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Running title: HCC and ill-being/well-being in young and old healthy females

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Abstract

Hair cortisol concentration (HCC) provides a retrospective measure of long-term (i.e. over a period of months) cortisol secretion and has been shown to be elevated in relation to chronic stress conditions. However associations in healthy participants with subjective ill-being are less clear and associations with well-being have not been explored. The current study examined HCC in relation to independent comprehensive measures of ill-being (stress, depression, anxiety) and well-being (subjective happiness, life satisfaction, psychological well-being) in healthy young and old females (mean ± SD: 19.5±2.2 years and 78.6±6.7 years respectively, total N = 115). The data supported evidence of increased total cortisol secretion with increased age. No association between ill-being and HCC was found in either the young or older group of participants. A positive association between HCC and well-being was found in the older participant group which was independent of ill-being and potential confounds. These findings do not support associations between HCC and ill-being in healthy young or old females. However the results suggest that HCC is able to distinguish levels of well-being in healthy older females.

Key words: Hair cortisol concentration; HCC; age; females; well-being; ill-being.

1. Introduction

Hair cortisol concentrations (HCC) provide a relatively new way of examining hypothalamic-pituitary-adrenal (HPA) axis secretory activity (Kirschbaum et al., 2009). Cortisol is assumed to be continuously incorporated into growing hair and HCC should thus represent a retrospective measure of integrated cortisol secretion over the period of hair growth, typically the past 2-6 months (Gow et al., 2010; Stalder & Kirschbaum, 2012). The measure has grown in popularity as it is complementary to more commonly used salivary cortisol analysis, providing data on long-term overall cortisol secretion, rather than short-term dynamic changes. ~~For example, the collection of hair samples is non-invasive, does not require repeated assessments or reliance on participant adherence to a strict timing protocol to obtain reliable data (Stalder et al., 2012). This is important as participant non-adherence is known to be a problem in ambulatory salivary or urinary cortisol assessments (Kudielka et al., 2003; Remer et al., 2008; Smyth et al., 2013)~~.

It is clear that high levels of HCC have been associated with demographic and health status, e.g. patients with Cushing's syndrome, markers of metabolic syndrome, smoking and alcoholism (Feller et al., 2014; Kuehl et al., 2015; Stalder et al., 2010; Thomson et al., 2010). In relation to psychosocial factors higher HCC levels have been found in the long-term unemployed (Dettenborn et al., 2012b), chronically stressed dementia caregivers (Stalder et al., 2014) and a range of stress-related psychiatric symptoms and disorders (reviewed in Wosu et al., 2013). These are all relatively severe conditions but the evidence linking HCC with subjective ill-being, (e.g. perceived stress) is mixed, with a majority showing no association (see Staufenbiel et al., 2013). Consequently a question remains about the sensitivity of HCC to sub-clinical levels of ill-being.

In addition to the study of ill-being and health, there is evidence that well-being confers benefit to concurrent and future health prospects (Pressman & Cohen, 2005). This has been demonstrated even after accounting for ill-being in the respective analyses (Chida & Steptoe, 2008; Kubzansky & Thurston, 2007). The comprehensive measurement of well-being involves assessment of different domains including subjective happiness (Lyubomirsky & Lepper, 1999), life satisfaction (Diener et al., 1985) and aspects of psychological well-being such as self-acceptance, environmental mastery, purpose in life and personal growth (Ryff, 1989). The evidence points to well-being as more than the mere absence of negative psychological symptoms and that well-being and ill-being function relatively independently and should be measured separately (Diener & Emmons, 1984; Huppert & Whittington, 2003; Russell & Carroll, 1999; Ryff et al., 2006). The HPA axis has been proposed to be a mediator of the relationship between well-being and health (Dockray & Steptoe, 2010). In line with this, the diurnal pattern of cortisol secretion has been linked with measures of well-being (Evans et al., 2007; Ryff et al., 2004; Simpson et al., 2008; Smyth et al., 2015; Steptoe & Wardle, 2005; Steptoe et al., 2005). However, to the best of our knowledge an investigation of HCC in relation to well-being is still outstanding.

