Hair cortisol measurement in older adults: Influence of demographic and physiological factors and correlation with perceived stress.

Joseph H. Lanfear[1], Clarissa D. Voegel, Tina M. Binz[2], *Richard A. Paul[1]

1. Bournemouth University, Faculty of Science and Technology, Poole, Dorset, BH12 5BB, UK.

2. Center for Forensic Hair Analytics, University of Zurich, Switzerland

*Corresponding author. Richard Paul (rpaul@bournemouth.ac.uk) Bournemouth University, Faculty of Science and Technology, Poole, Dorset, BH12 5BB, UK.

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Abstract

Aims

This study aimed to investigate correlation between hair cortisol levels and perceived stress scale (PSS) in addition to a range of demographic and physiological factors in a sample of older adults.

Experimental

Hair cortisol concentrations were established from 42 older adults aged between 60 and 80 years old. Age, sex, hair colour, smoking status, employment status, daytime sleeping, medication, waist to hip ratio (WHR) and PSS scores were assessed through a questionnaire. Hair cortisol concentration was assessed through liquid chromatography coupled to tandem mass-spectrometry (LC-MS/MS).

Results

Amongst the older adult group there was no statistically significant correlation between hair cortisol concentration and age, employment status, daytime sleep duration, WHR or PSS. When compared to previous data assessing hair cortisol in toddlers (7 months to 3 years old), adolescents (12-17 years old) and adults (18-60 years old) it is observed that there is a trend for higher hair cortisol in older adults (60-80 years old). Hair cortisol concentrations were significantly higher in males (n = 20) than in females (n = 22) and in participants with dark brown hair (n = 8). No relationship was investigated between hair cortisol concentration and smoking status or medication intake.

Conclusions

The results confirm that hair samples are a useful alternative to the current mediums that are used to analyse biological cortisol. The results also validate the use of LC-MS/MS as an effective analytical method for the quantitation of hair cortisol concentrations.

Keywords

Cortisol; hair; Age; Stress; Perceived Stress; older adults

1. Introduction

The physiological impact of long-term stress exposure on the human body has been of interest to scientists and researchers for a number of years. Perhaps the age group most at risk of health implications arising from long-term or chronic stress is older adults due to potential life-long exposure.

Stress is widely acknowledged as an emotion that is encountered when an individual is faced with experiences that are both emotionally and psychologically challenging (McEwen 2007). When faced with these experiences, the body activates an acute stress response. The acute stress response is a natural and healthy function of the human body (Engert et al. 2018) and has been behaviourally characterised as the "fight or flight" response (Taylor et al. 2000). The fight or flight response is a process in which the body creates a state of readiness in reaction to a stressor by releasing hormones such as adrenaline and cortisol (Oxington 2009, p.56). The release of these hormones causes a number of physiological changes in the body which is referred to as allostasis. These changes include an increased heart rate and blood flow caused by adrenaline, and the release of glucose from the liver caused by cortisol (McCarty 2016, p.34). Allostasis is the process of achieving physiological or psychological stability through physiological or behavioural changes (Sterling & Eyer 1988). An increase in blood flow increases the amount of oxygen circulating in the body, allowing for the body to maintain high levels of alertness, thus achieving stability. Once the threat of the stressor has subsided, elevated levels of the hormones involved in the acute stress response reduce back to normal.

Chronic stress arises as the result of a prolonged exposure to the acute stress response, and therefore prolonged levels of elevated hormones (McEwen 2007). This can have significant psychological and physiological effects on the body, for example, a decreased attention span and low energy levels (Parker 2007, p.6). The negative effects of chronic stress is referred to as allostatic overload which serves no purposes and predisposes individuals to serious pathophysiology and disease (McEwen & Wingfield 2003). Seeman et al. (2001) found a relationship between allostatic overload and an increased risk of suffering from cardiovascular disease (CVD), most notably in older adults aged 70–79.

The effects of chronic stress on older adults has been the subject of extensive research. Studies have demonstrated that chronic stress in an older population can result in a variety of different issues including health complications and cognitive impairment. Older adults are highly at risk of infection, illness and death caused by the influenza virus (Wilhelm 2018). Research has demonstrated that chronic stress in older adults aged 65 and over alters the immune system response to the influenza virus vaccine (Kiecolt-Glaser et al. 1996). This has the potential to cause significant health issues. Impaired cognitive performance was found to be related to elevated cortisol levels and therefore to chronic stress (Lupien et al. 1994). Furthermore, prolonged exposure to stress, often over a number of years, results in the loss of neurons in the brain which consequently results in the deterioration of memory (McEwen & Sapolsky 1995).

Elevated cortisol levels have shown to be strongly associated with increased prevalence of ill-health and diseases such as hypertension, type 2 diabetes mellitus, Cushing's syndrome (CS) and Metabolic syndrome (MetS) (Manenschijn et al. 2011; Stalder et al. 2013; Feller et al. 2014; Wester et al. 2017). CS develops as a result of a benign pituitary adenoma which causes an excess secretion of adrenocorticotropic hormone (ACTH), stimulating the production and release of excess glucocorticoids into the body's circulatory system (Newell-Price et al. 2006). CS exhibits as clinical features such as hypertension, menstrual irregularity and weight gain (Newell-Price et al. 2006).

Cortisol is often measured via the analysis of serum, saliva and urine (El-Farnhan et al. 2017). The fundamental limitation of the current biological matrices in which cortisol levels are measured in is the lack of capability to exhibit retroactive cortisol levels; therefore, there is an inability to indicate the extent or length of exposure to stress. When analysing serum, saliva and urine, only glucocorticoids circulating in the body at the time of sample collection are represented in the results. Since hormone secretion is consistent with a circadian rhythm, cortisol concentrations in these mediums is expected to fluctuate over the course of the day. As a result, concentrations in these fluids change from day-to-day and from hour-to-hour within each day. In recent years, hair analysis as a means of analysing and measuring cortisol concentration has seen increasing popularity.

