Title: Unemployment is associated with lower cortisol awakening and blunted dehydroepiandrosterone responses

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Abstract

Previous research has investigated the endocrinological consequences of unemployment as a likely pathway behind chronic stress and negative health outcomes. Despite these early attempts at delineating the neuroendocrine consequences of the chronic stress experienced by the unemployed, identifying a consistent and stable effect has remained elusive. Here we sought to strengthen existing knowledge into the effect of the stress of employment status on cortisol by improving on the methodological weaknesses of earlier studies and extend this line of enquiry by measuring the steroid hormone Dehydroepiandrosterone-Sulfate (DHEAS). Saliva samples were collected from unemployed and employed participants at four time points across two days. As expected, unemployed people reported higher stress, lower social support and lower self-esteem. Unexpectedly, the unemployed showed lower overall cortisol output, a likely consequence of a higher cortisol awakening response (CAR) in the employed. However, they also had a higher DHEA output across the day, albeit the diurnal pattern across the day was more dysregulated compared to that seen in those employed with a blunted response evident in the evening; the cortisol;DHEAS ratio was also lower in the unemployed group. Further, these hormone differences were correlated with self-esteem and stress. Taken together these results suggest that the relationship between employment status and endocrine responses is far more complicated than previously thought. We have shown for the first time that unemployed people have a lower CAR, but also show a blunted DHEA response relative to those employed and we suggest that this may be a feature of chronic stress exposure or perhaps dependent on the prevailing socio-economic context.

Keywords: Chronic Stress; Cortisol; DHEAS; Employment; Stress; Unemployment

Abbreviations: DHEA-S = Dehydroepiandrosterone-Sulfate

1. Introduction

Chronic stress causes negative health outcomes via its dysregulation of autonomic, endocrine, and immune system functioning (Segerstrom and Miller 2004, Morey *et al.* 2015). Despite decades of research, an established and consistent model of chronic stress in humans has remained elusive, necessitating more detailed and precise measurements of individual and contextual factors that may either mask or define relationships between chronic stress and health (Gallagher et al. 2009, Lovell et al. 2011, Segerstrom and O'Connor 2012). Unemployment is one source of chronic stress that has received comparatively little endocrinological research, particularly in recent years, despite the rising unemployment figures globally due to the latest economic crisis. Unemployment has been shown to be damaging to health, but has inconsistent associative patterns with health outcomes (McKee-Ryan *et al.* 2005, Roelfs *et al.* 2011), including cortisol (Claussen, 1994; Ockenfels et al., 1995). To date, evidence in this area has focussed largely on assessing overall cortisol, or diurnal rhythm differences only; with no assessment of the awakening response, which has been specifically related to a range of sources of chronic stress (Fries *et al.* 2009). Even with the assessments that have been undertaken, suggestions are that unemployment does affect cortisol secretion; however the methods employed by previous studies were not standardised and very broad (e.g. lack of control for gender, medication, and cortisol collection) making the results difficult to interpret. The present study aims to build upon and extend on these earlier studies.

Dehydroepiandrosterone (DHEA) is a steroid hormone of adrenal origin that has been receiving attention in recent years, as its role in health and disease is still being uncovered (Maninger *et al.* 2009). It and its sulphated form, DHEA-Sulfate (DHEAS), are measured similarly to cortisol - with salivary assessment - providing accurate comparability to circulating serum levels (Hucklebridge *et al.* 2005). It is immunoprotective (Bauer 2005, Buford and Willoughby 2008), has been related to higher levels of resilience (Morgan III *et al.* 2009, Petros *et al.* 2013), and is a protective factor against the damaging effects of excessive cortisol excretion (do Vale *et al.* 2011). Further, the ratio of cortisol to DHEAS has also been associated with health and disease outcomes, with a higher ratio being associated with a greater risk of mortality (Phillips *et al.* 2010). Circulating DHEA/DHEAS levels decline naturally over time with ageing (Bauer 2005, Maninger *et al.* 2009), however they also decline after both brief and prolonged exposure to stress (Izawa *et al.* 2012, Lennartsson *et al.* 2012). Using the chronic stress model of caregiving, DHEAS has been shown to be higher in non-caregivers; even in a young population where immunosenescence does not confound findings (Vedhara *et al.* 2002).

To date no assessments of DHEA/DHEAS have been carried out using unemployment as a model of chronic stress, and only very limited data is available on cortisol in the same (Arnetz *et al.* 1991,

Ockenfels *et al.* 1995, Dettenborn *et al.* 2010) implying that further investigation of dysregulation of the hypothalamic-pituitary-adrenal axis endocrinology is needed. Moreover, given that unemployment has been associated with immunomodulation (Hughes *et al.* 2015), cardiovascular disease (Gallo *et al.* 2004), and all-cause mortality (Browning and Heinesen 2012), it implies that there is likely a mechanistic relationship between this status and health decrements. Further, as previous research examining cortisol in unemployment has been fraught with methodological issues, it is possible that clearer differences may be uncovered with more controlled methodology. The present study sought to advance previous findings of cortisol dysregulation in unemployed participants, by comparing employed and unemployed participant groups. Moreover, the assessment of DHEAS in these participants was also undertaken to understand this important element of stress-induced health decrement. Based on the premise that chronic stress is damaging to the body and existing literature on unemployment, we hypothesised that unemployed participants would have lower cortisol awakening response, higher cortisol output and a higher cortisol to DHEAS ratio (cortisol:DHEAS) relative to those who were employed . Further, given that stress, social support, and self-esteem are important psychological mediators of unemployment stress, health and endocrine function (Segerstrom and Miller 2004, Pruessner *et al.* 2005, Uchino 2006, O'Donnell *et al.* 2008) we also wanted to confirm that our unemployed group were highly stressed and also tested whether they were associated with cortisol and DHEAS responses in these groups.

