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Abstract

Chronic stress can be important in the pathology of chronic disease. Hair cortisol concentrations (HCC) are proposed to reflect long term cortisol secretion from exposure to stress. To date, inconsistencies in the relationship between HCC and self-reported stress have been attributed to variation and limitations of perceived stress measurement. We report data from employees of two large public sector worksites (n=132). Socio-demographic, health, lifestyle, Perceived Stress Scale (PSS), and work-related Effort Reward Imbalance (ERI) were collected at baseline. Participants were asked to respond to mobile text messages every two days, asking them to report current stress levels (Ecological Momentary Assessment, EMA), and mean stress was determined overall, during work hours, and out of work hours. At 12 weeks, the Appraisal of Stressful Life Events Scale (ALES) was completed and 3cm scalp hair samples were taken, from which HCC was determined (to reflect cortisol secretion over the past 12 weeks). Mean response rate to EMA was 81.9±14.9%. Associations between HCC and the various self-reported stress measures (adjusted for use of hair dye) were weak (all <.3). We observed significant associations with HCC for EMA measured stress responses received out of work hours (ρ=.196, p=.013) and ALES Loss subscale (ρ=.241, p=.003), and two individual items from ERI (relating to future work situation). In regression analysis adjusting for other possible confounders, only the HCC-ALES Loss association remained significant (p=.011). Overall, our study confirms that EMA provides a useful measurement tool that can gather perceived stress measures in real-time. But, there was no relationship between self-reported stress collected in this way, and HCC. The modest association between HCC and stress appraisal does however, provide some evidence for the role of cognitive processes in chronic stress.

1. Introduction

Chronic stress is an important contributor in chronic disease pathology (Chrousos, 2009; Kristenson et al., 2004). When an individual is stressed, metabolic and physiological changes allow immediate physical exertion (fight-or-flight). However, *chronic* stimulation of the stress response through daily stress results in allostatic load (McEwen and Stellar, 1993); non-adaptive reactions that alter baseline physiology and increase the likelihood of disease (Brunner and Marmot, 2006; Goldman, 2001). Therefore, stress biomarkers can provide valuable information regarding stress and, in turn, future disease risk.

The present study explored hair cortisol concentration (HCC) as a marker of chronic stress. Cortisol is secreted via the hypothalamic-pituitary-adrenal (HPA) axis, which is activated in response to stressors, and has become a central marker of stress. Traditional collection through saliva, blood or urine is limited by sensitivity to diurnal cortisol changes, acute stress and consumption. HCC from a 3cm sample of scalp hair can reflect the past 3 months of cortisol secretion, offering a stable and feasible measure of chronic stress (Gow et al., 2010; Russell et al., 2012; Stalder and Kirschbaum, 2012).

Understandably, there is considerable and growing interest in HCC as an objective biomarker of chronic stress. Evidence is accumulating for the potential application of HCC in clinical and epidemiological studies (Wester and van Rossum, 2015; Wosu et al., 2013) and various relationships have been demonstrated between HCC and, for example, Cushing's Syndrome (Manenschijn et al., 2012), cardiovascular disease (Manenschijn et al., 2013), depression and mood disorders (Dettenborn et al., 2012b), and anxiety (Steudte et al., 2011). However, the association between HCC and subjectively measured stress remains unclear. A review of HCC with subjective stress-related psychological measures reported significant associations in less than half of studies (Staufenbiel et al., 2013). Reasons posited by the review authors included heterogeneity of study populations and diverse stress questionnaires (that cover different periods of time), and variable lengths of hair (which also cover different periods of time).