Activity of the HPA axis is known to vary across the lifespan (Lupien et al., 2009). Increasing age has consistently been shown to be related to differences in both the diurnal pattern and levels of cortisol secretion (Deuschle et al., 1997; Knoops et al., 2010; VanCauter et al., 1996) and there is evidence suggesting increasing HCC with age (Dettenborn et al., 2012b; Feller et al., 2014; Stalder et al., 2013). However further investigation is justified to explore how well-being and ill-being are related to HCC across age groups. In addition the relationship between stress and measures of salivary cortisol concentration have been shown to vary with sex (Roe et al., 2013). For HCC, although the majority of studies find no difference between the sexes (reviewed in Wosu et al., 2013), there is one notable exception in which levels of HCC were higher in 18-49 year old males compared to females, but not between 50-91 year old participants (Dettenborn et al., 2012b).

It is clear that the study of HCC is relatively new and further investigation is warranted. The aim of the current study was to use hair analysis to examine cortisol levels over the 3-month period before sampling (3cm scalp-near hair segments) in healthy female participants in relation to comprehensive measures of ill-being and well-being assessed at the time of sample collection, whilst accounting for known confounds. We chose to study two distinct age groups to examine potently different relationships within and between the different age groups. We chose to study females only as the relationship between stress and cortisol secretion has been reported to differ between the sexes (Dettenborn et al., 2012a; Roe et al., 2013). We hypothesised that HCC levels would be higher in the old age group but made no predictions about associations with ill-being and well-being.

2. Materials and Method

2.1. Participants

Two distinct age groups of healthy females were studied. In the younger group, 88 females were recruited from within the academic community at the University of Westminster. Participant age ranged between 18-27 (mean±SD: 19.5±2.2) years. Volunteers were awarded course credits for participating in the study. In the older group, 27 females were recruited from the University of the Third Age or the Women's Institute. Participant age ranged between 67-91 (mean±SD: 78.6±6.7) years. Volunteers were offered a small monetary award of a £10 high street voucher (only 7 participants accepted the voucher). All participants were selected on the basis that they were not pregnant, they had not taken any corticosteroid medication or suffered from adrenocortical dysfunction in the last year and they had not taken any illicit drugs in the last 6 months (ascertained by self-report). The University of Westminster ethics committee approved the protocol and all participants provided informed written consent.

Information about demographic variables (age, smoking status and ethnicity), health variables (medication, oral contraceptives use) and hair-specific characteristics (washes per week, hair treatments: bleach/colour/perm) were obtained via self-report from a self-developed questionnaire. As an index of subjective socioeconomic status (SES), participants rated where they stood in society in terms of education, occupation and wealth using a 1-10 ladder (Adler et al., 2000). The top of the ladder represented a higher social standing. A dichotomous smoking status variable was used contrasting smokers (current, occasional) vs. non/ex-smoker smokers.

Participants rated their health on a 1-5 scale ranging from poor health to excellent health.

2.2. Procedure

Ethics approval was obtained from the University of Westminster Ethics Committee. Following informed consent, participants attended a one-to-one research session with the lead researchers (NS or MB). During this one-to-one session the researchers collected the hair samples and details regarding participants' demographic, health and hair characteristics. Participants completed the self-report well-being and ill-being questionnaires online either one week prior or following the research session. The older group completed the questionnaires during the research session.

2.3. Self-report measures of well-being and ill-being

2.3.1. Subjective happiness

Participants completed the 4-item Subjective Happiness Scale (SHS; Lyubomirsky & Lepper, 1999), a measure of global subjective happiness. Each item is measured on a seven-point scale and following reverse scoring, scores are averaged. Scores range between 1 and 7 with higher scores reflecting higher happiness.

2.3.2. Life satisfaction

The 5-item Satisfaction with Life Scale (SWLS; Diener et al., 1985) measures individual's global cognitive judgments of aspects of general life satisfaction. Items are rated on a seven-point scale ranging from 'strongly disagree' to 'strongly agree'. Items are summed to give a total score, which ranges from 5 to 35, with higher scores indicating greater life satisfaction.

2.3.3. Psychological well-being scales

The present study used dimensions of the Ryffs Psychological Well-being Scales (Ryff, 1989). The dimensions used were Environmental Mastery (EM), Purpose in Life (PIL), Personal Growth (PG) and Self Acceptance (SA). The mid-length version of the scale was used which consists of 54 items (9 per dimension). Items are rated on a six-point scale ranging from 'strongly disagree' to 'strongly agree'. After reverse scoring, items for each dimension are summed, with possible scores ranging between 6-64 and higher scores indicating better psychological well-being.

