THE GERONTOLOGICAL SOCIETY OF AMERICA®

Journals of Gerontology: Biological Sciences
cite as: *J Gerontol A Biol Sci Med Sci*, 2020, Vol. 75, No. 2, 257–265
doi:10.1093/gerona/gly258
Advance Access publication November 9, 2018

OXFORD

Original Article

The Association Between Hair Cortisol, Hair Cortisone, and Cognitive Function in a Population-Based Cohort of Older Adults: Results From The Irish Longitudinal Study on Ageing

Joanne C. Feeney, PhD,[1] Aisling M. O'Halloran, PhD,[2] and Rose Anne Kenny, MD[2]

[1]The Irish Longitudinal Study on Ageing (TILDA), Department of Medical Gerontology, The University of Dublin, Trinity College, Dublin 2, Ireland. [2]Centre for Public Health, Institute of Clinical Sciences, Royal Victoria Hospital, Queen's University, Belfast BT12 6BA, UK

Received: May 14, 2018; Editorial Decision Date: November 1, 2018

Decision Editor: David Le Couteur, MBBS, FRACP, PhD

Abstract

Experimental evidence to date largely supports an association between the stress hormone cortisol and cognitive performance. Older adults, in particular, may be vulnerable to the neurotoxic effects of prolonged increases in cortisol; however, the assessment of chronic hormone levels has previously been challenging. Hair cortisol analysis has advantages over other cortisol metrics for this purpose as it facilitates the assessment of total hormone secretion over several months. Cortisol and cortisone were measured in the scalp hair of 1,876 older adults from The Irish Longitudinal Study on Ageing. Participants underwent a battery of cognitive tests assessing global function, memory, executive function, and processing speed. After adjustment for hair characteristics, demographics, metabolic risk factors, cardiovascular conditions, and depression, regression analysis revealed an inverse relationship of hair glucocorticoids to immediate (cortisol: $\beta = -.12$, $p = .032$; cortisone: $\beta = -.021$, $p = .036$) and delayed (cortisol: $\beta = -.13$, $p = .003$; cortisone: $\beta = -.23$, $p = .006$) word recall performance. They were also associated with more errors on the Mini-Mental State Examination (cortisol: incidence rate ratio (IRR) = 1.06, $p = .008$; cortisone: IRR = 1.14, $p = .002$) and Montreal Cognitive Assessment (cortisone: IRR = 1.06, $p = .015$). Higher hair glucocorticoids are inversely associated with memory and global cognition in a population-based sample of older adults. Future work should explore the prognostic significance of these findings.

Keywords: Glucocorticoids, Memory, Global cognition, Stress

There is good evidence to show that prolonged exposure to increased psychological and biological stress can have a detrimental effect on health. The brain may be particularly vulnerable to cellular damage from high circulating levels of stress hormones owing to its high metabolic demand and the high density of glucocorticoid (GC) receptors therein. The finding that these receptors are mostly located in the hippocampus, prefrontal cortex, and amygdala, and mediate both rapid nongenomic and slower genomic signaling (see ref. (1) for a review), points to a potential role for GCs in aspects of cognitive function. Indeed, the experimental evidence to date largely supports an association between acute GC levels and performance on tests of memory and, to a lesser extent, executive function (eg, (2–7)). However, considerable heterogeneity exists in the age of the individuals included in these studies. Furthermore, evidence regarding cognitive function in relation to longer-term corticosteroid concentrations is rather lacking. Older adults may be more vulnerable than younger individuals to the neurotoxic effects of stress owing to age-related changes in brain structure, function, and cellular metabolism. There is some empirical evidence in support of this (8–11); however, the impact of chronically elevated (or suppressed) GCs on the aged brain is still an area that has been comparatively underexplored, in part due to the difficulties in assessing levels over the medium to long term. Saliva, blood, and urine all require prolonged, repeated sampling at set time points to estimate stress hormone levels over a duration of weeks or months, which often is not feasible at all or results in low compliance. Recently, the development of hair steroid hormone profiling has enabled the quantification of the average levels of the hormone over a period of months by taking a sample of hair at a single point in time (12). Analysis of clinical groups with hypercortisolemia (13), comparison between hair and repeated saliva sampling (14,15),

and the high test–retest reliability of the measure across a period of several months to a year (16), all suggest that hair cortisol is a valid indicator of total cortisol output over the medium to long term.

To date, to the best of our knowledge, only two published studies have investigated the relationship between hair cortisol levels and cognitive function in healthy adults, and the findings have been contradictory. Pulopulos and colleagues (17) found that higher hair cortisol levels were related to better performance on short- and long-term verbal memory in a small sample of healthy older adults (n = 57). This was in sharp contrast to their results regarding the association between salivary cortisol and cognition in the same sample, which showed the expected inverse relationship. McLennan and colleagues (18) sampled hair cortisol from a small cohort of female nurses, ranging in age from young adults to young–old age. They found no association between cortisol and performance on a large battery of cognitive tests, even after examining potential moderators such as age, sex, or general well-being. The two previous studies differed with respect to sample characteristics (notably age, occupational, and health status of the participants) and yielded results that not only conflicted with one another, but were not in concordance with the majority of related studies using saliva or other cortisol metrics. Thus, an investigation of hair cortisol and cognitive function in a large, population-based cohort of older adults is warranted, further taking into account potential confounders such as vascular risk factors and hair treatment characteristics (19–21).

Hair cortisone is another biomarker of potential utility in the assessment of endogenous GC levels. Cortisone is an inactive metabolite of cortisol in humans. Cortisol is converted to cortisone by the action of the enzyme 11β-hydroxysteroid dehydrogenase type 2, leading to reduction in GC activity. However, cortisol can be regenerated from cortisone by the action of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). Only a handful of recent studies have investigated the relationship between hair cortisone and health indicators, but these point to a potentially meaningful role for its measurement in the determination of total GC levels within tissues (21–23). Moreover, it may be pertinent to assess cortisone levels in older adults in relation to risk factors for cognitive decline. In the adult brain, 11β-HSD1 is widely expressed in the hippocampus and forebrain. This expression has been shown to be markedly increased in the aged brain and is a biologically plausible mechanism through which the brain may become vulnerable to GC excess (24).

With these considerations in mind, we sought to examine the association between hair cortisol, hair cortisone, and cognitive function in a large, population-based sample of older adults. We hypothesized that any association between GCs and cognitive performance would run in a negative direction and would be most pronounced on tests of memory and executive functions.