Binz et al. (2018) found that age, sex, and hair colour all affected baseline cortisol levels in toddlers, adolescents and adults. Staufenbiel et al. (2013) found mental illnesses such as depression, post-traumatic stress disorder (PTSD) and physical stress also affect cortisol levels in hair. Smoking cigarettes is also associated with elevated salivary cortisol levels (Steptoe & Ussher 2006; Badrick et al. 2007). Increased hair cortisol levels have found to be associated with unemployed individuals (Dettenborn et al. 2010), and also those who are employed and significantly over-worked (Schulz et al. 1998). Wiebel et al. (1995) found that cortisol levels did not fluctuate significantly following daytime sleeping. A study conducted by Manenschijn et al. (2011) found a positive correlation between waist to hip ratio (WHR) and hair cortisol in younger populations.

The purpose of the current study is to identify physiological and demographic factors which influence the incorporation of cortisol into the hair of older adults. From this, we hope to identify older adults at risk from chronic stress through hair cortisol testing.

2. Experimental

2.1 Study Population

A total of 42 participants aged between 60 and 80 were recruited for the study. The sample is representative of a population of male and female older adults in good general physical health in the South West of England. Participants were made aware of inclusion and exclusion criteria before participation. Inclusion criteria were for participants to be over the age of 60 and in good general physical health. Exclusion criteria were a diagnosed psychiatric condition, the use of medication that impacts cortisol levels which includes opioids or oral steroids, and bleached or dyed hair, although uncoloured sections of highlighted hair were allowed.

2.2 Questionnaire & Self-assessment

Participants were asked to complete a questionnaire to capture demographic and physiological data. These were age, sex, hair colour (our study reports on natural hair colour, not dyed colour), smoking status, employment status, time and duration of daytime sleep, prescribed medication, and WHR.

Participants were also instructed to complete a Cohen Perceived Stress Scale (PSS) as a form of self-assessment on their perceived personal stress levels from the month preceding the date of participation. The Cohen PSS is an instrument that is used internationally to measure the degree to which an individual considers situations in their lives as stressful (Cohen et al. 1983).

The score is calculated through participants rating how regularly they feel stressed about a particular event. The score has a corresponding level of stress. A score of 0–13 is considered to be indicative of low perceived stress levels, 14–26 of moderate stress levels, and 27–40 of high stress levels. In this study participants completed the PSS-10, a ten-item version of the fourteen-question PSS-14, as research has highlighted the enhanced reliability and validity of the PSS-10 compared to the PSS-4 and PSS-14 (Mitchell et al. 2008; Karam et al. 2012). Cohen and Williamson (1988) conclude that the PSS-10 is as good a measure of perceived stress as the PSS-14 and significantly more reliable than the PSS-4.

2.3 Sample Collection

Hair samples were cut with scissors from the vertex posterior region of the head, as close to the scalp as possible. The samples cut were approximately the thickness of a pencil, or approximately 100 strands. Once cut, the sample was enclosed in a foil strip wrap with the proximal end which was closest to the scalp clearly indicated. The foil strip wrap containing the hair sample was contained within an envelope that clearly indicated the date of collection, the participant's name, and their signature of consent to proceed with analysis. Sample envelopes were stored at room temperature before being sent to the Zurich Institute of Forensic Medicine at the University of Zurich for analysis.

2.4 Hair Cortisol Extraction & LC-MS/MS Analysis

Cortisol extraction and analysis followed the stages described in Binz et al. (2016) using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Hair samples were washed to remove external contaminants, dried, and cut into snippets. Hair cortisol was extracted in methanol and then prepared for injection onto LC-MS/MS for quantitative analysis.

2.5 Statistical Analysis

Kolmogorov-Smirnov test revealed that the data set was not normally distributed. Therefore, non-parametric tests were used in the statistical analysis. The association between hair cortisol and participant age, WHR, and Cohen PSS scores was assessed using Spearman correlations. The association between hair cortisol and participant sex and daytime sleeping habits was assessed using Mann-Whitney *U* test. The association between hair cortisol and participant hair colour and employment status was assessed by Kruskal-Wallis *H* test.

2.6 Ethical Approval

This study received ethical approval from the Bournemouth University ethics committee (reference 22148). All participants gave informed consent.

3. Results

3.1 Participant Information

Lifestyle variables and cortisol concentrations in hair were assessed in a total of 42 participants. Of the total participants, 52% were female. The age of participants ranged from 60 to 80 years old, with a mean age of 68.1 years and a standard deviation of 5.3 years. The hair cortisol concentration levels of participants ranged from 1.4 to 82.5 pg/mg with a median concentration of 5.65 pg/mg, a mean concentration of 10.5 pg/mg and a standard deviation of 13.6 pg/mg.

A summary of participant information and corresponding hair cortisol levels is shown in Table 1

3.2 Physiological and Demographic Results

Statistical analysis was performed on the physiological and demographic data gathered from participants via the questionnaire to assess associations between lifestyle factors and hair cortisol levels. A summary of physiological and demographic results is shown in Table 2.

Within the cohort (n = 42) of older adults in this study, the Spearman correlation between participant age and hair cortisol levels was 0.099, $P = 0.53$ and not statistically significant.
Mann-Whitney *U* Test showed a significant difference in hair cortisol between males and females ($P = 0.018$, significant at $P < 0.05$). Mean hair cortisol in males ($n = 20$) was 14.4 pg/mg. Mean hair cortisol in females ($n = 22$) was 6.8 pg/mg. Participants with red hair ($n = 1$) and black hair ($n = 2$) were not included in this analysis as the sample sizes were not large enough to be statistically significant.