[Intro: 711/1000 words]

2. Methods

2.1 Participants

As part of a larger study, participants both employed and unemployed were recruited from across Ireland in a convenience sample using government agencies providing services to the unemployed, recruitment agencies, social media and print media advertising, and were offered €10 for participation. A total of 110 participants (69.1% female; 59% employed) that satisfied the inclusion criteria and were enrolled to take part completed the saliva testing and survey. Inclusion criteria were: being of working age (i.e. 18><65 years), being resident in Ireland and healthy *i*.e. not taking medications such as glucocorticoids or immunosuppressant's. Exclusion criteria were based on physiological and employment status parameters. Candidates were excluded in the case of: pregnancy, chronic illness (immune, endocrine, psychological/psychiatric, cardiovascular, or neurological), or oral/periodontal disease. Additionally, those candidates that self-identified as home-makers, were retired, or who were unemployed and receiving disability/incapacity benefit

were also excluded. This was to ensure that the unemployed sample was comprised of individuals who would self-identify as being unemployed and without vocational roles, and that were unemployed but otherwise able to work. *The project was approved by our University's Research Ethics Committee, and was conducted in accordance with the Declaration of Helsinki. Each participant gave informed consent before participation.*

2.2 Procedure

Prior to saliva collection, participants were asked to complete an internet-based or postal survey for demographic information, health behaviours (e.g. smoking, alcohol consumption, sleeping), and psychological questionnaires. After considering best practice guidelines on cortisol collection, and in particular attending to reducing bias on the assessment of the cortisol awakening response (Clow et al. 2010; Dockray et al. 2008; Stadler et al. 2016) participants were provided with eight saliva collection tubes (Sarstedt Ltd., Leicester, UK), and a diary log to note the date and time the samples were taken and a general written guide on how to collect their samples, along with a link to a video showing saliva collection specific to the protocol; they were also provided with a stamped addressed envelope for returning the samples. For example, the importance of the first awakening sample was emphasised by providing the following textual information 'Awake' is the first sample you take when you immediately wake in your usual way (alarm or natural waking): This should be when your eyes are wide open and you are ready to get up. This sample must be taken when you are lying down in bed. 'As shown in the YouTube video clip, it is very important that sampling and timing are done with accuracy.' Further, the YouTube clip not only showed how to collect the sample, it again emphasised accuracy and timing and the implications if not done correctly; the clip also showed a visual graph displaying the cortisol diurnal rhythm to reiterate the importance of adhering to the protocol. Participants were instructed to take four samples each day for two days; immediately upon waking (T1), thirty minutes after waking (T2), at midday (T3), and at eight in the evening (T4). As per the recommended guidelines above, two days of collection were implemented to ensure a more reliable assessment of the hormones. Participants were instructed to put the cotton swab in the salivette in their mouth for two-minutes and let the saliva collect naturally, *but* also not to eat or drink anything during taking the first two samples, and to avoid eating and drinking for at least 30 minutes before each other collection. Participants were provided with new tubes upon request if the procedure was not adhered to (n=8, with no differences between groups). Samples were refrigerated by participants upon collection, and, after returning to the laboratory, were frozen at -20˚C until centrifugation and assay.

2.3 Psychological Materials

As part of the participant survey, the following psychological scales were administered. The short Perceived Stress Scale (PSS-4) (Cohen *et al.* 1983) which assessed perceived stress over the previous month; a single-item self-esteem scale (SISE), that has been validated against the Rosenberg Self-Esteem Scale (Rosenberg 1965, Robins *et al.* 2001); and three items from the subscale "emotional/informational support" of Medical Outcomes Study Scale (MOS-3; "someone you can count on to listen to you when you need to talk"; " someone to give you good advice about a crisis"; and "someone to share your most private worries and fears with") (Sherbourne and Stewart 1991). This subscale was selected due to the associations of this type of support with psychological distress outcomes in unemployment (Bjarnason and Sigurdardottir 2003). The number of friends question was also taken from the scale; however this was not related to any other variable and so was not used for further analysis. To assess any contribution of individual differences in the sample we used the Ten Item Personality Inventory (TIPI) (Gosling *et al.* 2003), as personality has been related to both psychological and physiological reactions to stress (LeBlanc and Ducharme 2005).