In this paper, we explore the associations between HCC and a number of self-reported stress measures. We used participants in employment as being in work has been shown to be associated with an enhanced cortisol response (Kunz-Ebrecht et al., 2004). There were three aims to our study. First, we address a call for further investigation of HCC with more elaborate stress assessment strategies, such as ecological momentary assessment (Stalder and Kirschbaum, 2012). Ecological momentary assessment (EMA) is not a single research method, but encompasses a range of methods and methodological traditions (Moskowitz and Young, 2006;Shiffman et al., 2008; Smyth et al., 2009). Shiffman et al. (2008) summarised features common to EMA approaches, which help to outline the main advantages:

1) Data are gathered as participants go about their lives (i.e., ecologically valid);
2) Participants' current state is assessed, rather than relying on recall (i.e., momentary);
3) Moments to be assessed can be strategically selected (e.g., specific times, random sampling, or other sampling methods) to suit the particular research enquiry;
4) Multiple assessments over time can be used to measure variation in experiences or behaviour, over time and across situations

Second, we considered stress appraisal. An individual's resilience or their ability to cope with stressors, can influence their stress response. For example, psycho-physiological responses to stress are different depending on whether an individual views the stress positively, as challenge, compared with negatively, as a threat (Blascovich and Mendes, 2000; Seery, 2011), with individuals able to change their psycho-physiological response to stress based on the task instructions they are given (e.g., Seery et al., 2009; Turner et al., 2014). Further, primary cognitive appraisal of a stressor has been found to account for 26% of variance in the stress response assessed by cortisol (Gaab et al., 2005). To date, there has been little consideration of such factors in relation to HCC.

Finally, we explored the relationship between the work environment and HCC through assessment of the relative effort-reward balance. The Effort-Reward Imbalance (ERI) concept was developed in the context of cardiovascular disease prediction (Siegrist et al., 1986). It is based on the principle of reciprocity; that employee investment of effort in the absence of perceptibly adequate reward, promotes a stress response. Continued activation of this stress response has deleterious health effects, with ERI being linked with negative outcomes for mental health (Ndjaboué et al., 2013) and physical health (Kuper et al., 2002; van Vegchel et al., 2005). There is not consistent evidence linking ERI with salivary cortisol measures (Eller et al., 2012). Only two studies to date have linked ERI with HCC; one in Chinese kindergarten teachers (Qi et al., 2014), and another study that found the perceived promotion prospects item within ERI was negatively associated with HCC in Bangladeshi garment workers (Steinisch et al., 2014).

To address the above aims we measured associations between HCC and: (i) EMA-measured stress; (ii) general perceived stress; (iii) stress appraisal; and (iv) the effort-reward imbalance, in generally healthy, adult employees.

2. Methods

2.1 Participants

Participants were recruited from two large public sector employers in the West Midlands region of the UK. Workplace 1 was a higher education establishment and Workplace 2 was a local authority organisation. Exclusion criteria were: non-ownership of a mobile phone; hair typically shorter than 2 cm; conditions known to affect cortisol levels (including pregnancy, Cushing syndrome, Addison disease). With a power of .8 and an alpha level of .05, a target sample 150 was deemed sufficient to detect relatively weak correlations ($r > .2$) between HCC and perceived stress measures.

Out of the 153 participants recruited, 132 were included in most inferential statistics with HCC (although fully adjusted linear regression was possible for 122). This final sample comprised more women than men (107 vs. 25), with an overall mean age of 41.4±11.4 (range 21-69 yr).

2.2 Procedure

Employees were invited to take part through an email, which included a link to a brief online screening questionnaire, with attached information sheets. Screening questions included: smoking status; mobile phone ownership (yes/no); currently pregnant (yes/no); length of your head hair (less than 1 cm, 1-2 cm, typically longer than 2 cm). Participants were offered £15 in retail vouchers on completion of the project as an incentive. All eligible individuals who completed the screening survey were contacted to arrange appointments for baseline data collection at the worksite. Participants were then sent text messages at random times, every other day for 12 weeks, after which, follow-up data collection appointments were arranged. This study was approved by the University ethics committee