There were three dominant hair colours across the participant group–grey, dark brown and light brown. The relationship between hair colour and cortisol was assessed using Kruskal-Wallis *H* Test. A significant difference was observed between the three groups ($P = 0.025$, significant at $P < 0.05$). Mean hair cortisol levels in participants with grey hair ($n = 22$) was 10.1 pg/mg with a median concentration of 4.5 pg/mg. Mean hair cortisol levels in participants with dark brown hair ($n = 8$) was 14.9 pg/mg. Mean hair cortisol levels in participants with light brown hair ($n = 9$) was 6.9 pg/mg. No statistical analysis was performed on the data to investigate a relationship between smoking status and cortisol levels in hair. There was an insufficient representation of cigarette smokers in the study population to observe a statistically significant result. There were three dominant employment statuses across the participant group–full-time employment, part-time employment, and retired. The relationship between employment status and cortisol was assessed using Kruskal-Wallis *H* Test. No significant difference was observed between the three groups ($P = 0.16$, significant at $P < 0.05$). Mean hair cortisol levels in participants in full-time employment ($n = 7$) was 6.9 pg/mg. Mean hair cortisol levels in participants in part-time employment ($n = 12$) was 8.9 pg/mg. Mean hair cortisol levels in retired participants ($n = 23$) was 12.3 pg/mg.

Participants were divided into categories based on whether they did or did not sleep during the day. Daytime sleeping duration ranged from 5 to 90 minutes, with a mean sleeping duration of 30.4 minutes. Mann-Whitney *U* Test did not show a significant difference in hair cortisol between participants who did and didn't sleep during the day ($P = 0.84$, significant at $P < 0.05$). Mean hair cortisol in participants who did not sleep during the day ($n = 29$) was 10.8 pg/mg. Mean hair cortisol in participants who did sleep during the day ($n = 13$) was 9.5 pg/mg. The Spearman correlation between WHR in participants ($n = 41$) and hair cortisol levels was 0.28, $P = 0.078$ and not statistically significant. The Spearman correlation between Cohen PSS score and hair cortisol levels was -0.18, $P = 0.27$ and not statistically significant.

3.3 Perceived stress and associated hair cortisol levels

A summary of the scores given by participants to each question of the Cohen PSS and the associated level of stress is summarised in Table 3. Table 4 shows a summary of hair cortisol levels and the corresponding level of stress compared to participant's Cohen PSS scores and the corresponding level of stress.

4. Discussion

The results of this study show that in our sample population of older adults there was no association between age and hair cortisol. We have previously studied hair cortisol levels (Binz et al 2018) across a wide age range of people (a toddler group aged 7 months to 3 years (n=14), adolescents aged 12-17 years (n=353) and adults aged 18-60 years (n=173), and when comparing these results with the present study it can be seen that whilst there is no significant difference between the adult group and the older adult group, there is however a clear trend for hair cortisol to be higher in the older adults (Fig 1).

There are a number of previous studies that have examined this issue. The results of the current study add to previous findings asserting that there is no association between age and hair cortisol (Raul et al. 2004; Manenschijn et al. 2011; Stalder et al. 2012). However, it is important to note that the populations of these studies were not solely comprised of older adults. Therefore, a true comparison cannot be made with these studies. The results of this study are at a variance with those of studies focusing exclusively on older adults. Deuschle et al. (1997) showed increased hair cortisol concentrations in older adults aged 67–85. Feller et al. (2014) showed the same to be true in older adults aged 47–82. The difference in findings between previous studies and the current one may result from the difference in sample size. The relatively small sample size of the current study meant that a suitably large representation of older adults could not be evaluated. As with the current study, Raul et al. (2004) did not find any significant relationship between age and hair cortisol in adults, and indeed older adults, but found statistically higher cortisol levels in participants aged 0–20. This is a recurring finding in many studies investigating hair cortisol levels. Dettenborn et al. (2012) showed that children under the age of 10 had higher hair cortisol concentrations. Noppe et al. (2014) showed the same for children aged 4–14. Binz et al. (2018) showed the same association in toddlers aged 0.6–3. The increased hair cortisol of children compared to adults could be attributed to the difference in hair structure or hormone status between the two age groups (Binz et al. 2018).

Our results show a statistically significant correlation between sex and hair cortisol. Hair cortisol concentrations were significantly higher in males than in females. A large number of existing studies suggest that sex is a highly influential variable on hair cortisol, with cortisol concentrations consistently being positively related to the male sex in older adults, as well as younger ages (Dettenborn et al. 2012; Manenschijn et al. 2013; Feller et al. 2014; Staufenbiel et al. 2015; Stalder et al. 2017; Binz et al. 2018). The results of the current study are in accordance with these previous findings; however, some studies did not find such effect to be true (Raul et al. 2004; Manenschijn et al. 2011; Fischer et al. 2017). Kudielka et al. (2004) speculated that the sex differences in cortisol levels could be attributed to corticosteroid-binding globulin (CBG) and free cortisol. Stolk et al. (1996) clarified this relationship by asserting that males have lower CBG levels than females. As a result of this, males have increased levels of free cortisol. Since it is free cortisol that is incorporated into the hair, this may explain why males have consistently higher hair cortisol

concentrations.

