limitations; first, the sample size was modest and the younger and middle-age groups had fewer participants than the older adults, which may have influenced the null findings in relation to stress and stress × exercise in these age groups. However, sensitivity analysis (data not shown) where young and middle-aged groups were collapsed together, did not reveal any significant findings in relation to these variables. 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). Further, the measures employed were suitable to discriminate between those who engaged in high and low levels of regular exercise. 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). Finally, 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. Future research should seek to create a fuller endocrine, and also immune profile, associated exercise and chronic stress in individuals of different ages. 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.

In conclusion, this present study finds associations between cortisol, DHEA, exercise and stress in relation to age. Young adults who engaged in more exercise had higher levels of DHEA across the day and a higher AUC. Older adults who reported more severe life events stress had a significantly higher cortisol:DHEA ratio. Lastly, in older adults experiencing higher number of stressful life events, exercise buffered against an increased cortisol:DHEA ratio.

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# CHAPTER SIX

## 6.0. SERUM DEHYDROEPIANDROSTERONE, DEHYDROEPIANDROSTERONE SULPHATE AND CORTISOL RESPONSES TO ACUTE EXERCISE IN OLDER ADULTS IN RELATION TO EXERCISE TRAINING STATUS AND SEX

## 6.1. ABSTRACT

The aim of the present study was to investigate resting measures of dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEA-S) and cortisol, and the response and recovery of these hormones to acute exercise, in male and female older adults of different exercise training status. Participants were 49 community dwelling older adults (23 females) aged between 60 - 77 years who were either: sedentary (n = 14), moderately active (n= 14), or endurance trained (n = 21). Participants undertook an acute bout of exercise in the form of an incremental submaximal treadmill test. The exercise lasted on average 23min 49sec (SD = 2min 8sec) and participants reached 76.5% (SD = 5.44) of predicted maximal HR. Blood samples were collected prior to exercise, immediately, and 1h post exercise. DHEA levels significantly increased immediately post exercise, however, DHEA-S levels only significantly increased in females. Cortisol significantly decreased immediately post exercise, and 1 h post exercise compared to pre. There were no significant differences in resting hormone levels, nor hormonal responses to exercise between training status groups. The findings suggest that exercise can stimulate DHEA production in older adults, and that hormonal responses to exercise differ between male and female older adults.

## 6.2. INTRODUCTION

Dehydroepiandrosterone (DHEA) and its sulphated metabolite, dehydroepiandrosterone sulphate (DHEA-S) are androgens produced by the adrenal cortex. DHEA/S has been proposed to affect various systems of the body and be anti-ageing (Chahal and Drake, 2007). It has been established that DHEA/S is immune enhancing, where cortisol, also produced by the adrenal cortex, is immunosuppressive if chronically elevated (Buford and Willoughby, 2005). DHEA and DHEA-S production peaks at age 20-30 and then declines progressively with age (Belanger et al., 1994; Labrie et al., 1997; Orentreich et al., 1992). In contrast, cortisol has been reported to increase with age (Deuschle et al., 1997; VanCauter et al., 1996), although counter evidence exists (Orentreich et al., 1992). Reductions in DHEA/S have been implicated in the disturbance of other physiological systems, such as the musculoskeletal system (Walston et al., 2006). Further, over-representation of cortisol compared to DHEA, and the consequent increase in the cortisol:DHEA ratio with ageing (Phillips et al., 2007), is associated with immune impairments and infection risk in older adults (Butcher et al., 2005). Exercise has been proposed as an intervention to protect against changes in the neuroendocrine system with ageing and improve immunity in older adults (Phillips et al., 2007).

DHEA-S has been found to be significantly higher in older men who are endurance trained (Tissandier et al., 2001) and who regularly cycled at a moderate intensity (Ravaglia et al., 2001). In contrast, DHEA-S levels have been found to be similar between older male runners and sedentary controls (Arai et al., 2006). These studies did not include women; however, other studies investigating $VO_{2max}$ and energy expenditure rather than exercise training status have. DHEA-S correlated positively with $VO_{2max}$ (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). In contrast, Abbasi et al (1998) reported an association between $VO_{2max}$ and DHEA-S in men but not women, although this finding did not withstand adjustment for age. In these studies participants were of average fitness levels and not endurance trained. In addition, those who take part in moderate activities have not been compared to those who are endurance trained within the same study, nor has DHEA-S been examined in parallel with DHEA. Thus it seems important to examine whether or not higher levels of habitual physical activity might have an effect on DHEA and DHEA-S levels in both men and women.

DHEA (Aldred et al., 2009; Cumming et al., 1986) and DHEA-S (Tremblay et al., 2004) have been shown to increase in response to acute exercise in younger adults. Endurance trained young males showed attenuated increases in hormone concentrations in response to exercise compared to resistance trained individuals (Tremblay et al., 2004). One study has compared hormonal responses to resistance exercise in middle aged strength trained and untrained men (Cadore et al., 2008). There were no differences in hormones between trained and untrained men at rest; however, untrained men demonstrated a significant increase in DHEA and cortisol in response to acute resistance exercise, where trained men did not. This study suggests trained and untrained middle aged individuals may elicit different hormonal responses to exercise, however, less is known regarding responses in elderly individuals. As older adults of different training status and fitness may vary in hormone levels at rest, they may also display different hormonal responses to acute exercise.

A small number of studies have investigated these hormones in older adults, but these have mainly been limited to postmenopausal females; less is known about males and older

individuals. For example, DHEA significantly increased in response to exercise in females aged up to 69. This was restricted to resistance exercise and was not observed with endurance exercise (Copeland et al., 2002). Early postmenopausal females demonstrated an increase in DHEA-S immediately and 2 h post a combined endurance and strength training session (Kemmler et al., 2003). Another study in postmenopausal females found an increase in DHEA, but not DHEA-S, in response to submaximal exercise (Giannopoulou et al., 2003). A more recent study of older men and women reported that neither DHEA or DHEA-S increased immediately after acute submaximal exercise (Aldred et al., 2009), although this study only tested seven participants and no samples were taken during the recovery period. The lack of clear consensus may be due to the different exercise protocols employed and participants studied. With regard to cortisol, one study examined older fit and unfit females' baseline levels of cortisol, response and recovery to acute submaximal exercise, and failed to observe any significant differences between groups (Traustadottir et al., 2004), although DHEA/S were not measured in this study. To our knowledge, responses of DHEA, DHEA-S and cortisol to acute exercise in older males and females in relation to different levels of training status, has yet to be examined.

If exercise is to be used as a possible intervention to buffer against age induced changes in the neuroendocrine system, such as the reduction in DHEA/DHEA-S, then it is important to establish: first, whether exercise training influences levels of these hormones in older age; and second, whether training or sex affect hormonal responses to exercise. Therefore, the aim of the present study was to investigate resting measures of DHEA, DHEA-S and cortisol, and their response to and recovery from acute exercise, in male and female older adults of different exercise training status. It was hypothesised first, that older adults who were

exercise trained would present with a more favourable hormonal profile: higher levels of DHEA/S and a lower cortisol:DHEA ratio. Second, it was hypothesised that sedentary individuals would have a greater hormonal response to acute exercise.

## 6.3. METHODS

### *6.3.1. Participants*

Participants were 49 community dwelling older adults (23 females) recruited from the West Midlands area aged between 60 - 77 years. Inclusion criteria were: no endocrine or immune disorder, no psychiatric illness, no eating disorder, and not taking glucocorticoid medication. Twenty percent of participants reported suffering from a chronic illness; hypertension and asthma, and 43% reported taking medication; anti-hypertensives, non-corticosteroid inhalers, statins, and gastrointestinal medications. All participants described themselves as "white" ethnicity, and in terms of socio-economic status, 86% of participants classified themselves as non-manual based on their previous or current occupation using the Registrar General's Classification of Occupations (Classification of Occupations, 1980). Participants were either sedentary (n = 14), moderately active (n= 14), or endurance trained (n = 21). The sedentary participants were recruited from the local community and were not currently involved in any regular exercise nor had they been for 5 years prior to the study. Moderately active participants were recruited from local rambling groups, keep fit classes, aqua-fit classes, and gymnasia. Endurance trained older athletes were recruited from local running clubs and at races. Details of the exercise behaviour of participants are described in relation to the exercise diary below. There was no significant difference in socio-economic status, chronic illness, or medication use between the exercise groups.

### *6.3.2. Study design*

This study was a cross sectional investigation of the DHEA, DHEA-S and cortisol response and recovery to acute exercise in untrained, moderately trained, and untrained older adults. It comprised an acute exercise bout and the completion of a 14 day exercise diary.
All participants gave written informed consent prior to the study, which was approved by the University Research Ethics Committee.

### *6.3.4. Exercise diary*

To confirm the allocation at recruitment to sedentary, moderately active and endurance trained groups, participants completed a consecutive 14 day exercise diary where they recorded: what activity they did, the duration of the activity, and the intensity of the exercise. The intensity of the exercise was determined using a 0-10 RPE scale (Borg, 1998) where 0 was rest and 10 was maximal effort; participants were briefed on the use of the RPE scale and instructions and examples were also provided in the diary. The diary was analysed to determine how long was spent in moderate and vigorous activity based on MET values for a given activity (Ainsworth et al., 2000) and RPE (Nelson et al., 2007). The activities of the moderate group were mainly rambling, golf, yoga, badminton, swimming, and keep fit classes. The endurance trained group were runners, and cycling, circuit training and karate were also reported among this group. Minutes spent in moderate and vigorous exercise over the 14 day period were averaged per week. From this, an exercise score was created using the criteria from the Whitehall study (Marmot et al., 1991). This uses a 0-5 categorical scoring system e.g. if they spent 1-2 h performing an activity they were awarded a score of 1, 3-5 h a score of 2 and so on. A combined exercise score was calculated by multiplying the category score by a weighting of 2 for moderate, and 3 for vigorous activity. As shown in Table 1, the

exercise groups had significantly different exercise scores and significantly varied in moderate and vigorous exercise.

**Table 1.** Mean (SD) exercise score and time participating in moderate and vigorous exercise per week for sedentary, moderately active and endurance trained older adults. * indicates significance from other groups.

| | Sedentary | Moderate | Trained | F(df) | *p* | $\eta^2$ |
|---|---|---|---|---|---|---|
| Exercise score diary | 0.00* (0.00) | 4.85* (2.31) | 7.38* (3.02) | 41.8 (2,46) | <.001 | .645 |
| Time spent in moderate activity per week (min) | | 281.6* (183.26) | 94.4* (102.14) | 20.7 (2,46) | <.001 | .474 |
| Time spent in Vigorous activity per week (min) | | | 197.8* (121.94) | 36.3 (2,46) | <.001 | .612 |

### *6.3.5. Blood samples and hormone analysis*

Three blood samples were collected: prior to exercise, immediately post exercise, and 1h post exercise. The first blood sample was taken between 8.30-9.30 am, this was on average 3h after participants had woken up. For each blood sample, a 6 ml venous blood was collected from an ante-cubital vein into plain tubes (BD Vacutainer, Plymouth, UK). Blood was allowed to clot at room temperature for 1h, and then centrifuged at 4000 rpm for 5 min, and the separated serum was stored at -20°C until analysis. DHEA, DHEA-S and cortisol were analysed in duplicate using ELISAs based on the principle of competitive binding (IBL International, Hamburg, Germany). The microtiter wells are coated with a polyclonal antibody directed towards DHEA/DHEA-S or cortisol. The hormone in the sample competes with horseradish peroxidase conjugate for binding to the coated antibody. After 60 min incubation the unbound conjugate is washed off. After addition of a substrate solution and further 15 min 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%.

### *6.3.6. Procedure*

*Pre study screening*

Prior to entry to the study, participants completed pre study questionnaires. Participants were asked: if they suffered from any chronic illness, any acute illness, and if they were taking any medication. Participants completed: a physical activity readiness questionnaire (PAR-Q) (Tharrett and Peterson, 1997) the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983), and the Life Events Survey from the West of Scotland Twenty-07 study

(Ford et al., 1994) to assess stressful life events exposure over the past year. No participants met the criteria for high probability of anxiety or depression, and there was no significant difference in stressful life events exposure between exercise groups. Participants were given a 14 day exercise diary and instructions on how to complete it. They were then given an appointment for the acute exercise trial.

*Acute exercise bout*

Participants were asked to refrain from exercise and alcohol 24 h prior, and food and caffeine 12 h prior to arriving at the laboratory. Participants arrived between 8-9am and, on arrival, had the timeline for the visit described and asked if they had any questions. Their height and weight was measured. They were then were fitted with a heart rate (HR) monitor (Polar, Electro Kempele, Finland). The procedure for Douglas bag gas analysis was explained and they watched a researcher demonstrate how to position the nose clips and insert the mouthpiece. For familiarisation, participants practiced using the nose clip and mouthpiece and breathed for 1 min in a seated position for familiarisation. They then sat quietly and rested for 15 min before the 1$^{st}$ blood sample was taken, on average this was taken 2 h 45 min after waking (SD = 33 min), and timing of this sample did not differ significantly between groups.

