Is social disadvantage a chronic stressor? Socioeconomic position and HPA axis activity among older adults living in England

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Competing interests

None declared.

Abstract

Introduction

Living in socioeconomic disadvantage has been conceptualised as a chronic stressor, although this contradicts evidence from studies using hair cortisol and cortisone as a measure of hypothalamus-pituitary-adrenal (HPA)[1] axis activity. These studies used complete case analyses, ignoring the impact of missing data for inference, despite the high proportion of missing biomarker data. The methodological limitations of studies investigating the association between socioeconomic position (SEP)[2] defined as education, wealth, and social class and hair cortisol and cortisone are considered in this study by comparing three common methods to deal with missing data: (1) Complete Case Analysis (CCA)[3], (2) Inverse Probability Weighting (IPW) [4]and (3) weighted Multiple Imputation (MI)[5]. This study examines if socioeconomic disadvantage is associated with higher levels of HPA axis activity as measured by hair cortisol and cortisone among older adults using three approaches for compensating for missing data.

Method

Cortisol and cortisone levels in hair samples from 4,573 participants in the 6th wave (2012-2013) of the English Longitudinal Study of Ageing (ELSA)[6] were examined, in relation to education, wealth, and social class. We compared linear regression models with CCA, weighted and multiple imputed weighted linear regression models.

Results

Social groups with certain characteristics (i.e., ethnic minorities, in routine and manual occupations, physically inactive, with poorer health, and smokers) were less likely to have hair cortisol and hair cortisone data compared to the most advantaged groups. We found a consistent pattern of higher levels of hair cortisol and cortisone among the most socioeconomically disadvantaged groups compared to the most advantaged groups. Complete case approaches to missing data underestimated the levels of hair cortisol in education and social class and the levels of hair cortisone in education, wealth, and social class in the most disadvantaged groups.

Conclusion

This study demonstrates that social disadvantage as measured by disadvantaged SEP is associated with increased HPA axis activity. The conceptualisation of social disadvantage as a chronic stressor may be valid and previous studies reporting no associations between SEP and hair cortisol may be biased due to their lack of consideration of missing data cases which showed the underrepresentation of disadvantaged social groups in the analyses. Future analyses using biosocial data may need to consider and adjust for missing data.

Keywords: Cortisol; Cortisone; Socioeconomic position; Missing data

[1] HPA axis: Hypothalamus Pituitary Adrenal axis
[2] SEP: Socio-economic Position
[3] CCA: Complete Case Analysis
[4] IPW: Inverse Probability Weighting
[5] MI: Multiple Imputation
[6] ELSA: English Longitudinal Study of Ageing

1. Introduction

Living in socioeconomic disadvantage has often been conceptualised in terms of a chronic stressor that results in dysregulation of stress-responsive physiological systems such as the sympathetic nervous system and hypothalamus–pituitary–adrenal (HPA) axis (Dowd *et al.,* 2009). The HPA axis is responsible for the neuroendocrine adaptation component of the stress response to stressors, resulting in the release of cortisol several hours after encountering the stressor (Miller *et al.,* 2007). The HPA axis is suggested to play an important role in 'transducing' social-environmental experience into physiological responses such as increased secretion of stress hormones like cortisol which may have long-term impacts on health. However, the empirical evidence linking socioeconomic position (SEP) (measured often as employment status, educational attainment, wealth, income, occupation, and other economic circumstances) with cortisol levels is weak (Dowd *et al.,* 2009). Cortisol has a pronounced diurnal rhythm and a short circulating half-life resulting in fluctuating levels of cortisol from blood samples. The measurement of cortisol from a single blood sample is an unreliable measure of chronic HPA axis activity, as it is strongly impacted by short-term stressors (Sugaya et al., 2020). Instead, HPA axis status has been assessed using salivary cortisol in a number of large-scale, population-based studies, even though there are considerable issues with the sample processing and laboratory analyses of salivary cortisol (Adam and Kumari, 2009). Cortisol collection from saliva is an invasive technique that is susceptible to non-response due to non-compliance by the participants (Kudielka *et al.,* 2003; Broderick *et al.,* 2004). Having an integrated measure of cortisol allows us to avoid the issue of diurnal variability in cortisol, which requires the accurate reporting of time since awakening and is notoriously hard to collect in surveys.

In recent years, cortisol measured in hair samples have been increasingly employed in large population studies, partly in response to the methodological issues in processing and analysing saliva samples. Hair cortisol is believed to provide a summated measure of overall activity of the HPA axis over several weeks/months and this allows for the assessment of the average cortisol levels over time (weeks or months). This avoids the moment-to-moment fluctuations in blood and saliva cortisol reflecting short-term stress, rather than chronic stress and the drawbacks in sample collection for salivary measurements.

However, despite the clear conceptualisation of low SEP or social disadvantage as a chronic stressor, a number of studies have reported null or no significant associations with SEP.

Poorer educational attainment was not related to higher levels of hair cortisol among pregnant women in Germany (Braig *et al.*, 2015), adult volunteers in China (Chen *et al.*, 2013), and adults representative of the Dutch population (Staufenbiel *et al.*, 2015). In contrast to other studies which did report such associations among pregnant women in the US (Schreier, *et al.*, 2016) and healthy young military conscripted men in Switzerland (Boesch *et al.*, 2015). Earning below the minimum wage was related to higher levels of hair cortisol among adult volunteers in Kenya (Henley *et al.*, 2014); similarly lower income levels and adverse changes in income were related to higher levels of hair cortisol (Serwinski *et al.*, 2016). However, lower employment grade among London based civil servants (Abell *et al.*, 2016) and lower objective socio-economic status (SES) among US adults (O'Brien *et al.*, 2013) and lower subjective socioeconomic status among Spanish adults (Pulopulos *et al.*, 2014) were not associated with hair cortisol. The association between occupational grade and hair cortisol showed the reverse direction (lower cortisol among lower grade employees) in the unadjusted analyses among London civil servants (Abell *et al.*, 2016). Among children, lower levels of maternal education were not associated with higher levels of hair cortisol among children in Sweden (Karlén *et al.*, 2013, 2015) and in the Netherlands (Bosma *et al.*, 2015). Maternal education was inversely associated with hair cortisol among Brazilian children (Martins *et al.*, 2023) and Canadian children, although parental income was not associated with hair cortisol (Vaghri *et al.*, 2013). See Table A1 in Supplement A for a summary of studies on SEP and hair cortisol.

The reasons for the discrepant findings in relation to SEP and hair cortisol are not clear. They could be related to methodological issues of the studies. Opportunistic samples (O'Brien *et al.*, 2013) and a lack of information on the sampling frame (Chen *et al.*, 2013), small study samples (100 participants or fewer) (Karlén *et al.*, 2013; Vaghri *et al.*, 2013; Pulopulos *et al.*, 2014; Bosma *et al.*, 2015) all make it harder to generalise to the wider population. Many studies did not report response rates for their studies (Serwinski *et al.*, 2016) or reported very low participation rates (Vaghri *et al.*, 2013; Pulopulos *et al.*, 2014; Boesch *et al.*, 2015; Bosma *et al.*, 2015; Staufenbiel *et al.*, 2015). Without this information, the potential methodological bias might be substantial. Some studies on adults used education as the

measure of SEP, even though they were analysing adults in mid-life or older (Chen *et al.*, 2013; Braig *et al.*, 2015; Staufenbiel *et al.*, 2015; Schreier *et al.*, 2016). Similarly, most studies among children used maternal education as the measure of SEP, even though household measures of SEP provide a more complete description of early life circumstances (Karlén *et al.*, 2013, 2015; Bosma *et al.*, 2015).

All the aforementioned studies use complete case analyses for modelling the impact of SEP on HPA axis activity, except for (Staufenbiel *et al.*, 2015) who used multiple imputation to compensate for missing values in health behaviours and physical characteristics.

Recent developments in liquid chromatography tandem-mass spectrometry have made the quantification of different glucocorticoids in hair possible. Cortisol is converted into inactive cortisone by 11 beta hydroxysteroid dehydrogenase type 2 (11 beta-HSD2) (Raul *et al.*, 2004) and the analysis of hair cortisone in parallel to hair cortisol may provide greater insights into the amount of active and inactive corticosteroids in the body (Staufenbiel *et al.*, 2015). Furthermore, a study showed that hair cortisone levels are around 3-4 times higher than hair cortisol (Stalder *et al.*, 2013). Another study found that after adrenal stimulation and hydrocortisone administration, salivary cortisone reflected the systemic cortisol better and at the same time remained unaffected by changes in circulating corticosteroid binding globulin (CBG) (Perogamvros *et al.*, 2010). Similar to hair cortisol, hair cortisone also predicts obesity (van der Valk *et al.*, 2021, 2022) and the metabolic syndrome (Stalder *et al.*, 2013; Kuckuck *et al.*, 2024). However, most existing studies on glucocorticoids from hair samples report results on hair cortisol only even though hair cortisone is another valid indicator of HPA axis activity.

These methodological issues in some of the studies on SEP and hair cortisol may have resulted in biased estimates of the association, casting doubt on the concept of low SEP and social disadvantage as a chronic stressor. The present study addresses some of the limitations of previous studies by analysing a probability sample of older adults and including three common indicators of SEP. This study aims and takes into account the inevitable missing data when combining biological with survey data. We also analyse the association of SEP with hair cortisone as an additional marker of HPA axis activity. Our overall research question is to examine how socioeconomic disadvantage is associated with higher levels of HPA axis activity, as measured by hair cortisol and cortisone, among older adults depending on the method to account for missing data.

We address the research question in conjunction with the mechanisms of missing data (Little and Rubin, 2002):

1. Missing completely at random (MCAR) are considered the missing data when the probability of missingness is unrelated to the outcome variable: Y_{obs} and Y_{mis}; $p(R|\phi)$, where R is a missing data indicator (R=1 defines missingness and 0 otherwise), ϕ is a parameter that rules when R takes on the value of one or zero and Y_{obs}, Y_{mis} are the observed and unobserved parts of the data, respectively.

In this case the missing data will only be considered MCAR, if we cannot identify any variables/covariates related to our model of interest which may explain the missingness in cortisol and cortisone.

2. Missing at Random (MAR) is considered if the probability of missingness is related to Y_{obs} but not to Y_{mis}; $p\ (R|Y_{obs,}\ \phi)$.

If we identify at least one variable/covariate related to missing data in cortisol and cortisone, then we will consider the missing data to be MAR.

3. Missing Not at Random (MNAR) is considered if the probability of missingness depends on Y_{obs} and Y_{mis}; $p\ (\ R|Y_{obs}, Y_{mis}, \phi)$.

This particular mechanism of missing data relies on untestable assumptions because the missingness in cortisol and cortisone measures would be related to the levels of cortisol and cortisone themselves. For example, this would be the case if participants with higher levels of cortisol and cortisone drop out of the study due to high cortisol and cortisone levels indicating ill-health. This hypothesis is beyond the scope of this study and therefore will not be considered in the analysis.