In addition, our results show an association between hair colour and hair cortisol in older adults. Hair cortisol levels were higher in dark brown hair and grey hair than in light brown hair, but lower in grey hair than dark brown hair. To the best of the author's knowledge, this study is the first that investigates the influence of hair colour on cortisol levels in a sample exclusively comprised of older adults. Previous studies have suggested hair colour has no link to hair cortisol levels at any age (Raul et al. 2004; Sauvé et al. 2007; Dettenborn et al. 2012). However, more contemporary studies negate this finding, suggesting that darker hair colours are associated with higher hair cortisol levels (Staufenbiel et al. 2015; Rippe et al. 2016; Binz et al. 2018). The reason for this may be that cortisol is more strongly bound to eumelanin in pigmented hair than pheomelanin in less-pigmented hair, thus accounting for higher cortisol levels in darker hair colours (Pragst & Balikova 2006; Dettenborn et al. 2012). Furthermore, Binz et al. (2018) suggest that cortisol may bind with different interactions in different hair colours. For example, cortisol may bind to eumelanin strongly by ionic bonding but to pheomelanin by weaker interactions such as hydrogen bonds or van der Waals forces; therefore, cortisol is more likely to become disassociated from melanin in lighter hair by processes such as hair washing. It was noted that in the current study the light brown hair sample group was composed exclusively of female participants. Since it has been previously stated that males have consistently higher hair cortisol concentrations, it is possible that the lower cortisol levels attributed to a light brown hair colour in this study may be the effect of sex rather than the hair colour.

This study was not able to statistically conclude any association between smoking status and hair cortisol concentrations as only one participant from the study population smoked cigarettes. Feller et al. (2014) show that there is a positive link between smoking cigarettes and hair cortisol in older adults. Smoking cigarettes has been attributed to a stimulation of the hypothalamic-pituitary-adrenal (HPA) axis and an increase in circulating cortisol levels, particularly in males (Wilkins et al. 1982). This offers an explanation as to the relationship between smoking and increased hair cortisol. In addition, Feller et al. (2014) state that this relationship may only be present in older adults as they are more likely to have been smoking for longer periods of time. The representative number of smokers in the current study was too low to make any conclusive association between smoking at an older age and hair cortisol levels.

To further investigate the effects of physiological and demographic variables on hair cortisol in older adults, the current study explored the effect of employment status on hair cortisol levels. Our results did not show any significantly positive link between employment status in older adults and hair cortisol. There is limited literature regarding employment status and hair cortisol levels in older adults, and indeed younger adults, on which to compare the results of the current study to.Dettenborn et al. (2010) highlight a positive relationship between unemployment and elevated hair cortisol content with a link to psychological and financial stress. Schulz et al. (1998) showed the same to be true for individuals who self-

assessed as feeling over- worked with a link to fear of aversive consequences and failure to complete tasks on time. As the mean cortisol concentration was highest amongst the retired participants, it may be that there is an association between retirement and hair cortisol levels in older adults but the relatively small sample size did not allow for this relationship to be deemed significant. The higher mean value may be attributed to stress- related boredom or loneliness that may come with retirement for certain individuals.

The current study did not find any association between daytime sleeping durations of ninety minutes or less and hair cortisol content in older adults. This result is at a direct variance with the suggestion that daytime sleeping is positively related to higher hair cortisol levels in older individuals (Feller et al. 2014). The difference in results may be recognised as being the effect of sleep duration. Since endogenous cortisol levels are elevated shortly after waking, known as the cortisol awakening response (Fries et al. 2009), it would be expected that hair cortisol concentrations would be higher in adults who are sleeping during the day. However, it is possible that this effect is only apparent in individuals who sleep for closer to, or longer than, 90 minutes as opposed to 5 or 10 minutes at a time. The population composition of this study did not allow for a comprehensive range of sleeping durations to be evaluated.

The current study explored the effect of medication use on hair cortisol levels in older adults. There are a number of medications known to influence cortisol levels across a range of different biological mediums such as saliva and hair. It has been shown that oral steroids reduce endogenous cortisol levels. Frerichs & Tornatore (2004) showed that the oral steroids prednisone and dexamethasone caused a decrease in cortisol levels in individuals taking these medications. Furthermore, Masharani et al. (2005) showed that steroid-based oral inhalers resulted in a decrease in salivary cortisol levels. Granger et al. (2009) assert that oral steroids have an antagonistic effect within the HPA axis. This means that oral steroids suppress the actions of the HPA axis leading to a repressed release of cortisol in individuals taking this class of medication. Research has also shown that opioids, such as morphine, inhibit cortisol secretion (McDonald et al. 1959; Morley 1981; Zis et al. 1984). Zis et al. (1984) propose that this occurs due to the presence of an inhibitory opioid mechanism which, according to Morley (1981), is primarily focused at the pituitary level resulting in the inhibition of ACTH release. This means that an individual taking opioid medication will have a lower hair cortisol level than if they were not taking the medication. Because of this, the use of medications of this nature formed an aspect of the exclusion criteria in this study. Despite the exclusion of oral steroids and opioid medications from the study, participants listed other medications that were being administered (Table 6), some of which are also known to have an influence on cortisol level. Many participants were on statin medications, for example atorvastatin, rosuvastatin and simvastatin. Interestingly, the Cohen PSS scores were either lower than, or the same as, the stress levels based on hair cortisol content in participants taking statin medications, with the exception of one participant. A large number of participants were also taking medication for the treatment of hypertension, for example amlodipine, bisoprolol and losartan.

Similarly to the participants taking statin medication, with the participants taking medication to treat hypertension the Cohen PSS scores were either lower than, or equal to, the stress levels based on hair cortisol content in participants, again with the exception of one participant.