2.3.4. Perceived Stress

Participants completed the Perceived Stress Scale (PSS; Cohen et al., 1983). It assesses the subjective appraisal of stress and reflects the degree to which individuals appraise their lives as unpredictable, uncontrollable and overloaded in the last month. The 4-item version was used and items were measured on a five-point (0-4) Likert scale (never, almost never, sometimes, fairly often, very often). After reverse scoring items are summed and total scores can range from 0 and 20, with higher scores indicating greater perceived stress.

2.3.5. Depression, Anxiety and Stress

Participants completed the Depression, Anxiety and Stress Scale (DASS; Lovibond & Lovibond, 1995) which measures three related negative emotional states of depression, anxiety and stress in the last month. The original scale consists of 42 items; each of the three dimensions consists of 14 items. On a 4-point Likert scale (0-4) respondents rate the severity or frequency to which they have experienced (did not apply to me, applied to me to some degree, or some of the time, applied to me to a considerable degree, or a good part of time, applied to me very much, or most of

the time) each statement. Individual scores are obtained for each dimension by summing the scores for the relevant items. A higher score for each dimension indicates higher negative emotional states. The shorter 21-item version (7 items make up each dimension) was used in this study.

All self-report measures had good internal consistency (α >.75).

2.4. Hair sample and collection preparation

Hair samples were cut as close as possible to the scalp from a posterior vertex position using fine scissors. Cortisol concentrations were determined from the 3-cm hair segment most proximal to the scalp. This was assumed to represent hair grown over the 3-month period prior to hair sampling, based on an average hair growth rate of 1-cm per month (Wennig, 2000). Hair samples were stored in labeled foil packages in a dry place. Samples were analysed by the laboratory of TU Dresden, Germany. The wash and steroid extraction procedures followed the laboratory protocol described in detail in Stalder et al. (2012). Cortisol levels were determined using a commercially available immunoassay with chemiluminescence detection (CLIA, IBL-Hamburg, Germany).

2.5. Statistical Analysis

Factor analysis by means of principle component analysis, with varimax rotation was conducted to explore the structure of the self-report psychosocial measures. Cortisol data was skewed and, therefore, a log-transformation was applied which effectively reduced the skewness statistic, and for three outliers (>3 standard deviations from

the mean) log transformed values were winsorised. For illustration purposes, untransformed data is presented in the table and figure. Group comparisons between the young and older participants were conducted for participant characteristics (demographic, health and hair variables), the well-being and ill-being factors and HCC. Pearson correlations or tests of differences were conducted to examine if any of the participant characteristics influenced HCC. Mixed regression modelling was used to examine effects of well-being, ill-being and age group on HCC. The analysis was repeated introducing potential confounding variables (i.e. those that have previously been shown to influence HCC and those that were different between the age groups). Due to power restrictions, each variable was entered one at a time in the model.

3. Results

To test the structure of the psychosocial measures exploratory factor analysis was conducted by means of principle component analysis. Two components with an eigenvalue greater than 1.0 were found. Table 1 displays the component matrix, which shows the loadings of each measure on components 1 and 2 prior to rotation. All of the measures were correlated with component 1 before rotation. Following rotation the well-being measures (SHS, SWLS, EM, PG, PIL and SA) were strongly associated with component 1 and the ill-being measures (PSS and DASS) were strongly associated with component 2. The two components accounted for 69% of the total variance explained by the solution to the factor analysis. Component 1 accounted for 53% and component 2 accounted for 16%.

Insert Table 1 about here.

Table 2 displays the descriptive group statistics for participant characteristics, HCC, and the factors of well-being and ill-being. As expected there were differences in participant characteristics between the age groups. There was a trend for higher self-reported social status in the older group compared with the young group. In the young group, ethnicity was mixed, whilst all females in the older group were white. The young group were less likely to be taking prescribed medication and their self-reported health was significantly higher compared to the older group. Expectedly, none of the older group was taking oral contraceptives and use of these was only 16% in the young group. None of the females in the older group smoked whilst 21% were smokers in the young group. Ill-being but not well-being differed between the two groups, with the younger group scoring higher on the ill-being factor. The younger group washed their hair more frequently compared to the older group.

HCC differed between the two groups, with the older group exhibiting higher HCC than the young group. There was no relationship between HCC and SES (p = .914), self-reported health status (p = .551) or number of washes per week (p = .101). HCC did not differ for ethnicity (p = .316), smoking status (p = .169), taking prescribed medication (p = .103) or oral contraceptives (p=.177), or hair treatments (p = .865).