Implementing complete case analysis (CCA) will produce unbiased results only under the assumption of the MCAR scenario, where every case/participant with missing data in covariates (i.e., cortisol, cortisone, SEP measures and other covariates) will be discarded. Inverse probability weighting (IPW) and weighted multiple imputation (MI) will produce unbiased results under the assumption of MAR, addressing the impact of missing patterns of the outcome variable. The impact of SEP on the two different HPA axis activity measures (hair cortisol and cortisone) will be compared across the different

methods to compensate for missing data. Further on, we will present these findings and discuss them with previous literature.

2. Methods

2.1 Study population

For this study we utilised data from the English Longitudinal Study of Ageing (ELSA), a multidisciplinary prospective cohort study of men and women aged 50 years and over, living in private households in England, who were followed and re-interviewed every two years The sample was refreshed at waves 3, 4, and 6 to improve the sample's representativeness of the population aged 50 and older in England. ELSA sample was selected from households that have previously responded to the Health Surveys for England (HSE) in 1998, 1999, and 2001. HSE is a cross-sectional household survey that follows a two-stage sampling strategy from people living in England. First, it is ensured that all addresses from the Postcode Address File (PAF) have equal chances of being included in the sample and then a fixed number of addresses are selected systematically from each postcode sector. Potential loss of representativeness before the ELSA first interview due to non-response, refusal, attrition between HSE and ELSA and in further waves of ELSA can be corrected by using survey weights derived for each stage of the data collection (i.e., main interview, nurse visit, and blood sample collection) (Taylor *et al.*, 2007; Banks *et al.*, 2023). The National Research Ethics Service approved the study, and all participants gave their informed consent. The ELSA data and documentation are publicly available from the UK Data Service (https://ukdataservice.ac.uk). We analysed data from the 6th Wave (data collected in 2012-13) for the subsample, that participated in the health examination data collection (n= 7,730). From 9,169 core member participants, 7,730 (84.3%) respondents agreed to participate in the health examination and 6,180 (80.2%) agreed to give blood sample. Hair cortisol data were collected for the first time in Wave 6 ELSA blood sample collection. We investigate the association between socioeconomic position (SEP) and the levels of hair cortisol and hair cortisone.

2.2 Hair sample collection

The hair sample was taken from an area on the back of the head (vertex posterior), because this particular area shows the most consistent levels of hair cortisol/cortisone compared to different areas of the scalp's hair growth (Sauvé *et al.*, 2007). The sample needed to be a minimum of 2 cms in length

and a minimum of 10 mg in weight. Based on an average monthly hair growth of approximately 1 cm, the scalp-nearest hair segment of 2 cm represents average cortisol accumulated over an approximate time span of 2 months before sampling (Kirschbaum *et al.*, 2009). The wash procedure and steroid extraction were undertaken using high performance liquid chromatography–mass spectrometry (Gao *et al.*, 2013).

In Wave 6 out of 7,730 core participants in the health examination, 1,899 (24.5%) were ineligible for hair sample. Out of 1,899, most of those (96.1%) had shorter than 2 cm hair. Other reasons for ineligibility were breastfeeding, certain scalp conditions, and inability to sit with head remaining still. Hair samples were obtained from 5,267 participants (90.4% out of 5,828 eligible). Sample of hair cortisol and cortisone was obtained from 5,141 participants. The sample was restricted to 4,796 participants for cortisol and to 5,077 participants for cortisone due to some undetectable values. Our final analytical sample with information on participants with both cortisol and cortisone data, after excluding missing information on covariates, comprised of 4,573 participants.

2.3 Measures

2.3.1 Stress-related biomarkers

Cortisol and cortisone were measured using the hair sample collection from the health examination. Hair sample collection was conducted by nurses after following protocols[7]. Hair cortisol and hair cortisone concentrations were analysed at the Technische Universität Dresden in Germany in two phases in 2015 and 2018. For this reason, we added the variable "phase" in our analysis to adjust for this difference. Hair cortisol and cortisone were skewed, and therefore were logarithmically transformed to approach normal distribution to satisfy the requirements for linear regression analysis (Figures B1-B4 in the supplementary material B).

2.3.2 Socioeconomic position (SEP) characteristics

Several variables are included to measuring SEP at different life cycle phases. **Early adulthood SEP:** Education was measured as the highest educational qualification obtained and was classified into 1. University degree (NVQ 5-4) and higher education but without degree, 2. High school (NVQ 3-1), and

[7] More information can be found in the ELSA Documentation - https://www.elsa-project.ac.uk/data-and-documentation

3. Foreign qualifications or other qualifications or no qualifications. **Middle and late adulthood SEP**: Total net wealth was measured at benefit unit level and categorised into tertiles. Financial assets such as savings and investments were included to estimate the wealth variable (Banks *et al.*, 2006). The National Statistics Socioeconomic Classification scheme (NS-SEC) (Rose *et al.*, 1997) was used to measure social class, which described conditions and types of employability. The social class variable was operationalised in three categories: 1. Managerial and Professional occupations, 2. Intermediate occupations in engineering and in other technical occupations, and small employers and own account workers, and 3. Routine and manual occupations, which included those in lower supervisory and technical occupations, and those in semi-routine and routine occupations alongside participants with other occupations[7].

2.3.3 Other Covariates

Age (was categorised into 6 groups: 1. 50-59, 2. 60-64, 3. 65-69, 4. 70-74, 5. 75-79, 6. 80 and over), gender (male or female), marital status (categorised into 1. Married, 2. Cohabiting, 2. Single, 3. Widowed and 4. Divorced/Separated) and ethnicity (Whites and Ethnic minorities) were collected in the main interview basic questionnaire in Wave 6 of ELSA. Participants were asked whether they dyed or received any hair treatment, and we recoded a dichotomous variable describing participants who either had dyed or treated their hair and otherwise. The ELSA questionnaire distinguishes between seven hair colour categories: Brown, Blonde, Red/Auburn/Ginger, White, Grey, Black, Mix of Grey and Other colours. We recoded a new variable with three categories by merging 1. Blonde/Ginger, 2. Brunette/Black, 3. Grey/White/Mixed Grey and others. The date of hair sample collection during the nurse visit was used to estimate the season of collection as temperature, humidity, and transpiration can have an effect on hair cortisol concentrations (Boesch *et al.*, 2015; Grass *et al.*, 2015; Staufenbiel *et al.*, 2015); Season variable was produced from the nurse visit month date and was categorised as following: 1. Winter (December- January- February), 2. Spring (March- April- May), 3. Summer (June- July- August), and 4. Autumn (September- October- November). The variable for hair analysis phase was categorised into Phase 1 (2015) and Phase 2 (2018).

2.4 Statistical modelling

2.4.1 Missing hair cortisol data

ELSA has three stages of data collection in wave 2,4,6 and 8: (1) the main interview, (2) health examination (nurse visit) and (3) blood and hair sample collection. The multiple stages of data collection at different time points in the ELSA study increase the possibility of non-response. Biomarker data, such as blood, saliva or hair samples have typically lower response rates compared to the data in the main interview and health data due to refusal and/or inability to provide such samples, which leads to unit-nonresponse. Unit-nonresponse in population-based surveys is typically addressed by applying survey weights that adjust the sample for potential non-response bias in order to achieve representativeness of the sample (Groves *et al.*, 2002; Little and Rubin, 2002; Groves and Peytcheva, 2008). Inverse probability weighting (IPW) is one way to compensate for the sequential (participants who have not accepted a health examination cannot give blood sample) unit non-response found in the ELSA study. This method corrects the distribution of the sample observations in an attempt to approximate the distribution of the population from which the sample was collected. Alternatively, in studies like ELSA, non-response in the subsequent stages of data collection (health examination and blood sample collection) can themselves be treated as item non-response and, therefore, multiple imputation methods can be applied. In multiple imputation method, every missing item is replaced with values that represent a distribution of possibilities (Carpenter *et al.*, 2006; Seaman *et al.*, 2012). See supplementary material in Appendix C for further information.

We aimed to explore the HPA axis activity in relation to education, wealth, and social class in order to shed light on some of the inconsistencies in the existing literature cited in previous sections of this study. Therefore, we modelled each sociodemographic characteristic separately from the rest of the characteristics, to show independent effects of each SEP characteristics on hair cortisol and hair cortisone.

All analyses were performed in Stata/MP v.16.1 (StataCorp, College Station, TX, USA). The analysis code for this study can be found https://github.com/GeorgiaChatzi/Is-social-disadvantage-a-chronic-stressor-.git .

2.4.2 Complete case analysis (CCA)

Linear regression was implemented to investigate the association between education, wealth, and social class, and hair cortisol and cortisone after adjusting for covariates. Covariates were age, gender, interaction between age and gender, ethnicity, marital status, hair treatment and hair colour, season of hair collection, phase of hair analysis. Every participant with missing values in the covariates was excluded apart from the participants who had missing information for cortisol/cortisone; hair colour; hair treatment; and phase of hair analysis. The total sample was comprised of 4,573 participants.

2.4.3 Inverse Probability Weighting (IPW)

Inverse probability weighting is a commonly used approach to correct for unequal sampling fractions and to reduce bias coming from CCA where cases with missing data are discarded. In this method, complete cases are weighted by the inverse of the probability of being a complete case (Seaman and White, 2013). For this weight to be estimated, a response model must be created with variables identified to predict missingness in the outcome of interest. This way we can explore the underlying reasons for missingness of a particular value and this is an important advantage of all methods addressing missing data issues (Little *et al.*, 2022).

We selected variables identified to act as predictors of missingness drawn from the technical reports provided from ELSA to describe the existing survey weights (Bridges *et al.*, 2015) and from the broader literature of survey research for sociodemographic characteristics (Uhrig, 2008; Watson and Wooden, 2009) and health conditions (Jones *et al.*, 2006). ELSA does not provide a specific survey weight to account for differential non-response in biomarkers cortisol and cortisone, therefore, we constructed a new weight after taking account key variables and interactions. Variables that predicted missing hair cortisol and cortisone data were the following: age and gender interaction term, ethnicity, educational level, social class, government office region, volunteering work, date of the main interview, newly diagnosed with high blood pressure and newly diagnosed with osteoporosis, economic activity, and smoking status.

In order to construct the new weight, a dichotomous variable was created indicating 1 for those participants who had hair cortisol/cortisone biomarker data and with 0 otherwise in Wave 6. Then, logistic regression was estimated using Wave 6 main interview weight to adjust for non-response from previous waves.

The new weight variable was then created after multiplying the ELSA Wave 6 main interview weight with the inverse of the predicted response probabilities from the logistic regression model. This is defined as IPW weight. A weighted complete case analysis was performed using the IPW weight in a weighted linear regression model based on "svy" command to examine the effect of SEP on hair cortisol/cortisone after accounting for covariates. The total sample size therefore is the same as for the CCA method with 4,573 participants.