Our results did not show any positive link between WHR and hair cortisol concentrations. The result of the current study is in contradiction with a body of evidence inferring a positive relationship between WHR and hair cortisol in both younger and older adults (Manenschijn et al. 2011; Stalder et al. 2013; Feller et al. 2014; Staufenbiel et al. 2015). Nieuwenhuizen & Rutters (2008) attribute this relationship to the association between a hyperactive HPA axis and obesity. The authors assert that elevated cortisol levels, produced as a result of a hyperactive HPA axis, are closely linked to central fat distribution. This is most prominent in the waist and hip areas and, as a result, visceral obesity is oftentimes used as a diagnostic marker of elevated cortisol levels. The variance in results between those of the current study and those of the majority of previous studies could be recognised as being the effect of inaccuracies during measurement.

Finally, the results of the current study did not show a statistically significant relationship between Cohen PSS scores and hair cortisol concentrations. This finding suggests that older adults may not be good at accurately predicting how stressed they truly are. This highlights the need for a diagnostic tool to allow for a diagnosis of elevated cortisol related to chronic stress. Due to the lack of existing literature on the association between perceived stress and hair cortisol in older adults, comparisons have been made between other studies that compare perceived stress and hair cortisol in a range of populations. Our results are at a variance with those shown by Kalra et al. (2007). The study showed a positive and significant correlation between Cohen PSS scores in the PSS- 10 and hair cortisol levels in a sample of pregnant volunteers. Karlen et al. (2011) found an inverse correlation between Cohen PSS scores in the PSS-14 and hair cortisol concentrations in young adults. Our results are in accordance with those of Van Uum et al. (2009) and Dettenborn et al. (2010). Van Uum et al. (2009) did not find any positive link between Cohen PSS scores and hair cortisol of chronic pain sufferers. Dettenborn et al. (2010) did not find any statistically significant association between Cohen PSS scores in the PSS-14 and hair cortisol levels of unemployed adults. The authors assert that the lack of association between the two variables may be due to a lag effect in HPA axis reactivity. This means that while an individual may perceive themselves as stressed, the delay in the reaction of the HPA axis in releasing cortisol means that elevated cortisol concentrations are not profiled in the hair. There may be a difference in associations found between Cohen PSS scores and hair cortisol levels depending on which model of the Cohen PSS is used. Another cause for the lack of association between Cohen PSS scores and hair cortisol levels may be attributed to the participant's interpretations of stress. One individual may feel more stressed at smaller issues than another but the HPA axis may not be stimulated any more frequently than in an individual who does not consider smaller issues stressful. The mind's psychological response to a stressor may not match the body's chemical response to it, resulting in a

lack of correlation between perceived stress levels and actual stress levels.

One participant had a remarkably high hair cortisol concentration of 82.5 pg/mg despite scoring as having a low stress level in the Cohen PSS. This raises a number of discussion points. There is no apparent explanation for this result regarding the physiological and demographic variables that this study investigated. However, it may be explained by the participant forgetting to list a medication they are taking that may cause a significant elevation of hair cortisol levels. Although unlikely, it could also be attributed to some form of hair treatment known to affect basal cortisol levels, such as dying with peroxide (Hoffman et al. 2014). This elevated result could be recognised as being the effect of excessive alcohol consumption, particularly in an older adult (Feller et al. 2014). As proposed by Chaudieu et al. (2008), it may be that this participant feels abnormally anxious about environmental stressors, resulting in a significantly increased hair cortisol content. This result could also be the effect of a recent negative life event which has been found to increase levels of hair cortisol (Wester & Van Rossum 2015). Alternatively, the high result could be the result of a combination of multiple factors that the current study did not investigate.

For example, the study did not investigate the effects of alcohol consumption, physical exercise, hair washing or BMI, as some other studies have done (Manenschijn et al. 2011; Dettenborn et al. 2012; Feller et al. 2014). Although it is known that these factors have an effect on hair cortisol levels in younger populations, the current study was unable to account for the effect that these variables may have had on the results produced in this study. Another limitation is the lack of related existing literature. There has been significant research regarding hair cortisol in a range of population groups; however, very little research has been conducted with older adults. It is important that such research continues to explore hair cortisol in older adults, as the technique offers promise as a future diagnostic bio-marker of chronic stress. To date the factors affecting hair cortisol in this population are not sufficiently understood to enable researchers to accurately propose cut-off levels with which to determine clinically relevant excessively high hair cortisol in older adults. With additional research on sufficiently large sample sizes though, this would be an exciting prospect as a diagnostic tool.

5. Conclusion

Our results did not show any statistically significant relationship between hair cortisol and age in the older age group, employment status, daytime sleeping, WHR or Cohen PSS score. However, our results did show a statistically significant higher hair cortisol content in males and in individuals with dark brown hair. When compared with our previous study, the results show a clear trend for higher hair cortisol in older adults. The analysis of cortisol allows for an insight into the functioning of the HPA axis of an individual and therefore the diagnosis of any alterations to cortisol release. Hair as a medium for cortisol analysis has many benefits. Hair samples are easy to collect due to their non- invasive nature and easy to store as hair does not allow for the degradation of incorporated hormones even when cut. The data presented from this study offers further research into hair cortisol in older adults and LC-MS/MS as a technique for cortisol quantitation.