2.4 Hormone Assay

After thawing, samples were centrifuged at 3000*g* for 10 minutes and were assayed using commercially available ELISA kits for high-sensitivity cortisol and DHEAS (DRG Diagnostics, Marburg, Germany). The assays were analysed using a Biotek ELX800 (Bio-Tek, Vermont, USA) plate reader and Gen5 software (Bio-Tek, Vermont, USA). Each sample was assayed in duplicate, with the mean value between wells being recorded as the sample value. Two days of saliva collection were obtained, and values for both hormones were meaned across the two days to control for differences in daily activity. There were no significant differences for either hormone across the two days of collection (p>.05). Intra-assay % coefficient of variation (%CV) for cortisol was 9.21%, and mean inter-assay %CV was 5.56%. These values for DHEAS were 10.88% and 14.86%, respectively.

Those samples that were too high for detection were re-assayed using dilutions (n=115, 86% DHEAS). Dilutions were processed with 1:2 mixtures (where possible) with the kit zero standard. Where limited remaining sample was available, dilutions of lower concentration were used (n=12, 66.6% DHEAS). Any samples that were still too high for detection after dilution were recorded as missing (n=16, 93.75% DHEAS). Any samples that provided insufficient centrifuged volume for both assays were used for cortisol assay only, and those that provided insufficient volume for either assay were recorded as missing. A total of 10 missing datapoints for cortisol (for samples T1-T4: 3, 2, 0, 5) and 28 (15, 5, 3, 5) for DHEAS were recorded for the first day; and 7 missing datapoints for cortisol (3, 2, 1, 1) and 26 (14, 8, 3, 1) for DHEAS were recorded for the second day. However, it is worth

mentioning that these were equally distributed across the two groups; all p's > .05. Thus, slight differences in degrees of freedom reflect missing data.

2.5 Data Analyses

Statistical analyses were undertaken using IBM SPSS version 22. Paired samples *t*-tests were used to assess differences between the hormone assessments across the two days of collection, with no significant differences observable. The mean values across both days for cortisol and DHEAS were positively skewed, and were therefore subject to $\log_{10}$ transformation prior to analysis. Area under the curve (AUC with respect to ground (AUC_G) was calculated using the trapezoid method (Pruessner *et al.* 2003) were carried out with non-transformed values. Only cortisol was examined for awakening response (CAR), as DHEAS does not increase after awakening (Hucklebridge *et al.* 2005). As we were interested in establishing a healthy CAR, analyses of difference (ANOVA) were conducted on T2 cortisol, controlling for T1 values as per recommended guidelines (Stadler et al. 2016). Group differences between the employed and unemployed groups were assessed using x^2 and one-way ANOVA. Repeated measures ANOVA (group by time) was used to assess the differences between the groups across the mean hormone levels across the day. All other assessments were analysed using between-subjects ANOVA. Exploratory Hierarchical Linear Regressions were run *post hoc* to assess the contribution of significant psychological and behavioural variables to the resultant hormone variance.

3. Results

3.1 Sample characteristics

Summaries of the demographic, psychological, and endocrine assessments of the two samples are presented in tables 1. As can be seen from table 1, the present sample is majority female, white, and were of comparable age across the employed and non-employed groups. Initially we set out to examine differences between more subgroups; using permanent employed, temporary or self-employed, short-term unemployed (<12 months) and long-term unemployed (>12 months), however there were insufficient differences between all four groups with regard to their endocrine data to warrant this. Those who were unemployed had a mean duration of unemployment of 27.1 months (SD=33.34, range: <0-129 months) and this was not associated with endocrine functioning; implying that duration of unemployment, in this particular sample, would not need to be considered as an explanatory confound in later analyses. The two groups did however differ on marital status ($X^2(2)=9.21$, $p=.01$), with unemployed people more likely to be not in a relationship; and in income ($X^2(8)=32.14$, $p<.01$), as expected majority of unemployed participants were earning less than

€20,000 (*n*=32). No differences were observable for age, sex, use of hormonal therapy or contraception use, education, number of dependents, days of saliva samples (weekday/weekend), timing of sampling, weekly alcohol consumption, and hours of sleep across the groups. As would be expected, our employed group were more likely to wake up earlier (7:21am) than our unemployed group (8:50am); thus, in line with recommended guidelines (Stalder et al. 2016) we controlled for this in our main CAR analyses. Further, given the sex-endocrine associations, we checked for differences between males and females on endocrine levels, which were all non-significant, CAR ($F_{(1)}$=0.447, p=.50; AUC_G Cortisol ($F_{(1)}$=0.009, p=.92); AUC_G DHEAS ($F_{(1)}$=0.13, p=.90), the cortisol/DHEAS ratio $F_{(1)}$=0.440, p=.50. However, as expected, differences were found between the groups for perceived stress (PSS-4), Social Support (MOS-3), and self-esteem (SISE); but not personality (TIPI). Those who were unemployed were more stressed, had lower social support and lower self-esteem (see table 1).