Insert table 2 about here

Mixed regression modelling analysis was conducted to examine whether there was a relationship between HCC and the well-being and ill-being factors and if this interacted with the age groups. The results of this model are presented in Table 3. Ill-being was not associated with HCC and there was no interaction between ill-being and the age groups on HCC. There was a significant positive relationship between the well-being factor and HCC, independent of ill-being. A significant interaction

between age group and the well-being factor on HCC emerged, signalling that higher well-being scores were associated with higher HCC in the old group but not the young group (see Figure 1). Again this relationship was independent of ill-being. These results were unchanged when potential confounding variables were entered separately into the model. Additionally, none of the variables were significantly related to HCC in the model: subjective SES (p=.239), ethnicity (p=.876), smoking status (p=.144), self-reported health (p=.594), medication use (p=.392), taking oral contraceptives (p=.353), hair washes per week (p=.193) or hair treatments (p=.700).

Insert table 3 about here

Insert Figure 1 about here

4. Discussion

Results showed that HCC was higher in the older compared to the young group. This finding is consistent with studies showing a positive association between age and HCC (Dettenborn et al., 2012b; Feller et al., 2014). There was no association between ill-being and HCC in either the young or old group of participants. This null finding is consistent with a majority of such studies in young people (Stalder & Kirschbaum, 2012; Staufenbiel et al., 2013) but is the first to demonstrate no relationship between HCC and ill-being in an old sample (mean age 78.6±6.7 years). A novel finding to emerge from the study, the first to examine HCC in relation to well-being in healthy participants, was the positive association between HCC and well-being in the old, but not the young age group. This positive association was independent of ill-being and potential confounds and may point to a beneficial role for increased HPA axis activation, within the normal range, in older females.

Increasing age has consistently been shown to be related to differences in both the diurnal pattern and levels of cortisol secretion, as assessed from plasma and saliva samples (Deuschle et al., 1997; Knoops et al., 2010; Ryff et al., 2006; VanCauter et al., 1996). This has not always been reflected in age-related higher HCC, which has to some extent been attributed to small sample sizes and limited variability in age range in some studies (reviewed in Wosu et al., 2013). However, in larger studies covering a relatively wide age range (Dettenborn et al., 2012b; Stalder et al., 2013) or conducted within an elderly population (Feller et al., 2014), HCC has been shown to be positively correlated with increased age. The study presented here examined differences between two groups of healthy females with very marked age differences, thus maximizing the opportunity for difference. The results suggest that HCC is able to detect age-related changes in HPA axis secretory activity, at least between the young and very old. These data concur with evidence suggesting elevated 24-h cortisol production rates as well as 24-h plasma free cortisol levels with aging (Purnell et al., 2004). In addition, this capacity may be related to the observation that in older adults the amplitude of the circadian pattern of cortisol is flattened and elevated with a strong impact of age upon increased night-time plasma cortisol levels, from 20.00 to 1.30 hours (Deuschle et al., 1997). Such a prolonged period of increased cortisol secretion, day after day, may increase the likelihood of being reflected in higher HCC.

This study was not able to demonstrate any relationship between ill-being and HCC in either young or older healthy females. This is despite the use of a comprehensive measure of ill-being encompassing perceived stress, depression and anxiety. However, these data are in line with the previous literature showing no or only inconsistent associations between self-reported ill-being and HCC in healthy participants (see Staufenbiel et al., 2013). It is clear that HCC can discriminate

relevant demographic and health status variables such as Cushing's syndrome, metabolic syndrome, smoking and alcoholism (Feller et al., 2014; Kuehl et al., 2015; Stalder et al., 2010; Thomson et al., 2010). In addition higher HCC levels are associated with severe stress and stress-related psychiatric symptoms (Dettenborn et al., 2010; Stalder et al., 2014; Wosu et al., 2013). These conditions share the capacity to exert sustained input to the HPA axis, resultant in hypersecretion of cortisol which is reflected in higher HCC.