2.4.4 Multiple imputations incorporating Wave 6 attrition weights (MI weighted)

Missing data methods such as MI which impute or fill the missing values in statistical models have the advantage, compared to CCA and IPW, to use observed values in the incomplete cases for statistical inference and not only to get the best predictions of the missing values (Little and Rubin, 2002; Little *et al*.,, 2022).The precision of MI is enhanced with the utility of auxiliary variables (Collins *et al.,* 2001; Hardt *et al.,* 2012). Variables that were in the model of interest and variables that were thought to predict or be associated with missing values were included in the multiple imputation model. Previous literature found additional auxiliary variables, which were associated with socioeconomic disadvantage and cortisol/cortisone variables. These include financial unit type (1. Single, 2. Couple, separate finances, 3. Couple, joint finances), Body Mass Index (BMI) (1. Underweight to Normal, 2. Overweight, and 3. Obese), and number of medications (1. None, 2. 1-2, 3. 3-5, 4. 6 or more). were included in the imputation model. We performed a weighted multiple imputation by chained equations (i.e., mi impute chained). Wave 6 main interview weight was used in two ways. First, we registered and used the weight as a covariate in the imputation model, then we used command "svyset" to designate the same weight and strata and we used command "svy" to estimate the multiple imputed regression models.

The multiple imputation method followed the combining rule (Rubin, 1987) in which every missing value is replaced with a set of plausible values that represent the uncertainty about the true value to impute. First, we created a hundred imputed datasets due to the large proportion of missing data in some variables (i.e., 43.9% for cortisol and cortisone). We imputed missing observations in variables cortisol and cortisone (n=3,572 – 43.9%); hair treatment (n=3,366 – 41.3%); hair colour (n=3,366 – 41.3%); season of hair collection (n=986 – 12.1%); BMI (n=1,300 – 15.9%), and number of medications (n=990 – 12.2%). Then standard procedures were implemented to analyse the multiple imputed data and

combine the results for statistical inference. We performed multiple imputation for 8,145 participants who responded in the ELSA Wave 6 main interview with full information on covariates. See Supplement C for further information on missing data analysis.

3. Results

3.1 Descriptive Statistics

Table 1 displays the unweighted means (and standard deviations) of log cortisol and log cortisone by the variables in the model of interest. Anova and t-test were carried out to assess the differences between categories in variables and log cortisol and log cortisone. The age-group differences showed a linear pattern in log cortisol, where higher levels of cortisol were found with increasing age but the youngest and the oldest age-groups had higher levels of log cortisone. Women had lower levels of log cortisol and log cortisone compared to men ($p<0.01$). There was no evidence that the difference between of ethnic minority and the white majority group in log cortisol and log cortisone was statistically significant ($p=0.41$ and $p=0.24$, respectively). Significantly different levels of log cortisone, but not log cortisol, were found for marital status ($p<0.01$) with single respondents having the highest levels of log cortisone. Hair treatment influenced the levels of both biomarkers, with those who received no treatment to have higher levels of both log cortisol and log cortisone ($p<0.01$).

There were significant differences in terms of hair colour with those having brunette/black or grey, mixed grey, white and other colour of hair having higher levels of both log cortisol and log cortisone ($p<0.01$) compared to those with lighter hair.

Samples taken during summer tended to have the highest log cortisol ($p=0.20$) and samples collected in autumn show the highest levels of log cortisone ($p=0.83$), although the differences were not significant. Participants whose hair sample was analysed in phase 1 (2015) had higher levels of log cortisol ($p<0.01$), but lower levels of log cortisone ($p<0.01$) compared to those participants with sample from phase 2.

Respondents with foreign or no qualifications had the highest levels of log cortisol ($p=0.23$) and log cortisone ($p=0.12$) but were not statistically different from other categories. Those in the lowest wealth tertile had the highest levels of cortisol and cortisone ($p<0.01$). Respondents in semi-routine and routine

classes had the highest levels of cortisol ($p<0.01$) and cortisone ($p<0.01$) followed by those in managerial and professional occupations.

Table 1 HERE

3.2 Missing data response model

Table 2 shows the response propensity logistic regression model (Odds ratios and 95%CIs) of characteristics of participants who had valid hair cortisol/cortisone data. In terms of sociodemographic characteristics, we found that men and women in the older age categories were more likely to have hair biomarker data compared to younger men. The ethnic minority group was less likely to have hair cortisol/cortisone data. High school graduates were more likely to have biomarker data whereas those working in routine and manual occupations were less likely to have biomarker data compared to more advantaged participants. Those living in specific regions of England (i.e., East of England, South-East, South-West) were more likely to have biomarker data compared to those in the North-East. Participants who were physically inactive either or not engaging in volunteering work were less likely to have biomarker data. Participants with specific newly reported health conditions (i.e., high blood pressure and osteoporosis) were, also, less likely to have hair cortisol/cortisone data. Self-employed and retired participants were more likely to have biomarker data compared to those employed while those with smoking habits were less likely to have these data compared to non-smokers. In terms of study-design related characteristics, those who had the main interview during July or August were more likely to have hair cortisol and hair cortisone data.

Table 2 HERE

3.3 Multivariable analyses

This section shows results of three statistical methods that compensate for missing data in hair samples for cortisol and cortisone biomarker data [i.e., complete case analysis (CCA), inverse probability weighting (IPW) and weighted multiple imputation (MI)]. Supplementary Tables D1-D6 (in supplement D) present the coefficients (SEs and p-values) of log cortisol and log cortisone regressed on the three measures of SEP in three separate models, each model controlling for age, gender, interaction term with age and gender, ethnicity, marital status, hair treatment and colour, season of hair collection and phase of hair sample analysis. Additional predicted levels (and 95% CIs) extracted from the regression models

are presented in Figures 1-3 and Table D7 in the supplementary material. We found that log cortisol and log cortisone were moderately correlated (Spearman's rho=0.41, p-value<0.01). Below we provide a description of our findings separately for each SEP indicator.

3.3.1 Education

Log cortisol levels were higher for respondents with foreign or no qualifications compared to those with degree level qualifications (Table D1), although the differences were marginally statistically significant only in the IPW method (b=0.13, p-value=0.05). Whereas log cortisone levels (Table D2) were significantly higher among respondents with foreign or no qualifications in all methods and effect sizes were slightly larger in IPW (b=0.08, p-value=0.02) and weighted MI method (b=0.09, p-value=0.01) compared to CCA (b=0.07, p-values=0.02). Predicted levels in Figure 1 also showed higher levels of log cortisol and log cortisone, although only in cortisone we found that 95% CIs of IPW and MI did not overlap with those of CCA which indicates difference between the methods.

3.3.2 Wealth

Participants in the lowest wealth tertile had significantly higher levels of log cortisol (Table D3) and log cortisone (Table D4) compared to the wealthiest participants, according to each of the three methods for compensating for missing data. However, effect sizes in cortisol was larger in CCA (b=0.22, p-value<0.01), compared to IPW (b=0.15, p-value=0.04) and MI (b=0.21, p-value<0.01). Whereas in cortisone, effect sizes were larger in MI (b=0.15, p-value<0.01) compared to CCA (b=0.14, p-value<0.01) and IPW (b=0.13, p-value<0.01).

Similarly, the predicted levels (Figure 2) showed higher levels of both log cortisol and log cortisone for the disadvantaged wealth groups but only in log cortisone we noticed non-overlapping 95% CIs between the methods, which indicate a significant difference between the IPW and MI methods compared to CCA.

3.3.3 Social class

Those participants in the routine and manual occupational class had significantly higher levels of log cortisol (Table D5) and log cortisone (Table D6), in comparison with those in managerial and professional occupation. This difference was statistically significant for all three methods. However, effect sizes in log cortisol were higher in IPW (b=0.17, p-value=0.01) and MI (b=0.15, p-value=0.01)

compared to CCA (b=0.13, p-value=0.02). Similarly, effect sizes in log cortisone were higher in MI (b=0.13, p-value<0.01) compared to CCA (b=0.10, p-value<0.01) and IPW (b=0.10, p-value<0.01). Predicted levels in Figure 3 also showed higher levels of log cortisol and log cortisone, although only in log cortisone we found that 95% CIs of IPW and MI did not overlap with those of CCA.

Overall, the standard errors using the IPW method were consistently larger in comparison to the standard errors generated by CCA and MI, reflected in the slightly larger confidence intervals in Figures 1-3.

3.3.4 Other covariates

There were also some differences in the association between log cortisol and the interaction between age-group and gender when comparing the three approaches to missing data (Tables D1, D3, and D5). Women between 60-64 were significantly more likely to have higher levels of log cortisol compared to men between 50-59 in the CCA and IPW in all three measures of SEP. However, in the MI analyses, the interaction between gender and age-group was not statistically significant. This is at least partially due to the fact that women between 60-64 were almost 6 times more likely than men, in the 50-59 group, to have hair cortisol/cortisone data (Table 2), therefore the significant interaction between age-group and gender in the CCA may have arisen because of fewer older men with hair cortisol data. Similar findings, with the oldest women (80+) could be found in log cortisone results (Tables D2, D4, and D6), whereas we found consistent statistically different results in terms of gender.

Ethnic minority groups had lower levels of both log cortisol and log cortisone in IPW but low levels only of log cortisone in CCA. In the MI analyses, these findings were not statistically different.

Marital status was associated only with log cortisone; our findings suggested that single and divorced or separated participants had higher levels of log cortisone, compared to those with a partner. However, in the model exploring wealth categories, we found statistically different results in IPW only for the divorced and separated participants.

There were some differences in the association between hair treatment and hair colour variables with log cortisol and log cortisone (Tables D1-D6). Respondents who had brunette or black hair had higher levels of log cortisol whereas those respondents who had their hair treated or who had darker or grey,

white, or mixed grey hair displayed higher levels of log cortisone (in each of the three methods for compensating for missing data).

Respondents whose hair sample was analysed in phase 2 (2018) had lower levels of log cortisol, but higher levels of log cortisone compared to those, whose hair sample was analysed in phase 1 (2015).

4. Discussion

This is the first study to examine the association between SEP and levels of HPA axis activity as measured by hair cortisol and hair cortisone among older adults that explicitly addresses methodological issues from missing data occurring from combining biological and survey data. This study analyses a probability sample of older adults living in England.

There was a consistent pattern in the results, regarding the association between socioeconomic disadvantage and higher levels of hair cortisol and cortisone among older adults. This association was found in the CCA, IPW and weighted MI approaches, dealing with missing data. We found larger differences between socioeconomic characteristics and cortisone and between methods, compared to cortisol. This is in accordance with the literature suggesting, that cortisone is more closely linked with cardiometabolic outcomes than cortisol (van der Valk *et al.*, 2022; Kuckuck *et al.*, 2024). Therefore, studies including cortisone measurements might offer more relevant insights into health compared to those focusing solely on cortisol.

CCA is still the dominant methods to handle missing data in biomarker research. However, we found some evidence that the levels of both cortisol and cortisone among the most disadvantaged socioeconomic groups were smaller in the CCA, in comparison with the IPW and weighted MI analyses. Therefore, we suggest that is important to account for missing data in biosocial research, as the assumption that the missing data are MCAR is not supported. Therefore, estimates based on CCA are potentially biased.