Methods

Participants and Design

Data from Wave 3 of The Irish Longitudinal Study on Ageing (TILDA) was analyzed. TILDA is a nationally representative prospective cohort study, examining the social, health, and economic characteristics of community-dwelling adults aged 50 and older, living in the Republic of Ireland. A total of 8,175 adults were surveyed at Wave 1 of the study. Detail of the study design has been published elsewhere (25,26). Briefly, participants were recruited through a two-stage clustered, stratified sampling design by geographic area and by household. Data collection comprises (i) a computer-assisted personal interview (CAPI) carried out in the respondent's home; (ii) a self-completion questionnaire (SCQ); and (iii) a health assessment, administered by trained nurses either in a dedicated health center or during a home visit. Participants in the study are surveyed every 2 years with respect to the CAPI and self-completion questionnaire components and are invited to a health assessment every 4–6 years. Wave 3 took place in 2014–2015. A total of 6,310 self-respondents aged 54 and older completed a CAPI, with 85% of these completing a self-completion questionnaire and 82% taking part in a health assessment. Ethical approval was obtained by the institutional review board, and all respondents provided signed informed consents before participation in the study.

Cognitive Assessment

A battery of neuropsychological tests was administered broadly indexing different aspects of cognitive function. Global cognitive performance was assessed by the Mini-Mental State Examination (MMSE; (27)) and the Montreal Cognitive Assessment (MoCA; (28)). The MMSE was administered as part of the CAPI, and the MoCA was carried out during the health assessment. Verbal memory was assessed during the CAPI using a 10-word list learning paradigm with immediate and delayed recall tests. Verbal fluency was assessed during the CAPI by asking participants to name as many animals as possible in 1 minute. Executive functions were also assessed during the health assessment by evaluating performance on the Color Trails Task 2 (CTT2; (29)). Sustained attention was assessed by the number of commission errors made on the sustained attention to response task (30). Cognitive processing speed was evaluated using a computer-based choice reaction time task (CRT). The aforementioned tests were also administered at Wave 1 of TILDA and further details have been published elsewhere (31).

Hair Sample Collection, Analysis, and Related Variables

Hair samples were collected as part of the health assessment by a trained nurse. Hair was sectioned off at the posterior vertex, and a sample of 3 cm in length was cut as close to the scalp as possible with a medical scissors. The scalp end of the sample was identified by a plastic tie, and each sample was placed inside an envelope and stored in a dark, dry place until shipping. Samples were analyzed by Dresden Lab Service GmbH at the Technische Universität Dresden, Germany. Steroid analysis was carried out using high-performance liquid chromatography–mass spectrometry, with wash and extraction procedures as described by Gao and colleagues (32). Only samples with a minimum weight of 7.5 mg were included in the analysis. A sample of approximately 3 cm in length is presumed to approximate the 3 months before sampling, based on an average growth rate of 1 cm per month (33). Hair cortisol and cortisone concentrations were expressed in pg/mg. A very brief additional questionnaire was administered to respondents at the time of hair sample collection to obtain information on hair-related factors that could influence hair steroid hormone concentrations. These included any use of steroid medications, and any current medical conditions that would likely affect GC levels and about which information was not already captured during the CAPI (eg, Cushing's disease), hair washing frequency, and application of hair dye or any other chemical treatment. Hair care is of potential relevance in the determination of any association between hair GCs and cognitive function, as it may both affect hair hormone concentrations (eg, (21)) and reflect personal grooming habits—changes in which can be a consequence of impaired cognitive function (34). The season during which the hair

sample was taken was also noted, as several studies have detected seasonal variation in GC levels (19,21,35), which may also have relevance for detecting relationships with cognitive status (36).

Sociodemographic, Lifestyle, and Health Factors

Information on sociodemographic indicators such as age, sex, education, and social class, was collected during the CAPI. Education was coded into three categories according to the highest level obtained: primary, secondary, or tertiary level. Social class was also coded into three levels: unskilled or semi-skilled, nonmanual or skilled, and professional or managerial. Data on anthropometric and cardiovascular health variables that have previously been shown to be associated with both hair GCs and cognitive function in older adults were also gathered (21,37). Body mass index (BMI) was derived from height and weight measures carried out during the health assessment and individuals were further coded into underweight (<18.5), healthy weight (18.5–24.9), overweight (25–29.9), and obese (≥30), for descriptive analysis. Individuals were classified as diabetic if they self-reported a doctor's diagnosis either in response to a question during the CAPI or in the additional questionnaire that accompanied the hair sampling. Individuals were asked about their smoking habits and were categorized as never, past, or current smokers. Other cardiovascular conditions ascertained by self-reported doctor's diagnosis were high blood pressure, angina, heart attack, heart failure, stroke or transient ischemic attack, high cholesterol, heart murmur, and irregular heart rhythm. These conditions were summed and coded into a three-level variable for analysis denoting participants with none, one, and two or more conditions. The Composite International Diagnostic Interview Short Form for major depression (38) was administered to respondents to screen for a major depressive episode within the last 12 months. A diagnostic algorithm is used to determine whether an individual meets the criteria for a major depressive episode or not (as per ref. (38) and detailed at https://www.hcp.med.harvard.edu/ncs/ftpdir/cidisf_readme.pdf). This yielded a dichotomous variable whereby individuals meeting the criteria for a major depressive episode were assigned a "1," and those who did not meet the criteria, a "0."

Statistical Analysis

One individual with Addison's disease and one with a pituitary tumor were excluded before analysis because of the impact of those conditions on the hypothalamic–pituitary–adrenal axis. We also excluded 278 individuals taking steroid medications from the analysis as preliminary investigation showed that these individuals had markedly elevated hair cortisol levels. A further 54 samples had undetectable cortisol levels and three samples had undetectable cortisone levels. Both cortisol and cortisone were heavily right-skewed and were log-transformed before analysis. Individuals with values +/–3 *SD* from the mean were excluded (n = 34 for cortisol, n = 15 for cortisone). This left 1,960 individuals with valid hair cortisol and cortisone for analysis, and a total of 1, 876 with no missing data on any covariates (see Figure 1 for a detailed breakdown).