[Insert Tables 1 around here]

3.2 Group differences in cortisol and DHEAS diurnal rhythm

Endocrine mean and SD values are displayed in table 1. In ANOVAs, the Greenhouse-Geisser correction was applied for DHEAS. No significant differences were detectable across the groups in relation to the collection times for cortisol ($F_{(3)}$=1.24, p=.297, η_p^2=.013), or DHEAS ($F_{(3)}$=2.26, p=.082, η_p^2=.028). Whilst the two groups overall diurnal patterns were not significantly different from each other for cortisol ($F_{(3, 95)}$=2.28, p=.082, η_p^2=.067) and DHEAS ($F_{(2.73, 215.13)}$=2.259, p=.08, η_p^2=.028), their cortisol and DHEAS trajectories are consistent with those reported throughout the literature. *After* controlling for age however, the interaction for cortisol became significant ($F_{(3, 94)}$=2.85, p=.04, η_p^2=.08). Polynomial contrasts revealed significant cubic interactions between time and unemployment groups, Cortisol ($F_{(1, 97)}$=6.982, p=.01, η_p^2=.068), and DHEAS ($F_{(1, 79)}$=5.578, p=.021, η_p^2=.067), respectively. The cortisol and DHEAS diurnal patterns are illustrated in Figure 1 (A and B, respectively). However, as can been seen in figure 1A the groups cortisol T2 levels differ significantly from each other ($F_{(1, 104)}$=13.50, p<.01, η_p^2=.115), with the employed producing higher levels; and DHEAS differs at T4 ($F_{(1, 102)}$=4.71, p=.03, η_p^2=.044), with the unemployed group producing a blunted response; *this was not altered by age.*

[Insert Figure 1 A&B around here]

3.3 Group differences in profiles of cortisol output

AUC_G was found to be significant difference between employment groups ($F_{(1,97)}$=4.82, p=.03, η_p^2=.047), with the employed showing a higher AUC_G output, which was still evident after controlling for age. To assess the difference in CAR, a univariate ANOVA assessing group differences in T2

cortisol, controlling for T1 levels, was processed. This showed a significant difference between the employment groups with respect to awakening cortisol rise, using the T1 data as a control ($F_{(2,99)}$=12.78, p<.01, η_p^2=.205), with the employed showing a higher morning reactivity. This also remained significant after controlling for between group differences in awakening response, and age, $F_{(4,97)}$=7.987, p=.006, η_p^2=.076.

3.4 Group differences in profiles of DHEAS output

Using the same AUC_G method, we found a significant difference between the groups ($F_{(1,79)}$=5.90, p=.02, η_p^2=.069), with higher means from the unemployed group. Mean DHEAS output was also calculated as with cortisol. Comparisons of these means across the two groups showed no significant difference overall, but a trend is indicated ($F_{(1,79)}$=3.23, p=.07, η_p^2=.039).

3.5 Group differences in Cortisol:DHEAS ratio

Cortisol:DHEAS ratio was derived by log10 the AUCg values and dividing cortisol by DHEA values as per recommend guidelines (Sollberger & Ehlert, 2016). A significant difference was observed across the employment groups ($F_{(1,79)}$=11.38, p<.001, η_p^2=.128) carried by a larger cortisol:DHEAS ratio in the employed group.

3.6 Contribution of psychosocial variables to group differences in cortisol

Several hierarchical linear regressions (see table 2) were carried out to explore the associations between the group differences in logged cortisol and DHEA parameters and those psychosocial variables that were found to differ significantly. In the first step, the predictor (employment group), and demographic, health and sampling variables (age[1], waking up times, marital status, income, and number of cigarettes smoked) were entered; followed by the psychological variables added simultaneously in the second step: self-esteem, stress and social support. To examine these associations with CAR, T1 cortisol was also entered at the first step, along with the predictor and other potential confounds, and the overall regression showed significant associations between the psychosocial variables and CAR ($F_{(10,101)}$=3.87, p<.001, R^2=.21). Although employment group remained significant, self-esteem, perceived stress, but not social support, were associated with the CAR, such that higher self-esteem and lower stress were associated with a higher awakening response; together they explained an additional 9% of the variance in the CAR. For cortisol AUC_G, the regressions for both models were not significant, but at step two both employment group and elf-esteem were significant predictors, explaining 6% of the variance (see table 2); higher self-esteem

[1] Age, although not significantly different across the two groups, was included as a trend was indicated and it is strongly associated with both cortisol and DHEAS output.

was associated with a higher cortisol AUC_G. For DHEAS AUC_G, the overall regression in step one, was significant ($F_{(6, 80)}$=2.51, p=.02) and a trend observed for step two ($F_{(9, 80)}$=1.96, p=.06, R^2=.20), with age being significantly associated. The final regression was for cortisol:DHEA ratio, the step two regression was not significant with only unemployment group was significant in the equation. R^2=.18). Given that the groups also differed for T4 DHEAS, we ran the regression again and a similar pattern was observed: no psychosocial variables were associated ($F_{(9, 94)}$= 1.67, p=.11, R^2=.14), but age was (β=-.24, t=-2.40, p=.02).