Both the IPW and weighted MI approaches resulted in higher estimated levels of hair cortisol and hair cortisone for disadvantaged socioeconomic groups in comparison with the complete case approach. Given the generally smaller standard errors (SEs) of the weighted MI models, in comparison with the IPW models, it may be useful for researchers to consider using the weighted MI approach to compensate for large amounts of missing data. Note that the SEs from the MI models were in some cases (i.e., age

and gender interaction estimates) larger in comparison with the CCA or the IPW models. This is expected because the multiple imputation process can build additional uncertainty into the estimates. In such cases IPW is the preferred missing data compensation method (Seaman and White, 2013).

However, for both IPW and weighted MI approaches, it is crucial to specify a response propensity model (the model for predicting missingness) that reflects the diversity of missing data mechanisms. For missing hair cortisol and hair cortisone data, we found that the data were not MCAR and that respondents with poorer health, who were physically inactive and not engaged in volunteering were more likely to have missing hair cortisol/cortisone data. Missing data methods set out to compensate for the underlying reasons why those data are missing. Ignoring those characteristics of participants who are less likely to have cortisol sample would underestimate the effects of SEP on cortisol if the sample consists predominantly of individuals from the most advantaged SEP groups who display generally better health. The selection of more socially engaged and advantaged respondents with better health into the complete case analyses is likely to bias any associations between socioeconomic factors and biomarkers of stress.

The association of social disadvantage with higher levels of the stress biomarkers was consistent for both cortisol and cortisone. Although both cortisol and cortisone levels are the products of HPA axis activity, they are only moderately correlated with each other. Therefore, there may be differences between them in the way they accumulate in hair. We found that hair treatment and lighter hair colour were associated with lower levels of hair cortisone, rather than hair cortisol. It is possible that any hair treatment including dyeing hair could result in lower levels of detectable cortisone. Hair cortisol may be more resistant to such hair treatments. This suggests that different factors may affect these biomarkers of stress and that combining them into a single measure (Quinkler and Stewart, 2003; Ferrari, 2010; Zhang *et al.*, 2017) may mask rather than elucidate the different processes that affect these biomarkers.

In this study, we also identified socioeconomic measures that could reflect relevant life course processes that are important in relation to stress related biomarkers. Previous studies suggest that there is no association between lower educational attainment and hair cortisol concentration in adults (Chen *et al.*, 2013; Braig *et al.*, 2015; Staufenbiel *et al.*, 2015) and children (Karlén *et al.*, 2013, 2015; Bosma *et al.*,

2015). Our findings, using the IPW with a specific non-response weight structured to compensate for hair cortisol missingness, contradict this literature and suggest that previous studies may have underestimated the effect of socioeconomic disadvantage on stress related biomarkers. Education may not be the best indicator of SEP for older adults (over 50 years of age) as it is determined by parental sociodemographic characteristics, and it is an indicator of early life SEP (Davey-Smith *et al.*, 1998; Galobardes e*t al.*, 2007). On the other hand, net total wealth includes household income, and assets (i.e., savings and investments) accumulated over the life-course, therefore, it is considered a well-established indicator to describe SEP over the life-course (Galobardes *et al.*, 2006). The association of wealth with HPA axis function may reflect the stressful living conditions of the poor, or alternatively may reflect the importance of accumulated wealth as a buffer against chronic stress. Wealth has been associated with several health outcomes in older adults (Demakakos *et al.*, 2016). The consistent pattern of associations between low wealth and higher levels of cortisol and cortisone may indicate that low wealth measures capture stressful social environments among older adults to a better extent than a lack of educational qualifications and routine occupational social class, measures from much earlier in the life course. Some research results from previous studies on socioeconomic position measures suggest that people with lower income have higher cortisol levels (Henley *et al.*, 2014; Serwinski *et al.*, 2016) while other studies suggest that there is no effect from income level (O'Brien *et al.*, 2013) and employment grade (Abell *et al.*, 2016) on cortisol levels.

This is the largest study using a probability sample of older adults living in England to explore the SEP effects on hair cortisol and hair cortisone. Furthermore, this study set out to address the methodological limitations in previous studies on SEP and hair cortisol where missing data was ignored. Inconsistencies in findings in previous research of SEP and cortisol led to explore the impact of missing data, since often large number of participants in population studies do not have cortisol sample. We also aimed to explore the characteristics of participants who are less likely to have a valid cortisol sample and - after taking into account these characteristics - to estimate if disadvantaged SEP is associated with higher levels of cortisol. Previous studies' limitations related to poor measurement of SEP and non-representative small samples are also explicitly addressed in our study. A key limitation of our study is

the cross-sectional study design. Therefore, we cannot rule out reverse causality and distinguish between whether disadvantaged SEP causes increased HPA axis activity, or if adversity earlier on in life determines both disadvantaged SEP and cortisol/cortisone. Data on hair cortisol and cortisone in later waves of ELSA would potentially offer more opportunities to explore this issue.

5. Conclusion

This study demonstrates that social disadvantage as measured by disadvantaged SEP is correlated with increased HPA axis activity, supporting the conceptualisation of social disadvantage as a chronic stressor. The lack of evidence for such an association between SEP and hair cortisol in previous studies is potentially based in the absence of compensation methods for missing data. Analyses of biomarker datasets with considerable amounts of missing data that rely on complete case analyses may underestimate the role of social environmental factors like socioeconomic position on biomarkers of stress.

Table 1: Means (SDs) of log cortisol and cortisone (log pg/ml) by covariates								
Variables			**Log Cortisol (pg/ml)**			**Log Cortisone (pg/ml)**		
Age categories	**N (%) (Total=4,573)**		**Mean (SD)**		**P-values***	**Mean (SD)**		**P-values***
50-59	928	20.3	2.02	1.38	<0.01	2.07	0.79	<0.01
60-64	910	19.9	2.02	1.51		1.92	0.79	
65-69	892	19.5	2.11	1.47		1.96	0.78	
70-74	680	14.9	2.17	1.45		1.96	0.76	
75-79	623	13.6	2.23	1.58		1.93	0.75	
80+	540	11.8	2.22	1.53		2.15	0.69	
Gender								
Male	1,496	32.7	2.21	1.51	<0.01	2.20	0.71	<0.01
Female	3,077	67.3	2.06	1.46		1.89	0.78	
Ethnicity								
Whites	4,473	97.8	2.11	1.48	0.41	1.99	0.77	0.24
Ethnic minorities	100	2.2	1.99	1.38		1.90	0.84	
Marital status								
Married/ Cohabiting	3,071	67.2	2.09	1.46	0.55	1.96	0.75	<0.01
Single	230	5.0	2.16	1.43		2.13	0.85	
Widowed	778	17.0	2.16	1.45		2.03	0.78	
Divorced/Separated	494	10.8	2.14	1.67		2.05	0.83	
Hair treatment								
Yes	1,834	40.1	2.01	1.44	<0.01	1.78	0.81	<0.01
No	2,739	59.9	2.18	1.50		2.14	0.71	
Hair colour								
Blonde/Ginger	789	17.3	1.94	1.45	<0.01	1.69	0.82	<0.01
Brunette/Black	1,194	26.1	2.11	1.41		2.03	0.79	
Grey/White/Mixed Grey/Other	2,590	56.6	2.16	1.52		2.07	0.73	
Season of hair collection								
Winter (Dec-Feb)	1,218	26.6	2.04	1.52	0.20	1.99	0.66	0.83
Spring (Mar-May)	310	6.8	2.05	1.45		1.99	0.78	
Summer (Jun-Aug)	1,096	24.0	2.17	1.50		1.98	0.86	
Autumn (Sep-Nov)	1,949	42.6	2.13	1.45		2.00	0.78	
Phase of hair analysis								
1(2015)	2,575	56.3	2.19	1.58	<0.01	1.84	0.78	<0.01
2(2018)	1,998	43.7	2.00	1.34		2.19	0.72	
Educational level								
Higher Education	1,401	30.6	2.09	1.42		2.01	0.74	
High school	1,493	32.7	2.08	1.50	0.23	1.96	0.79	0.12
Foreign or no qualifications	1,679	36.7	2.16	1.51		2.01	0.79	
Wealth tertiles								
Highest tertile	1,619	35.4	2.05	1.45	<0.01	1.94	0.73	<0.01
Middle tertile	1,521	33.3	2.05	1.43		1.96	0.78	
Lowest tertile	1,433	31.3	2.25	1.56		2.09	0.80	
Social class								
Managerial & Professional	1,598	34.9	2.08	1.46	0.01	1.99	0.76	<0.01
Intermediate	1,226	26.8	2.03	1.44		1.91	0.77	
Routine & Manual Occupations	1,749	38.3	2.19	1.53		2.04	0.78	

Note: *Statistical test at levels of 5% and 1% include T-test and ANOVA wherever appropriate.

Table 2: Response model for hair Cortisol/Cortisone (N=8,145)				
	Odds Ratios	**Standard Errors**	**95% Confidence Intervals**	
Age categories with gender				
50-59 Male	1(ref)			
50-59 Female	4.86	0.58	3.84	6.14
60-64 Male	1.64	0.20	1.29	2.09
60-64 Female	5.72	0.72	4.47	7.32
65-69 Male	1.38	0.18	1.07	1.78
65-69 Female	5.53	0.73	4.26	7.17
70-74 Male	1.45	0.20	1.10	1.91
70-74 Female	4.86	0.70	3.67	6.45
75-79 Male	1.69	0.25	1.26	2.25
75-59 Female	4.80	0.70	3.60	6.38
80+ Male	1.36	0.21	1.00	1.84
80+ Female	3.84	0.56	2.88	5.12
Ethnicity				
Whites	1(ref)			
Ethnic Minorities	0.42	0.07	0.30	0.59
Educational level				
Higher Education	1(ref)			
High school	1.17	0.09	1.00	1.35
Foreign or no qualifications	0.93	0.07	0.80	1.08
Social class				
Professional & Managerial Occupations	1(ref)			
Intermediate	0.92	0.07	0.79	1.07
Routine & Manual Occupations	0.85	0.06	0.74	0.98
Government Office Region				
Northeast	1(ref)			
Northwest	1.04	0.14	0.79	1.35
Yorkshire	1.05	0.14	0.81	1.36
East Midlands	1.04	0.14	0.79	1.35
West Midlands	0.73	0.10	0.56	0.94
East of England	1.46	0.20	1.12	1.91
London	0.84	0.12	0.63	1.11
Southeast	1.35	0.17	1.05	1.74
Southwest	1.72	0.24	1.32	2.25
Volunteering Work				
No	1(ref)			
Yes	1.17	0.08	1.03	1.33
Physical Activity				
More than once a week	1(ref)			
Once a week	0.91	0.09	0.75	1.11
One to three times a month	0.90	0.15	0.66	1.24
Hardly ever or never	0.74	0.07	0.60	0.90
Date of the interview				
January/February	1(ref)			
March/April	0.65	0.15	0.41	1.03
May/June	1.25	0.15	0.99	1.58
July/August	1.23	0.12	1.02	1.50
September/October	1.09	0.10	0.91	1.31
November/December	1.08	0.10	0.89	1.31
Diagnosed with High Blood Pressure (newly reported)				

Not mentioned	1(ref)			
Mentioned	0.76	0.09	0.60	0.97
Diagnosed with Osteoporosis (newly reported)				
Not mentioned	1(ref)			
Mentioned	0.66	0.13	0.44	0.98
Economic Activity				
Employed	1(ref)			
Self-employed	1.38	0.17	1.09	1.75
Retired	1.30	0.11	1.10	1.53
Unemployed	1.20	0.14	0.95	1.50
Smoking status				
Never smoked	1(ref)			
Ex-smoker	0.95	0.06	0.84	1.07
Current smoker	0.83	0.08	0.69	0.99
Intercept	0.35	0.06	0.25	0.49

Notes: 1. Total sample size (N=8,145)
2. Logistic regression model with Odds Ratios (ORs), Standard Errors (SEs) and 95%Confidence Intervals (CIs).
3. Binary outcome variable of 0: not having hair Cortisol/Cortisone data (n=3,572), and 1: having hair Cortisol/Cortisone data (n=4,573).
4. Odds are expressed relative to a reference category. Odds ratios greater than 1 indicate higher odds while ratios lower than 1 indicate lower odds.
5. Only variables significant in the levels of 5% and 1% were included.
6. We used Wave 6 main interview weight to adjust for non-response and selection bias from previous waves.