Participants then undertook an acute bout of exercise in the form of an incremental submaximal treadmill test. A researcher demonstrated how to walk on the treadmill for those who had not used one before. Participants then began walking on the treadmill and speed was gradually increased until the participant reached a pace they considered "brisk walking". Once this pace was reached the test commenced comprising 4 min stages. Every 4 min the

gradient increased between 2 - 3.5%, depending on the participants HR. During the final minute of each 4 min stage expired air samples were collected into Douglas bags (Cranlea, Birmingham, UK) for the determination of oxygen consumption. HR was monitored continuously throughout exercise and recorded every 15 sec during the final min of each stage, and RPE was obtained at the end of each stage and prior to the termination of the exercise. The test was terminated once the participant had reached 75% of their predicted maximum HR, as determined by the formula 208 – (age × 0.7) (Tanka et al., 2001). In a few cases, participant's HR did not increase proportionally with the exercise, in which case the exercise was terminated once they reached 'hard' on the RPE scale. Once the exercise was finished, participants were seated immediately for another blood sample, and a final blood sample was taken 1 h post exercise.

Carbon dioxide production and oxygen consumption were determined from Douglas bag samples using an infrared carbon dioxide analyser and a paramagnetic oxygen analyser (Analyser Series 1440, Servomex, Crowborough, East Sussex, UK). Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and corrected to standard temperature and pressure. Maximal oxygen consumption ($VO_{2max}$) was predicted using a regression equation created from plotting the relationship between HR and oxygen consumption during the final 3 stages of exercise.

### *6.3.7. Statistical analyses*

Univariate ANOVA was used to examine differences between exercise groups for: age, BMI, $VO_2$max, and HR, RPE and exercise duration. Repeated measures ANOVA was used to examine the DHEA, DHEA-S and cortisol response to exercise, test any main effects of

group, and finally to examine any group × time interactions. Repeated measures ANOVA was also used to investigate any sex differences in hormone responses. Where significant effects emerged, subsequent ANCOVA was performed to adjust for any potential confounding variables, such as age. Greenhouse-Geisser corrections were applied with the repeated measures analyses and partial $\eta^2$, a measure of effect size, is reported throughout.

## 6.4. RESULTS

### *6.4.1. Group characteristics*

Table 2 displays age, BMI and predicted $VO_{2max}$ for the exercise groups. Age, $F(2,46) = 5.06$, $p = .01$, $\eta^2 = .180$, and BMI, $F(2,46) = 8.94$, $p < .001$, $\eta^2 = .280$, differed significantly between exercise groups. $VO_{2max}$ also differed significantly between groups, $F(2,39) = 22.14$, $p < .001$, $\eta^2 = .532$, and this group effect withstood adjustment for age. On average, males had a significantly higher $VO_{2max}$ compared to females, $F(1,40) = 15.185$, $p < .001$, $\eta^2 = .275$. In comparison to $VO_{2max}$ criteria for sex and age group (McArdle et al., 2001) males in the untrained, moderate, and trained groups were classified as having average, good and excellent aerobic fitness for their age group, respectively. Females in the untrained and moderate groups were classed as average, and trained females were classed as excellent for their age group.

**Table 2.** Mean (SD) age, BMI and $VO_{2max}$ for exercise training status and sex. * Indicates a significant difference compared to other training groups for both sexes, $p$ <.05.

| | Sedentary | | Moderate | | Trained | |
|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females |
| Age | 66.8 (3.48) | 70.6 (4.63) | 67.5 (5.36) | 67.6 (3.25) | 65.6 (4.82) * | 62.3 (4.23) |
| BMI | 26.3 (3.40) | 25.4 (3.61) | 27.9 (2.75) | 25.4 (3.07) | 23.5 (1.93) * | 21.3 (1.71) |
| VO2max ($ml \bullet kg^{-1} \bullet min^{-1}$) | 35.4 (3.53) | 27.0 (5.89) | 40.1 (7.69) | 29.5 (3.53) | 48.1 (6.30) * | 44.4(6.35) |

### *6.4.2. Acute exercise*

The mean duration of the incremental exercise test was 23min 49sec (SD = 2min 8sec) and the mean final RPE obtained at the end of exercise was 5.1 (SD = 1.08), which was equivalent to 'hard'. The final HR achieved at the end of the exercise was 132.7 (SD = 9.91) bpm, this was equivalent to 76.5% (SD = 5.44) of predicted maximal HR. There were no significant differences between exercise training status groups or sex for: exercise duration; final RPE, final HR, or percentage HR.

### *6.4.3. DHEA, DHEA-S and cortisol*

*Training status*

There were no significant group differences in any of the hormone parameters, at any of the time points. Accordingly, all subsequent results are reported for participants as a whole. $VO_{2max}$ was positively associated with DHEA-S levels in women, although this was not statistically significant, r(18) = .42, $p$ = .07. There were no other trends for $VO_{2max}$ and hormone levels.

*DHEA*

There was a significant main effect of time for DHEA where DHEA levels increased immediately post exercise, F(2,92) = 6.62, $p$ =.004, $\eta^2$ = .126. This effect is displayed in Figure 1A.

*Cortisol*

As shown in Figure 1B, there was a significant main effect of time for cortisol which decreased immediately post exercise, and 1 h post exercise compared to pre, F(2,92) = 19.58, $p$ <.001, $\eta^2$ = .299. Males had significantly higher overall cortisol levels than women, F(1,47) = 4.06, $p$ = .05, $\eta^2$ = .079, (Table 3).

*DHEA-S*

There was a trend for DHEA-S to increase immediately post exercise, but this failed to reach statistical significance ($p$ = .07). However, the effect was significant for females, F(2,42) =

4.37, $p$ =.02, $\eta^2$ = .172. Compared to pre, females had a significant increase in DHEA-S immediately post exercise. Although they did not exhibit a response to exercise, males had significantly higher overall DHEA-S levels than women, F(1,43) = 4.48, $p$ = .04, $\eta^2$ = .094. Descriptive statistics of hormone levels for males and females are displayed in Table 3.

*Cortisol:DHEA/DHEA-S ratio*

There was a significant main effect of time for the cortisol:DHEA ratio, F(2,90) = 20.04, $p$ <.001, $\eta^2$ = .308, where the ratio decreased immediately post and 1 h post exercise compared to pre (Figure 2A). The cortisol:DHEA-S ratio also decreased significantly immediately post and 1 h post exercise compared to pre, F(2,78) = 19.08, $p$ <.001, $\eta^2$ = .329 (Figure 2B).

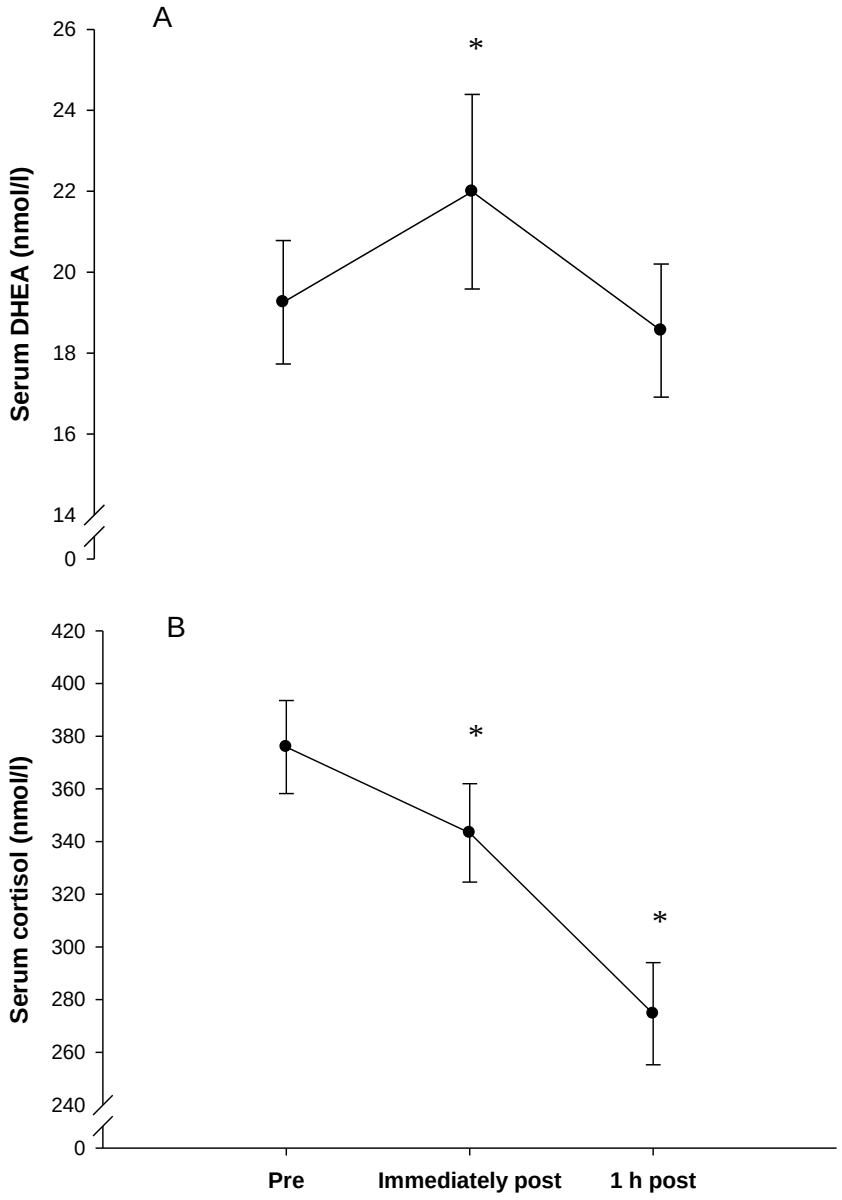


**Figure 1. A** Serum DHEA response to acute exercise in older adults. DHEA values immediately post exercised increased significantly from pre values, * p = .004. **B** Serum cortisol response to acute exercise in older adults. Significantly different from pre values, * *p* <.001.

**Table 3.** Mean (SD) hormone values (nmol/l) for males and females, overall, pre, immediately post and 1 h post exercise. † indicates significant difference between sexes, $p < .05$. * indicates a significant within sex difference from pre, $p < .05$.

| Hormone variable | Males | Females |
|---|---|---|
| DHEA (nmol/l) | 18.9 (12.23) | 20.8 (12.70) |
| Pre | 18.9 (8.61) | 19.5 (12.72) |
| Immediately post | 20.2 (9.66) | 23.6 (22.44) |
| 1 h post | 17.6 (8.38) | 19.2 (14.24) |
| DHEA-S overall (nmol/l) | 2649.8 (971.25) † | 2036.9 (971.24) |
| Pre | 2650.3 (1028.99) | 2002.4 (923.70) |
| Immediately post | 2659.4 (1028.99) | 2096.8 (935.97) * |
| 1 h post | 2639.7 (1007.09) | 2011.5 (939.47) |
| Cortisol (nmol/l) | 362.6 (107.22) † | 300.8 (108.24) |
| Pre | 402.6 (132.77) | 355.3 (110.46) |
| Immediately post | 375.6 (130.80) | 308.4 (118.40) |
| 1 h post | 309.6 (141.1) | 238.6 (112.47) |

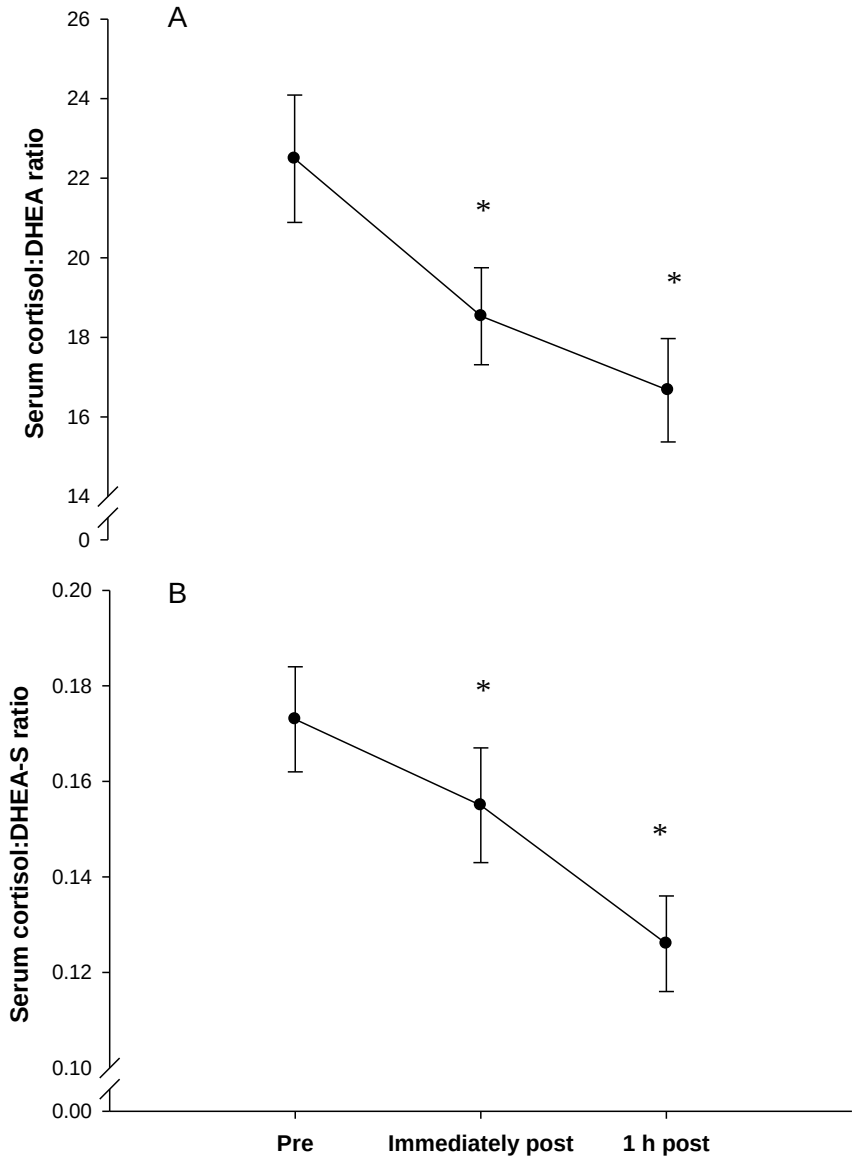


**Figure 2. A** Serum cortisol:DHEA ratio response to acute exercise in older adults. Significantly different from pre values, * p <.001. **B** Serum cortisol:DHEA-S ratio response to acute exercise in older adults. Significantly different from pre values, * p <.001.