Associations between cortisol, cortisone, and potential covariates were explored by regressing log-transformed cortisol or cortisone on the variable of interest. The relationship between cortisol or cortisone and cognitive function, adjusting for covariates, was analyzed with a model appropriate to the distribution of the cognitive variable in each case. Specifically, visualization of the data, and further inspection of model residuals revealed that several of the cognitive performance variables were not normally distributed and violated the assumptions of ordinary least squares (OLS)

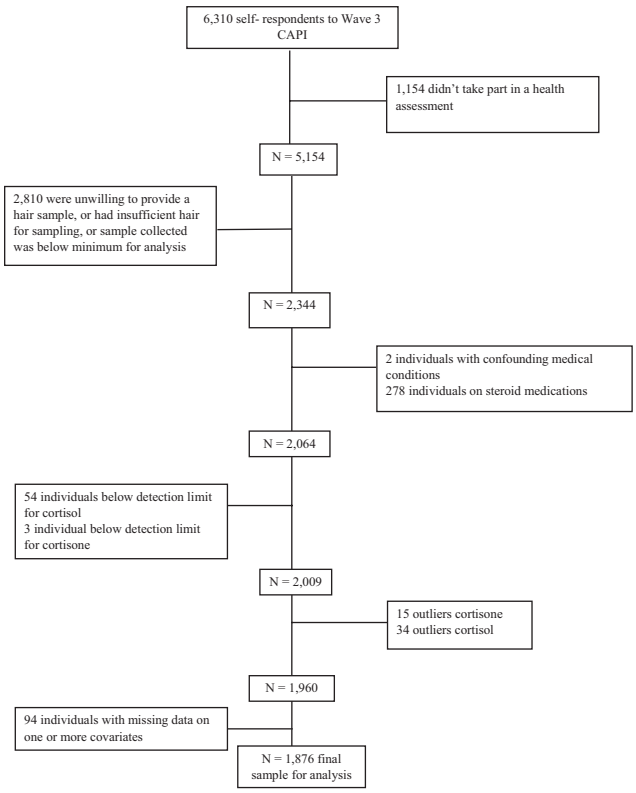

Figure 1. Flow chart detailing participants excluded from analysis and the pattern of missing data.

regression, even after the removal of any outliers. The MMSE and the MoCA were analyzed using negative binomial regression (due to overdispersion) with the outcome in each case being the number of errors made (30—raw score). Coefficients should be interpreted as the change in the log count of MMSE or MoCA errors associated with a unit increase in the predictor. Sustained attention to response task errors of commission were also modeled using negative binomial regression. Performance on the CRT still violated assumptions for OLS regression even after applying various transformations and thus was analyzed by ordinal logistic regression with quintiles of reaction time as the outcome variable. Verbal fluency and time to complete CTT2 were analyzed using OLS regression after applying a logarithmic transformation. Immediate verbal recall and delayed verbal recall were both negatively skewed, with the residuals from the OLS regression models also exhibiting a slight skew. However, creating a variable representing number of errors on both tests and applying a negative binomial regression model, resulted in almost identical results to the OLS models. Thus, for ease of interpretation, the results from the OLS regression analyses are presented.

Three models were tested in all cases. In Model 1, cognitive performance was regressed on hair cortisol and hair cortisone (separate models) with no adjustment. Model 2 was adjusted for age, sex, education, chemical treatment, season of hair sampling, smoking, diabetes, cardiovascular conditions, and BMI. BMI was included as a continuous variable in the model (15 individuals who were underweight were excluded). A final third model included an additional adjustment for the recent occurrence of a major depressive episode. In all models, standard errors were corrected for the stratified-clustered survey design. Data were analyzed using Stata, version 14 (StataCorp, College Station, TX).

To account for the number of individual tests carried out, an adjustment for multiple comparisons was made by controlling the false discovery rate as per Benjamini and Yekutieli (39), facilitated by the Smileplot package in Stata.

Finally, differences between individuals included and excluded from these analyses were investigated. Individuals excluded were those who did not attend a health assessment, those who attended a health assessment but would not consent to hair sampling or had insufficient hair for analysis, and those with missing data on covariates.

Results

Determinants of Hair GC Concentrations

The geometric mean (95% CI) of cortisol in the sample was 6.63 (6.28 to 7.01) and cortisone was 9.57 (9.27 to 9.88). The mean age of the participants was 66.5 years (range 54–94). Women comprised 74% of the sample; 40% of the sample were educated to tertiary level or higher. A full description of the participant characteristics can be found in Table 1.

Age and male sex were both positively associated with hair cortisol and cortisone ($p < .001$). Investigation of the association between hair characteristics and GC levels revealed that chemical treatment of any sort was associated with lower hair cortisol and cortisone ($p < .001$). Frequency of hair washing was not significantly associated with either hair cortisol or hair cortisone. Investigation of seasonal variation revealed that hair cortisol and cortisone were highest among individuals sampled in the autumn months (September–November).

Socioeconomic status was associated with hair GCs. Specifically, having attained secondary- or tertiary-level education (relative to primary level only or no formal education) was associated with lower hair cortisol ($p = .003$ and $p = .040$, respectively) and hair cortisone ($p < .001$). Individuals with occupations classed as unskilled or semi-skilled had higher hair cortisol than those in the nonmanual or skilled group ($p = .028$), but their cortisol levels did not differ significantly from those in the managerial or professional group. Individuals in the unskilled or semi-skilled group did, however, have higher hair cortisone than those in the managerial or professional classes ($p = .020$). A comparison of educational attainment and social class showed education to be the stronger predictor of hair cortisol and cortisone concentrations. Furthermore, there was a greater degree of missingness on the social class variable. As the two variables were very closely related, for the previous reasons education was chosen over social class for inclusion in the analysis of hair GCs and cognitive function.

BMI was positively associated with both hair cortisol and hair cortisone. Obese individuals had significantly higher cortisol than those of healthy weight ($p = .008$). In the case of hair cortisone, both individuals who were overweight and those who were obese had higher cortisone than individuals in the healthy weight range ($p < .001$). Having a doctor's diagnosis of diabetes was also associated with higher hair cortisol ($p = .014$) and cortisone ($p < .001$). Both past and current smokers had higher hair cortisone, relative to those who had never smoked ($p < .001$); whereas higher hair cortisol was associated with past smoking only ($p = .001$). Higher hair GCs were observed for individuals with cardiovascular conditions in a dose-dependent manner, being highest for individuals reporting two more cardiovascular conditions (relative to none) for both cortisone and cortisol ($p < .001$). Having suffered from a major depressive episode in the past 12 months was also associated with higher cortisol ($p = .032$) and cortisone ($p = .030$). Full details can be found in Supplementary Table 1.