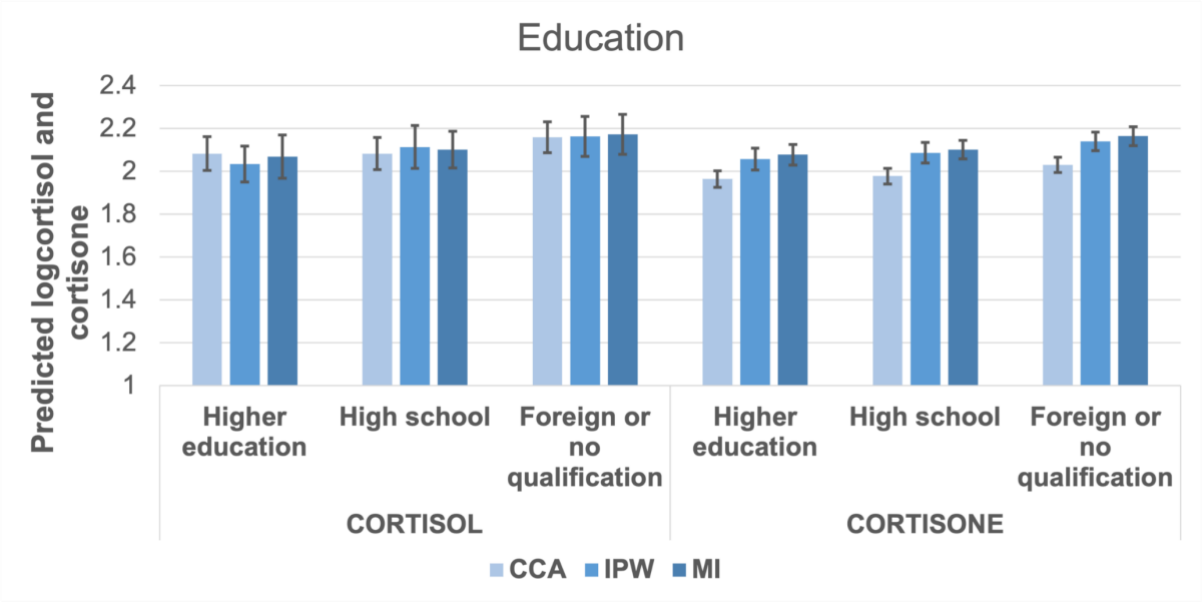

Figure 1: Predicted levels of log cortisol and log cortisone by education in three statistical methods estimated from Table D1 and D2.
Note: Complete Case Analysis (CCA), Inverse Probability Weighting (IPW), and Multiple Imputation (MI). Full description of values in Table D7.

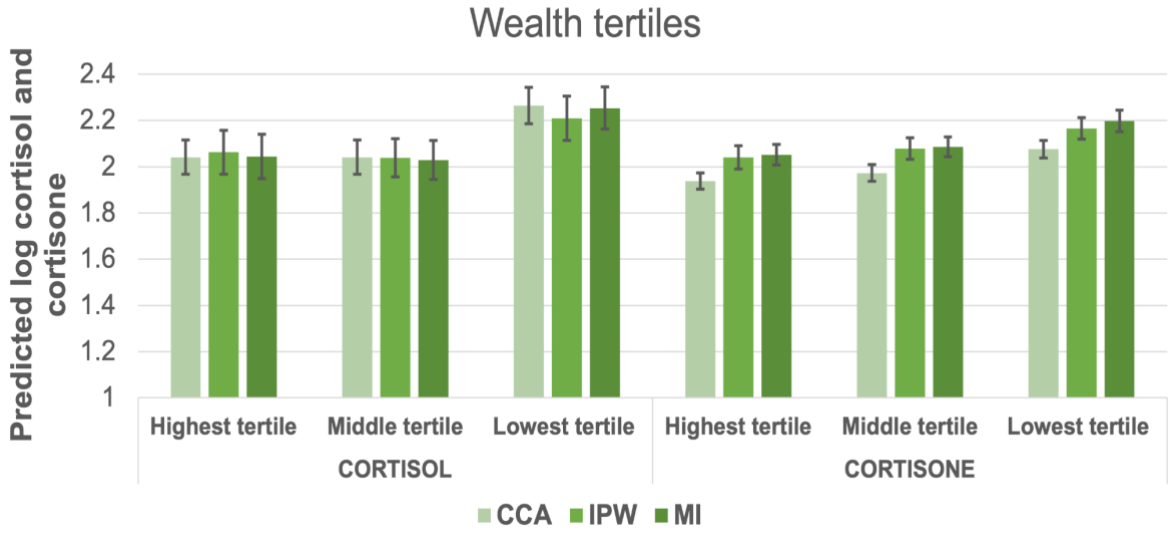

Figure 2: Predicted levels of log cortisol and log cortisone by wealth tertiles in three statistical methods estimated from Tables D3 and D4.
Note: Complete Case Analysis (CCA), Inverse Probability Weighting (IPW), and Multiple Imputation (MI). Full description of values in Table D7.

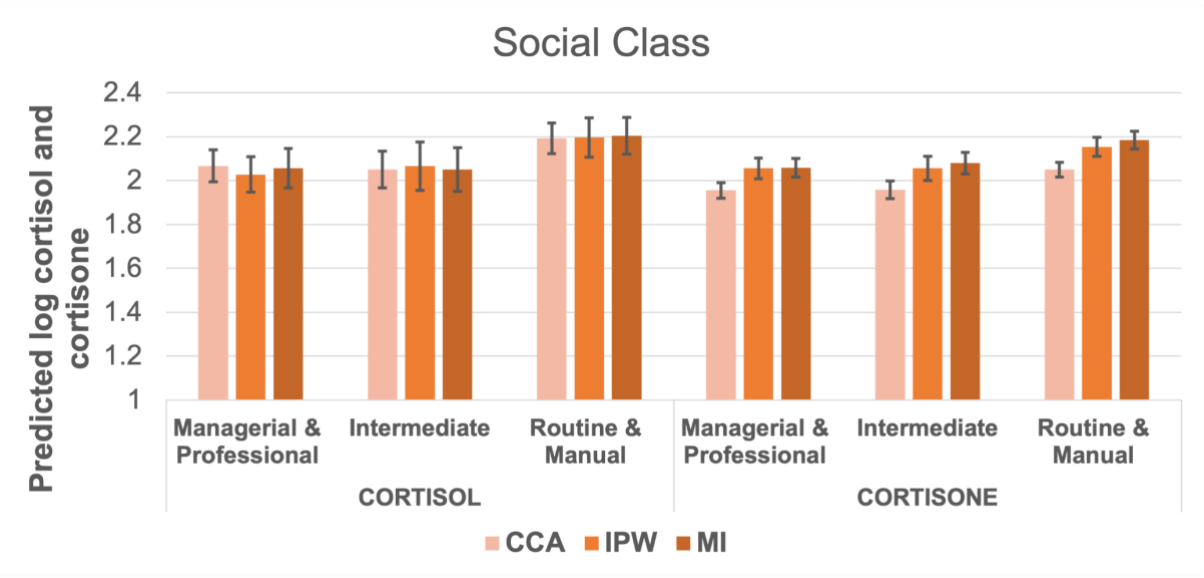

Figure 3: Predicted levels of log cortisol and log cortisone by social class in three statistical methods estimated from Tables D5 and D6.
Note: Complete Case Analysis (CCA), Inverse Probability Weighting (IPW), and Multiple Imputation (MI). Full description of values in Table D7.

Funding sources

NS, TC, AC, and TH were supported by the United Kingdom Economic and Social Research Council, National Centre for Research Methods [ES/L008351/1]. GC was supported by the United Kingdom Economic and Social Research Council, National Centre for Research Methods [ES/J500094/1]. The views and opinions are those of the authors and not necessarily reflect those of the Economic and Social Research Council and the National Centre for Research Methods. ELSA is funded by the National Institute on Aging (R01AG017644), and by UK Government Departments coordinated by the National Institute for Health and Care Research (NIHR).

Ethical approval

ELSA was approved by the London Multicentre Research Ethics Committee (MREC/01/2/91), and informed consent was obtained from all participants.

Author contributions

NS, TC, GC, and AC participated in the study conceptualisation. GC conducted the analysis and drafted the manuscript. TC drafted part of the introduction and literature review. NS, TC, AC, and TH contributed to the design of the work, data interpretation and critically revised the manuscript for important intellectual content. All authors have read and approved the final manuscript. GC has full access to the data and takes responsibility for the integrity of the data and accuracy of the data analyses.

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Supplementary material A

Table A1: Summary of studies on socioeconomic position and hair cortisol.