## 6.5. DISCUSSION

### *6.5.1. Hormonal responses to acute exercise*

Older adults demonstrated a significant increase in DHEA immediately post exercise. DHEA has been previously shown to increase in response to submaximal aerobic (Giannopoulou et al., 2003) and resistance exercise (Copeland et al., 2002) in postmenopausal women, and this study extends previous findings to older males. Despite a similar exercise protocol, the present findings contrast with those from a recent study which failed to find an increase in DHEA with exercise in individuals aged between 65 - 75 years (Aldred et al., 2009). However, this could reflect the small sample size employed in this previous investigation.

Cortisol decreased immediately post and 1 hour post exercise compared to pre exercise. Independent activation of the zona fasciculata and zona reticularis has been noted previously (Velardo et al., 1991). A decrease in cortisol post exercise has also been noted in postmenopausal females (Kemmler et al., 2003), this decrease continued throughout the 2 h recovery period. As with the present study, Kremmler et al (2003) exercised their participants in the morning, however, the authors suggested that their observed decreases were above that of normal diurnal decline. It is difficult to separate the effects of exercise from the effects of diurnal variation. However, the first blood sample taken in the present study was nearly 3 h after waking; therefore, participants would have already experienced the large decrease in cortisol that occurs following the cortisol awakening response. This is suggested by cortisol diurnal rhythm data collected on average a week before acute exercise testing in the present participants (unpublished data). Further, the present finding is in contrast to Traustadottir et al. (2004), who reported an increase in cortisol in older females

after 15 minutes of cycling, this exercise bout also took place in the morning period, suggesting time of day is not responsible for this difference.

As a result of the decrease in cortisol, which was not apparent with DHEA/S, the cortisol:DHEA/S ratio significantly decreased immediately and 1 h post exercise. This represents a more favourable endocrine profile, although it may be that this only occurs if exercise takes place in the morning period. Identical exercise in the afternoon would be required to determine whether this is the case. If the effect is limited to morning exercise, it could be that this is because it is the optimal time to alter the balance between cortisol and DHEA/S and thus promote greater protection against decrements in immunity linked to higher cortisol levels. However, this is highly speculative, and exercise of a longer duration or a higher intensity in the morning may still produce an increase in cortisol. It is known that only high intensity or exhaustive exercise results in increases in cortisol, with a threshold of 60% $VO_{2max}$ required for its release (Bishop, 2006; Pedersen and Hoffman-Goetz, 2000). Therefore, although participants were exercised to 75% of their predicted maximum capacity, as the exercise was graded, and not at a predetermined intensity, it is possible that they did not exercise for a sufficient period at 60% $VO_{2max}$ or above to elicit an increase in cortisol.

To our knowledge, this is the first study to examine the responses of DHEA/S alongside cortisol to acute exercise in relation to exercise training status in elderly participants. The present findings suggest that sedentary, moderately trained and endurance trained older adults do not vary in their hormonal responses to exercise. This implies that older adults, regardless of training status, are able to produce a DHEA response to exercise. This finding is in contrast to those of Cadore et al (2008) who found that trained middle-aged men required a

greater exercise stimulus to produce a hormonal response. However, this study used strength trained individuals and investigated acute resistance exercise in middle aged, not elderly individuals. Although no effects of training status were found, the response of DHEA-S to exercise did differ between sexes. Females showed a significant increase in DHEA-S immediately post exercise whereas males did not. This increase in DHEA-S is consistent with prior research in early postmenopausal females (Kemmler et al., 2003), although males were not tested in this previous study.

Possible mechanisms for exercise-induced increases in DHEA/S have been outlined previously, with increased secretion rate by the adrenal cortex as a result of ACTH stimulation (Johnson et al., 1997; Keizer et al., 1989; Keizer et al., 1987) and decreased metabolic clearance due to a reduction in hepatic blood flow during exercise (Ponjee et al., 1994) being the most commonly cited. The mechanism responsible for the observed increase in DHEA-S in females but not in males is not clear. DHEA-S is found in higher and more stable concentrations due to its longer half life, it has been suggested that larger increases in DHEA-S are required to observe significant changes (Johnson et al., 1997). Therefore, as women have significantly lower levels of DHEA-S at baseline, they may have greater potential to exhibit a significant increase in response to exercise. Resting DHEA-S levels have been shown to be associated with physical activity in females but not males within the same study (Bonnefoy et al., 1998; Kostka et al., 2002); consequently, it could be speculated that females may be more sensitive to exercise induced changes. The ability of an acute exercise bout to increase DHEA/S in older adults may afford a non-pharmacological method for increasing anabolic hormones. However, as levels returned back to baseline within an hour, it is debatable how beneficial such a short term increase is. Exercise of a longer

duration and higher intensity may be required in order to elevate and maintain DHEA/S levels for a significant period post exercise, although this needs to be balanced against what exercise protocols older adults can realistically perform.

### *6.5.2. Hormone levels and exercise training status*

Acute exercise performed on a regular basis over a period of years does not appear to influence resting hormone levels, as interestingly, there were no differences among older adults in relation to their exercise training status. This is consistent with Arai et al (2006) who found no significant difference in DHEA-S between older male runners and those who were sedentary, and Traustadottir et al (2004) who reported no differences in cortisol levels between fit older women and those who were of average fitness. Although this present finding is in contrast to other studies which suggest DHEA/S is higher in endurance trained (de Gonzalo-Calvo et al., 2011; Tissandier et al., 2001) and moderately trained (Ravaglia et al., 2001) older men. In these previous and current investigations, blood samples were collected at similar times under the same conditions (fasted, rested etc). However, differences could be due to variation in the participants studied, in terms of fitness, training load and how long they have been exercising for.

Our current findings suggest that long term exercise training, be it at a vigorous or moderate intensity, does not lead to a more favourable hormonal profile among older adults compared to those who do not exercise. All participants in the present study were healthy older adults with no endocrine or immune disorders; it is possible that the sedentary group were healthier and fitter than the average older adult and this is why no differences emerged between exercisers and non exercisers. They were of relatively high socio-economic status, as is

characteristic of study volunteers and is associated with better health (Anderson and Armstead, 1995). It may be that long term exercise training does not impact upon hormone levels of healthy older adults, but it could have the potential to have a positive effect on more vulnerable older adults, for example, those who are frail, suffer from an illness, depression, or who are experiencing chronic stress (Phillips et al., 2007).

In healthy older adults, 6 months of resistance (Häkkinen et al., 2000) or endurance training (Hersey et al., 1994) did not elicit an increase in DHEA/S. However, exercise intervention studies in this area are currently lacking, especially those of a long duration and also in unhealthy populations. Even if long term exercise turns out not to be beneficial from a hormonal perspective, it should still be encouraged as evidence suggests that it has the potential to decelerate immunosenescence (Arai et al., 2006; Nieman et al., 1993; Shinkai et al., 1995), as well as to maintain aerobic capacity and muscular strength, and thereby preserve physical function with ageing.

### *6.5.3. Limitations and conclusions*

The present study suffers from several limitations. Firstly, as a $VO_{2\ max}$ test was not performed, and aerobic fitness was estimated from a submaximal test. However, maximal exercise testing in older adults raises a number of issues (Hugget et al., 2005) not least the ethics of such testing. As a result of not obtaining a $VO_{2\ max}$, exercise was graded in intensity and was not steady state, which may have produced a different response. Investigating hormonal responses to exercise of different durations and intensities in this population would be valuable; although the feasibility of multiple trials and what demands can be placed on participants may be a limiting factor.

In conclusion, older adults demonstrated a significant increase in DHEA in response to acute submaximal aerobic exercise, whereas only older females showed a significant increase in DHEA-S. Further, neither resting hormone levels, nor the response to exercise was influenced by exercise training status. Although these findings suggest that long term exercise may not affect the hormonal status of older adults, future research involving longitudinal studies and exercise interventions are required to definitively determine whether exercise can be used to maintain or improve the ageing endocrine system.

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# CHAPTER SEVEN

## 7.0. DISCUSSION

## 7.1. SUMMARY OF MAIN FINDINGS

The research in this thesis was concerned with the relationships between cortisol, DHEA/S and psychosocial and behavioural factors in healthy young, middle-aged and older adults.

The first two papers from study one examined differences in the cortisol diurnal rhythm between young adults and community dwelling older adults. This study demonstrated that older adults had a flattened diurnal amplitude, due to reduced cortisol production in the morning period. The first paper presents findings in relation to depression, anxiety and social support, where it was found younger adults experiencing symptoms of depression had a higher cortisol levels upon awakening and an exaggerated increase upon waking. Both depression and anxiety were also associated with an increased CAR and AUC; in addition, lower social support was associated with a higher AUC and CAR. Interestingly, lower social support had the opposite effect in older adults who exhibited a lower AUC. This paper presents novel findings that age may mediate the relationship between psychosocial factors and diurnal patterns of cortisol secretion. Paper two also confirmed that age may influence how certain factors relate to cortisol diurnal rhythm: this time in the form of health behaviours. Younger adults who had a poorer diet had significantly lower cortisol in the morning period which resembled the flattened diurnal amplitude of older adults; diet did not have an impact on cortisol secretion in older adults.

The aim of paper three in chapter four was to examine the diurnal rhythms of cortisol and DHEA in relation to age and physical function in older adults. Adults aged 72-86 presented with a raised cortisol profile, a blunted DHEA profile and increased cortisol:DHEA ratio in comparison to older adults aged 65-71. The results of this study supported the hypothesis that

flatter rhythms of cortisol and DHEA would relate to poorer levels of physical function. The diurnal amplitude of DHEA was abolished in individuals whose independence in carrying out daily activities had been compromised, a novel finding in an area previously lacking investigation.

The fourth paper presented in chapter five examined stressful life events and habitual exercise behaviour in relation to the diurnal rhythms of cortisol and DHEA and the cortisol:DHEA ratio among different age groups: outcomes previously not investigated in conjunction with this form of stress and exercise in the context of age. The results of this study found that younger adults who engaged in more exercise demonstrated higher levels of DHEA and a more dynamic diurnal pattern. Older adults experiencing more severe life events stress had a higher cortisol:DHEA ratio, suggesting that this form of stress might accelerate age-associated hormonal changes. Further, as life events stress did not affect younger or middle-aged adults, this provides support for the view that older adults may be more vulnerable to stress. In addition, older individuals who reported greater life events exposure and who did not participate in regular aerobic exercise had a significantly higher cortisol:DHEA ratio, whereas those with high stress exposure but who exercised were not significantly different from those experiencing low stress. This provides preliminary evidence to support the hypothesis that exercise may buffer against the potential negative effects of stress on the neuroendocrine system of older adults.

The aim of the last study in chapter six was to establish if exercise training status of older adults influenced resting measures of DHEA/S and cortisol, and how these hormones responded to acute exercise. This study was more comprehensive than previous studies, as it

included individuals who participated in both moderate intensity exercise and those who were endurance trained, both male and females were examined and both serum sulphated and un-sulphated DHEA were measured alongside cortisol. This study found that exercise can stimulate DHEA production in older adults, and DHEA-S production in older females. Training status did not influence resting hormone parameters or how they responded to exercise, nor did it influence the diurnal rhythms of cortisol and DHEA in saliva, which were also measured but not presented in this paper (see Appendix 8.1). This suggests that long term exercise training does not protect against age related changes in these adrenal hormones.