More subtle changes in HPA axis activation associated with ill-being, as reflected in dysregulation in the diurnal pattern of cortisol secretion (Adam et al., 2014; Evans et al., 2007; Garcia-Banda et al., 2014; Mannie et al., 2007; Oskis et al., 2011; Smyth et al., 2015) may not impact upon HCC. For example a flattened diurnal cortisol profile may present with lower morning levels but higher evening levels. This type of profile would not affect the overall levels of cortisol secreted and hence not be detectable in HCC. It is clear that HCC is not comparable to measures of the diurnal pattern of cortisol secretion, assessed from multiple saliva sampling over several days (Kuehl et al., 2015; Pulopulos et al., 2014; Steudte et al., 2011). Assessment of HCC and diurnal cortisol regulation should be best viewed as complementary strategies for the assessment of HPA axis activation.

The finding of a positive relationship between HCC and well-being within the older group is new, although not surprising given that the participants were all healthy females. For example in a sample of which 87% were middle-aged females, general anxiety disorder was associated with lower HCC (Steudte et al., 2011). Similarly in a study with 75% healthy older females (age range 56-77 years) lower HCC was associated with worse cognitive function (Pulopulos et al., 2014). These studies appear to indicate benefit from relatively higher HCC in largely female samples. It is

not possible to deduce whether this finding reflects higher cortisol secretion at specific times of day (e.g. morning, evening, night-time). However, there is evidence that low overall diurnal levels of cortisol (determined from multiple saliva sampling) is associated with a flattened cortisol slope across the day (i.e. day decline) which has been interpreted as an indicator of stress-related hypocortisolemia (Roe et al., 2013). Certainly, a low flat cortisol day cycle has been associated with exhaustion and chronic stress (Giese-Davis et al., 2004; Jerjes et al., 2005; Witteveen et al., 2010). Furthermore, such stress-related hypocortisolemia has been more closely linked to females than males (Roe et al., 2013).

Although further corroboration is required, it is conceivable that the positive association between well-being and higher HCC in this healthy old female population indicates a beneficial underlying diurnal pattern of cortisol secretion (Roe et al., 2013). However, the reason why this was true for well-being with no associations for ill-being remains speculative. It is plausible that relatively higher HCC levels found in this healthy sample reflected the beneficial energy-mobilising properties of cortisol. It could plausibly be argued that measures of well-being are more closely related to energy-related thoughts and activities than the measures of ill-being (e.g. environmental mastery, purpose in life, personal growth vs. negative emotional states of depression, anxiety and stress). In line with this, previous research has indicated that increased manifestation of the cortisol awakening response can be observed on days characterized by a higher level of anticipated activity (see Law et al., 2013). Particularly in elderly individuals, such increased activity levels may be viewed as a marker of a healthy, energised lifestyle and higher well-being. The failure to observe a positive association between HCC and well-being in the young age group may reflect the more resilient nature of HPA axis functioning in earlier life,

with increasing age exposing the consequences of a lifetime's wear and tear (Lupien et al., 2009).

There was no relationship between HCC and SES, self-reported health, ethnicity, smoking status, taking prescribed medication or hair treatments or number of hair washes per week, which is counter to some published outcomes (Wosu et al., 2015). Although there were differences between the age groups on some of these measures (e.g. SES, ethnicity, medication and smoking) inclusion of these correlates in the analyses did not affect the reported results.

This study has several strengths the most notable of which is separate assessment of well-being and the extensive and thorough evaluation of both ill-being and well-being as independent factors. More than one validated measure was used for each domain and the robustness of the dimensions was tested with factor analyses which confirmed and justified two distinct factors for separate analysis. A potential limitation was that the ill-being measures were used in the validated form asking participants to assess their stress, depression and anxiety over the previous month (not the three month period assumed to correspond with the HCC measure). The use of a subjective, rather than objective, measure of SES is a further limitation. The sample sizes are consistent with several published reports on HCC (Stalder et al., 2014), although the unequal samples is a limitation. Another limitation was difference in group characteristics, in addition to age, although the study assessed and accounted for a wide range of these potentially confounding factors in the statistical analyses. The study was designed to examine young and old females only, the results may not be generalizable to similar male populations.

4. Conclusions

The data supports previous evidence of increased total cortisol secretion with increased age. Additionally, these findings correspond to previous evidence failing to show clear a relationship between HCC and self-reported ill-being, in either young or old healthy females. However, results suggested that HCC is able to distinguish different levels of well-being in healthy older females, a finding that merits further investigation. Although HCC is unable to detect subtle changes in the pattern of HPA axis activation, if the results presented here are confirmed HCC's convenient, retrospective and non-invasive properties may make it an attractive complementary study medium in future research of aging and well-being.

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