First Author	Year	Countries	Sample	Response Rate /Participation Rate	SEP measure	Association With hair cortisol
O'Brien	2012	USA	135 adults	NA	Lower Tertiles of SES derived from Income and Education	No association
Chen	2013	China	103 adults	NA	Lower Education	No association
Karlen	2013	Sweden	100 children aged 1, 3, 5, and 8 years	RR=74%	Lower Maternal Education	No association
Vaghri	2013	Canada	333 children aged 5	RR=30.3%	Lower Maternal Education	Higher hair cortisol levels
Vaghri	2013	Canada	275 children aged 5	RR=30.3%	Lower Paternal Income	No association
Henley	2014	Kenya	108 adult volunteers	RR=47%	Lower income	Higher hair cortisol levels
Pulopulos	2014	Spain	57 healthy older people aged 56 to 77 years old)	PR=37%	Lower Subjective Social Status	No association
Bosma	2015	Netherlands	33 children aged 10-12	RR=16.8%	Lower Maternal Education	No association
Boesch	2015	Switzerland	177 young healthy men	PR=26%	Lower Education	Higher hair cortisol levels
Braig	2015	Germany	768 pregnant mothers	RR=49%	Lower Education	No association
Karlen	2015	Sweden	209 children aged 8	PR=11%	Living in a Flat vs House	Higher hair cortisol levels
Staufenbiel	2015	Netherlands	1,141 adults (mean age 46)	PR=33%	Lower Education	No association
Abell	2016	UK	3,507 adults (aged 59-83)	PR=56%	Lower Employment Grade	No association
Gidlow	2016	UK	132 healthy employed adults	NA	Area Deprivation	Higher hair cortisol levels
Serwinski	2016	UK & Hungary	164 working w omen (aged 26-65)	NA	Lower income	Higher hair cortisol levels

Schreier	2016	USA	180 pregnant women	RR=80%	Lower Education	Higher hair cortisol levels
Ouellet-Morin	2021	Canada	556 adolescents	PR=48.3%	Household income Parental education Occupational class	U shaped function
Martins	2023	Brazil	3235 children and 3102 mothers	RR=80.7% and 77.4%, respectively	Lower maternal education	Higher hair cortisol levels

Note: NA=Not Available

Supplementary material B

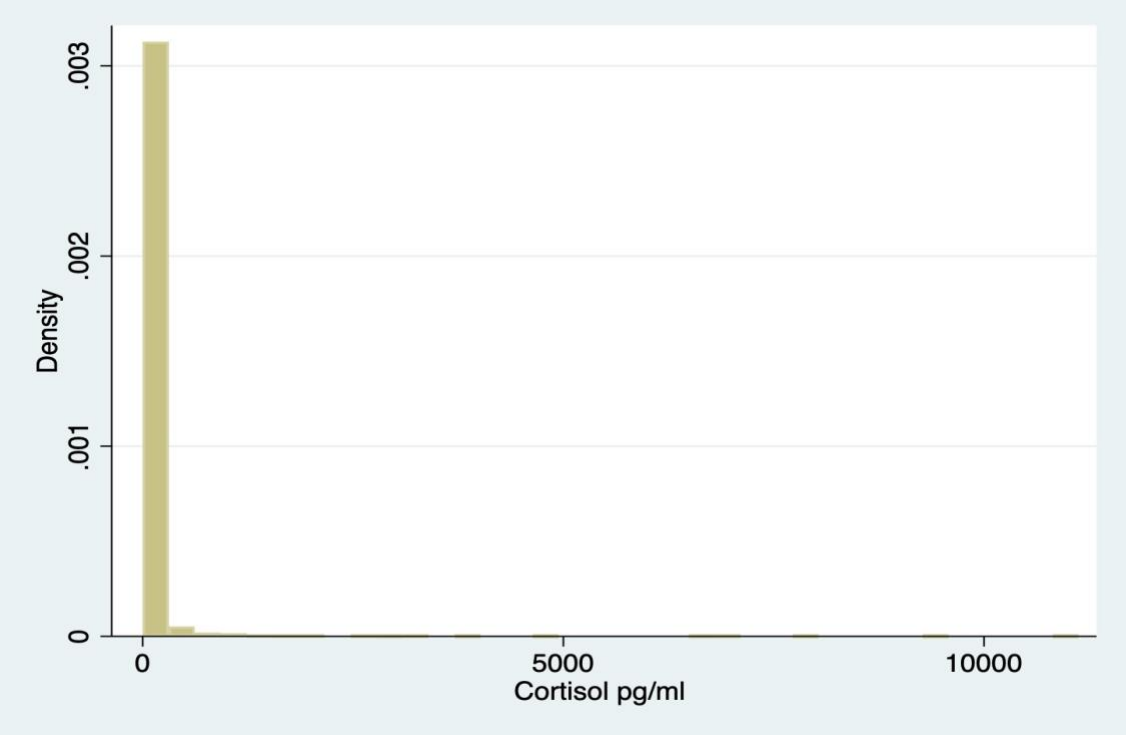

Figure B1: Cortisol distribution before log transformation (n=4,573)

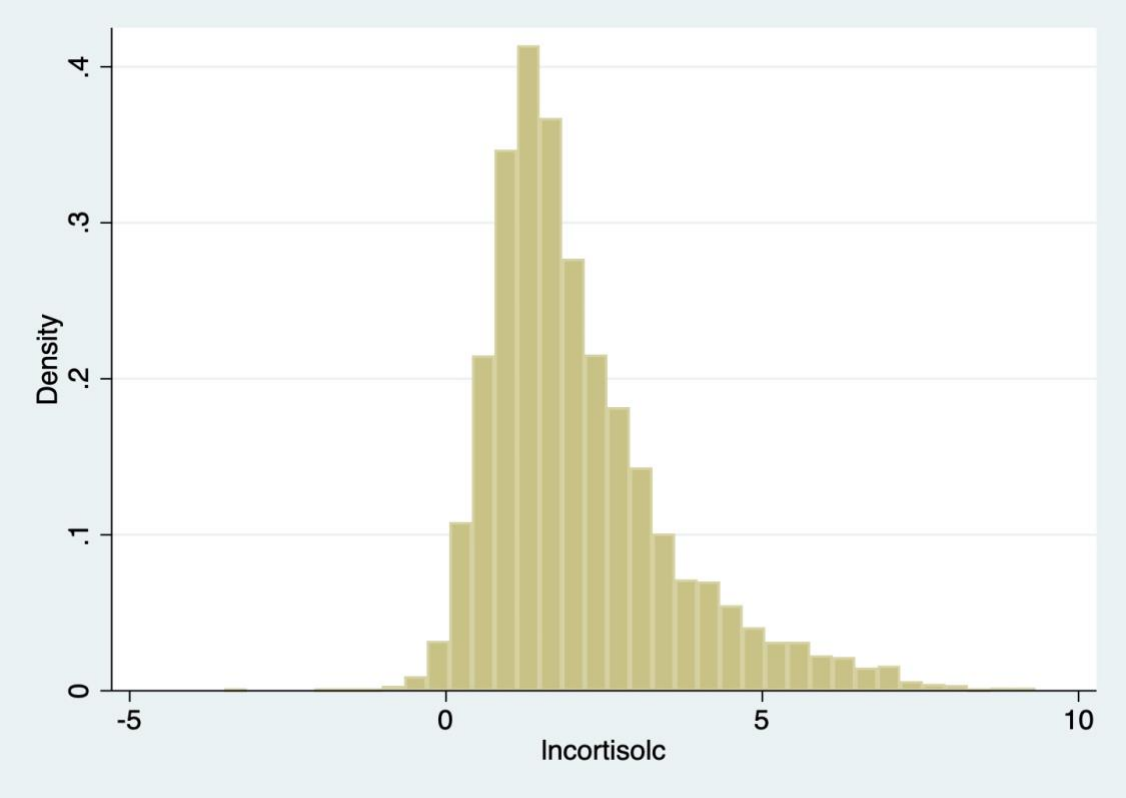

Figure B2: Cortisol distribution after log transformation (n=4,573)

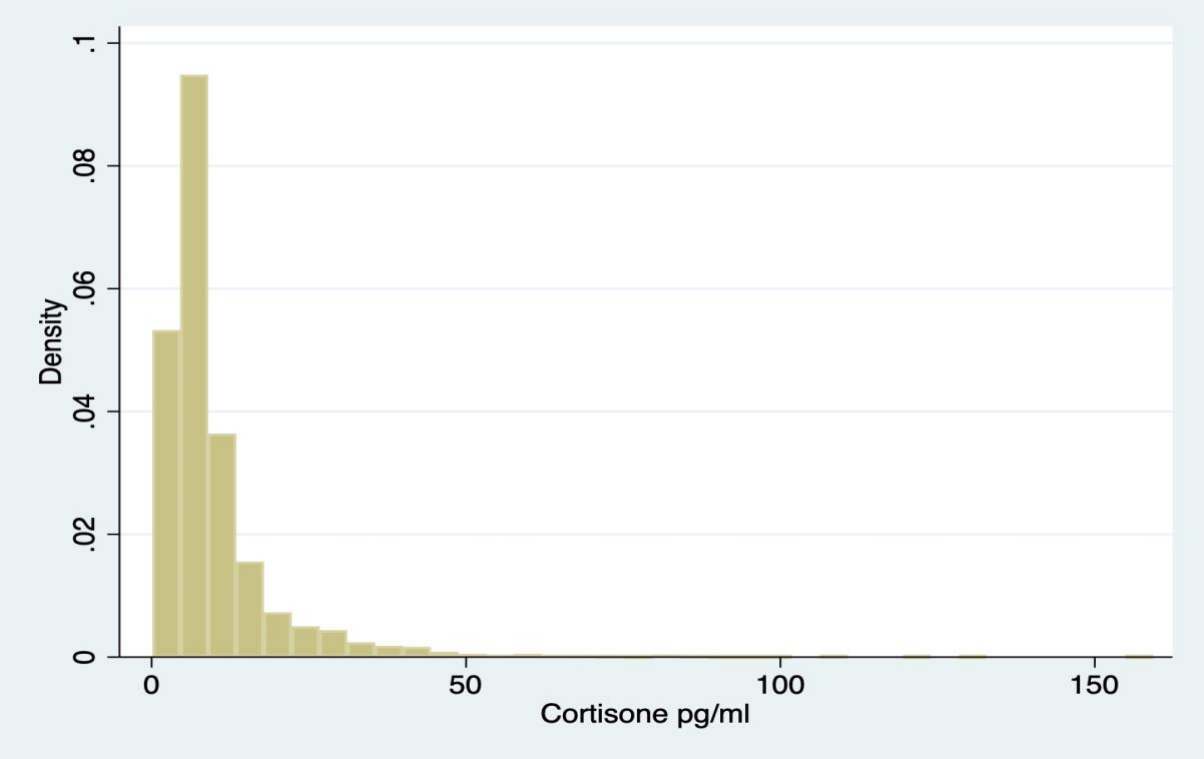

Figure B3: Cortisone distribution before log transformation (n=4,573)