4. Discussion

The present work was designed to build on prior research and as expected we confirmed that our unemployed group are highly stressed as indexed by their psychological data and CAR response. We also found that both stress and self-esteem were associated with cortisol responses, but not DHEA which is similar to that in other studies of stress and endocrine functioning (Pruessner *et al.* 2005). However, our remaining cortisol and DHEA findings were not as expected. Overall, we present rather curious findings that appear to tell two different stories: that of hormone levels, and that of hormonal dysregulation. Our findings suggest that those who are employed have a higher cortisol output, and those that are unemployed have a higher DHEAS output. These unexpected patterns are extended by the findings of higher cortisol:DHEAS ratio in the employed, a profile that is more harmful to health (Phillips *et al.* 2010), and is associated with chronic stress (Izawa *et al.* 2012), meaning our employed sample may be physiologically more stressed than our unemployed sample. At all points across the day, the unemployed participants in the present sample secrete higher levels of DHEAS than do the employed, which is associated with better health outcomes (Hazeldine *et al.* 2010). Further, the unemployed have a lower cortisol:DHEAS ratio, a factor that is protective to health and immunity (Phillips *et al.* 2010). Why such differences should be apparent is not immediately clear, and not easily explainable. The variation in cortisol:DHEAS is almost twice as large amongst the employed than the unemployed. This is indicative of larger differences in physiological stress amongst the employed group, a finding in keeping with the broader literature (Lundberg 2005).

However, when looking at the diurnal rhythms of the hormones we see a pattern that is more typical of the view that unemployment is a chronic stressor that damages health through endocrine dysregulation. Whilst the employed do seem to have higher levels of cortisol, they also exhibit a healthier awakening response, and steeper slope of decline than those who are unemployed. In fact, chronic stress is characterised by a flatter diurnal rhythm of cortisol (Miller *et al.* 2007), and this is

also pronounced in the awakening response (Chida and Steptoe 2009). In fact, it could also be that the lower cortisol in the unemployed group could be due to a "blunting" of HPA output as a consequence of being chronically stressed (e.g., see Miller et al. 2007). Thus, whilst our findings do appear to go against the wider literature in unemployment research insomuch as the unemployed have lower cortisol than the employed, we also find that they exhibit a lower awakening response – a finding more in keeping with the view that unemployment is a chronic stressor (Miller *et al.* 2007). This is supported by the psychological findings herein, where the unemployed report higher perceived stress and distress, and lower emotional/informational social support. These findings are consistent with the wider literature in unemployment (McKee-Ryan *et al.* 2005). Interestingly, we observe what appears to be blunting at Time 4 in DHEAS in the unemployed compared to the employed. This pattern is similar to that reported in ageing samples (Heaney *et al.* 2012). It is also worth noting that in animal studies of chronic stress, DHEA has been shown to rise as opposed to decline after stress repeated exposure (Maninger *et al.* 2010). Higher levels have also been observed in studies of patients experiencing psychopathology (Erbay and Kartalci, 2015), suggesting that higher levels of DHEA are also associated with negative health outcomes. For example, comorbid depression post-traumatic stress disorder (PTSD) has been associated with higher levels of DHEA when compared to those with PTSD and without depression (Gill *et al.* 2008), and healthy controls (Jergović *et al.* 2015). Moreover, higher levels of DHEA, and lower cortisol:DHEA ratios have been associated with depressed individuals experiencing recurrence of depressive episode (Mocking *et al.* 2015). It is also worth noting that some researchers have suggested that there is still some uncertainty surrounding the role of these biomarkers in the aetiology of some health conditions and that further investigation is needed (Aggarwal *et al.* 2014).

Another reasonable explanation for the unexpected findings of the unemployed sample exhibiting lower cortisol and cortisol:DHEAS ratio and higher DHEAS would be related to the social-economic context in which this study was carried out. Whilst the unemployment rate in Ireland is currently at 8.9%, at its peak it was 15% in 2012/13 and was between 10-11% during our data collection period – which is still a high prevalence rate. Given the widespread effect of the global recession on unemployment, it became normative and individuals were more likely to blame the system rather than themselves for their position. It is, therefore, possible that the experience of unemployment within this context is qualitatively different from other contexts, especially given that social welfare payments vary across countries, and are not time-restricted as in other welfare states (e.g, Spain); and the welfare system in Ireland may be more generous, and perhaps health protective, than those found in other welfare states. This notion could be tested in future study designs. In fact, the higher the level of unemployment, the more the status is seen as normal and less shameful, and the more

social support there may be from others out of work (Roelfs *et al.* 2015). The parallel research in this area relating to health and mortality has both confirmed (Tapia Granados *et al.* 2014) and denied (Roelfs *et al.* 2015) this theory. Logically, the reverse would also hold for those who are employed during a recession – increasing their feelings of insecurity, and burdening them with the need to justify their employ constantly to retain their jobs. Whilst our unemployed group were more stressed than our employed group, it also worth noting that these are higher than recent general population norms for the PSS-4 in (Warttig et al. 2013), indicating that our employed group are significantly more stressed (Mean 6.11 vs Mean of 10.3, $p < .01$). However, given that our unemployed reported higher stress, it still does not really explain the between biological group differences observed here. Also speculative, it may be these unmeasured factors may be contributing to the patterns found here, although researchers using other models of chronic stress have argued that consideration of context is critical to understanding the psychophysiological correlates (Gallagher et al. 2009; Lovell & Wetherell, 2011).