We suggest that further study in this field should be concerned with establishing basal hair cortisol concentrations in a larger cohort of older adults in order to form more generalisable conclusions regarding the effects of physiological and demographic variables on hair cortisol in older age. In addition to this, the effects of additional variables known to have an effect in younger populations, such as mental health and alcohol consumption, could also be investigated. Further work could also follow the same concept that formed the basis of this study but review participants on a monthly basis to observe any changes in hair cortisol levels and potential variables that may be causing this effect. This may be beneficial in identifying individuals with continually increasing hair cortisol concentrations who may be becoming at risk of the deleterious effects of chronic stress. Ultimately a better understanding of the different variables that influence hair cortisol concentrations in older adults is essential to establishing the extent to which cortisol can be relied upon as a diagnostic biomarker of chronic stress. This can then allow for an effective and accurate method of monitoring stress levels through hair analysis in older adults to be developed with the ultimate goal of reducing stress and improving well- being.

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Tables

Table 1. Summary of the age, sex and hair colours of participants, with corresponding hair cortisol concentrations (*n* = 42)

Participant number	Cortisol (pg/mg)	Age	Sex*	Hair colour
1	6.5	64	F	Red
2	3.6	62	M	Grey
3	14.9	63	M	Dark Brown
4	7.2	66	M	Grey
5	22.4	60	M	Dark Brown
6	4.6	65	M	Grey
7	1.8	71	F	Grey
8	1.7	60	M	Brown
9	4.3	70	F	Light Brown
10	10.6	64	F	Dark Brown
11	2.7	62	F	Grey
12	1.4	61	F	Grey
13	20.5	67	M	Brown
14	27.5	66	F	Grey
15	4.5	68	M	Grey
16	6.0	63	F	Light Brown
17	1.9	62	F	Grey
18	3.2	67	F	Grey
19	4.2	72	F	Grey
20	2.1	71	F	Brown
21	17.7	72	M	Grey
22	4.9	72	F	Dark Brown
23	4.5	65	M	Grey
24	3.7	72	F	Grey
25	6.8	62	M	Dark Brown
26	14.3	68	M	Black
27	3.7	75	M	Grey
28	17.6	75	M	Grey
29	5.3	68	F	Grey
30	27.2	64	M	Dark Brown
31	12.1	72	M	Black
Participant number	**Cortisol (pg/mg)**	**Age**	**Sex***	**Hair colour**
32	4.4	71	F	Light Brown

33	12.5	74	F	Dark Brown
34	10.1	67	F	Light Brown
35	23.6	67	F	Light Brown
36	6.1	71	M	Grey
37	2.7	80	F	Grey
38	82.5	75	M	Grey
39	8.5	79	M	Grey
40	5.1	78	F	Light Brown
41	7.7	72	M	Grey
42	5.1	67	F	Light Brown

*Sex is denoted as either M (Male) or F (Female)

Table 2. Summary of the participant physiological and demographic results (*n* = 42)

Participant number	Smoking status*	Cigarette quantity	Employment status	Daytime sleep?*	Sleep Duration (mins)	WHR
1	N	N/A	Part-Time	N	N/A	0.86
2	N	N/A	Full-Time	N	N/A	1.04
3	N	N/A	Full-Time	N	N/A	1
4	N	N/A	Part-Time	Y	5–10	0.9
5	N	N/A	Full-Time	N	N/A	1.02
6	N	N/A	Part-Time	N	N/A	0.85
7	N	N/A	Part-Time	Y	30	0.83
8	N	N/A	Full-Time	N	N/A	0.95
9	N	N/A	Part-Time	N	N/A	0.81
10	N	N/A	Part-Time	N	N/A	0.74
11	N	N/A	Full-Time	N	N/A	1
12	N	N/A	Full-Time	N	N/A	0.75
13	N	N/A	Part-Time	N	N/A	0.83
14	N	N/A	Retired	N	N/A	0.91
15	N	N/A	Retired	Y	30–60	0.89
16	N	N/A	Part-Time	Y	20	0.84
17	N	N/A	Full-Time	N	N/A	0.84
18	N	N/A	Retired	Y	10	0.91
19	N	N/A	Retired	Y	5	0.91
20	N	N/A	Retired	N	N/A	0.76
21	N	N/A	Part-Time	Y	20	0.95
22	N	N/A	Retired	Y	20	0.85
23	N	N/A	Retired	N	N/A	0.85
24	N	N/A	Retired	Y	30–60	0.87
25	N	N/A	Retired	N	N/A	0.89
26	N	N/A	Retired	N	N/A	0.94
27	N	N/A	Retired	N	N/A	0.98
28	N	N/A	Part-Time	Y	30	0.94

Participant number	Smoking status*	Cigarette quantity	Employment status	Daytime sleep?*	Sleep Duration (mins)	WHR
29	N	N/A	Part-Time	N	N/A	0.86
30	N	N/A	Retired	Y	60	1.05
31	N	N/A	Retired	N	N/A	0.94
32	N	N/A	Retired	N	N/A	0.86
33	Y	11-15	Retired	N	N/A	0.86
34	N	N/A	Retired	N	N/A	N/A
35	N	N/A	Retired	Y	60	0.78
36	N	N/A	Retired	N	N/A	0.89
37	N	N/A	Retired	Y	30	0.80
38	N	N/A	Retired	N	N/A	0.91
39	N	N/A	Retired	N	N/A	0.98
40	N	N/A	Retired	N	N/A	0.70
41	N	N/A	Retired	N	N/A	0.96
42	N	N/A	Part-Time	N	N/A	0.80

*Responses are denoted as either (N) No or (Y) Yes

Table 3. Participant self-assessment Cohen PSS scores and the corresponding level of stress (*n* = 42)