Table 1. Sample Characteristics ($N = 1,876$)

	Mean (*SD*)/frequency (%)
Age, mean (*SD*)	66.4 (8.7)
Sex (female), *n* (%)	1,396 (74.4)
Education, *n* (%)	
None or primary[a]	412 (22.0)
Secondary[b]	712 (37.9)
Tertiary or higher[c]	752 (40.1)
Social class, *n* (%)	
Unskilled or semi-skilled	318 (18.5)
Nonmanual or skilled	812 (47.3)
Managerial or professional	586 (34.1)
BMI, mean (*SD*)	
Underweight	15 (0.8)
Normal	450 (24.0)
Overweight	821 (43.7)
Obese	591 (31.5)
Smoking, *n* (%)	
Never smoked	941 (50.1)
Past smoker	726 (38.7)
Current smoker	209 (11.1)
Diabetes, *n* (%)	150 (8.0)
Cardiovascular conditions, *n* (%)	
None	716 (38.2)
1	688 (36.7)
2 or more	472 (25.2)
Frequency of hair washing, *n* (%)	
Once per week or less	710 (37.9)
2–4 times per week	867 (46.2)
>4 times per week	299 (15.9)
Chemical treatment of hair (dye, bleach, perm), *n* (%)	1,033 (55.1)
Season of hair sampling	
Winter	347 (18.5)
Spring	339 (18.1)
Summer	581 (31.0)
Autumn	609 (32.5)
Depression (%)	95 (5.1)
Cortisol (log), mean (*SD*), pg/mg	1.9 (1.2)
Cortisone (log), mean (*SD*), pg/mg	2.3 (0.7)
Delayed word recall score, mean (*SD*)	6.3 (2.5)
Immediate recall total score, mean (*SD*)	14.1 (3.1)
Animal naming score, mean (*SD*)	19.0 (5.6)
MoCA score, mean (*SD*)	25.3 (3.8)
MMSE score, mean (*SD*)	28.7 (1.8)
CTT2 time (s), mean (*SD*)	109.6 (40.5)
CRT (ms), mean (*SD*)	516.2 (199.7)
SART errors, mean (*SD*)	3.1 (2.8)

Note: CTT2 = Color Trails Task 2; CRT = choice reaction time; MMSE = Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; SART = sustained attention to response task; *SD* = standard deviation.

[a]"None or primary" corresponds to 0–8 years education. [b]"Secondary" corresponds to 12–14 years of education. [c]"Tertiary or higher" corresponds to 16–21 years of education.

Relationship Between Hair GCs and Cognitive Function

Simple regression analysis without adjustment for covariates (Model 1) revealed that both hair cortisol and hair cortisone were inversely associated with performance on the immediate recall task (cortisol: $B = –0.25$, CI = –0.37 to –0.13, $p < .001$; cortisone: $B = –0.57$, CI = –0.76 to –0.38, $p < .001$), delayed recall task (cortisol: $B = –0.24$, CI = –0.34 to –0.15, $p < .001$; cortisone:

B = –0.52, CI = –0.68 to –0.36, p < .001), MoCA (cortisol: B = 0.05, CI = .02,0.08, p = .001; cortisone: IRR = 0.12, CI = 0.07 to 0.17, p<.001), and MMSE (cortisol: B = 0.10, CI = 0.05 to 0.15, p < .001; cortisone: B = 0.22, CI = 0.14 to 0.30, p < .001). Hair cortisone was marginally inversely associated with performance on the verbal fluency task (B = –0.36, CI = –0.71 to –0.001, p =.049); however, there was no statistically significant relationship between cortisol and verbal fluency score (B = –0.16, CI = –0.36 to 0.04, p = .113). Higher levels of both GCs were associated with slower time to complete CTT2 (cortisol: B = 0.02, CI = 0.01 to 0.04, p < .001; cortisone: B = 0.05, CI = 0.03 to 0.07, p < .001). Neither hair cortisol nor hair cortisone was significantly associated with sustained attention to response task errors (cortisol: B = 0.01, CI = –0.03, 0.05, p = .596; cortisone: B = 0.01, CI = –0.06 to 0.07, p = .824) or CRT performance in the case of cortisol (OR = 1.05, CI = 0.97 to 1.13, p = .191), though the association between CRT and cortisone was marginally significant (OR = 1.14, CI = 1.01 to 1.29, p = .041).

For some of the cognitive performance variables, the association between hair cortisone and hair cortisol was attenuated after adjustment for covariates in Model 2 and Model 3 and was no longer significant. However, several other associations persisted even after adjustment for all covariates (Model 3). Specifically, an inverse relationship of hair cortisol and hair cortisone with immediate recall (cortisol: p = .032; cortisone: p = .036) and delayed recall performance (cortisol: p = .003; cortisone: p = .006) remained. The association of MMSE errors with both hair cortisol (p = .008) and hair cortisone (p =.002) also persisted. However, only hair cortisone remained significantly associated with increased errors on the MoCA (p = .015). The multivariable associations of hair cortisol and hair cortisone with verbal fluency, CRT, sustained attention to response task, and CTT2 performance did not reach statistical significance. Table 2 shows the full results of Model 3—the regression of task performance on hair GCs adjusting for age, age^2 (to account for the curvilinear relationship between age and cognitive function), sex, education, chemical hair treatment, season, BMI, smoking, diabetes, cardiovascular conditions, and depression. Figure 2 displays the fully adjusted marginal mean scores on the delayed word recall task and MMSE (number of errors) by cortisol and cortisone. This represents the predicted mean cognitive performance across the range of cortisol and cortisone values observed in the sample, holding all covariates at their average values. It shows a difference in recall performance of approximately one word, between individuals with the lowest and highest cortisol and cortisone values in our sample, when covariates are accounted for. For performance on the MMSE the difference those between low and high GCs is approximately half a point.

Given that each cognitive outcome was treated independently and a number of tests were carried out, we used a correction for multiple comparisons using a method of controlling the false discovery rate to minimize the risk of type I error (39). This yielded a corrected p value of .016. The associations of both hair cortisol and hair cortisone with delayed word recall and MMSE performance remained statistically significant after this correction, as did the association of cortisone with MoCA.

Sensitivity analysis: effect modification by sex

As evidence suggests there may be sex differences in how the brain responds to stress (40), coupled with the fact that in our study GC concentration differed by sex, and the majority of individuals in the sample were female, we carried out a sensitivity analysis to investigate whether the relationships with cognitive function varied by sex. Only one notable sex difference was found and that was in the relationship between hair cortisone and immediate recall performance. A significant effect of a Sex × Cortisone interaction term was observed (B = –0.54, p = .016), with simple effects analysis showing that the association between cortisone and immediate recall performance existed only for women (men: B = 0.20, p =.320; women: B = –0.33, p = .004). There was no evidence of effect modification by sex for the association between cortisone and the other cognitive variables, nor for the association between cortisol and any of the cognitive variables.

Missing Data Analysis

Comparison of the individuals included and excluded from the analyses showed that individuals included were younger (66.4 vs 67.4) and more likely to have been educated to tertiary level (40.1% vs 31.7%), than those excluded. They were also much more likely to be female (74.4% vs 45.9%). There was a slightly lower prevalence of diabetes among individuals included, and they were slightly less likely to be obese. They did not differ significantly in terms of number of chronic or other cardiovascular conditions, but those included in the analysis did have a slightly higher cognitive test scores than those excluded (MMSE mean [*SD*] = 28.7 [1.8] vs 28.3 [2.3]). Full details of differences can be found as Supplementary Table 2.