## 7.2. THE DIURNAL RHYTHMS OF CORTISOL AND DHEA AND AGE

Chapters two to five examined age differences in cortisol and DHEA. The first study presented in chapters two and three examined the diurnal rhythm in younger and older adults, in which older adults displayed a blunted CAR and a flatter diurnal profile. Chapter five examined the diurnal rhythms of cortisol and DHEA in young, middle-aged and older cohorts. Interestingly, no significant variations were found in relation to age and cortisol; however, young adults had the highest overall levels of DHEA, followed by middle-aged adults, with older adults having the lowest levels of DHEA. This resulted in a increase in the cortisol:DHEA ratio with age, where older adults presented with a significantly higher ratio compare to the young and middle-aged cohorts. Chapter four explored diurnal differences in cortisol and DHEA among older adults. Participants aged 72-86 showed higher diurnal cortisol levels at certain periods of the day and a higher AUC. They also had lower DHEA levels overall, and with increasing age, the DHEA AUC was attenuated and the slope of decline became less steep, and finally these older old adults had a higher cortisol:DHEA ratio compared to older adults aged 65-71.

The field of study examining cortisol in relation to age presents varied findings. The findings of study one highlighting attenuation in the CAR and flattened cortisol is in line with certain previous studies (Deuschle et al., 1997; Kudielka and Kirschbaum, 2003; Luz et al., 2003; VanCauter et al., 1996; Yen and Laughlin, 1998). The flattened profile was a product of lower morning cortisol, consistent with Maes et al (1994) and Ahn et al (2007), rather than increased evening cortisol observed in other studies (Deuschle et al., 1997; Luz et al., 2003; VanCauter et al., 1996). Studies examining simply the level of cortisol, rather than its diurnal rhythm, have also suggested a decrease in cortisol with age (Orentreich et al., 1992; Straub et al., 2000). In addition, some studies have shown no association with age (Edwards et al., 2001a; Wolf et al., 2002).

The results of study one seem to add to this picture of mixed results; further, they also differ from study two where no differences were observed between cohorts in cortisol. As pointed out in chapters two and three, the lack of consistency in findings may be due to individual variability in cortisol, which has been shown with ageing longitudinally (Lupien et al., 1996), and also in studies with younger adults (Smyth et al., 1997). Therefore, individual differences in the participants studied may contribute to this lack of consensus. Although there may be a general shift towards a flatter diurnal profile, this may be characterised through either an increased nadir or blunted secretion in the morning.

The story for DHEA is more straightforward. This hormone peaks at age 20-30 and then declines progressively with age (Belanger et al., 1994; Labrie et al., 1997; Orentreich et al., 1992). Further, an increase in the cortisol:DHEA ratio in older adults has been well established (Phillips et al., 2007). Findings from the second study in this thesis are consistent

with previous findings; however, paper four extends age differences to within older adults and also to the diurnal profile of DHEA. The more elderly individuals had lower DHEA and a flatter diurnal profile, suggesting that age differences not only occur between older and younger adults, but significant changes also occur among older adults as a result of ageing and within a relatively short time frame. In addition, changes not only take place in relation to the overall level, but the rhythm of secretion is also reduced.

The potential mechanisms of age-related changes in cortisol and DHEA were briefly outlined in the introduction to this thesis. It has been proposed that the elevation of cortisol in relation to DHEA is a compensatory mechanism by the adrenal cortex (Straub et al., 2001). It may be that the ageing adrenal cortex cannot maintain the levels of all hormones, and opts to support cortisol synthesis at the expense of other hormones such as DHEA. Alternatively, this may reflect increased stimulation of the HPA axis from inflammatory cytokines with ageing. The relative increase in cortisol has been proposed to be an adaptive response, aimed to suppress immune activity and pro-inflammatory cytokines to counteract age-associated inflammation (Sergio, 2008).

## 7.3. HEALTH BEHAVIOURS, CORTISOL AND AGE

The findings of chapter three imply that the relationship between health behaviours and diurnal cortisol may be mediated by age. Younger adults with a poor quality of food intake exhibited a reduction in the amplitude of the cortisol diurnal profile, which was mainly driven by reduced cortisol in the morning period. This builds upon previous literature that has found a flatter cortisol rhythm in individuals with a higher intake of saturated fat (Garcia-Prieto et al., 2007); that study however was conducted in middle-aged women. This study, to

our knowledge, is the first we are aware of to report an association between habitual dietary intake, diurnal cortisol and age.

A bi-directional relationship exists between cortisol and metabolism (Lasikiewicz et al., 2008). Enhanced cortisol clearance, diminished circulating cortisol levels and cortisol responses to CRF have been cited in obese individuals (Kopelman et al., 1988; Müssig et al., 2010). Further, the macronutrient content of the diet has been shown to influence metabolic clearance of cortisol, independently of weight loss (Stimson et al., 2007). In the present study, the flattened diurnal profile observed in relation to diet was independent of body mass index and all younger participants had body mass indices within the normal range. Nevertheless, it is possible that a flattened diurnal profile and HPA axis dysregulation could be a marker of susceptibility to obesity or metabolic disturbance later in life. However, the mechanisms underlying, and the direction of the association between increased clearance and reduced cortisol are yet to be fully understood, and longitudinal investigations are required to properly test this hypothesis. In addition to this possible health implication, as the young individuals with poor diet imitated the diurnal pattern of older adults, it could be suggested that this flattened profile may indicate vulnerability to other adverse outcomes. Interestingly, no associations with diet were found among the older adults. It may be that cortisol rhythms are no longer susceptible to this aspect of health behaviour in older age.

On the flipside, it could be argued that the low fat, high fruit and vegetable intake groups represent people on diets and thus restricting energy intake. In which case, it may not be those with a poorer quality of diet who have decreased cortisol output, but rather those with 'healthier' diets who have increased cortisol. A study has shown that low calorie dieting

increases cortisol output across the day (Tomiyama et al., 2010). However, this study was a short term intervention and therefore not based on habitual eating behaviour and associations with stress also emerged. Further, no participants reported being on a diet in the present study in response to a question in the health behaviours questionnaire.

## 7.4. PSYCHOSOCIAL FACTORS, CORTISOL, DHEA, AND AGE

Various studies have previously examined depression or anxiety in relation to cortisol; however, the findings presented in chapter two build on previous literature by simultaneously examining depression, anxiety and also social support, and comparing relationships between young and older adults.

The results suggest that the relationship between diurnal cortisol and these psychosocial factors vary between young and older adults. In younger adults, a higher CAR characterised those with depressive symptomatology. The present finding among the undergraduate students supports evidence from Prussner et al (2003) where a higher CAR was found in male university students with higher depressive scores. An elevated CAR has also been found in relation to diagnosed depression in the community (Bhagwager et al., 2005; Vreeburg et al., 2009). This study suggests that it is only the morning period and the CAR that is mainly altered with depression, and cortisol levels are similar to non-depressed individuals thereafter, a conclusion comparable to that drawn by Bhagwager et al. (2005). However, the overall rhythm has been shown to be altered (Sjogren et al., 2006) and CAR blunted (Stetler and Miller, 2005) in other studies. Therefore, the effects of depression in the community appear to be characterised by dysregulation, rather than a specific directional change (Stetler and Miller, 2005). Although it seems anxiety is not associated with diurnal cortisol in

adolescents (Adam, 2006), anxiety was associated with a higher CAR and AUC in current sample of university students, further suggesting age influences the relationship between cortisol and negative affect, in addition to the finding that neither anxiety or depression were related to cortisol among the older participants. To our knowledge, the current study is one of the limited few that have explored cortisol in relation to anxiety in older adults; therefore future studies are required among this age group to confirm the present findings, and expose reasons for potential differences in associations between age groups.

Age also influenced the relationship between social support and cortisol. Individuals with lower social support had a reduced AUC in older adults, where it was associated with an increased AUC and CAR in the younger adults. The influence of social support on immune response has been shown to differ between young and old age groups previously (Gallagher et al., 2008; Phillips et al., 2006); the present finding implies that this may also be the case for the endocrine system.

The nature of the relationship between depression and cortisol was discussed in chapter three, where it was concluded that the association may be bi-directional and influenced by multiple factors. Regardless of the mechanisms underlying the relationship, this study further highlights the importance of considering age as a factor that may affect associations between psychosocial factors and physiological measures. This theme also emerged in study two, this time in relation to another aspect of psychosocial health: life events stress.

The results from chapter five showed that older adults experiencing more severe life events stress had a significantly higher cortisol:DHEA ratio than those reporting lower stress levels.

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 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. This study not only supports the theory that stress may accelerate features of ageing, but provides initial support for the hypothesis that older adults may be more at risk from the effects of chronic stress (Graham et al., 2006; Kiecolt-Glaser and Glaser, 2001; Phillips et al., 2007). However, larger scale studies comparing the impact of stress across individuals of different ages are required to confirm this proposition. The implications of an increased cortisol:DHEA ratio may have far reaching consequences for the psychological and physiological health of ageing individuals (Straub et al., 2001).

The findings of chapters two and five suggest that the relationship between psychosocial factors and cortisol and DHEA may vary depending on age group. A significant relationship with depression, anxiety and cortisol was only evident in younger adults. The opposite was true in relation to stressful life events, where significant associations emerged among the older adults. Collectively these findings propose that the relationship between psychosocial factors and cortisol and DHEA may be determined by age and also type of psychosocial variable. Further, associations may occur in relation to different aspects of the diurnal rhythm or outcome variables of these hormones, depending on the psychosocial factor considered.

## 7.5. CORTISOL, DHEA AND PHYSICAL FUNCTION IN OLDER ADULTS

The results of chapter four demonstrate that lower levels of cortisol and a flattened diurnal rhythm of DHEA are associated with poorer physical function. Cortisol levels were significantly reduced overall, but markedly in the morning period in relation to balance. This is comparable to findings that attenuated cortisol concentrations upon awakening predicted higher levels of fatigue later that day (Adam et al., 2006). In contrast, higher levels of cortisol have been associated with frailty, assessed by chair stands, a tandem stand and walk test (Peeters et al., 2007). This relationship was with serum morning cortisol and tandem stand test in women, but evening salivary cortisol and chair stand and walk test in men. Higher levels and also blunted diurnal variation of cortisol have also been related to frailty assessed using different outcome measures; weight loss, exhaustion, slowness, weakness, and inactivity (Varadhan et al., 2008). In the present study, those less independent in carrying out activities of daily living displayed lower levels of DHEA in the morning period generating a flat diurnal rhythm. This direction of association between DHEA and physical function is consistent with previous findings in relation to DHEA-S (Berkman et al., 1993; Voznesensky et al., 2009).

The results presented in this paper extend previous associations with physical function to salivary DHEA and illustrates that the diurnal rhythm of DHEA may also be altered among individuals with lower levels of function, once again highlighting the importance of the pattern of hormonal secretion for health and wellbeing. Further, the present findings suggest that a lower output of both cortisol and DHEA is associated with lower functioning in older adults. Although high levels of cortisol may influence physical function via increased risk of sarcopenia as a result of catabolism, a lack of cortisol may operate through different

mechanisms. Inflammation and inflammatory mediators have been proposed as a pathway to impaired functioning and presentation of frailty (Cohen, 2000; Fried et al., 2005; Walston et al., 2006). Mediators of inflammation such as IL-6, TNF-α, C-reactive protein increase during the process of ageing (Straub et al., 2001), although higher levels of these factors have also been associated with markers of frailty (Cohen et al., 1997; Ershler and Keller, 2000), and indeed mortality in the elderly (Harris et al., 1999). Inflammation has been implicated, both directly and indirectly, as a mechanism of age related sarcopenia (Boire, 2009; Jones et al., 2009), and consequently may be involved in impaired muscle strength and balance. As cortisol (Sergio, 2008) and DHEA (Daynes et al., 1993) can inhibit inflammatory markers associated with ageing, lower levels of these hormones may increase the presence of inflammation, and consequently its effects on ageing muscle. However, as these mechanisms were not tested as part of this thesis, the processes by which cortisol and DHEA relate to physical function can only be speculated upon. Interestingly, no findings emerged for the cortisol:DHEA ratio. It may be that the balance between other hormones, for example cortisol:growth hormone ratio (Nass and Thorner, 2002), is more a more important factor in relation to muscle related outcome variables.

Neuroendocrine dysregulation may be a pathway to the onset and development of frailty (Joseph et al., 2005; Walston et al., 2006). It could be proposed that physical function deterioration may be bridging the gap between hormonal changes and the presentation of frailty. Therefore, those individuals who demonstrated poorer functioning may be at risk of frailty in the future either through deterioration of function over time, or through factors such as falls (Joseph et al., 2005) and subsequent hospital admission (Donaldson et al., 1990). Potentially, changes in these hormones and their diurnal rhythms could be used as a marker to

highlight those at risk of frailty. However, longitudinal research monitoring these hormones alongside physical function over time would be required to support this notion.