2.3 Measures

2.3.1 Baseline

- Demographic: age, gender, ethnicity, deprivation (rank based on Index of Multiple Deprivation of home neighbourhood, where higher rank indicates less deprivation).
- Hair-related: washing frequency (from 1 to >7 times/week), use of hair dye / treatment (yes/no).
- Mobile phone- number (for text message follow-up).
- Stress: Perceived Stress Scale, PSS-10 (Cohen et al., 1983), as a measure of general stress.
- Effort-Reward Imbalance (ERI): 16-items that provide summary scores for effort (3–15), reward (7–35) and over-commitment scale (6–24), and the effort–reward ratio, where higher ERI indicates greater stress potential (Leineweber et al., 2010). Sensitivity analysis was also run for individual ERI items.

2.3.2 Three-month follow-up

- Stressful life event appraisal: Appraisal of Life Events Scale (ALES) (Ferguson et al., 1999), where individuals were asked to 'describe briefly the most stressful event that you experienced in the last three months' and then appraise it by rating 16 items using a 6-point scale (where 0 = not at all to 5 = very much so). Summary scores were calculated for three primary appraisal dimensions of threat, challenge and loss.
- Holiday periods: Any days in the past three months when participants were on annual leave (holiday) from work were identified to be classified as 'non-work' periods (for EMA data processing).
- Hair sample: at least 3 cm of hair were taken from the posterior vertex position of the scalp. Based on an average hair growth rate of 1 cm per month (Wennig, 2000), this is thought to represent hair grown in the 12 weeks between baseline and follow-up and, therefore, is assumed to reflect cumulative cortisol secretion in that period.

2.3.3 Ecological Momentary Assessment (EMA)

Text messages were sent to all participants every other day (by Silk Business Systems Ltd), for 12 weeks, asking for them to indicate current stress levels: '*Before you read this message, how STRESSED were you feeling? Reply with a number between 1 and 5*' (Response categories: 1=Not at all, 2=A little, 3=Moderately, 4=A lot, 5=Extremely). Messages were sent at random times during waking hours (Monday-Friday 0800h-2200h; Saturday-Sunday 0900h-2200h). Mean values were determined for each participant over the 12-week period and categorised as: *Overall* (from all responses); *Work* (0900h-1700h Monday-Friday); *Out of work* (after 1700h Monday-Friday, weekend and any days where participants reported to have taken annual leave). This estimate of *working hours* was based on those typical for the two employers, although it is possible that some participants working hours did not align perfectly (e.g., if in part-time employment).

2.4 Hair sampling and analysis

Samples were analysed by Salimetrics Ltd, Cambridge, UK. Only 136 participants provided hair samples; all were analysed in duplicate, 15 with a double extraction (i.e., two samples analysed from the same hair sample) and 121 with single extraction. The cortisol result from the ELISA (µg/dL) was corrected for the individual hair weight of the sample, the amount of methanol used for extraction and the reconstitution volume, and the resulting HCC is reported in pg/mg. Duplicate analysis was conducted by independently weighing out two samples of hair from the same participant and then independently extracting cortisol (washing in isopropanol, grinding, methanol incubation and a portion of the methanol evaporated until dry and then reconstituted and analysed in the ELISA). The Pearson correlation of the HCC between duplicates of r=.95 was comparable to the r=.97 reported by Kirschbaum et al. (2009) for duplicate extractions.