Table 2. Multivariable Association Between Hair Cortisol, Hair Cortisone and Cognitive Function

Dependent variable	Cortisol (log)			Cortisone (log)		
	B/OR	95% CI	*p*	B/OR	95% CI	*p*
Immediate word[a] recall	–0.12	–0.22 to –0.01	**.032**	–0.21	–0.40 to –0.01	**.036**
Delayed word recall[a]	–0.13	–0.22 to –0.04	**.003**	–0.23	–0.39 to –0.06	**.006**
Verbal fluency[a]	–0.05	–0.24 to 0.14	.633	–0.23	–0.60 to 0.14	.229
MoCA errors[b]	0.02	–0.00 to 0.05	.089	0.06	0.01 to 0.11	**.015**
MMSE errors[b]	0.06	0.01 to 0.10	**.008**	0.13	0.05 to 0.22	**.002**
Log CTT2 time (s)[a]	0.01	–0.00 to 0.02	.101	0.02	–0.00 to 0.04	.113
SART errors[b]	0.001	–0.03 to 0.04	.944	–0.01	–0.08 to 0.06	.764
CRT (quintiles)[c]	1.02	0.95 to 1.10	.524	1.10	0.95 to 1.27	.195

Note: BMI = body mass index; CRT = choice reaction time; CTT2 = Color Trails Task 2; MMSE = Mini-Mental State Examination; MoCA = Montreal Cognitive Assessment; OR = odd ratio; SART = Sustained Attention to Response Task. Bold values represent significance level p = .016.

[a]Ordinary least squares regression (B). [b]Negative binomial regression. [c]Ordered logistic regression (OR). Models were adjusted for age, age^2, sex, education, chemical hair treatment, season, BMI, smoking, diabetes, cardiovascular conditions, and depression.

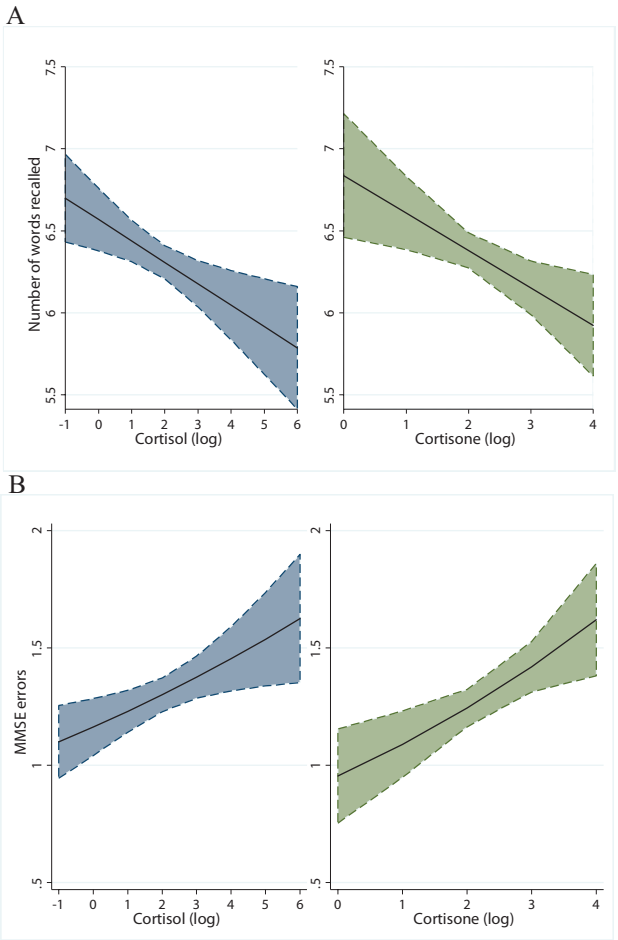

Figure 2. Estimated marginal mean (A) delayed word recall score and (B) number of errors on the Mini-Mental State Examination (MMSE), by increasing hair cortisol and hair cortisone. Estimates are adjusted for age, age^2, sex, hair treatment, season, education, smoking, diabetes, body mass index, cardiovascular conditions, and depression. Shaded areas correspond to 95% confidence interval bands. Full color version is available within the online issue.

Discussion

This study examined the relationship between hair GCs and cognitive function, in a large, population-based sample of older adults. We found that higher levels of hair cortisone and hair cortisol were associated with poorer performance on several of the cognitive performance variables tested, after adjustment for covariates. Specifically, associations with immediate and delayed word recall, MMSE and MoCA (hair cortisone only) persisted after adjustment for demographic characteristics, hair treatment, cardiovascular health and depression.

To the best of our knowledge, this is the first study to examine both hair cortisol and cortisone levels in association with cognitive function in older adults. Furthermore, it is the first study to examine the relationship between hair cortisol and cognitive function in a large, population-based sample. The fact that the results of this study are at odds with the only two previous studies to investigate this relationship in adults is unsurprising. Both earlier studies had small sample sizes and differed markedly both from each other and from this study in terms of the characteristics of the individuals sampled. Our results are in keeping with the literature on cognitive function and cortisol as assessed by repeat salivary or urinary cortisol measurement (eg, (8–10)), particularly with respect to the finding that individuals with higher cortisol have poorer memory performance.

The current results add further to the literature by providing evidence that hair cortisone may be a useful additional marker for assessing the impact of endogenous GCs on cognition. Several studies have examined determinants of hair cortisone in adults and found them to be largely similar to those of hair cortisol (21,41), which is also supported by the current results. Stalder and colleagues noted, however, that hair cortisone showed a stronger relationship with individual cardiometabolic variables than hair cortisol, and this is again consistent with our data. Of note, our data also replicate the observations made by Stalder and colleagues and Staufenbiel and colleagues that extreme outliers were more likely in hair cortisol then hair cortisone data. Hair cortisone may be a more accurate measure of systemic GC levels, in keeping with the finding that salivary cortisone not cortisol most closely approximates unbound (biological active) cortisol in serum (42) after adrenal stimulation. It is also plausible that local activity of the 11β-HSD1 enzyme could play a role. 11β-HSD1 activity in the skin dermis and epidermis increases with age (43), which may result in local conversion of cortisone to cortisol. Although it is largely presumed that hair GCs entirely reflect systemic hormone concentrations, this has been disputed by some and therefore it is possible that cortisol on the surface of the skin and in sweat may have a small effect on concentrations in hair (eg, (44), but see ref. (45)). It also appears that the hair follicle itself can secrete small amounts of cortisol (46). Further work is required to better elucidate the local as well as systemic influences on the production and incorporation of cortisone and cortisol into hair.