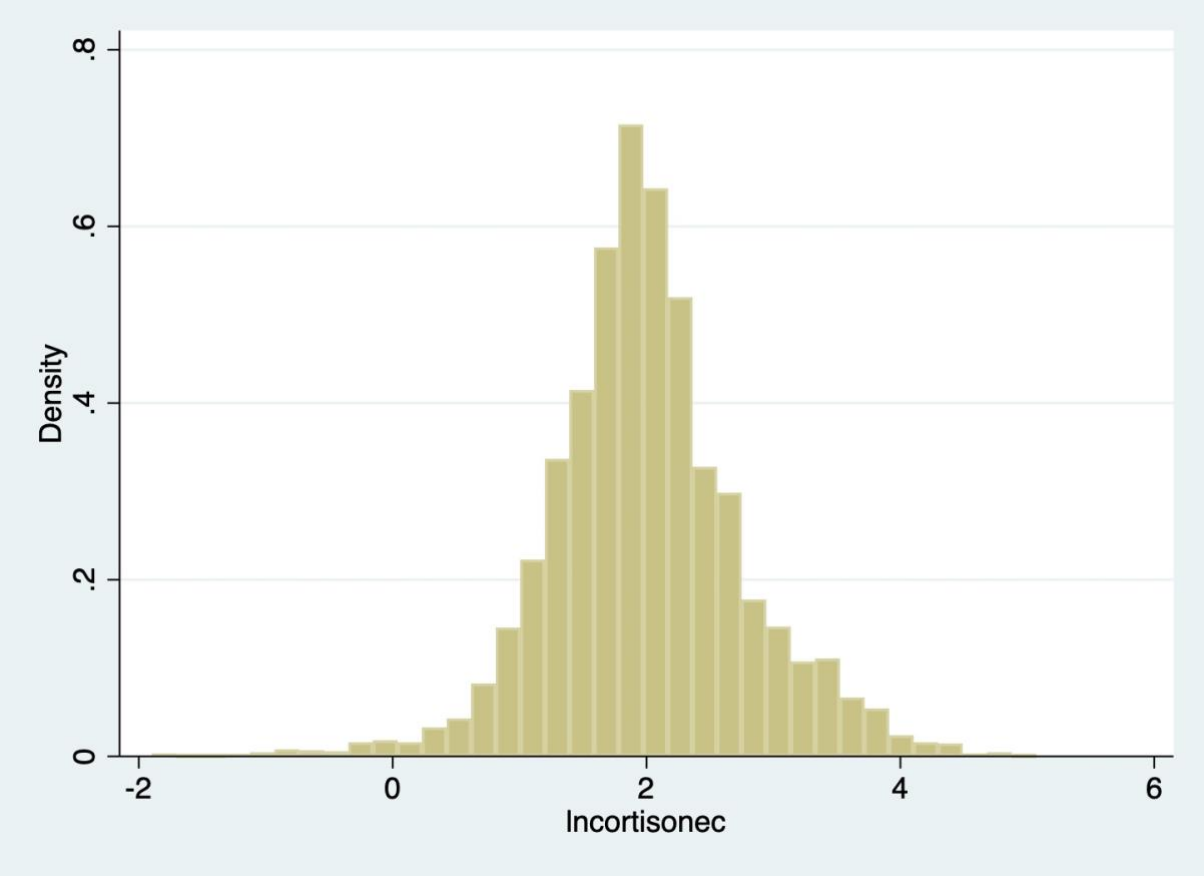

Figure B4: Cortisone distribution after log transformation (n=4,573)

Supplementary material C

Missing data analysis and missing data methods (additional material)

Out of 7,730 ELSA participants in the nurse data collection, there were 4,796 participants with hair cortisol and 5,077 with hair cortisone data. This is partly because some people were ineligible for the data collection (having less than 2 cm of hair). Others refused to give hair samples, mainly for reasons related to appearance. We generated a binary variable indicating whether or not the participant had hair cortisol/cortisone data and used a logistic regression model to identify variables predicting missingness. In addition to the variables used by the ELSA report to derive the nurse visit weights, we also explored other variables that could be associated with missingness. As baldness predominantly affects men, we may expect gender to be associated with having hair cortisol data. Furthermore, given the association of ageing with hair loss, we may expect younger participants to be more likely to have hair cortisol data. We also examined whether respondents with poorer health were less likely to have hair cortisol data and whether those who volunteered were more likely to have hair cortisol data.

There was an interaction between gender and age-group: men were less likely to have hair cortisol data compared to women, and men from all age groups were more likely to have hair cortisol/cortisone sample apart from the youngest age group (50-59). Volunteering was associated with having hair cortisol data, as was British/Irish ethnicity, non-winter nurse visit months, participants living in some south parts of England, those physically active, and those who never smoked.

Some methodological issues with multiple imputations

There is a rapid uptake of MI the last 15 years and it is highlighted that the vast majority of papers using MI do not include imputation diagnostics and very rarely run sensitivity checks for modelling decisions (Hayati Rezvan *et al.,* 2015). MI cannot differentiate which variables are outcomes and which are predictors, and therefore, it is considered very important to include all the variables from the model of interest (analysis model) in the imputation model (Austin et al., 2021). MI have the advantage compared to other imputation methods to account for uncertainty by allowing to calculate a between variance of the statistics from the imputed datasets and this variance is accounted for in the final analysis (Little and Rubin, 2002).

In our analyses, we included all the variables from the model of interest, variables that we identified to predict missingness for hair cortisol and cortisone data and auxiliary variables which were associated in the literature with either our exposures or outcomes from the model of interest (Galobardes *et al.*, 2006).

MI was implemented using the "mi impute chained" command in Stata/MP v.16.1 and we generated 100 imputations. There are no specific guidelines for the number of imputations that need to be generated but there is a rule of thumb that the number of imputations should be at least equal to the percentage of incomplete cases (White *et al.,* 2011; Nguyen *et al.,* 2017). In our case because we had large amounts of missing data (i.e., from 43.9% to 12,1%) in five variables with our outcomes included, we decided to opt in to 100 imputations. In term of diagnostics, some researchers choose to compare observed values with imputed values of the variables from the imputation model. We found that there are some minor differences in the distributions between observed and imputed values (results not shown) but it is not necessarily problematic since under Missing At Random (MAR) assumption we should expect some differences (Nguyen *et al.,* 2017). Furthermore, some simulation studies suggest that imputed values should not fall within plausible or possible ranges as MI are not implemented to replace missing values but to enable valid inferences (von Hippel, 2013; Rodwell *et al.*, 2014).

Supplementary material D

Table D1: Log cortisol regressed on education adjusted for covariates using three methods to compensate for missing data

	Complete Case Analysis (CCA)			Inverse Probability Weighting (IPW)			Multiple Imputation (MI)		
	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values
Variables									
Education level									
Higher education	ref			ref			ref		
High school	0.00	0.06	0.99	0.08	0.07	0.24	0.03	0.07	0.62
Foreign or no qualification	0.08	0.06	0.17	0.13	0.07	0.05	0.10	0.07	0.13
Age categories									
50-59	ref			ref			ref		
60-64	-0.34	0.12	0.01	-0.25	0.13	0.05	-0.14	0.12	0.25
65-69	0.10	0.12	0.41	0.16	0.15	0.28	0.10	0.12	0.44
70-74	0.12	0.13	0.35	0.10	0.14	0.48	0.12	0.13	0.34
75-79	0.27	0.14	0.05	0.37	0.15	0.02	0.22	0.13	0.10
80+	0.19	0.15	0.20	0.32	0.16	0.05	0.26	0.15	0.09
Gender									
Male	ref			ref			ref		
Female	-0.07	0.11	0.53	-0.13	0.12	0.27	-0.09	0.12	0.42
Age X Gender									
50-59 Male	ref			ref			ref		
60-64 Female	0.41	0.15	0.01	0.43	0.15	0.01	0.21	0.14	0.12
65-69 Female	-0.12	0.15	0.44	-0.10	0.17	0.55	-0.06	0.14	0.65
70-74 Female	-0.09	0.16	0.60	0.01	0.16	0.97	-0.03	0.15	0.82
75-79 Female	-0.24	0.17	0.14	-0.27	0.18	0.14	-0.14	0.16	0.36
80+ Female	-0.06	0.18	0.74	-0.06	0.20	0.75	-0.04	0.17	0.80
Ethnicity									
Whites	ref			ref			ref		
Ethnic minorities	-0.12	0.15	0.44	-0.41	0.19	0.03	-0.15	0.20	0.46
Marital Status									
Married/Cohabiting	ref			ref					
Single	0.06	0.10	0.57	0.17	0.14	0.23	0.11	0.13	0.37
Widowed	0.06	0.07	0.40	0.00	0.08	0.98	0.03	0.08	0.70
Divorced/Separated	0.09	0.07	0.20	0.13	0.10	0.22	0.08	0.09	0.41
Hair Treatment									
Yes	ref			ref			ref		
No	0.10	0.06	0.09	0.09	0.07	0.20	0.09	0.07	0.23
Hair colour									
Blonde/Ginger	ref			ref			ref		
Brunette/Black	0.15	0.07	0.04	0.22	0.08	0.01	0.19	0.08	0.02
Grey/White/Mixed Grey/Other	0.07	0.07	0.34	0.09	0.08	0.30	0.09	0.08	0.30
Season of hair collection									
Winter (Dec-Feb)	ref			ref			ref		
Spring (Mar-May)	0.07	0.09	0.49	0.06	0.11	0.58	0.03	0.11	0.80
Summer (Jun-Aug)	0.09	0.06	0.15	0.16	0.08	0.04	0.07	0.08	0.35
Autumn (Sep-Nov)	0.07	0.05	0.22	0.08	0.07	0.26	0.06	0.06	0.34
Phase of hair analysis									
1(2015)	ref			ref			ref		
2(2018)	-0.19	0.05	<0.01	-0.16	0.06	0.01	-0.18	0.05	<0.01
Intercept	1.96	0.13	<0.01	1.86	0.13	<0.01	1.93	0.14	<0.01

Notes: 1. Sample size for CCA and IPW is 4,573, for MI is 8,145

2. Cortisol and cortisone were log transformed.

3. Linear regression model with Coefficients, Standard Errors (SEs), and p-values

Table D2: Log cortisone regressed on education adjusted for covariates using three methods to compensate for missing data.