There are several limitations to the present study that warrant caution in interpreting the findings. The sample itself was relatively modest, and hormone data were further limited due to insufficient saliva volumes in some samples; although, our sample size is larger than previous studies in the area (Dettenborn et al., 2010). Similarly, there were 28 data points on day 1 of sampling and 26 data points on day 2 missing from our DHEAS sample which raises the risk of statistical errors or incorrect conclusions regarding differences between groups; however, the missing data points did not differ across groups. Although the present study used ELISA kits that did not recommend controlling for salivary flow rate for calculation of DHEAS, it must be acknowledged that there is some debate and no consensus in the literature on whether or not one should control for salivary flow rate. The use of salivettes for collection of DHEA has also been questioned (Gallagher et al., 2006); however, this is the key reason why we opted to measure DHEAS as this has been validated using salivates (Whetzel & Klein, 2010). Moreover, as is common in psychological research, the sample was majority female. As cortisol levels and their reactions to stress vary by sex (Kudielka and Kirschbaum 2005), it is possible that there are sex/gender effects in the relationship between the stress from unemployment and these steroid hormones that we were unable to detect. Similarly, although there were no group differences on contraceptive or HRT use, a lack of measurement of ovulatory phase in our female participants is a potential limitation here. Further, given that our endocrine data is somewhat inconsistent with some of the wider literature, in particular regarding the impact of chronic stress on the cortisol/DHEA ratio, we have speculated a number of reasons for this such as higher DHEA are also seen in other studies, most notably in studies of psychopathology. As we do not have this data for the present sample, this is speculative and suggests that further research is

clearly warranted. Of importance is the implication of self-esteem as a potential buffer. The assessment of self-esteem herein was a single-item measure, which whilst being correlated to the well-established Rosenberg Self-Esteem Scale (Robins *et al.* 2001) is still limited. It is clear that there may be a relationship between self-esteem and the biological stress of unemployment, but there exists an unmeasured variance in our predictor that may tie this concept to the context of unemployment being more normative in times of recession. This limits our ability to draw clear reasons for some of our more unexpected findings, and so it will be important to define this in the future. Finally, the normality of unemployment itself may be a limiting factor here, as it is possible that higher levels of unemployment in a recession may make unemployment more normal, and less stressful, than during times of economic growth or across different welfare states a question that could be investigated in future studies.

5. Conclusions

The present findings would support the conjecture that the stress of unemployment is both complex and multifaceted. We present findings that go against both theory and prior research, and show a profile of higher biological stress in the employed in terms of overall levels of cortisol and DHEAS. Conversely, when considering diurnal rhythm, it is the opposite – and we see a less healthy CAR, and the appearance of blunting in diurnal slope of DHEAS in the unemployed. This is echoed in the subjective experience of the unemployed, who report higher levels of stress and distress than do the employed. It is possible that these differences can be attributed to the social context, particularly as Europe is struggling to emerge from the latest global recession. The blunting of the diurnal rhythm of DHEAS is a new finding in a young, stressed sample, and suggests that unemployment may cause dysregulation – something that is potentially harmful and is associated with ageing. What is of importance is the suggestion that the stressful effects of unemployment may well be highly contextual, particularly in terms of the socio-economic environment. It is, therefore, important that research into the stress and health effects of unemployment be continued not just in times of recession and high unemployment. In fact, it could be that the commonality of unemployment provides some level of protection against its potentially harmful stress; therefore making its research during boom times more important.

Conflicts of Interest: The authors declare no conflicts of interest.

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[Discussion: 1696/2000 words]

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Table 1. Demographic, health-related, psychosocial characteristics and endocrine levels of employed and unemployed participants.