## 7.6. HABITUAL EXERCISE, CORTISOL, DHEA AND DHEA-S

Chapters five and six examined cortisol and DHEA/S in relation to habitual exercise behaviour. Chapter five investigated the diurnal rhythms of cortisol and DHEA in saliva in younger, middle-aged and older adults. It was found that undergraduate students who participated in more exercise had higher DHEA across the day and larger diurnal amplitudes. They also had a significantly lower cortisol:DHEA ratio; however, it should be conceded that the ratio finding did not withstand statistical adjustment.

This is the first study to our knowledge to investigate diurnal DHEA in the context of habitual exercise in younger adults. The positive relationship between exercise and DHEA fits in with research in related fields. For example, in exercise immunology it has been hypothesised that those who take part in regular moderate exercise are at reduced risk of infection, compared to sedentary or those engaging in a high volume of training at a high level of exertion. It has been suggested that, although immune system changes return to baseline within a few hours of exercise, acute exercise increases immune surveillance that reduces risk over time. DHEA increases in response to an acute exercise in younger adults (Aldred et al., 2009; Cumming et al., 1986), and decreases back to baseline shortly after. It may be that short term boosts in DHEA from regularly performed acute exercise may result in elevated diurnal levels over time in this population.

The lower levels of DHEA and flatter rhythm shown in the younger adults who did not participate in as much exercise may have implications for other aspects of health in the future, be it physiological or psychological. Alternatively, as suggested in chapter five, those with lower exercise scores may not have lower levels of DHEA *per se*, rather those with higher scores have managed to boost their DHEA levels above normal values. All younger adults in the chapter five participated in at least mild and moderate exercisers and there were no individuals who did not exercise. In addition, only a small number of adults were featured and thus this study represents only preliminary evidence for associations between DHEA and habitual exercise. More data are required from younger adults with a wide variation in exercise behaviour.

Exercise was not associated with hormone parameters independently among the older adults, but it did interact with stress in this age cohort. Older adults who reported a higher number of stressful events 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. This finding suggests that exercise can protect against an age-related increase in the cortisol:DHEA ratio and its associated risks in older adults who have experienced stress within the past year. This is consistent with previous research that has shown exercise may protect against the effects of stress on other age-related physiological changes, such as telomere shortening and functional decline (Puterman et al., 2010; Unger et al., 1997). The present finding extends the role of exercise as a buffer against stress to the cortisol:DHEA ratio, an outcome which, as mentioned previously, may be particularly important for the health of ageing individuals. There was no difference between those who did and did not exercise in the low stress group, suggesting that exercise may only

exert a protective effect when individuals have relatively high stress exposure; this is parallel to previous research that has shown potential buffers may act only under conditions of stress. For example, social support buffered against mortality in middle-aged men only in those with high stressful life events exposure (Rosengren et al., 1993). Overall, the results from chapter five offer support to the proposal that exercise could be used as a possible intervention to protect the neuroendocrine system from the impact of stress in older adults (Phillips et al., 2007).

In chapter five, it was suggested that exercise effects which were independent of stress may not have emerged in the older cohort as a result of them not participating in exercise at a high enough intensity to have a beneficial effect on DHEA. Therefore, one of the aims of the final study of this thesis presented in chapter six was to examine highly trained older individuals who participated in high intensity habitual exercise, in comparison to those who engaged in more moderate activities and sedentary individuals. The findings of this final study are comparable to those from chapter five in that habitual exercise, whether endurance or moderate, performed over a number of years, does not appear to influence resting hormone levels. This was true of cortisol, DHEA and DHEA-S in serum, as well as cortisol and DHEA, and their diurnal rhythms, in saliva (see Appendix 8.1.). Previously, no differences were found between older male runners and sedentary controls in DHEA-S levels (Arai et al., 2006), nor between fit and averagely fit older women in cortisol levels (Traustadottir et al., 2004). However, this present finding is in contrast to other studies which suggest DHEA/S is higher in endurance trained (de Gonzalo-Calvo et al., 2011; Tissandier et al., 2001) and moderately trained (Ravaglia et al., 2001) than in untrained older men. Differences in findings may be due to variation in the participants studied, in terms of fitness, training load

and how long they have been exercising for. The present study improves upon previous literature by including both sexes, endurance trained individuals alongside moderately trained in comparison to sedentary, and also DHEA and DHEA-S alongside cortisol.

The findings of chapters five and six suggest that habitual exercise, be it at a vigorous or moderate intensity, does not necessarily lead to a more favourable hormonal profile, be it in serum at rest or in saliva across the day, among older adults compared to those who do not exercise. No associations emerged in relation to stress, or stress and exercise in the final study. These data were not presented as this was not the focus of this study; however, the stress variables clustered even more so at the lower end of the scales and means and inter-quartile ranges of stress scores in this sample were lower than those found in the study reported in chapter four. Therefore, it may be that individuals examined in the final study were not experiencing as much stress as those in chapter five and this may account for the lack of associations with stress.

It may be that exercise only has an impact on hormone levels of older individuals who are in a compromised state, such as those experiencing stress in chapter five, or those who are frail or suffering from a chronic illness. Further, although exercise training and fitness appears not to be beneficial from a hormonal perspective, it should still be encouraged as evidence suggests that it has the potential to decelerate immunosenescence (Arai et al., 2006; Nieman et al., 1993; Shinkai et al., 1995). Further, exercise has been associated with higher physical functioning in older adults (Berkman et al., 1993); accordingly, preservation of aerobic capacity and muscular strength through exercise may offer benefits into old age.

## 7.7. ACUTE EXERCISE, CORTISOL, DHEA, AND DHEA-S IN OLDER ADULTS

The final study in this thesis presented in chapter six demonstrated that an acute bout of aerobic exercise stimulates an increase in serum DHEA in older adults. This is in line with previous studies that also found an increase in response to both aerobic (Giannopoulou et al., 2003) and resistance exercise (Copeland et al., 2002). These previous investigations took place in postmenopausal women; the current study extends their findings to both male and female older adults. Further, this is the first study, to our knowledge, that has examined adrenal hormone responses in relation to exercise training status in elderly participants. There were no differences in hormonal responses between sedentary, moderately trained and endurance trained older adults; all older adults appear to be able to elicit an increase in DHEA post-exercise, and training status was not a factor which determined the response of these hormones to exercise. Nevertheless, it appears that sex is a factor which influences the hormonal response to exercise in older adults. Females showed a significant increase in DHEA-S immediately post-exercise whereas males did not. This increase in DHEA-S is consistent with prior research in early postmenopausal females (Kemmler et al., 2003), although there were no males for comparison in this previous study.

Exercise induced increases in DHEA/S have been attributed to increased secretion as a result of ACTH stimulation (Johnson et al., 1997; Keizer et al., 1989; Keizer et al., 1987) and also a decrease in metabolic clearance (Ponjee et al., 1994). The observed sex difference in the DHEA-S response may be due to the significant difference at baseline between males and females. It has been suggested that as DHEA-S is found in higher and more stable concentrations compared to DHEA, larger increases in DHEA-S are required to observe significant changes (Johnson et al., 1997). Therefore, as women had significantly lower

levels of DHEA-S at baseline, they may have greater potential for showing a significant increase in response to exercise. Resting DHEA-S levels have been previously shown to be associated with physical activity in females but not males within the same study (Bonnefoy et al., 1998; Kostka et al., 2002); consequently, it could be speculated that older females may be more sensitive to exercise induced changes.

In contrast to DHEA, cortisol decreased both immediately post- and 1 hour post-exercise compared to pre-exercise. Independent activation of the zona fasciculata and zona reticularis has been noted previously (Velardo et al., 1991). A decrease in cortisol post-exercise has also been reported in post-menopausal women (Kemmler et al., 2003). One reason given for the decrease in cortisol in chapter six was possibly due to diurnal variation as the exercise took place in the morning period. However, it was argued that this may not be the case based on when the exercise took place in relation to waking, such that the initial steep decline would already have passed prior to exercise. In addition, diurnal variation may also be discounted on the basis of previous research that found an increase in cortisol in older females at the same time of day (Traustadottir et al., 2004). It is more likely that, although participants were exercised to 75% of their predicted maximum capacity, as the exercise was graded, and not at a predetermined intensity, they did not exercise for a sufficient period at 60% $VO_{2max}$ or above required to stimulate an increase in cortisol (Bishop, 2006; Pedersen and Hoffman-Goetz, 2000).

As a result of the decrease in cortisol, which was not apparent with DHEA/S, the cortisol:DHEA/S ratio significantly decreased immediately and 1h post-exercise. This represents a more favourable endocrine profile; however, this effect may be limited to the

morning period, and to exercise protocols of this nature. The ability of an acute exercise bout to increase DHEA/S in older adults may afford a non-pharmacological method for increasing anabolic hormones. However, as levels returned back to baseline within an hour, the benefits of such an increase are debatable. Exercise of a longer duration and higher intensity may be required in order to elevate and maintain DHEA/S levels for a significant period post-exercise, although this may also be accompanied by an increase in cortisol. Studies investigating exercise bouts at different times of day and at different intensities/durations are required to determine if there is an optimal period and type of exercise for older adults perform from a hormonal perspective.

## 7.8. LIMITATIONS

The studies in this thesis suffer from several limitations. Small sample size is a common problem among all studies, which may have limited the power to detect statistically significant effects in some cases. Significant findings did emerge from all studies but they may have been found in relation to more variables examined if more individuals were recruited. However, studies in this area of research often suffer from small sample size; this is most likely due to challenges in recruiting older adults from the community. In addition, the difficulties in recruiting older adults who do not suffer from conditions which could potentially affect the HPA axis and thus confound the findings. On the other hand, the sample size of studies of this nature is also restricted by the expense of hormone analysis. Therefore, even if recruitment was to continue for extended periods to capture more participants, resources may be the limiting factor. Further, as hormones such as cortisol have been shown to be subject to seasonal variation and perceived seasonality (Persson et al., 2008; Thorn et al., 2009), it is important to collect data within the same season. Further limitations in

relation to the participants in these studies also exist. Chapters two and three included extreme age groups only and would have benefited from a middle-aged cohort for further age comparisons. Chapter four originally aimed to include frail and non-frail older adults, but older adults still living in the community were generally not especially frail; thus differences in physical function as a precursor to frailty were examined as an alternative. In addition, participants were generally of a relatively high socio-economic status, as is characteristic of study volunteers and is associated with better health (Anderson and Armstead, 1995). Therefore, these findings cannot be generalised to populations of a lower socio-economic status, or who are not white in ethnicity.

A limitation which applies to chapters two through to five, is that compliance of saliva sampling was measured subjectively using a sampling diary and oral self report. In order to maximise compliance, older adults were provided with wristbands where they could write reminders and young adults were asked to put reminders in their mobile phones. When sampling instructions and packs were given to participants, honesty in reporting when samples were taken and sampling delays was stressed. Devices are emerging in psychobiological research that allow automated recording of sampling, but the use of such devices was beyond the scope of the resources available for research reported in this thesis. Further, ideally more samples would have been collected to capture all hours of waking. In hindsight, papers one to four may have benefited from a sample prior to participants sleeping, as 12h may not have been long enough to include all hours of waking. However, this could have been problematic in study one which included undergraduate students, who have a tendency to go out in the later hours of the evening and consume alcohol. Ideally, if financial resources permitted, samples would have been collected over a two day period and then

averaged, as recognised in the Discussion sections of chapter one to four; some studies have shown that the CAR is influenced by state factors and therefore subject to day-to-day variability (Hellhammer et al., 2007; Stalder et al., 2009; Thorn et al., 2009). However, there is also evidence there is evidence that the diurnal profile of cortisol and DHEA (Edwards et al., 2001a; Edwards et al., 2001b; Hucklebridge et al., 2005) and the CAR (Clow et al., 2004; Pruessner et al., 1997; Wüst et al., 2000) show reasonable temporal stability and do not significantly differ when measured over a two day period.

A limitation of the final study, presented in this thesis in chapter six, was the lack of maximal exercise testing, and therefore the inability to prescribe a steady state bout of exercise. As mentioned in this chapter, maximal exercise testing in older adults raises a number of issues (Hugget et al., 2005) and is difficult to obtain in practice. Not only may a maximal exercise testing protocol struggle to gain ethical approval, it might also have deterred the sedentary elderly from participating.

## 7.9. FUTURE DIRECTIONS

The associations demonstrated in this thesis could be used in future research in various ways. Certain aspects of this thesis require replicating in larger studies to support the current findings; this issue aside, there are numerous ways in which the present research could be taken forward. One route which could be particularly valuable would be intervention studies with older adults, be it to reduce the impact of stress or to protect against or reverse changes in physical function, and exercise would be an ideal candidate. The findings of study two suggest that habitual exercise may buffer against stress; therefore it would be interesting to see whether or not an exercise intervention would be able to reduce the cortisol:DHEA ratio

in stressed older adults who do not currently exercise. Interventions with a psychosocial focus, such as stress management techniques, might also be deployed to buffer against stress in older populations.