2.5 Statistical analysis

Hair cortisol data were significantly positively skewed and so were log transformed. All inferential analyses were carried out on transformed data, but means and standard deviations are provided in original units (pg/mg). To explore possible differences in population characteristics between worksites, independent samples t-tests (normally distributed continuous variables), Mann-Whitney (non-normal continuous data) and chi-squared tests (categorical data) were used. Given the lack of significant differences between the two worksites in terms of age, gender, HCC and most perceived stress measures, they were treated as a single sample for all analysis (Supplementary file 1). Kappa (κ) statistic was calculated to test for the significant associations between dichotomous variables (e.g., gender and use of hair treatment), where κ<.20 showed poor agreement, κ =.21-.40 fair, κ=.41-.60 good, κ=.61-.80 substantial and κ=.80-1.00 almost perfect (Landis and Koch, 1977). Wilcoxon Signed Rank tests were used to determine within-subject differences in EMA scores (work vs. out of work hours). Associations between HCC and self-reported stress measures were explored using Spearman's rank correlations (perceived stress measures were not normally distributed), which were classified as weak where the correlation coefficient (rho, ρ) was <.3, moderate if .3 to .49, or strong if >.5 (Cohen, 1988). These were then repeated as partial correlations, controlling for a significant HCC confounder (use of hair dye/treatment). Variables that remained significantly correlated with HCC were transformed (Box-Cox transformation) and entered into linear regression models, adjusting for other possible confounders.

3. Results

3.1 Sample characteristics

Out of those recruited at baseline (n=153), as a result of loss-to-follow-up (n=16), failure to provide an adequate hair sample (n=1), and exclusions as HCC data were extreme outliers (>4 standard deviations above the mean), our final sample was 132. Fully adjusted linear regression was possible for 122 participants.

Detailed sample characteristics overall and by workplace are provided in Supplementary file 1. Overall, there were more women than men (107 vs. 25), mean age was 41.4±11.4 (range 21-69 yr), there was a good distribution across deprivation quintiles from most (Q1=18.0%) to least deprived (Q5=21.1%), with the majority educated to degree level or higher (78.8%). Data confirmed a relatively healthy sample in terms of self-reported health (92.4% Good to Excellent), mean HCC (10.8±9.4 pg/mg) and BMI (25.1±4.8 kgm^{-2}), with overall PSS towards the higher end of normative values (16.6±7.2).

To explore possible bias in loss to follow-up, we compared final sample (n=132) versus those lost to follow-up / excluded: age (mean 41.4±11.4 vs. 41.8±11.6, t=.138, p=.890); gender (% female 81.1% vs. 81.0%, chi-sq=<.001, p=.991); and PSS (mean 16.6±7.2 vs. 18.6±7.3, t=1.156, p=.250). These indicated that bias in loss to follow-up was not an issue.

3.2 Hair cortisol concentration

Mean HCC concentration was in the range of values that might be expected for a non-patient population of employed adults (10.8±9.4 pg/mg). Confounders of HCC considered elsewhere were explored (Wells et al., 2014). There was no evidence for correlations between HCC and: age (ρ=.017, p=.850); BMI (ρ=.101, p=.256); or frequency of hair washing (ρ=-.161, p=.068), which is consistent with some other findings (Dettenborn et al., 2010; Manenschijn et al., 2011). There was some evidence of: lower HCC in females compared with males (10.21±8.28 vs. 13.41±12.56 pg/mg, t(130)=2.006, p=.047); lower mean HCC in those who did use hair dye/treatment, compared with those who did not (9.94±8.58 vs. 12.40±10.25, t=-2.27(128), p=.026); higher HCC with higher alcohol consumption (r=.176, p=.046); higher HCC with more deprivation approaching significance (r=-.170, p=.055). Further exploration of these patterns identified that of the 130 people with complete data for hair treatment use and HCC, all those who did use treatment were female (79 females, 0 males). The resulting strong association between gender and use of hair dye (κ=.418, p<.001), meant that the latter was used as a covariate in partial correlations to further explore associations between HCC and perceived stress measures.

3.3 Ecological Momentary Assessment

Mean response rate to text messages was 81.9±14.9%. To explore possible response bias, we compared EMA scores across tertiles based on response rate, and found no significant differences (overall, during work, or out of work; data not shown). Self-reported stress levels indicated that, overall, participants self-reported being ‘A little’ stressed (Mdn=1.91). Stress reported during work hours was higher than out of work (Mdn 2.0 vs. 1.6, Z=-8.178, p<.001).