We found that cosmetic chemical treatment of hair (the majority of which comprised hair dying) had a significant effect of hormone levels. This is consistent with the majority of recent studies (eg, (19,21,39,47)) and suggests that perhaps individuals should be asked to abstain from hair treatments in advance of future research studies of similar nature.

Our data show seasonal variation in hormone levels with highest hair cortisol and cortisone among participants whose hair samples were obtained during autumn, corresponding to a growth period covering summer or autumn. A seasonal variation in hair GCs previously reported has been mixed with a couple of studies finding highest levels in hair samples reflecting growth during spring or summer (21) and summer or autumn (48), and a recent study finding highest levels in winter (19). In addition to seasonal variation in hypothalamic–pituitary–adrenal axis activity—the nature of which is still under debate—temperature and humidity are additional factors that may influence hair GC levels in particular (49). More research is warranted in light of the differences reported between studies.

The initial hypotheses were partially supported by the findings, clearly so in relation to memory. This is most likely because of the high density of GC receptors in the hippocampus and the pivotal role of this region in hypothalamic–pituitary–adrenal axis regulation (1,11). Although the association between hair cortisol and cortisone and performance was also observed for global tests of cognition, which include items tapping executive functions such as cognitive control and working memory, the observed relationship between hair GCs and stand-alone tests of executive function such as the CTT2 was largely attenuated after adjustment for covariates, somewhat contrary to our hypothesis. Although task-specific variation occurs—as different tests assess different executive functions—it may also be the case that the relationship with global cognitive performance is driven by other items within the scale in question, though we did not directly test this in this analysis.

There are several possible mechanisms underpinning the observed associations. The first is that greater total cortisol output over a number of years or even decades has enacted or contributed to a process of neuronal decay or accelerated age-related neuroanatomical change. This, in turn, is manifested by poorer performance in certain areas of cognitive function. Although this hypothesis has historically been difficult to test, it has some support from the small, extant literature examining long-term salivary cortisol levels or chronic stress, cognitive function, and brain integrity. Two studies that have measured cortisol levels repeatedly over a period of years rather than over shorter timescales have shown that persistently high or increasing cortisol levels in mid-to-late life are related to poorer memory performance (9,50). Also in support of this, self-reported chronic stress in midlife and persistence of elevated stress over time predicts decreased hippocampal atrophy (51,52) and increased risk of dementia (52–54) in later life. A second potential mechanism concerns the potential for cognitive deficits to arise from a deleterious effect of increased cortisol levels more acutely (as a consequence of a recent period of stress). This latter mechanism has a lot of empirical support from the salivary or serum cortisol literature (eg, (5,6,8,55), but see ref. (56) to the contrary) in humans showing that short-term differences in stress hormones are related to memory performance and (to a lesser extent) other cognitive functions. A third possibility also exists whereby cognitive functioning and cortisol levels in later life might both be, in part, determined by shared genetic traits or early-life environmental factors, perhaps mediated by individual differences in regional brain volume (eg, (57)). Longitudinal follow-up will shed further light on these separate, but not necessarily unrelated, mechanisms. It is also noteworthy that adjusting the models for depression did not change the results, suggesting that the associations observed are not a consequence of the well-established relationship between depression and cognitive impairment. Elevated hair GCs over the medium to long term may constitute a modifiable risk factor for poor cognitive performance and as such these results highlight the potential of therapeutic approaches to reduce or mitigate the impact of stress.

Our results also contribute to the field by confirming the association of several other demographic-, hair-, and health-related variables previously observed in smaller samples or occupational cohorts, with hair cortisone (in addition to cortisol) in a population-based study. A further strength of the study is the fact that we excluded individuals taking steroid medications from the analysis. Few previous studies of hair GCs and health indicators have taken into account the use of steroid medications. Evidence suggests that these do alter cortisol levels, but the direction of the effect reported has varied, and may depend on the type of corticosteroid, route of administration and the assay used to quantify GC levels (12,18,58,59). Interestingly, in preliminary analyses we found substantially elevated cortisol (but not cortisone) among those individuals taking steroid medications (not reported here).

Limitations of this study include the fact that findings are cross-sectional in nature and thus we cannot confirm the causality. Further, the sample included in the analyses differed from the parent sample with respect to several key demographic and health-related characteristics, so that those included in the analysis represented the younger and healthier participants. We speculate that the relationships observed here may be more pronounced in those individuals who were excluded by necessity, given that they were, on average, older and in poorer physical and cognitive health. The majority of the sample were female. However, sensitivity analysis testing effect modification by sex revealed only one notable sex difference, and that was in the relationship between hair cortisone and immediate recall performance. A significant effect of a Sex × Cortisone interaction term was observed, with simple effects analysis showing that the association existed only for women. Why this should have been the case for immediate recall but not delayed recall or performance on any of the other tasks, and for cortisone but not cortisol, is unclear. Nonetheless, it may be an avenue for future investigation, given the evidence supporting a role for estrogen depletion in age-related memory loss in women (60,61).

In conclusion, this study is the first to report a negative association of hair cortisol and cortisone with cognitive function, and the first to investigate this relationship in a large, population-based study of older adults. Future work will focus on the longitudinal relationship between these variables, and further exploration of the utility of hair cortisone as a neuroendocrine biomarker.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Funding

This work was supported by the Irish Government, the Atlantic Philanthropies, and Irish Life Assurance PLC. J.C.F. and A.M.O.H. were funded by the Centre for Ageing Research and Development in Ireland (CARDI) Leadership in Ageing Fellowships.

Acknowledgements

We are grateful to all of the The Irish Longitudinal Study on Ageing (TILDA) respondents for participating in the study. J.C.F. contributed to the study design, implementation, analysis, and preparation of the manuscript. A.M.O.H. and R.K. contributed to the study design, implementation, and revision of the manuscript. Researchers interested in using TILDA data may access the data for free from the following sites: Irish Social Science Data Archive at University College Dublin http://www.ucd.ie/issda/data/tilda/; Inter-university Consortium for Political and Social Research at the University of Michigan http://www.icpsr.umich.edu/icpsrweb/ICPSR/studies/34315.

Conflict of Interest

None declared.