Participant number	Q1	Q2	Q3	Q4*	Q5*	Q6	Q7*	Q8*	Q9	Q10	PSS score	Stress level**
1	3	3	3	3 (1)	3 (1)	4	2 (2)	2 (2)	4	2	25	M
2	1	1	1	3 (1)	2 (2)	1	3 (1)	3 (1)	2	1	12	L
3	2	2	2	4 (0)	4 (0)	3	3 (1)	2 (2)	4	3	19	M
4	0	2	1	3 (1)	2 (2)	1	3 (1)	4 (0)	3	0	11	L
5	1	0	1	4 (0)	4 (0)	1	4 (0)	4 (0)	1	0	4	L
6	1	0	1	4 (0)	1 (3)	0	4 (0)	4 (0)	2	1	8	L
7	2	1	2	3 (1)	2 (2)	1	3 (1)	2 (2)	2	0	14	M
8	0	0	0	4 (0)	3 (1)	1	3 (1)	3 (1)	1	0	5	L
9	4	3	3	3 (1)	2 (2)	2	3 (1)	2 (2)	3	3	24	M
10	2	2	2	3 (1)	3 (1)	2	3 (1)	3 (1)	1	1	14	M
11	4	3	3	3 (1)	3 (1)	2	2 (2)	2 (2)	2	3	23	M
12	1	0	2	4 (0)	3 (1)	2	3 (1)	3 (1)	1	0	9	L
13	0	0	0	4 (0)	4 (0)	0	4 (0)	4 (0)	0	0	0	L
14	1	2	2	1 (3)	3 (1)	0	3 (1)	4 (0)	4	0	14	M
15	1	1	3	4 (0)	4 (0)	1	4 (0)	4 (0)	2	1	9	L
16	1	1	2	2 (2)	2 (2)	1	3 (1)	3 (1)	1	1	13	L
17	4	3	2	2 (2)	1 (3)	3	2 (2)	2 (2)	3	2	26	M
18	1	1	2	1 (3)	3 (1)	2	3 (1)	3 (1)	2	1	15	M
19	3	0	3	1 (3)	2 (2)	2	2 (2)	2 (2)	2	2	21	M
20	1	0	1	4 (0)	3 (1)	1	3 (1)	4 (0)	2	0	7	L
21	1	0	0	4 (0)	3 (1)	0	3 (1)	3 (1)	4	0	8	L
22	1	2	2	3 (1)	2 (2)	2	3 (1)	3 (1)	2	1	15	M
23	1	0	2	4 (0)	3 (1)	0	4 (0)	4 (0)	1	0	5	L
24	0	1	2	4 (0)	3 (1)	2	3 (1)	3 (1)	2	0	10	L

Participant number	Q1	Q2	Q3	Q4*	Q5*	Q6	Q7*	Q8*	Q9	Q10	PSS score	Stress level**
25	1	2	1	4 (0)	3 (1)	1	4 (0)	4 (0)	2	1	9	L
26	1	0	0	4 (0)	4 (0)	0	3 (1)	4 (0)	1	0	3	L
27	2	1	2	3 (1)	2 (2)	2	3 (1)	3 (1)	2	1	15	M
28	2	2	2	1 (3)	3 (1)	2	3 (1)	3 (1)	3	2	19	M
29	2	2	2	3 (1)	3 (1)	2	3 (1)	3 (1)	1	2	15	M
30	0	0	1	4 (0)	3 (1)	0	3 (1)	4 (0)	2	0	5	L
31	3	2	2	3 (1)	1 (3)	1	3 (1)	1 (3)	3	1	20	M
32	3	2	3	3 (1)	2 (2)	1	1 (3)	2 (2)	0	0	17	M
33	2	1	1	3 (1)	3 (1)	2	2 (2)	3 (1)	2	1	14	M
34	2	2	3	3 (1)	2 (2)	2	2 (2)	2 (2)	2	2	20	M
35	2	1	2	2 (2)	2 (2)	2	2 (2)	2 (2)	1	1	17	M
36	0	0	0	4 (0)	3 (1)	4	3 (1)	4 (0)	0	1	7	L
37	2	2	3	3 (1)	2 (2)	1	4 (0)	2 (2)	2	1	16	M
38	1	1	2	4 (0)	3 (1)	1	3 (1)	3 (1)	0	1	9	L
39	0	1	1	4 (0)	2 (2)	0	3 (1)	2 (2)	3	0	10	L
40	0	3	3	2 (2)	2 (2)	0	1 (3)	1 (3)	4	2	22	M
41	1	0	1	4 (0)	3 (1)	1	3 (1)	3 (1)	2	0	8	L
42	1	3	0	3 (1)	2 (2)	2	1 (3)	3 (1)	2	1	16	M

*The scores given to answers to questions 4, 5, 7 and 8 are reversed. The reversed scores are situated within the brackets. For example, a score of 4 is reversed to (0). The sum of all scores across the 10 questions is calculated to determine the stress level

**Stress levels are assessed as either Low (L) 0–13, Moderate (M) 14–26, or High (H) 27–40

Table 4. Comparison of participant's hair cortisol and stress levels, and Cohen PSS scores and stress levels (*n* = 42)

Participant number	Cortisol (pg/mg)	Stress level*	PSS score	Stress level**
1	6.5	M	25	M
2	3.6	L	12	L
3	14.9	M	19	M
4	7.2	M	11	L
5	22.4	H	4	L
6	4.6	M	8	L
7	1.8	L	14	M
8	1.7	L	5	L
9	4.3	M	24	M
10	10.6	M	14	M
11	2.7	L	23	M
12	1.4	L	9	L
13	20.5	H	0	L
14	27.5	H	14	M
15	4.5	M	9	L
16	6.0	M	13	L
17	1.9	L	26	M
18	3.2	L	15	M
19	4.2	M	21	M
20	2.1	L	7	L
21	17.7	H	8	L
22	4.9	M	15	M
23	4.5	M	5	L
24	3.7	L	10	L
25	6.8	M	9	L
26	14.3	M	3	L
27	3.7	L	15	M
28	17.6	H	19	M
29	5.3	M	15	M
30	27.2	H	5	L
31	12.1	M	20	M
32	4.4	M	17	M