3.4 HCC versus perceived stress

Unadjusted correlation analysis showed a lack of association between HCC and PSS. EMA-measured stress also showed weak, non-significant associations with HCC (ρ=.043 to .138, all p>.120; Table 1). For appraisal of stress, positive correlations approaching significance were observed between HCC and the Loss and Challenge sub-scales of the ALES (the latter not in the expected direction); but both were weak (<.3). For ERI, no associations with HCC were observed for any of the summary scores (ρ=.024 to .079, all p>.377; Table 1), with weak, non-significant associations for two individual items towards higher HCC with greater expectations of undesirable changes to work situation (ρ=.153, p=.089) and lower perceptions of the adequacy of promotion prospects (ρ=-.165, p=.068; Supplementary file 2).

To account for the potential confounding effect of hair treatment, non-parametric partial correlations (controlling for use of hair dye) were run for each perceived stress measures and HCC (Table 2). In general, this strengthened associations, reaching statistical significance for EMA stress responses for out of work hours and ALES Loss score, but all remaining weak. Similarly for the two individual ERI items, the associations remained weak, but reached statistical significance (expected undesirable changes to work situation ρ=.182, p=.021; adequate promotion prospects ρ=-.-154, p=.044; Supplementary file 2).

Linear regression analysis were performed for each of the variables that showed significant associations with HCC (which were first transformed), adjusting for use of hair dye/treatment, age, gender, deprivation, alcohol consumption. Data revealed that the HCC-ALES Loss association remained significant, independent of other confounders (β=.225, p=.011, Adj R^2=.126), whereas the relationship for EMA out of work hours was attenuated beyond significance (β=.162, p=.072, Adj R^2=.101). Significant associations between HCC and individual ERI items also disappeared in regression analysis (expected undesirable changes to work situation β=.049, p=.591, Adj R^2=.080; adequate promotion prospects β=.101, p=.266, Adj R^2=.091).

There were significant associations between various the subjective stress measures. For example, overall EMA-measured stress was significantly positively correlated with PSS, ERI effort, over-commitment and effort:reward, and ALES Threat and Loss scores (all ρ>.3, p<.001).

4. Discussion

Data from our sample of healthy employed adults showed EMA to be a useful tool for measuring self-reporting stress, with high compliance. We found limited evidence of an association between HCC and subjective stress measures from EMA, general perceived stress levels and effort-reward imbalance. We did observe a modest association between HCC and stress appraisal, and perceived work situation.

As far as we know, this was the first comparison of HCC and EMA-measured stress, whereby participants self-reported their level of stress in real-time throughout the 12-weeks corresponding to the HCC accumulation period, thus reducing limitations associated with recall methods. The generally high response to EMA text messages confirms this as a low burden method for real-time measurement with relatively high compliance (82% response to texts messages). Our low loss-to-follow up (approximately 10%) could be attributed in some part to the continued engagement with participants throughout the 12-week period as a result of text message contact.

The only significant EMA-HCC association was for self-reported stress when people were outside work hours. In general individuals reported less stress outside of work (Kunz-Ebrecht et al., 2004). The positive association between self-reported stress outside of work and HCC may be reflective of a more profound effect on chronic HPA axis stimulation (leading to higher HCC) when people are not able to *switch off* and de-stress outside work. Either because of the pervasive and ongoing nature of their work, or that they have a number of different stressors in their home and personal life that are more closely related to chronic HPA axis stimulation. However, it is also worth noting the association was weak (<.3) and attenuated when other possible confounders were considered. This accords with previous research that stress self-report measures are often inconsistent with behavioural and physiological measures (e.g., Weinberger et al., 1979). It also accords with the 2013 review of HCC studies, which discussed the '*often found ''lack of psychoendocrine covariance*''' (Staufenbiel et al., 2013; p.1230); i.e., the dissonance between HCC and self-reported stress. Explanations proposed included divergence in time due to lag effects caused by different timing of stress responses (Schlotz et al., 2008), differences between the four-week recall period of the typically used PSS (Cohen et al., 1983) and hair samples reflecting 12-week cortisol secretion, and recall or social desirability biases associated with such measures (Staufenbiel et al., 2013). Indeed, our observed association between HCC and PSS did not reach significance. Real-time EMA-monitored stress when participants were outside normal work hours did correlate with HCC, but the difference in strength of associations was not striking, and overall, and during work hours, EMA values were weaker and non-significant.