Although long term exercise did not influence hormone levels in the final study, this study included individuals who were healthy. It may be that exercise interventions may be most relevant to older adults in a poor state of health or at risk of future adverse health outcomes. Therefore, exercise is a strategy that should be explored among such individuals to see if it can maintain or improve the ageing endocrine system. Further, exercise may be a valuable tool to preserve or reverse changes in physical function and consequently reduce the risk of frailty in older age. Of course, interventions have to be designed so that they are of a sufficient time course and contain exercise programmes likely to achieve benefits to an individual. In addition to intervention studies, mechanisms underlying the associations presented were alluded to, although could only be speculated upon. Future research could take a mechanistic and longitudinal approach in order to more strongly indicate causality and better understand the reasons for these relationships. In addition, longitudinal studies would allow the future impact of these associations for health to be observed. This thesis focused on the HPA axis; however, other aspects of the neuroendocrine system may also be associated with behavioural and psychosocial variables. Future research should examine how alterations in various endocrine parameters impact upon other physiological systems in the context of health and wellbeing.

## 7.10. CONCLUSION

In conclusion, the present studies demonstrate a range of associations between behavioural and psychosocial factors and cortisol and DHEA among individuals of different ages. The findings in relation to diurnal cortisol and DHEA suggest that the level of these hormones in the morning period may be particularly important. Generally, it was alterations at this time of day that were associated with negative variables, be it depression, reduced physical function, poorer diet, or less engagement in exercise. This feature of diurnal profiles may be significant, as secretion in the morning period could pre-determine levels for the rest of the day. The findings support evidence that these hormones and their diurnal rhythms are central to various aspects of health and wellbeing, and this relationship may be affected by age. Although it is important to establish patterns of hormonal regulation in identification with behavioural or psychosocial measures, individual variability should not be forgotten. In addition, both flatter diurnal profiles and increases in amplitude in relation to certain parameters were observed, suggesting that general deviations away from an individual's normal pattern of secretion of these hormones may be meaningful, regardless of the nature of their appearance. Finally, exercise interacts with cortisol:DHEA and psychosocial factors, whereby it may exert a protective effect on the cortisol:DHEA ratio against stress in older adults. Although long term exercise training did not alter baseline hormonal levels or attenuate age-associated changes in healthy older adults, an acute bout of exercise can affect hormonal levels in older populations and this appears to be influenced by sex.

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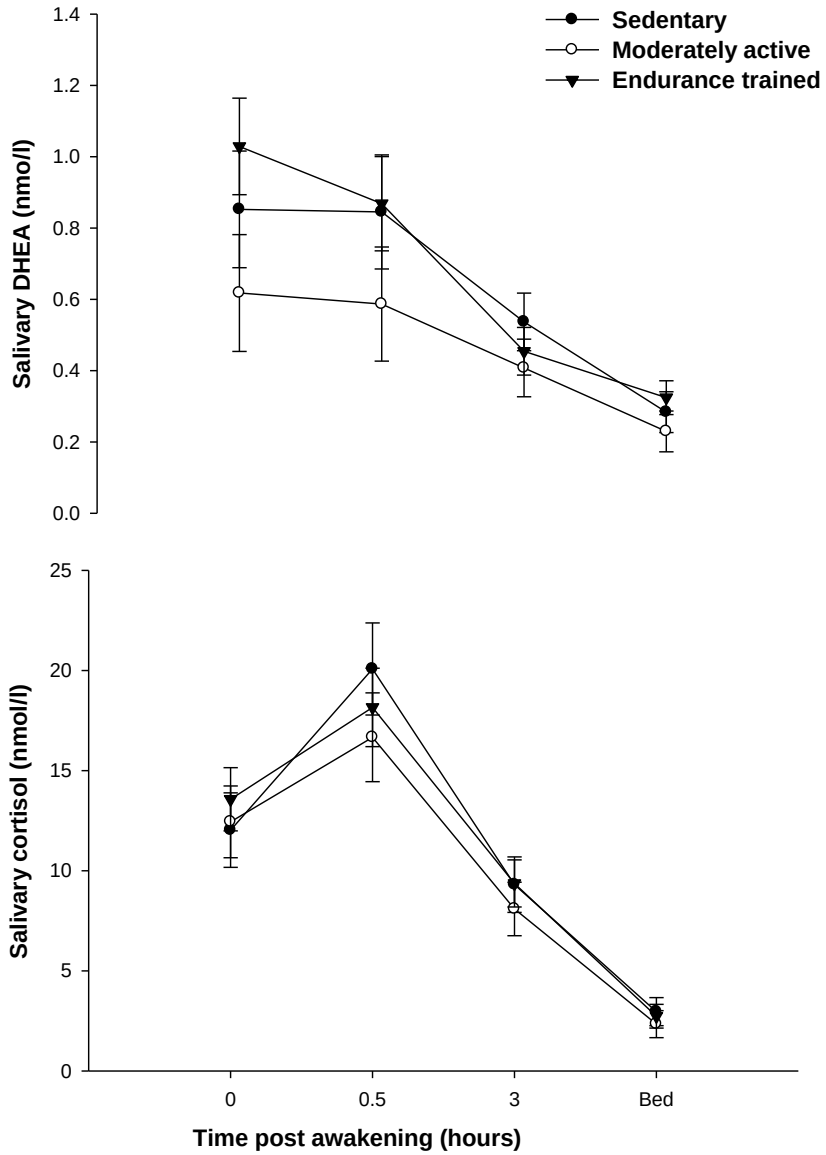
# 8.0. APPENDIX

## 8.1. ADDITIONAL FINDINGS FROM CHAPTER SIX

The below figures show the diurnal rhythms of salivary cortisol and DHEA collected from participants of study 3, presented in Chapter Six. There were no significant differences between exercise training status groups. Saliva was collected via a 4 minute passive drool and processed and analysed as described in Chapters Four and Five.



## 8.2. HEALTH BEAVIOURS QUESTIONNAIRE

**Please circle the appropriate answer**

| | | | | | | |
|---|---|---|---|---|---|---|
| Over the last year, how many cigarettes, on average, did you smoke per day? | None | 1-5 | 6-10 | 11-20 | 21+ | 40+ |
| Over the last year, on average, how often have you taken an alcoholic drink? | Never | Special Occasions only | 1-2 per month | 1-2 per week | Almost daily | 2 per day or more |

For the following question, please base your answers on the following:

**1 unit = ½ pint of beer, 1 small glass of wine, 1 measure of spirit**

Remember that home poured measures are likely to be larger

**1 bottle of wine = 6 glasses, 1 average bottle of spirits = 27 measures**

| | | | | | | |
|---|---|---|---|---|---|---|
| Over the last year, on average, how many units did you drink per week? | None | 1-5 | 6-10 | 11-20 | 20-40 | 41+ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Over the last year, how many hours, on average did you sleep per night? | 0-3 | 4-5 | 6-7 | 8-9 | 10-11 | 12+ |
| Over the last year, how often have you taken vitamin/mineral supplements? | Never | Once a month | Once a week | A few per week | Every day | More than one per day |

**Please answer the following questions with reference to your diet over the last year.**

**Answer as honestly and accurately as possible.**

| | | | | | |
|---|---|---|---|---|---|
| Are you on a special diet of any sort? | No | Vegetarian | Vegan | Weight-loss | Other |

If other, please state…….

| | | | | | |
|---|---|---|---|---|---|
| How often do you eat breakfast? | Every day | Most days (3-6) | Once or twice a week | Less than once a week | Never |

Apart from breakfast, how many main/cooked meals do you usually have during the day?

**.....**

How many cups/cans of caffeinated drink (coffee/tea/cola) do you usually drink in a day?

**.....**

In a typical week, please indicate how often you have eaten each of the following foods **over the past year.**

| | Never | Less than once a week | 1 or 2 a week | Most days (3-6) | Once a day | 2-3 times a day | 4 or more times a day |
|---|---|---|---|---|---|---|---|
| Fresh fruit/salad/raw veg. | | | | | | | |
| Cooked veg. (not potatoes) | | | | | | | |
| Chips/fried food | | | | | | | |
| Potatoes/pasta/rice | | | | | | | |
| Bread (2 slices=one portion) | | | | | | | |
| Crisps/similar | | | | | | | |
| Tea | | | | | | | |
| Sweets/Chocolate | | | | | | | |
| Breakfast cereal | | | | | | | |
| Biscuits/cakes/puddings | | | | | | | |
| Low fat snack bars | | | | | | | |
| Full fat dairy products | | | | | | | |
| Reduced fat dairy products | | | | | | | |
| Fish/seafood (not fried) | | | | | | | |
| Poultry (not fried) | | | | | | | |
| Processed meat (e.g. pasties, | | | | | | | |
| Beef/lamb/pork/ham/ bacon | | | | | | | |
| Soft drinks (non-caffeinated) | | | | | | | |
| Pure fruit juice | | | | | | | |

## 8.3. HOSPITAL ANXIETY AND DEPRESSION SCALE

This questionnaire is designed to help us understand how you feel. Read each item and circle the reply which comes closest to how you have been feeling in the past week.

Don't take too long over your replies, your immediate reaction to each item will probably be more accurate than a long thought-out response.

| | | | | |
|---|---|---|---|---|
| **1. I feel tense or 'wound up'** | Most of the time | A lot of the time | From time to time, occasionally | Not at all |
| **2. I still enjoy the things I used to enjoy** | Definitely as much | Not quite so much | Only a little | Hardly at all |
| **3. I get a sort of frightened feeling as if something awful is about to happen** | Very definitely & quite badly | Yes, but not too badly | A little, but it doesn't worry me | Not at all |
| **4. I can laugh and see the funny side of things** | As much as I always could | Not quite so much now | Definitely not so much now | Not at all |
| **5. Worrying thoughts go through my mind** | A great deal of the time | A lot of the time | From time to time but not too often | Only occasionally |
| **6. I feel cheerful** | Not at all | Not often | Sometimes | Most of the time |
| **7. I can sit at ease and feel relaxed** | Definitely | Usually | Not often | Not at all |
| **8. I feel as if I am slowed down** | Nearly all the time | Very often | Sometimes | Not at all |
| **9. I get a sort of frightened feeling like 'butterflies' in the stomach** | Not at all | Occasionally | Quite often | Very often |
| **10. I have lost interest in my appearance** | Definitely | I don't take as much care as I should | I may not take quite as much care | I take just as much care as ever |
| **11. I feel restless as if I have to be on the move** | Very much indeed | Quite a lot | Not very much | Not at all |
| **12. I look forward with enjoyment to things** | As much as ever I did | Rather less than I used to | Definitely less than I used to | Hardly at all |
| **13. I get sudden feelings of panic** | Very often indeed | Quite often | Not very often | Not at all |
| **14. I can enjoy a good book, radio or TV programme** | Often | Sometimes | Not often | Very seldom |

## 8.4. MEDICAL OUTCOMES STUDY SOCIAL SUPPORT SURVEY

About how many close friends and close relatives do you have (people you feel at ease with and can talk to about what is on your mind)? _____

| People sometimes look to others for companionship, assistance, or other types of support. How often is each of the following kinds of support available to you if you need it? | None of the time | A little of the time | Some of the time | Most of the time | All of the time |
|---|---|---|---|---|---|
| **1.** Someone to help you if you were confined to bed | | | | | |
| **2.** Someone you can count on to listen to you when you need to talk | | | | | |
| **3.** Someone to give you good advice about a crisis | | | | | |
| **4.** Someone to take you to the doctor if you needed it | | | | | |
| **5.** Someone who shows you love and affection | | | | | |
| **6.** Someone to have a good time with | | | | | |
| **7.** Someone to give you information to help you understand a situation | | | | | |
| **8.** Someone to confide in or talk to about yourself or your problems | | | | | |
| **9.** Someone who hugs you | | | | | |
| **10.** Someone to get together with for relaxation | | | | | |
| **11.** Someone to prepare your meals if you were unable to do it yourself | | | | | |
| **12.** Someone whose advice you really want | | | | | |
| **13.** Someone to do things with to help you get your mind off things | | | | | |
| **14.** Someone to help with daily chores if you were sick | | | | | |
| **15.** Someone to share your most private worries and fears with | | | | | |
| **16.** Someone to turn to for suggestions about how to deal with a personal problem | | | | | |
| **17.** Someone to do something enjoyable with | | | | | |
| **18.** Someone who understands your problems | | | | | |
| **19.** Someone to love and make you feel wanted | | | | | |