References

1. McEwen BS, Nasca C, Gray JD. Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex. *Neuropsychopharmacology*. 2016;41:3–23. doi: 10.1038/npp.2015.171
2. Butler K, Klaus K, Edwards L, Pennington K. Elevated cortisol awakening response associated with early life stress and impaired executive function in healthy adult males. *Horm Behav*. 2017;95:13–21. doi: S0018-506X(16)30499-8
3. de Quervain DJ, Roozendaal B, McGaugh JL. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature*. 1998;394:787–790. doi: 10.1038/29542
4. Evans P, Hucklebridge F, Loveday C, Clow A. The cortisol awakening response is related to executive function in older age. *Int J Psychophysiol*. 2012;84:201–204. doi: 10.1016/j.ijpsycho.2012.02.008
5. Kirschbaum C, Wolf OT, May M, Wippich W, Hellhammer DH. Stress- and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. *Life Sci*. 1996;58:1475–1483. doi: 002432059600118X

6. Lupien SJ, Gaudreau S, Tchiteya BM, et al. Stress-induced declarative memory impairment in healthy elderly subjects: relationship to cortisol reactivity. *J Clin Endocrinol Metab*. 1997;82:2070–2075. doi: 10.1210/jcem.82.7.4075
7. Roozendaal B. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. *Neurobiol Learn Mem*. 2002;78:578–595. doi: S1074742702940803
8. Lee BK, Glass TA, McAtee MJ, et al. Associations of salivary cortisol with cognitive function in the Baltimore memory study. *Arch Gen Psychiatry*. 2007;64:810–818. doi: 10.1001/archpsyc.64.7.810
9. Li G, Cherrier MM, Tsuang DW, et al. Salivary cortisol and memory function in human aging. *Neurobiol Aging*. 2006;27:1705–1714. doi: 10.1016/j.neurobiolaging.2005.09.031
10. Lupien S, Lecours AR, Lussier I, Schwartz G, Nair NP, Meaney MJ. Basal cortisol levels and cognitive deficits in human aging. *J Neurosci*. 1994;14(5 Pt 1):2893–2903. doi:10.1523/JNEUROSCI.14-05-02893.1994
11. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci*. 2009;10:434–445. doi: 10.1038/nrn2639
12. Wright KD, Hickman R, Laudenslager ML. Hair cortisol analysis: a promising biomarker of HPA activation in older adults. *Gerontologist*. 2015;55(suppl 1):S140–145. doi: 10.1093/geront/gnu174
13. Wester VL, Reincke M, Koper JW, et al. Scalp hair cortisol for diagnosis of Cushing's syndrome. *Eur J Endocrinol*. 2017;176:695–703. doi: 10.1530/EJE-16-0873
14. Short SJ, Stalder T, Marceau K, et al. Correspondence between hair cortisol concentrations and 30-day integrated daily salivary and weekly urinary cortisol measures. *Psychoneuroendocrinology*. 2016;71:12–18. doi: 10.1016/j.psyneuen.2016.05.007
15. Zhang Q, Chen Z, Chen S, Xu Y, Deng H. Intraindividual stability of cortisol and cortisone and the ratio of cortisol to cortisone in saliva, urine and hair. *Steroids*. 2017;118:61–67. doi: 10.1016/j.steroids.2016.12.008
16. Stalder T, Steudte S, Miller R, Skoluda N, Dettenborn L, Kirschbaum C. Intraindividual stability of hair cortisol concentrations. *Psychoneuroendocrinology*. 2012;37:602–610. doi: 10.1016/j.psyneuen.2011.08.007
17. Pulopulos MM, Hidalgo V, Almela M, Puig-Perez S, Villada C, Salvador A. Hair cortisol and cognitive performance in healthy older people. *Psychoneuroendocrinology*. 2014;44:100–111. doi: 10.1016/j.psyneuen.2014.03.002
18. McLennan SN, Ihle A, Steudte-Schmiedgen S, Kirschbaum C, Kliegel M. Hair cortisol and cognitive performance in working age adults. *Psychoneuroendocrinology*. 2016;67:100–103. doi: 10.1016/j.psyneuen.2016.01.029
19. Abell JG, Stalder T, Ferrie JE, et al. Assessing cortisol from hair samples in a large observational cohort: the Whitehall II study. *Psychoneuroendocrinology*. 2016;73:148–156. doi: S0306-4530(16)30461–9
20. Stalder T, Steudte-Schmiedgen S, Alexander N, et al. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. *Psychoneuroendocrinology*. 2017;77:261–274. doi: S0306-4530(16)30611–4
21. Staufenbiel SM, Penninx BW, de Rijke YB, van den Akker EL, van Rossum EF. Determinants of hair cortisol and hair cortisone concentrations in adults. *Psychoneuroendocrinology*. 2015;60:182–194. doi: 10.1016/j.psyneuen.2015.06.011
22. Scharlau F, Pietzner D, Vogel M, et al. Evaluation of hair cortisol and cortisone change during pregnancy and the association with self-reported depression, somatization, and stress symptoms. *Stress*. 2018;21:43–50. doi: 10.1080/10253890.2017.1392507
23. Wang W, Deng H, Wang L, Cao C, Xu H, Zhang J. Hair cortisone level is associated with PTSD's dysphoric arousal symptoms in highly traumatized Chinese females. *J Affect Disord*. 2015;182:18–22. doi: 10.1016/j.jad.2015.04.036
24. Chapman K, Holmes M, Seckl J. 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev*. 2013;93:1139–1206. doi: 10.1152/physrev.00020.2012
25. Donoghue OA, McGarrigle CA, Foley M, et al. Cohort Profile Update: The Irish Longitudinal Study on Ageing (TILDA). *Int J Epidemiol*. 2018;47:1398–1398I. doi: 10.1093/ije/dyy163
26. Whelan BJ, Savva GM. Design and methodology of the Irish Longitudinal Study on Ageing. *J Am Geriatr Soc*. 2013;61 (suppl 2):S265–S268. doi: 10.1111/jgs.12199
27. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189–198. doi: 0022-3956(75)90026-6
28. Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53:695–699. doi: 10.1111/j.1532-5415.2005.53221.x
29. D'Elia LF, Satz P, Uchiyama CL, White T. *Color Trails Test: Professional Manual*. Odessa, FL: Psychological Assessment Resources; 1996.
30. Robertson IH, Manly T, Andrade J, Baddeley BT, Yiend J. 'Oops!': performance correlates of everyday attentional failures in traumatic brain injured and normal subjects. *Neuropsychologia*. 1997;35:747–758. doi:10.1016/S0028-3932(97)00015-8
31. Feeney J, Finucane C, Savva GM, et al. Low macular pigment optical density is associated with lower cognitive performance in a large, population-based sample of older adults. *Neurobiol Aging*. 2013;34:2449–2456. doi: 10.1016/j.neurobiolaging.2013.05.007
32. Gao W, Stalder T, Foley P, Rauh M, Deng H, Kirschbaum C. Quantitative analysis of steroid hormones in human hair using a column-switching LC-APCI-MS/MS assay. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2013;928:1–8. doi: 10.1016/j.jchromb.2013.03.008
33. Wennig R. Potential problems with the interpretation of hair analysis results. *Forensic Sci Int*. 2000;107:5–12. doi: 10.1016/S0379-0738(99)00146-2
34. Mlinac ME, Feng MC. Assessment of activities of daily living, self-care, and independence. *Arch Clin Neuropsychol*. 2016;31:506–516. doi: 10.1093/arclin/acw049
35. King JA, Rosal MC, Ma Y, et al. Sequence and seasonal effects of salivary cortisol. *Behav Med*. 2000;26:67–73. doi: 10.1080/08964280009595753
36. Arsenault-Lapierre G, Chertkow H, Lupien S. Seasonal effects on cortisol secretion in normal aging, mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging*. 2010;31:1051–1054. doi: 10.1016/j.neurobiolaging.2008.07.011
37. Samieri C, Perier MC, Gaye B, et al. Association of cardiovascular health level in older age with cognitive decline and incident dementia. *JAMA*. 2018;320:657–664. doi: 10.1001/jama.2018.11499
38. Kessler RC, Andrewsw G, Mroczek D, Ustun B, Wittchen H. The world health organization composite international diagnostic interview short-form (CIDI-SF). *Int J Methods Psychiatr Res*. 1998;7:171–185.
39. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Stat*. 2001;29:1165–1188. doi: 10.1214/aos/1013699998
40. McEwen BS, Gray JD, Nasca C. 60 years of neuroendocrinology: redefining neuroendocrinology: stress, sex and cognitive and emotional regulation. *J Endocrinol*. 2015;226:T67–T83. doi: 10.1530/JOE-15-0121
41. Stalder T, Kirschbaum C, Alexander N, et al. Cortisol in hair and the metabolic syndrome. *J Clin Endocrinol Metab*. 2013;98:2573–2580. doi: 10.1210/jc.2013-1056
42. Perogamvros I, Keevil BG, Ray DW, Trainer PJ. Salivary cortisone is a potential biomarker for serum free cortisol. *J Clin Endocrinol Metab*. 2010;95:4951–4958. doi: 10.1210/jc.2010-1215
43. Tiganescu A, Walker EA, Hardy RS, Mayes AE, Stewart PM. Localization, age- and site-dependent expression, and regulation of 11β-hydroxysteroid dehydrogenase type 1 in skin. *J Invest Dermatol*. 2011;131:30–36. doi: 10.1038/jid.2010.257
44. Russell E, Koren G, Rieder M, Van Uum SH. The detection of cortisol in human sweat: implications for measurement of cortisol in hair. *Ther Drug Monit*. 2014;36:30–34. doi: 10.1097/FTD.0b013e31829daa0a
45. Grass J, Kirschbaum C, Miller R, Gao W, Steudte-Schmiedgen S, Stalder T. Sweat-inducing physiological challenges do not result in acute changes in hair cortisol concentrations. *Psychoneuroendocrinology*. 2015;53:108–116. doi: 10.1016/j.psyneuen.2014.12.023