The only association with HCC that remained significant in the final regression model, was for stress appraisal, which indicated higher HCC with the higher *loss* appraisal, reflecting greater propensity to consider the potential for suffering and sadness of stressors (Ferguson et al., 1999). Staufenbiel et al. (2013) noted the importance of considering the role of individual's cognitive stress appraisal in relation to HCC. Stress need not be perceived negatively by an individual and similar levels of self-reported stress can be associated with contrasting physiological responses, for example depending on whether the situation is perceived as a challenge or a threat (Seery et al., 2009; Turner et al., 2014). It is possible that a stronger effect would have been detected in a more chronically stressed group, or perhaps with a more generic stress appraisal measure to reflect general attitudes to stress and ability to deal with ongoing daily stressors that contribute to chronic stress.

Effort-reward imbalance was not related to HCC; there were no associations for summary scores and the significant partial correlations for two ERI items (expected negative changes in work situation; promotion prospects), did not persist in regression analysis with confounders. This is somewhat contrary to findings from studies of non-Western populations that have explored this relationship (Qi et al., 2014; Steinisch et al., 2014). Qi et al. (2014) reported a modest correlation linking higher HCC with higher ERI (r=.33, p=.006) in female, Chinese kindergarten teachers. Although sample HCC levels reported by authors (Mdn 11.8 pg/mg) were similar to those presented here, there are other sample differences that might explain our different findings. Specifically, they comprised only female teachers from three kindergartens, which would have been more homogeneous in terms of the type of daily effort-rewards associated with the role (despite 14 working at kindergarten for children with developmental disorders). This might have improved the ability to detect associations through reducing possible confounders. Steinisch et al. (2014) combined a seven-item ERI measure and five setting-specific items to explore work-related stress in a sample of Bangladeshi garment workers who had very low HCC levels (mean 3.27±2.58 pg/mg). 'Work-related values', a composite of items three items (job security, promotion prospects, and job latitude), was a significant predictor of HCC in regression analysis (β=.209, p=.021), which was largely explained by an association between HCC and promotion prospects (β=.230, p=.007). The counter-intuitive direction of this relationship was attributed to the study context; promotion within that specific industry was rare and could represent an unfavourable change associated with greater stresses and exceptional demands. This has some synergy with our data. The partial correlations we observed (controlling for hair treatment) suggested that HCC was higher in employees who perceived less adequate promotion prospects or anticipated detrimental changes to their working situation. Similar to the Steinisch et al. (2014) study, these suggest a link between HCC and expectations or optimism about one's personal working future. As the associations were no longer significant in our adjusted regression analysis, further examination in larger samples, with more statistical power might be warranted to discern if a genuine, independent association exists.