## 8.5. THE LIFE EVENTS SCALE FOR STUDENTS (LESS)

| IN THE LAST YEAR...Has this event happened to you? | | | | If yes, please indicate how stressful you found the event | | | |
|---|---|---|---|---|---|---|---|
| | | **Yes** | **No** | **Not at all** | **A little** | **Moderately** | **Very** |
| 1 | Death of Parent | | | | | | |
| 2 | Major personal injury | | | | | | |
| 3 | Major argument with parents | | | | | | |
| 4 | Beginning an undergraduate or graduate program at university | | | | | | |
| 5 | Moving away from home | | | | | | |
| 6 | Getting an unjustified low mark on a test | | | | | | |
| 7 | Failing a number of courses | | | | | | |
| 8 | Minor violation of the law (e.g. speeding ticket) | | | | | | |
| 9 | Getting kicked out of college | | | | | | |
| 10 | Seeking psychological or psychiatric consultation | | | | | | |
| 11 | Vacation alone/with friends | | | | | | |
| 12 | Pregnancy (either yourself or being father) | | | | | | |
| 13 | Minor car accident | | | | | | |
| 14 | Seriously thinking about dropping college | | | | | | |
| 15 | Getting your own car | | | | | | |
| 16 | Jail term (self) | | | | | | |
| 17 | Moving out of town with parents | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 18 | Vacation with parents | | | | | | |
| 19 | Establishing a new steady relationship with partner | | | | | | |
| 20 | Finding a part time job | | | | | | |
| 21 | Sex difficulties with boy/girlfriend | | | | | | |
| 22 | Failing a course | | | | | | |
| 23 | Major change in health in close family member | | | | | | |
| 24 | Major car accident (car wrecked, people injured) | | | | | | |
| 25 | Death of your best or very good friend | | | | | | |
| 26 | Family get-togethers | | | | | | |
| 27 | Break-up of parent's marriage | | | | | | |
| 28 | Losing part-time job | | | | | | |
| 29 | Major and/or chronic financial problems | | | | | | |
| 30 | Major argument with boy/girlfriend | | | | | | |
| 31 | Parent losing job | | | | | | |
| 32 | Switch in program within same college or university | | | | | | |
| 33 | Losing a good friend | | | | | | |
| 34 | Change of job | | | | | | |
| 35 | Break-up with boy/girlfriend | | | | | | |
| 36 | Minor financial problems | | | | | | |

## 8.6. LIFE EVENTS SURVEY

In each section below, there are examples of the sort of worrying things that might have happened in different areas of your life. I would like you to tick if the event happened to you *in the **past YEAR.***

For each event that happened either to you, I would like you to give it a score of how serious (stressful, worrying, disruptive) a problem it was on a scale of 1 to 10, where 1 is something really small and unimportant and 10 is the worst thing that could ever happen to you.

For example, if you crashed your car since your recent vaccination and this was the worst thing that ever happened to you, you would tick "happened to me" and circle number 10.

*Example*

| | | ✓ |
|---|---|---|
| *Crashed car* | *Happened to me* | ✓ |
| | *Didn't happen* | |

**How serious?**

**Not at all** **Worst**

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|

***Health***

**How serious?**

**Not at all** **Worst**

| | | | |
|---|---|---|---|
| 1. An unexpected illness | Happened to me | | 1 2 3 4 5 6 7 8 9 10 |
| | Didn't happen | | |
| 2. Period in hospital | Happened to me | | 1 2 3 4 5 6 7 8 9 10 |
| | Didn't happen | | |
| 3. An operation | Happened to me | | 1 2 3 4 5 6 7 8 9 10 |
| | Didn't happen | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4. Serious illness diagnosed | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 5. An existing condition got worse | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 6. Depression or nerves | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 7. Painful or upsetting treatment | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 8. Serious accident causing injury | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 9. Developing a handicap | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 10. A period of poor health | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 11. Other worries about health | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 12. Any other health problems | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

## Marriage / Cohabitation

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 13. Living apart, divorce or split from long term partner | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 14. Serious row or disagreement | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 15. Difficult spells | Happened to me | | → | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

| | | |
|---|---|---|
| in the marriage or partnership | Didn't happen | |
| 16. Other problems in the marriage or partnership | Happened to me | |
| | Didn't happen | |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|

***Relationships***

| | | |
|---|---|---|
| 17. Serious disagreement within family | Happened to me | |
| | Didn't happen | |
| 18. Serious disagreement with friends | Happened to me | |
| | Didn't happen | |
| 19. End of a relationship | Happened to me | |
| | Didn't happen | |
| 20. Seeing much less of family | Happened to me | |
| | Didn't happen | |
| 21. Seeing much less of friends | Happened to me | |
| | Didn't happen | |
| 22. Problems with children | Happened to me | |
| | Didn't happen | |
| 23. Other problems with relationships | Happened to me | |
| | Didn't happen | |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|

***Deaths***

| | | |
|---|---|---|
| 24. Spouse / partner died | Happened to me | |
| | Didn't happen | |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 25. Other household member died | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 26. Other close family (parent, child, sibling) | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 27. Other more distant family died | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 28. Friend died | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

***Work***

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 29. Made redundant or changed work | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 30. On strike | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 31. Un-employment | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 32. Enforced retirement | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 33. Change for worse at work | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 34. Serious rows at work | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 35. Difficulty in business venture | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 36. Other work problems | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

***Housing***

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 37. Problems moving house | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 38. Worries over poor housing | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 39. Problem with landlord or council | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 40. Difficulties over mortgage or rent | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 41. Damage or repairs to house | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 42. Problems with neighbours | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 43. Problems in the neighbour-hood | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 44. Other housing problems | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

***Finances***

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 45. Problems paying bills | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 46. A drop in income | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 47. Difficulties in paying a loan | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 48. Other financial problems | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

***General***

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 49. Having to give up an activity | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 50. Burglary or theft | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 51. Losing something important | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 52. Violence, being attacked | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 53. Problems with officials | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 54. Legal or police problems | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 55. Problems gambling or | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| drinking | Didn't happen | | | | | | | | | | |
| 56. Problems driving or on the road | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 57. Giving someone bad news | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 58. Seeing something distressing | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

***Other***

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 59. Any other events? If so, what?……. | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |
| 60. Any other events? If so, what?……. | Happened to me | | ⇨ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| | Didn't happen | | | | | | | | | | | | |

## 8.7. OLDER ADULT LIFE EVENTS QUESTIONNAIRE

We know you have already answered questions like these, but we are trying to validate a new stress scale for older adults. We would be very grateful if you would tell us which of these events have happened to you and rate how stressful they are if they have happened to you in the **last year**.

| Has this event happened to you in the last year? | | | **If yes, please indicate how stressful you found the event** | | | |
|---|---|---|---|---|---|---|
| **Please tick the appropriate answer** | **Yes** | **No** | **Not at all** | **A little** | **Moderately** | **Very** |
| **Health** | | | | | | |
| 1. Problem with medication<br>2. e.g. side effects, forgetting to take it, changes in medication etc. | | | | | | |
| 3. Been to hospital or doctors for tests | | | | | | |
| 4. Been to hospital or doctors for treatment or operation | | | | | | |
| 5. Had regular checkups at doctor | | | | | | |
| 6. New health problem or condition diagnosed | | | | | | |
| 7. Terminal illness diagnosed | | | | | | |
| 8. Current health problem got worse | | | | | | |
| 9. Short term illness | | | | | | |
| 10. Poor service when in hospital e.g. poor medical care, poor nursing care, poor accommodation etc. | | | | | | |
| **Family** | | | | | | |
| 11. Had to sort out finances of a family member | | | | | | |
| 12. Deterioration in health of a family member | | | | | | |
| 13. Deterioration of health of spouse or partner | | | | | | |
| 14. Seen family less often than would like | | | | | | |
| 15. Tension over a special occasion (e.g. wedding, Christmas) | | | | | | |
| 16. Family member diagnosed with a serious illness | | | | | | |

| | Yes | No | Not at all | A little | Moderately | Very |
|---|---|---|---|---|---|---|
| 17. Had to make or change a Will | | | | | | |
| 18. Had to travel long distance to see family | | | | | | |
| 19. Frequently listened to other people's problems | | | | | | |
| 20. Argument(s) with family members | | | | | | |
| 21. Problem with pet (e.g. pet dies, illness, expensive treatment, disruptive behaviour, mess) | | | | | | |
| 22. New family issue caused increased worry | | | | | | |
| **Home & Neighbourhood** | | | | | | |
| 23. Moved or moving house<br>24. e.g. high cost, complications, disruptions, forced to move house, dealing with solicitors, | | | | | | |
| 25. Problem with neighbours | | | | | | |
| 26. Crime in neighbourhood | | | | | | |
| 27. Mugging or attempted mugging | | | | | | |
| 28. Burglary of my home | | | | | | |
| 29. Vandalism e.g. graffiti in neighbourhood, damage to my home | | | | | | |
| 30. Needed to get help in the home e.g. handyman for repairs | | | | | | |
| **Marriage & Relationships** | | | | | | |
| 31. Divorce | | | | | | |
| 32. Separation | | | | | | |
| 33. Had a negative change in your relationship with your partner | | | | | | |
| 34. Loss of a friendship | | | | | | |
| 35. Friend diagnosed with a serious illness | | | | | | |
| **Care** | | | | | | |
| 36. Had to move into a care home | | | | | | |
| 37. Friend or family member put into a care home | | | | | | |
| 38. Had to care for spouse or partner with illness or disability | | | | | | |

| | Yes | No | Not at all | A little | Moderately | Very |
|---|---|---|---|---|---|---|
| 39. Had to care for child with illness or disability | | | | | | |
| 40. Had to care for a friend or neighbour | | | | | | |
| 41. Change in my ability to care for myself | | | | | | |
| 42. Change in my ability to care for others | | | | | | |
| **Bereavement** | | | | | | |
| 43. Death of close family member (child, grandchild, sibling, parent) | | | | | | |
| 44. Death of spouse or partner | | | | | | |
| 45. Death of friend | | | | | | |
| 46. Attended increased number of funerals | | | | | | |
| 47. Death of other person (more distant family or distant friend) | | | | | | |
| **Finance** | | | | | | |
| 48. Problem paying bills | | | | | | |
| 49. Living costs increased e.g. insurance, council tax, cost of travel | | | | | | |
| 50. Had little or no pension | | | | | | |
| 51. Difficulty finding access to or getting financial help or benefits | | | | | | |
| | | | | | | |
| **Work** | | | | | | |
| 52. Retirement problem e.g. having to retire, missing colleagues, miss work | | | | | | |
| 53. Problem at work e.g. too much work, deadline pressure, relationships with colleagues, time management issue | | | | | | |
| 54. Difficulty juggling work and other commitments | | | | | | |
| 55. Difficult changes at work | | | | | | |
| 56. Problem with partner's work | | | | | | |
| **Ageing** | | | | | | |
| 57. Deterioration of hearing | | | | | | |

| | Yes | No | Not at all | A little | Moderately | Very |
|---|---|---|---|---|---|---|
| 58. Deterioration of eye sight | | | | | | |
| 59. Dental problems | | | | | | |
| 60. Reduced mobility e.g. unable to walk far or drive or get out of the bath or go up stairs | | | | | | |
| 61. Memory problems got worse | | | | | | |
| 62. Difficulty getting insurance because of age | | | | | | |
| 63. People misjudged capabilities because of age | | | | | | |
| 64. Change in attitude from others because of age e.g. lack of respect | | | | | | |
| 65. Problem with driving (e.g. having to give up, traffic) | | | | | | |
| 66. Difficulty using technology (e.g. computer, internet, mobile phone) | | | | | | |
| **Outside agencies** | | | | | | |
| 67. Needed to sort out a problem (e.g. with bank, social services) | | | | | | |
| 68. Threat of legal action | | | | | | |
| 69. Needed help with daily tasks but difficult to get support | | | | | | |
| 70. Difficulties with telephone (e.g. problems with phone centres, being put on hold, hassle from companies phoning the house). | | | | | | |

## 8.8. THE BERG BALANCE SCALE

Please document each task and/or give instructions as written. When scoring, please record the lowest response category that applies for each item.

In most items, the subject is asked to maintain a given position for a specific time.

Progressively more points are deducted if:

• the time or distance requirements are not met

• the subject's performance warrants supervision

• the subject touches an external support or receives assistance from the examiner

Subject should understand that they must maintain their balance while attempting the tasks. The choices of which leg to stand on or how far to reach are left to the subject. Poor judgment will adversely influence the performance and the scoring.

**SITTING TO STANDING**

INSTRUCTIONS: Please stand up. Try not to use your hand for support.

( ) 4 able to stand without using hands and stabilize independently

( ) 3 able to stand independently using hands

( ) 2 able to stand using hands after several tries

( ) 1 needs minimal aid to stand or stabilize

( ) 0 needs moderate or maximal assist to stand

**STANDING UNSUPPORTED**

INSTRUCTIONS: Please stand for two minutes without holding on.

( ) 4 able to stand safely for 2 minutes

( ) 3 able to stand 2 minutes with supervision

( ) 2 able to stand 30 seconds unsupported

( ) 1 needs several tries to stand 30 seconds unsupported

( ) 0 unable to stand 30 seconds unsupported

If a subject is able to stand 2 minutes unsupported, score full points for sitting unsupported. Proceed to item #4.

**SITTING WITH BACK UNSUPPORTED BUT FEET SUPPORTED ON FLOOR OR ON A STOOL**

INSTRUCTIONS: Please sit with arms folded for 2 minutes.