Participant number	Cortisol (pg/mg)	Stress level*	PSS score	Stress level**
33	12.5	M	14	M
34	10.1	M	20	M
35	23.6	H	17	M
36	6.1	M	7	L
37	2.7	L	14	M
38	82.5	H	9	L
39	8.5	M	10	L
40	5.1	M	22	M
41	7.7	M	8	L
42	5.1	M	16	M

*Stress levels are assessed as either Low (L) 0–4 pg/mg, Moderate (M) 4–15 pg/mg, or High (H) >15 pg/mg (hair cortisol levels as proposed by Binz et al 2018)

**Stress levels from PSS score are assessed as either Low (L) 0–13, Moderate (M) 14–26, or High (H) 27–40

Table 5. Published data on the factors affecting basal hair cortisol levels, arranged by number of study participants

Source of cortisol	Number of participants	Analytical technique*	Age of participants	Factors investigated	Study	Summary of conclusions
Hair	654	CLIA	47–82	Age, sex, smoking status, alcohol consumption, occupation, daytime sleep, medical conditions, WHR, BMI	Feller et al. (2014)	Cortisol levels significantly higher with increased age, excess alcohol consumption, WHR, duration of daytime sleep, and in males than in females. HCC (mean ± SD) in participants 60–70 years was 34.1 ± 27.7 pg/mg; and >70 years 40.5 ± 40.5 pg/mg. Levels also positively associated with smoking, type 2 diabetes, and retirement. No relationship observed between cortisol levels and BMI.
Hair	554	LC-MS/MS	0.6–70	Age, sex, hair colour	Binz et al. (2018)	Cortisol levels highest in toddlers and lowest in adolescents. HCC in adults > 60 (n=11) was approx. mean 8.8 pg/mg. Levels higher in adult males than in adult females. Levels higher in black hair than in blond hair.
Hair	360	CLIA	1–91	Age, sex, hair colour, hair washing, contraception and smoking status	Dettenborn et al. (2012)	HCC in adults aged 50-91 (n=31) was approx. mean 23 pg/mg (males) and 22 pg/mg (female). Cortisol levels higher in children than in adults, and in males than females. No association between cortisol levels and hair colour, contraception or smoking status. Cortisol levels decreased in distal segments of the hair with hair washing frequency.
Hair	195	ELISA	18–63	Age, blood pressure, BMI, WHR	Manenschijn et al. (2011)	Cortisol levels positively correlated with WHR. No association between cortisol levels and age, blood pressure or BMI.

Hair	42	LC-MS/MS	60–80	Age, sex, hair colour, smoking status, employment status, daytime sleeping, medication, WHR	**This study**	Cortisol levels higher in males than females, and in dark brown hair than light brown or grey hair. No association between cortisol levels and age, employment status, daytime sleep, WHR or medication. Insufficient data to observe any relationship between cortisol levels and smoking status.

*Abbreviation legend: CLIA = Chemiluminescence Immunoassay, LC-MS/MS = Liquid Chromatography Tandem Mass Spectrometry, ELISA = Enzyme Linked Immunosorbent Assay.

Table 6. Summary of the different medications listed as being taken by participants, the number of participants on each medication and its use.

Medication	Number of Participants Using	Use*
Atorvastatin	3	Lower Cholesterol
Rosuvastatin	2	Lower Cholesterol
Simvastatin	2	Lower Cholesterol
Amlodipine	2	Treatment of hypertension
Bendroflumethiazide	1	Treatment of hypertension
Beta-blockers (Unspecified)	1	Treatment of hypertension
Bisprolol	2	Treatment of hypertension
Carvedilol	1	Treatment of hypertension
Enalapril	1	Treatment of hypertension
Indapamide	1	Treatment of hypertension
Losartan	4	Treatment of hypertension
Nifedipine	1	Treatment of hypertension
Moxonodine	1	Treatment of hypertension
Ramipril	2	Treatment of hypertension
Amitriptyline	2	Antidepressant
Clomipramine	1	Antidepressant
Venlafaxine	1	Antidepressant
Canaglifloxin	1	Treatment of type 2 diabetes
Metformin	1	Treatment of type 2 diabetes
Sitagliptin	1	Treatment of type 2 diabetes
Clenil	1	Treatment of asthma symptoms
Salbutamol	2	Treatment of asthma symptoms
Seretide	1	Treatment of asthma symptoms
Clopidogrel	3	Prevention of blood clots
Rivaroxaban	1	Prevention of blood clots
Allopurinol	2	Prevention of gout and kidney stones
Furosemide	1	Increase urine production
Glucosamine	1	Treatment of joint pain
Hormone Replacement Therapy	1	Treatment of menopausal symptoms
Lansoprazole	3	Prevention of gastroesophageal reflux

Medication	Number of Participants Using	Use*
Letrozole	1	Treatment of breast cancer
Levothyroxine	4	Treatment of thyroid deficiency
Omeprazole	1	Reduction of stomach acid
Rotigotine	1	Treatment of Parkinson's disease
Tamsulosin	2	Treatment of an enlarged prostate
Tegretol	1	Treatment of epilepsy

*All medication uses are taken from National Health Service or National Institute for Health and Care Excellence

Figure legends

Figure 1. Hair cortisol concentration across four age groups: toddler, adolescent, adult and older adults. The category 'older adults' displays our previous research data (Binz et al 2018) n = 11, in addition to the current study (N=42).

Figures

Figure 1.