Our data, in the context of the growing HCC literature, add to the evidence that the relationship between HCC and self-reported stress is inconsistent, in some cases, contradictory (Staufenbiel et al., 2013), and may vary across different population groups. Out of 15 studies, Staufenbiel noted positive associations in six, no association in seven, and inverse associations in two studies. This inconsistency has continued to emerge. For example, a study of reportedly healthy middle-aged female nurses and librarians found significant positive associations of HCC with perceived stress (r=.20), and the Hospital Anxiety Depression (HAD) scale (r=.23) (Faresjö et al., 2014). However, both were weak (r <.3) and the HCC values appear high (median=34.9 pg/mg), which suggest that the cohort was more chronically stressed than other general population samples, including that reported here. Conversely, Oullette et al. (2015) reported lower HCC in more chronically stressed participants, which is consistent with the findings of lower HCC in those who with Post Traumatic Stress Disorder (PTSD) or reporting trauma (Luo et al., 2012; Steudte et al., 2013). To further add to the complexity of this area, analysis of pooled data from five small cohorts (total n=324), identified a significant curvilinear relationship between HCC and PSS score, such that HCC increased with higher perceived stress, but then reduced at the highest levels (Wells et al., 2014). This latter sample mostly comprised people in some way linked with mental health problems/substance misuse (personal, family members or volunteers), with 70 participants from the general population. So again, that nature of the population is a likely contributor.

In contrast to these studies, our data highlight a lack of consistent associations between self-reported and HCC measured stress in generally healthy, non-patient sample. Of note our mean HCC levels (10.6±9.0 pg/mg) were lower than most other non-patient populations or control groups in other published HCC research, which range from 15 to 34.9 pg/mg (Dettenborn et al., 2012a; Dettenborn et al., 2010; Faresjö et al., 2014; Stalder et al., 2010; Stalder et al., 2012; Steudte et al., 2011). However, our sample mean HCC levels ranged from 2.3 to 62.9 pg/mg. EMA data indicated that overall, participants felt 'a little' stressed and the mean PSS score was 16.6, ranging up to 32 (out of a maximum of 40), which is towards the higher end of normative values for the general population (Cohen and Williamson, 1988). So although we did not appear to have a highly chronically stressed group, there was variation within the sample that should have allowed differences and associations to be detected. Indeed, associations were detected between the different subjective stress measures which provided confidence in the concurrent validity of the self-report measures, and that the lack of or relatively weak associations with HCC were genuine, rather than artefacts of problems with specific perceived stress measures.

It, therefore, seems more likely that as suggested elsewhere (Staufenbiel et al., 2013), HCC may provide a better marker of clinical conditions (or their development) known to be linked with chronic stress or altered cortisol levels. For example, HCC has been validated in conditions known to be associated with altered corstiol secretion, such as Cushing Syndrome (Manenschijn et al., 2012; Thomson et al., 2010) and pregnancy (Kirschbaum et al., 2009; Steptoe et al., 2003); HCC appears higher in chronically stressed dementia caregivers who report depressive symptoms (Stalder et al., 2014), and has been linked with cardiovascular disease (Manenschijn et al., 2013). In the general population, in the absence of such manifest (or developing) conditions, HCC could still provide a useful biomarker of stress, but its association with perceived stress is perhaps not a useful means of *validation*.

Strengths of our study include the use of multiple subjective measures of stress, including recall, real-time EMA, appraisal and work-specific, which were correlated with one another (moderate-strong) in expected directions. A number of study limitations are acknowledged. First, our sample size compares favourably with many others in the field, but challenges in recruitment prevented a larger sample that would have provided greater statistical power and, perhaps, greater range in stress levels. Sample size has often been a limited in HCC studies, but some larger studies have been published (Dettenborn et al., 2012a; Manenschijn et al., 2013; Wells et al., 2014), and more are needed. Second, PSS data were collected at baseline as a marker of general stress, with EMA providing the more novel measure of perceived stress for the time period corresponding with the 12-week HCC period. Therefore, the four-week recall period of PSS preceded the 12-week HCC period, so relies on the assumption that this was representative of general stress levels, including during the 12-weeks represented by HCC. This is unlikely to have been a considerable problem given consistent correlations between PSS and other subjective stress measures, and the functionality of PSS as a measure of general stress level. Third, our stress appraisal measure focused on a specific stressful event within the study period and is, therefore, dependent on the occurrence of sufficiently stressful event. A more generic measure might be more useful to reflect general attitudes to stress and ability to deal with ongoing daily stressors that contribute to chronic stress. Fourth, a degree of sample selection bias is inevitable, which could explain why our sample was not highly chronically stressed. While this should not necessarily be a problem for exploration of perceived and physiological stress measures, it is a limitation on generalisability of our associations (or lack of) to more stressed populations.