( ) 4 able to sit safely and securely for 2 minutes

( ) 3 able to sit 2 minutes under supervision

( ) 2 able to able to sit 30 seconds

( ) 1 able to sit 10 seconds

( ) 0 unable to sit without support 10 seconds

**STANDING TO SITTING**

INSTRUCTIONS: Please sit down.

( ) 4 sits safely with minimal use of hands

( ) 3 controls descent by using hands

( ) 2 uses back of legs against chair to control descent

( ) 1 sits independently but has uncontrolled descent

( ) 0 needs assist to sit

**TRANSFERS**

INSTRUCTIONS: Arrange chair(s) for pivot transfer. Ask subject to transfer one way toward a seat with armrests and one way toward a seat without armrests. You may use two chairs (one with and one without armrests) or a bed and a chair.

( ) 4 able to transfer safely with minor use of hands

( ) 3 able to transfer safely definite need of hands

( ) 2 able to transfer with verbal cuing and/or supervision

( ) 1 needs one person to assist

( ) 0 needs two people to assist or supervise to be safe

**STANDING UNSUPPORTED WITH EYES CLOSED**

INSTRUCTIONS: Please close your eyes and stand still for 10 seconds.

( ) 4 able to stand 10 seconds safely

( ) 3 able to stand 10 seconds with supervision

( ) 2 able to stand 3 seconds

( ) 1 unable to keep eyes closed 3 seconds but stays safely

( ) 0 needs help to keep from falling

**STANDING UNSUPPORTED WITH FEET TOGETHER**

INSTRUCTIONS: Place your feet together and stand without holding on.

( ) 4 able to place feet together independently and stand 1 minute safely

( ) 3 able to place feet together independently and stand 1 minute with supervision

( ) 2 able to place feet together independently but unable to hold for 30 seconds

( ) 1 needs help to attain position but able to stand 15 seconds feet together

( ) 0 needs help to attain position and unable to hold for 15 seconds

**REACHING FORWARD WITH OUTSTRETCHED ARM WHILE STANDING**

INSTRUCTIONS: Lift arm to 90 degrees. Stretch out your fingers and reach forward as far as you can. (Examiner places a ruler at the end of fingertips when arm is at 90 degrees. Fingers should not touch the ruler while reaching forward. The recorded measure is the distance forward that the fingers reach while the subject is in the most forward lean position. When possible, ask subject to use both arms when reaching to avoid rotation of the trunk.)

( ) 4 can reach forward confidently 25 cm (10 inches)

( ) 3 can reach forward 12 cm (5 inches)

( ) 2 can reach forward 5 cm (2 inches)

( ) 1 reaches forward but needs supervision

( ) 0 loses balance while trying/requires external support

**PICK UP OBJECT FROM THE FLOOR FROM A STANDING POSITION**

INSTRUCTIONS: Pick up the shoe/slipper, which is place in front of your feet.

( ) 4 able to pick up slipper safely and easily

( ) 3 able to pick up slipper but needs supervision

( ) 2 unable to pick up but reaches 2-5 cm(1-2 inches) from slipper and keeps balance independently

( ) 1 unable to pick up and needs supervision while trying

( ) 0 unable to try/needs assist to keep from losing balance or falling

**TURNING TO LOOK BEHIND OVER LEFT AND RIGHT SHOULDERS WHILE STANDING**

INSTRUCTIONS: Turn to look directly behind you over toward the left shoulder. Repeat to the right. Examiner may pick an object to look at directly behind the subject to encourage a better twist turn.

( ) 4 looks behind from both sides and weight shifts well

( ) 3 looks behind one side only other side shows less weight shift

( ) 2 turns sideways only but maintains balance

( ) 1 needs supervision when turning

( ) 0 needs assist to keep from losing balance or falling

**TURN 360 DEGREES**

INSTRUCTIONS: Turn completely around in a full circle. Pause. Then turn a full circle in the other direction.

( ) 4 able to turn 360 degrees safely in 4 seconds or less

( ) 3 able to turn 360 degrees safely one side only 4 seconds or less

( ) 2 able to turn 360 degrees safely but slowly

( ) 1 needs close supervision or verbal cuing

( ) 0 needs assistance while turning

**PLACE ALTERNATE FOOT ON STEP OR STOOL WHILE STANDING UNSUPPORTED**

INSTRUCTIONS: Place each foot alternately on the step/stool. Continue until each foot has touch the step/stool four times.

( ) 4 able to stand independently and safely and complete 8 steps in 20 seconds

( ) 3 able to stand independently and complete 8 steps in > 20 seconds

( ) 2 able to complete 4 steps without aid with supervision

( ) 1 able to complete > 2 steps needs minimal assist

( ) 0 needs assistance to keep from falling/unable to try

**STANDING UNSUPPORTED ONE FOOT IN FRONT**

INSTRUCTIONS: (DEMONSTRATE TO SUBJECT) Place one foot directly in front of the other. If you feel that you cannot place your foot directly in front, try to step far enough ahead that the heel of your forward foot is ahead of the toes of the other foot. (To score 3 points, the length of the step should exceed the length of the other foot and the width of the stance should approximate the subject's normal stride width.)

( ) 4 able to place foot tandem independently and hold 30 seconds

( ) 3 able to place foot ahead independently and hold 30 seconds

( ) 2 able to take small step independently and hold 30 seconds

( ) 1 needs help to step but can hold 15 seconds

( ) 0 loses balance while stepping or standing

**STANDING ON ONE LEG**

INSTRUCTIONS: Stand on one leg as long as you can without holding on.

( ) 4 able to lift leg independently and hold > 10 seconds

( ) 3 able to lift leg independently and hold 5-10 seconds

( ) 2 able to lift leg independently and hold ≥ 3 seconds

( ) 1 tries to lift leg unable to hold 3 seconds but remains standing independently.

( ) 0 unable to try of needs assist to prevent fall ( ) TOTAL SCORE (Maximum = 56)

## 8.9. NOTTINGHAM INDEPENDENCE IN ACTIVITIES OF DAILY LIVING INDEX

For each activity, please tick the response which most describes your ability

| Can you…..? | **Not at all** | **With help** | **Alone with difficulty** | **Alone easily** |
|---|---|---|---|---|
| **MOBILITY** | | | | |
| - walk around outside? | | | | |
| - climb stairs? | | | | |
| - get in and out of the car? | | | | |
| - walk over uneven ground? | | | | |
| - cross roads? | | | | |
| - travel on public transport? | | | | |
| **IN THE KITCHEN** | | | | |
| - manage to feed yourself? | | | | |
| - make yourself a hot drink? | | | | |
| - take hot drinks from one room to another? | | | | |
| - do the washing up? | | | | |
| - make yourself a hot snack? | | | | |
| **DOMESTIC TASKS** | | | | |
| - manage your own money when out? | | | | |
| - wash small items of clothing? | | | | |
| - do your own shopping? | | | | |
| - do a full clothes wash? | | | | |
| **LEISURE ACTIVITIES** | | | | |
| - read newspapers and books? | | | | |
| - use the telephone? | | | | |
| - write letters? | | | | |
| - go out socially? | | | | |
| - manage your own garden? | | | | |
| - drive a car? | | | | |

## 8.10. HANDGRIP STRENGTH CRITERIA

If an individual has a grip strength value below that outlined for their sex and BMI then they would meet the criteria for frailty on this outcome (Amed et al., 2007).

| **MALE** | | **FEMALE** | |
|---|---|---|---|
| **BMI** | **GRIP STRENGTH** | **BMI** | **GRIP STRENGTH** |
| ≤ 24 | ≤ 29 | ≤ 23 | ≤ 17 |
| 24.1 – 26 | ≤ 30 | 23.1 – 26 | ≤ 17.3 |
| 26.1 - 28 | ≤ 30 | 26.1 – 29 | ≤ 18 |
| > 28 | ≤ 32 | >29 | ≤ 21 |

## 8.11. EXERCISE QUESTIONNARES

### *8.11.1. Exercise questionnaire from the Whitehall study used to generate exercise scores*

Over the last year, how many hours per week on average, have you spent participating in activities which are:

| | | | | | | |
|---|---|---|---|---|---|---|
| Mildly energetic e.g. walking? | 0 | 1-2 | 3-5 | 6-8 | 9-10 | 11+ |
| Moderately energetic e.g. leisurely swimming, golf | 0 | 1-2 | 3-5 | 6-8 | 9-10 | 11+ |
| Vigorously energetic e.g. running, squash | 0 | 1-2 | 3-5 | 6-8 | 9-10 | 11+ |

### *8.11.2. Exercise questionnaire from the West of Scotland study used to assign physical activity levels*

Choose one activity category that best describes your usual pattern of exercise, related to transportation, occupation, exercise and wellness, and leisure or recreational purposes

| | |
|---|---|
| **Level 1** | Inactive or little activity other than usual activities of daily living |
| **Level 2** | Regularly (≥ 5 days per week) participate in physical activity requiring a low level of exertion that result in slight increases in breathing and heart rate for at least 10 minutes at a time |
| **Level 3** | Participate in aerobic exercise such as brisk walking, jogging or running, cycling, swimming, or vigorous sports at a comfortable pace or other activities requiring similar levels of exertion for **20 to 60 minutes** per week |
| **Level 4** | Participate in aerobic exercises such as brisk walking, jogging or running at a comfortable pace, or other activities requiring similar levels of exertion for **1 to 3 hours** per week |
| **Level 5** | Participate in aerobic exercises such as brisk walking, jogging or running at a comfortable pace, or other activities requiring similar levels of exertion for **over 3 hours** per week |

## 8.12. 14 DAY EXERCISE DIARY

# Sport/Exercise History

1. Do you have a sport/sports that you participate in regularly? For example, you consider yourself a runner

_____

2. How long have you been participating in your sport/sports?

_____

3. At what level do you participate in this sport? For example, at a competitive level or for leisure?

If at a competitive level, please describe which level e.g. club, regional, national

_____

4. Did you used to take part in a sport regularly, but no longer due to injury or other reasons? If so, please say what you used to do and how long ago you gave it up

_____

# Diary

We would like you to keep a diary over the next two weeks (**14 consecutive days**) recording what physical activity you do. In this diary we would like you to record: what **type of activity** you did, the **duration** of the activity and the **intensity**. How to record these are explained on the next few pages, followed by the diary we would like you to keep.

**1. Types of physical activity**

This is simply the physical activity you participated in. For example: running, swimming, golf, walking, cycling, tennis, yoga, squash, lawn bowls, weight training, aerobics

In addition to naming the activity we would like you to say what **type** it was for individual activities (as opposed to team sports), such as running, cycling etc. Examples of training are below.

**Continuous**: This is activity without rest intervals

**Interval**: This involves bursts of work. Usually high intensity exercise is alternated with periods of rest or low activity

**2. Duration**

This is simply how long you participated in the activity for

**3. Intensity – Session RPE (rating of perceived exertion)**

If you participate in an activity we would like you to rate how intense you thought it was **using the scale and instructions** that follow. Please rate the **overall intensity** of the whole session and not just certain aspects of the session. Ideally, we would like you to complete this 30 minutes after you have completed the activity.

## INSTRUCTIONS

We ask you to rate your perceived exertion, that is, how effortful, strenuous and fatiguing the exercise felt to you.

This depends mainly on how hard you had to drive your legs and/or arms, and how heavy your breathing was.

10, *"Maximal" effort*, is the main anchor. It is the highest effort you have ever experienced.

When rating your perceived exertion, start with a *verbal expression* and then choose a number. If your perception of effort was *Very light*, rate 1; if it is *Moderate*, rate 3; and so on.

It is very important that you answer what you perceived and not what you believe you ought to answer. Be as honest as possible and try not to overestimate or underestimate the intensity of your effort.

**Examples**

| | |
|---|---|
| 1 | Is very light, like walking slowly at your own pace or lifting a small weight. |
| 3 | Is not especially hard; it feels fine, and it is no problem to continue. |
| 5 | You are tired, but you don't have any great difficulties. |
| 7 | You can still go on but have to push yourself very much; you are very tired. |
| 10 | You are working at your maximum effort. This is as hard as most people have ever experienced in their lives. |

## Rating of Perceived Exertion

| | |
|---|---|
| 0 | Rest |
| 1 | Very very easy |
| 2 | Easy |
| 3 | Moderate |
| 4 | Somewhat hard |
| 5 | Hard |
| 6 | |
| 7 | Very hard |
| 8 | |
| 9 | Very, very hard |
| 10 | “Maximal” effort |

# 14 Day Diary

**Example**

| Day | Activity and type | Duration | Session RPE (intensity) |
|---|---|---|---|
| 1 | Swimming, interval training | 20 minutes | 8 |
| 2 | Golf | 1 hr 30 minutes | 3 |

| **Day** | **Activity and type** | **Duration** | **Session RPE (intensity)** |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |
| 5 | | | |
| 6 | | | |
| 7 | | | |
| 8 | | | |
| 9 | | | |
| 10 | | | |
| 11 | | | |
| 12 | | | |
| 13 | | | |
| 14 | | | |