46. Ito N, Ito T, Kromminga A, et al. Human hair follicles display a functional equivalent of the hypothalamic-pituitary-adrenal axis and synthesize cortisol. *FASEB J*. 2005;19:1332–1334. doi: 10.1096/fj.04-1968fje
47. Jackson SE, Kirschbaum C, Steptoe A. Hair cortisol and adiposity in a population-based sample of 2,527 men and women aged 54 to 87 years. *Obesity (Silver Spring)*. 2017;25:539–544. doi: 10.1002/oby.21733
48. Braig S, Grabher F, Ntomchukwu C, et al. Determinants of maternal hair cortisol concentrations at delivery reflecting the last trimester of pregnancy. *Psychoneuroendocrinology*. 2015;52:289–296. doi: 10.1016/j.psyneuen.2014.12.006
49. Boesch M, Sefidan S, Annen H, et al. Hair cortisol concentration is unaffected by basic military training, but related to sociodemographic and environmental factors. *Stress*. 2015;18:35–41. doi: 10.3109/10253890.2014.974028.
50. Lupien SJ, de Leon M, de Santi S, et al. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci*. 1998;1:69–73. doi: 10.1038/271
51. Gianaros PJ, Jennings JR, Sheu LK, et al. Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus. *Neuroimage*. 2007;35:795–803. doi: 10.1016/j.neuroimage.2006.10.045
52. Johansson L, Skoog I, Gustafson DR, et al. Midlife psychological distress associated with late-life brain atrophy and white matter lesions: a 32-year population study of women. *Psychosom Med*. 2012;74:120–125. doi: 10.1097/PSY.0b013e318246eb10
53. Johansson L, Guo X, Waern M, et al. Midlife psychological stress and risk of dementia: a 35-year longitudinal population study. *Brain*. 2010;133(pt 8):2217–24. doi: 10.1093/brain/awq116
54. Sindi S, Hagman G, Håkansson K, et al. Midlife work-related stress increases dementia risk in later life: the CAIDE 30-year study. *J Gerontol B Psychol Sci Soc Sci*. 2017;72:1044–1053. doi: 10.1093/geronb/gbw043
55. MacLullich AM, Deary IJ, Starr JM, Ferguson KJ, Wardlaw JM, Seckl JR. Plasma cortisol levels, brain volumes and cognition in healthy elderly men. *Psychoneuroendocrinology*. 2005;30:505–515. doi: 10.1016/j.psyneuen.2004.12.005
56. Harris MA, Cox SR, Brett CE, Deary IJ, MacLullich AMJ. Cognitive ability across the life course and cortisol levels in older age. *Neurobiol Aging*. 2017;59:64–71. doi: 10.1016/j.neurobiolaging.2017.07.012
57. Kremen WS, O'Brien RC, Panizzon MS, et al. Salivary cortisol and prefrontal cortical thickness in middle-aged men: a twin study. *Neuroimage*. 2010;53:1093–1102. doi: 10.1016/j.neuroimage.2010.02.026
58. Wells S, Tremblay PF, Flynn A, et al. Associations of hair cortisol concentration with self-reported measures of stress and mental health-related factors in a pooled database of diverse community samples. *Stress*. 2014;17:334–342. doi: 10.3109/10253890.2014.930432
59. Wester VL, van Rossum EF. Clinical applications of cortisol measurements in hair. *Eur J Endocrinol*. 2015;173:M1–10. doi: 10.1530/EJE-15-0313
60. Peper JS, van den Heuvel MP, Mandl RC, Hulshoff Pol HE, van Honk J. Sex steroids and connectivity in the human brain: a review of neuroimaging studies. *Psychoneuroendocrinology*. 2011;36:1101–1113. doi: 10.1016/j.psyneuen.2011.05.004
61. Vest RS, Pike CJ. Gender, sex steroid hormones, and Alzheimer's disease. *Horm Behav*. 2013;63:301–307. doi: 10.1016/j.yhbeh.2012.04.006