			Employed (*n*=59)	Unemployed (*n*=51)	Test of Difference
Mean Age (SD) - years			39.8 (11.91)	35.4 (12.67)	$F_{(1, 108)}$=3.73, *p*=.06
Sex (female)			41 (69.5%)	35 (68.6%)	X^2(1)=0.01, *p*=.92
Marital status (partnered)			36 (61.0%)	19 (37.3%)	**X^2(2)=9.21, *p*=.01**
Ethnicity (Caucasian)			59 (100)	49 (96.1)	X^2(1)=2.36, *p*=.13
Income	Less than €10,000 (%)		4 (6.8)	18 (35.3)	**X^2(8)=32.14, *p*<.01**
	€10,000 to €19,999 (%)		7 (11.9)	14 (27.5)	
	€20,000 to €29,999 (%)		10 (16.9)	8 (15.7)	
	€30,000 to €39,999 (%)		9 (15.3)	5 (9.8)	
	€40,000 to €49,999 (%)		7 (11.9)	-	
	€50,000 to €74,999 (%)		8 (13.6)	1 (2.0)	
	€75,000 to €99,999 (%)		7 (11.9)	1 (2.0)	
	€100,000 or more (%)		3 (5.1)	-	
	Undisclosed (%)		4 (6.8)	4 (7.8)	
Level of Education	Primary (%)		-	1 (2.0)	X^2(4)=4.99, *p*=.28
	Secondary	Exit at 16 (%)	3 (5.1)	4 (7.8)	
		Exit at 18 (%)	7 (11.9)	11 (21.6)	
	Vocational Qualification (%)		11 (18.6)	10 (19.6)	
	Undergraduate (%)		22 (37.3)	19 (37.3)	
	Postgraduate (%)		12 (20.3)	5 (9.8)	
	Doctoral/Professional (%)		4 (6.8)	1 (2.0)	
Mean Number of Dependents (SD)			1.7 (1.06)	1.5 (.92)	$F_{(1, 108)}$=1.42, *p*=.24
Smoking behaviour – number of cigarettes per day		None (%)	51 (86.4%)	28 (54.9%)	**X^2(4)=14.74, *p*<.01**
		1-5 (%)	3 (5.1%)	10 (19.6%)	
		6-10 (%)	2 (3.4%)	5 (9.8%)	
		11-20 (%)	3 (5.1%)	5 (9.8%)	
		21+ (%)	-	3 (5.9%)	
Mean alcohol consumption per week - units		None (%)	17 (28.8%)	11 (21.6%)	X^2(5)=2.77, *p*=.74
		1-5 units (%)	24 (40.7%)	21 (41.2%)	
		6-10 units (%)	10 (16.9%)	8 (15.7%)	
		11-20 units (%)	2 (3.4%)	5 (9.8%)	
		20-40 units (%)	3 (5.1%)	4 (7.8%)	
		41+ (%)	3 (5.1%)	2 (3.9%)	
Mean hours of sleep per night (SD)			6.8 (1.69)	6.8 (1.13)	$F_{(1, 94)}$=0.01, *p*=.99
Hormonal contraceptive use (yes)			11 (18.6%)	9 (17.6%)	X^2(3)=0.56, *p*=.91
Other hormonal medication use (yes)			1 (1.7%)	1 (2.0%)	X^2(3)=0.53, *p*=.91
Mean PSS-4 (SD)			10.3 (2.87)	12.6 (3.49)	**$F_{(1, 108)}$=14.2, *p*<.01**
Mean MOS-3 (SD)			11.7 (3.36)	9.7 (3.59)	**$F_{(1, 108)}$=9.29, *p*<.01**
Mean SISE Score (SD)			2.6 (0.77)	2.2 (0.90)	**$F_{(1, 108)}$=5.63, *p*=.02**
Mean TIPI – Extraversion (SD)			8.6 (2.92)	8.6 (2.59)	$F_{(1, 108)}$=0.01, *p*=.95
Mean TIPI – Agreeableness (SD)			10.3 (2.14)	9.9 (2.30)	$F_{(1, 108)}$=1.08, *p*=.30
Mean TIPI – Conscientiousness (SD)			11.1 (2.07)	10.5 (2.63)	$F_{(1, 108)}$=1.85, *p*=.18
Mean TIPI – Emotional Stability (SD)			9.2 (2.41)	8.4 (2.67)	$F_{(1, 108)}$=2.94, *p*=.09
Mean TIPI – Openness (SD)			10.4 (2.10)	10.4 (2.19)	$F_{(1, 108)}$=0.01, *p*=.93
Mean Cortisol Cortisol AUC_G (SD)			1.8 (0.19)	1.7(0.23)	**$F_{(1, 102)}$=5.33, *p*=.02**
Mean CAR (SD)			1.1 (0.22)	0.9 (0.25)	**$F_{(1, 99)}$=12.78, *p* <.01**
Mean Cortisol:DHEAS ratio (SD)			1.3 (0.91)	0.8 (0.48)	**$F_{(1, 79)}$= 11.61, *p* <.01**
Mean DHEAS AUC_G (SD)			1.7 (0.14)	1.8 (0.18)	**$F_{(1, 79)}$=4.42, *p*=.03**

*Significant differences highlighted in **bold**

Figure 1. The patterns of secretory activity of (a) cortisol and (b) DHEAS by (un)employment group. The values are mean (log_{10}) with standard errors.