5. Conclusions

Overall, our study confirms that EMA provides a useful measurement tool that can gather perceived stress measures in real-time and, perhaps, can help to limit loss-to-follow-up. Further the relationship between HCC and stress appraisal does provide evidence for the role of cognitive processes in chronic stress. But, ultimately, we concur with the conclusions of Wells et al. (2014), that the relationship between self-reported stress and stress biomarkers remains unclear. The apparent lack of perceived stress-HCC associations in the general population should not be a reason to doubt the utility of HCC. Larger, prospective cohort studies with follow-up of disease end-points would help to determine the relative predictive power of HCC and perceived stress, and the role of HCC as a physiological predictor of stress-related physical and mental health conditions in the general population. As evidence accumulates linking HCC with chronic conditions (e.g., CVD), it would be preferable to confirm HCC as useful, non-invasive, objective marker of disease risk, regardless of self-reported measures.

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Table 1. Correlation matrix of HCC and perceived stress measures

		HCC (log)		PSS		EMA						ERI								ALES			
						Overall		Work		Out of work		Effort		Reward		Over-commitment		Effort:reward		Threat		Challenge	
		rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p	rho	p
PSS		.148	.107																				
EMA	Overall	.067	.449	.502	<.001																		
	Work	.043	.625	.499	<.001	.972	<.001																
	Out of work	.138	.120	.420	<.001	.834	<.001	.732	<.001														
ERI	Effort	.019	.829	.393	<.001	.376	<.001	.367	<.001	.307	<.001												
	Reward	.025	.783	-.083	.366	-.066	.460	-.106	.235	-.083	.349	-.060	.503										
	Over-commitment	.095	.292	.476	<.001	.383	<.001	.384	<.001	.312	<.001	.541	<.001	-.091	.307								
	Effort:reward	.024	.788	.362	<.001	.337	<.001	.356	<.001	.285	.001	.874	<.001	-.499	<.001	.481	<.001						
ALES	Threat	.093	.292	.414	<.001	.347	<.001	.346	<.001	.360	<.001	.229	.009	<.001	.999	.201	.023	.203	.021				
	Challenge	.164	.063	-.121	.186	-.075	.397	-.079	.372	-.082	.353	-.080	.367	-.068	.449	-.060	.504	-.059	.505	-.164	.061		
	Loss	.168	.057	.460	<.001	.362	<.001	.360	<.001	.335	<.001	.328	<.001	.013	.887	.275	.002	.271	.002	.645	<.001	-.327	<.001

HCC, mean hair cortisol concentration; PSS, perceived stress scale; EMA, ecological momentary assessment of stress from text message responses; ALES, Appraisal of Life Events Scale; ERI, Effort-Reward Imbalance questionnaire.

Table 2. Partial correlations of HCC (Lg10) with perceived stress measures, controlling for hair treatment

			Unadjusted correlation		Partial correlation[a]	
		n	rho	p	rho	p
PSS		119	.148	.107	.134	.072
EMA	Overall	129	.067	.449	.110	.109
	Work	129	.043	.625	.084	.173
	Out of work	129	.138	.120	.196	.013
ERI	Effort	126	.019	.829	.087	.167
	Reward	126	.025	.783	.031	.365
	Over-commitment	126	.095	.292	.111	.109
	Effort:reward	126	.024	.788	.067	.229
ALES	Threat	130	.093	.292	.133	.066
	Challenge	130	.164	.063	.132	.068
	Loss	130	.168	.057	.241	.003

[a]Adjusted for use of hair treatment