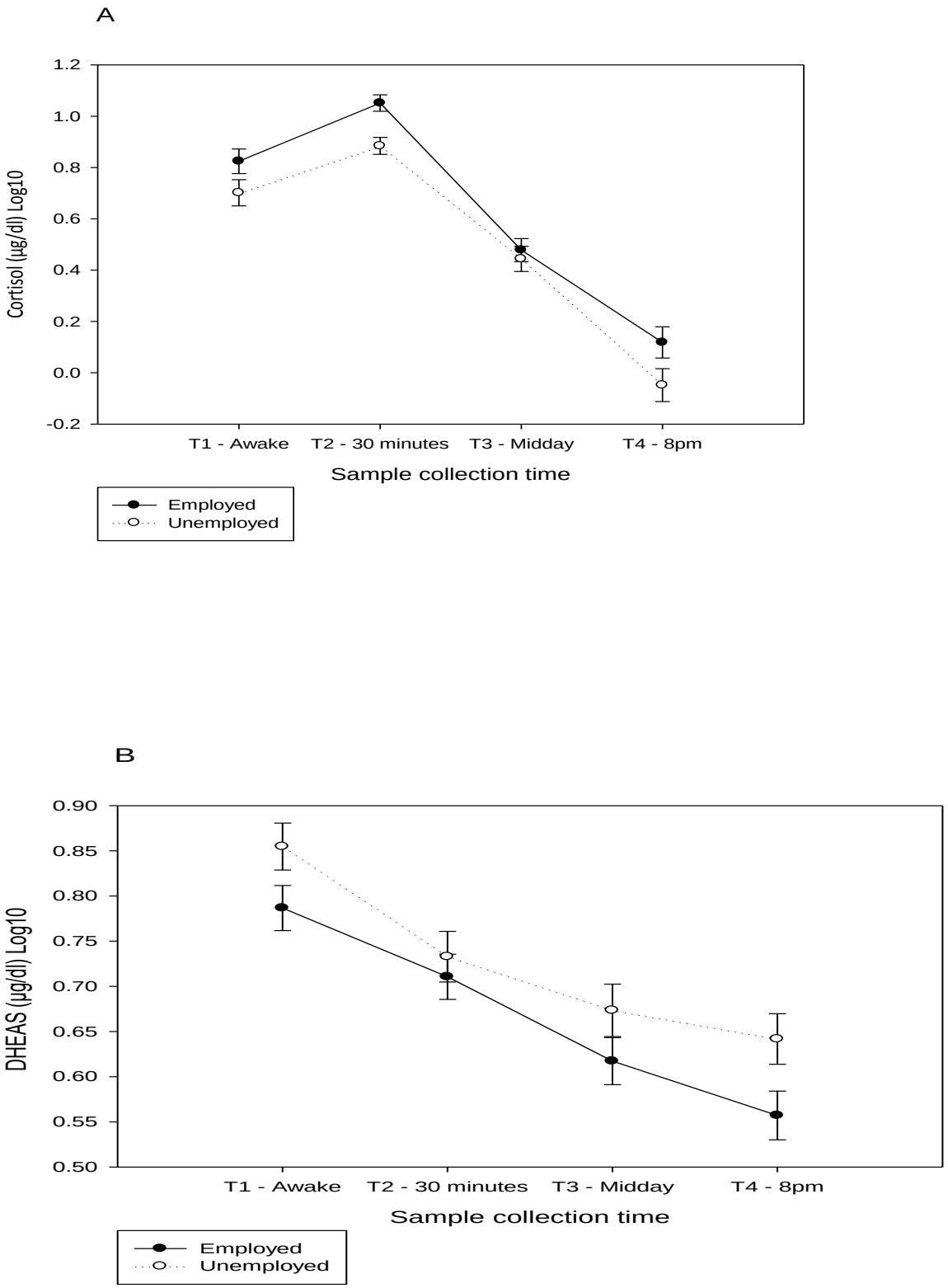

Table2. Summary of hierarchical regressions of psychosocial variables predicting CAR, Cortisol AUC_G, DHEAS AUC_G, and cortisol:DHEA

Variable	β	t	*p*	95 % CI	R^2	ΔR^2
CAR						
Step 1						
Unemployed group	- .28	-2.40	**.01**	-.26, -.02		
Age	.05	0.47	.63	-.03, .00		
Marital status	-.04	-0.43	.66	-.04, .03		
Income	.05	0.45	.65	-.01, .02		
Cigarettes	.03	0.33	.74	-.03, .05		
Awakening times	.01	0.17	.86	.03, .04		
T1 Cort (log_{10})	.29	3.16	**.00**	.07, .33		
					.21	
Step 2						
Self-esteem	.33	3.06	**.003**	.03, .16		
Stress	-.29	-2.66	**.009**	-.03, -.005		
Social support	-.01	- 0.12	.90	-.01, .10		
						.09
Cortisol AUC_G						
Step 1						
Unemployed group	- .23	-1.75	.08	-.21, .01		
Age	.02	0.20	.83	-.03, .00		
Marital status	-.02	-0.18	.85	-.04, .03		
Income	.01	0.12	.90	-.01, .02		
Cigarettes	-.01	-.17	.86	-.04, .03		
Awakening times	.03	0.25	.80	.03, .04		
					.05	
Step 2						
Self-esteem	.27	2.29	**.02**	.01, .13		
Stress	-.17	-1.34	.16	-.03, .005		
Social support	-.10	- 0.95	.34	-.01, .007		
						.06
DHEAS AUC_G						
Step 1						
Unemployed group	.31	2.24	**.02**	.01, .20		

Age	-.30	-2.51	.63	-.03, .00		
Marital status	-.21	-1.89	.66	-.04, .03		
Income	.18	1.24	.65	-.01, .02		
Cigarettes	-.12	-0.80	.74	-.03, .05		
Awakening times	-.05	-0.13	.86	.03, .04		
					.17	
Step 2						
Self-esteem	-.02	-.017	.86	-.06, .05		
Stress	.01	0.10	.91	-.01, .01		
Social support	-.18	- 1.58	.11	-.01, .002		
						.03
cortisol:DHEA						
Step 1						
Unemployed group	.32	2.23	**.02**	.01, .16		
Age	-.12	-1.10	.27	-.004, .001		
Marital status	-.01	-0.12	.90	-.02, .02		
Income	.06	0.47	.63	-.01, .01		
Cigarettes	.10	0.91	.36	-.01, .04		
Awakening times	.06	0.48	.63	.01, .03		
					.17	
Step 2						
Self-esteem	-.17	-0.84	.40	-.07, .02		
Stress	.12	0.83	.40	-.007, .01		
Social support	-.02	- 0.16	.87	-.01, .008		
						.01

*Significant associations are highlighted in **bold**