	Complete Case Analysis (CCA)			Inverse Probability Weighting (IPW)			Multiple Imputation (MI)		
	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values
Variables									
Education level									
Higher education	ref			ref			ref		
High school	0.01	0.03	0.64	0.03	0.04	0.41	0.02	0.03	0.47
Foreign or no qualification	0.07	0.03	0.02	0.08	0.03	0.02	0.09	0.03	0.01
Age categories									
50-59	ref			ref			ref		
60-64	-0.02	0.06	0.68	0.03	0.07	0.64	0.00	0.06	0.96
65-69	-0.03	0.06	0.65	0.02	0.07	0.73	-0.03	0.06	0.61
70-74	-0.02	0.06	0.74	0.03	0.07	0.71	-0.03	0.06	0.59
75-79	-0.15	0.07	0.03	-0.07	0.08	0.38	-0.10	0.06	0.10
80+	-0.23	0.07	<0.01	-0.18	0.07	0.01	-0.11	0.07	0.14
Gender									
Male	ref			ref			ref		
Female	-0.30	0.06	<0.01	-0.26	0.07	<0.01	-0.29	0.06	<0.01
Age X Gender									
50-59 Male	ref			ref			ref		
60-64 Female	0.00	0.07	0.99	-0.04	0.08	0.60	-0.02	0.07	0.73
65-69 Female	0.00	0.07	0.98	-0.06	0.09	0.48	-0.01	0.07	0.83
70-74 Female	-0.03	0.08	0.72	-0.10	0.09	0.26	-0.03	0.08	0.72
75-79 Female	0.11	0.08	0.19	0.03	0.09	0.77	0.03	0.07	0.66
80+ Female	0.31	0.09	<0.01	0.29	0.08	<0.01	0.14	0.08	0.08
Ethnicity									
Whites	ref			ref			ref		
Ethnic minorities	-0.17	0.07	0.02	-0.28	0.12	0.02	-0.13	0.10	0.20
Marital Status									
Married/Cohabiting	ref			ref			ref		
Single	0.08	0.05	0.09	0.16	0.07	0.04	0.13	0.06	0.03
Widowed	0.05	0.03	0.13	0.04	0.04	0.30	0.06	0.04	0.13
Divorced/Separated	0.11	0.04	<0.01	0.14	0.05	0.01	0.11	0.04	0.01
Hair Treatment									
Yes	ref			ref			ref		
No	0.24	0.03	<0.01	0.22	0.03	<0.01	0.22	0.04	<0.01
Hair colour									
Blonde/Ginger	ref			ref			ref		
Brunette/Black	0.24	0.03	<0.01	0.23	0.04	<0.01	0.23	0.04	<0.01
Grey/White/Mixed Grey/Other	0.15	0.04	<0.01	0.12	0.04	0.01	0.15	0.04	<0.01
Season of hair collection									
Winter (Dec-Feb)	ref			ref			ref		
Spring (Mar-May)	0.02	0.05	0.68	0.04	0.05	0.51	0.01	0.06	0.83
Summer (Jun-Aug)	0.01	0.03	0.67	0.07	0.04	0.09	0.02	0.04	0.59
Autumn (Sep-Nov)	0.03	0.03	0.21	0.06	0.03	0.05	0.04	0.03	0.25
Phase of hair analysis									
1(2015)	ref			ref			ref		
2(2018)	0.41	0.02	<0.01	0.40	0.03	<0.01	0.42	0.03	<0.01
Intercept	1.69	0.06	<0.01	1.65	0.07	<0.01	1.69	0.07	<0.01

Notes: 1. Sample size for CCA and IPW is 4,573, for MI is 8,145

2. Cortisol and cortisone were log transformed.

3. Linear regression model with Coefficients, Standard Errors (SEs), and p-values

Table D3: Log cortisol regressed on wealth adjusted for covariates using three methods to compensate for missing data									
	Complete Case Analysis (CCA)			Inverse Probability Weighting (IPW)			Multiple Imputation (MI)		
	Coef (b)	SEs	p-values	**Coef (b)**	SEs	p-values	**Coef (b)**	SEs	p-values
Variables									
Wealth tertiles									
Highest tertile	ref			ref			ref		
Middle tertile	0.00	0.05	1.00	-0.02	0.06	0.70	-0.01	0.06	0.80
Lowest tertile	0.22	0.06	<0.01	0.15	0.07	0.04	0.21	0.07	<0.01
Age categories									
50-59	ref			ref					
60-64	-0.33	0.12	0.01	-0.26	0.13	0.05	-0.12	0.12	0.34
65-69	0.12	0.12	0.34	0.17	0.15	0.25	0.13	0.12	0.31
70-74	0.15	0.13	0.27	0.12	0.14	0.39	0.16	0.13	0.22
75-79	0.28	0.14	0.04	0.39	0.15	0.01	0.25	0.13	0.06
80+	0.21	0.15	0.16	0.35	0.17	0.03	0.29	0.15	0.06
Gender									
Male	ref			ref			ref		
Female	-0.10	0.11	0.40	-0.14	0.12	0.24	-0.09	0.12	0.42
Age X Gender									
50-59 Male	ref			ref			ref		
60-64 Female	0.44	0.15	<0.01	0.45	0.15	<0.01	0.22	0.14	0.11
65-69 Female	-0.09	0.15	0.55	-0.08	0.17	0.66	-0.05	0.14	0.73
70-74 Female	-0.06	0.16	0.72	0.03	0.16	0.85	-0.02	0.15	0.87
75-79 Female	-0.21	0.16	0.20	-0.25	0.18	0.17	-0.13	0.16	0.43
80+ Female	-0.04	0.18	0.81	-0.04	0.20	0.82	-0.04	0.17	0.83
Ethnicity									
Whites	ref			ref			ref		
Ethnic minorities	-0.12	0.15	0.44	-0.42	0.19	0.03	-0.16	0.20	0.42
Marital Status									
Married/Cohabiting	ref			ref			ref		
Single	0.01	0.10	0.96	0.13	0.14	0.36	0.05	0.13	0.68
Widowed	0.02	0.07	0.82	-0.03	0.08	0.74	-0.01	0.08	0.90
Divorced/Separated	0.02	0.07	0.83	0.09	0.10	0.41	0.00	0.09	0.96
Hair Treatment									
Yes	ref			ref			ref		
No	0.10	0.06	0.11	0.08	0.07	0.22	0.08	0.07	0.26
Hair colour									
Blonde/Ginger	ref			ref			ref		
Brunette/Black	0.14	0.07	0.04	0.22	0.08	0.01	0.19	0.08	0.02
Grey/White/Mixed Grey/Other	0.07	0.07	0.33	0.08	0.08	0.33	0.09	0.08	0.30
Season of hair collection									
Winter (Dec-Feb)	ref			ref			ref		
Spring (Mar-May)	0.07	0.09	0.47	0.07	0.11	0.53	0.03	0.11	0.76
Summer (Jun-Aug)	0.08	0.06	0.19	0.16	0.08	0.05	0.07	0.08	0.38
Autumn (Sep-Nov)	0.06	0.05	0.27	0.07	0.07	0.30	0.05	0.06	0.39
Phase of hair analysis									
1(2015)	ref			ref			ref		
2(2018)	-0.20	0.05	<0.01	-0.17	0.06	0.01	-0.19	0.05	<0.01
Intercept	1.95	0.12	<0.01	1.90	0.14	<0.01	1.92	0.14	<0.01

Notes: 1. Sample size for CCA and IPW is 4,573, for MI is 8,145
2. Cortisol and cortisone were log transformed.
3. Linear regression model with Coefficients, Standard Errors (SEs), and p-values

Table D4: Log cortisone regressed on wealth adjusted for covariates using three methods to compensate for missing data									
	Complete Case Analysis (CCA)			Inverse Probability Weighting (IPW)			Multiple Imputation (MI)		
	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values
Variables									
Wealth tertiles									
Highest tertile	ref			ref			ref		
Middle tertile	0.03	0.03	0.18	0.04	0.03	0.27	0.03	0.03	0.25
Lowest tertile	0.14	0.03	<0.01	0.13	0.04	<0.01	0.15	0.03	<0.01
Age categories									
50-59	ref			ref			ref		
60-64	-0.02	0.06	0.70	0.03	0.07	0.65	0.01	0.06	0.81
65-69	-0.02	0.06	0.74	0.03	0.07	0.65	-0.01	0.06	0.87
70-74	-0.01	0.06	0.86	0.04	0.07	0.58	-0.01	0.06	0.87
75-79	-0.15	0.07	0.03	-0.06	0.08	0.45	-0.09	0.06	0.17
80+	-0.22	0.07	<0.01	-0.17	0.07	0.02	-0.08	0.07	0.25
Gender									
Male	ref			ref			ref		
Female	-0.32	0.06	<0.01	-0.27	0.07	<0.01	-0.29	0.06	<0.01
Age X Gender									
50-59 Male	ref			ref			ref		
60-64 Female	0.02	0.07	0.81	-0.02	0.08	0.78	-0.02	0.07	0.79
65-69 Female	0.02	0.07	0.83	-0.04	0.08	0.64	0.00	0.07	0.95
70-74 Female	-0.01	0.08	0.92	-0.08	0.09	0.38	-0.02	0.08	0.79
75-79 Female	0.13	0.08	0.11	0.05	0.09	0.61	0.05	0.07	0.53
80+ Female	0.33	0.09	<0.01	0.30	0.08	<0.01	0.15	0.08	0.06
Ethnicity									
Whites	ref			ref			ref		
Ethnic minorities	-0.17	0.07	0.02	-0.28	0.12	0.02	-0.13	0.10	0.18
Marital Status									
Married/Cohabiting	ref			ref			ref		
Single	0.05	0.05	0.31	0.12	0.08	0.10	0.09	0.06	0.12
Widowed	0.03	0.03	0.44	0.02	0.04	0.63	0.03	0.04	0.40
Divorced/Separated	0.07	0.04	0.07	0.11	0.05	0.04	0.07	0.04	0.11
Hair Treatment									
Yes	ref			ref			ref		
No	0.23	0.03	<0.01	0.22	0.03	<0.01	0.21	0.04	<0.01
Hair colour									
Blonde/Ginger	ref			ref			ref		
Brunette/Black	0.23	0.03	<0.01	0.23	0.04	<0.01	0.23	0.04	<0.01
Grey/White/Mixed Grey/Other	0.15	0.04	<0.01	0.12	0.04	0.01	0.15	0.04	<0.01
Season of hair collection									
Winter (Dec-Feb)	ref			ref			ref		
Spring (Mar-May)	0.02	0.05	0.65	0.04	0.05	0.45	0.02	0.06	0.77
Summer (Jun-Aug)	0.01	0.03	0.75	0.06	0.04	0.10	0.02	0.04	0.63
Autumn (Sep-Nov)	0.03	0.03	0.27	0.06	0.03	0.06	0.03	0.03	0.30
Phase of hair analysis									
1(2015)	ref			ref			ref		
2(2018)	0.41	0.02	<0.01	0.40	0.03	<0.01	0.41	0.03	<0.01
Intercept	1.67	0.06	<0.01	1.64	0.07	<0.01	1.67	0.06	<0.01

Notes: 1. Sample size for CCA and IPW is 4,573, for MI is 8,145

2. Cortisol and cortisone were log transformed.

3. Linear regression model with Coefficients, Standard Errors (SEs), and p-values

Table D5: Log cortisol regressed on social class adjusted for covariates using three methods to compensate for missing data									
	Complete Case Analysis (CCA)			Inverse Probability Weighting (IPW)			Multiple Imputation (MI)		
	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values
Variables									
Social class									
Managerial & Professional	ref			ref			ref		
Intermediate	-0.02	0.06	0.77	0.04	0.07	0.60	-0.01	0.07	0.93
Routine & Manual Occupations	0.13	0.05	0.02	0.17	0.06	0.01	0.15	0.06	0.01
Age categories									
50-59	ref			ref			ref		
60-64	-0.34	0.12	0.01	-0.26	0.13	0.04	-0.14	0.12	0.26
65-69	0.10	0.12	0.43	0.15	0.15	0.32	0.10	0.12	0.43
70-74	0.12	0.13	0.38	0.08	0.14	0.56	0.12	0.13	0.35
75-79	0.27	0.14	0.05	0.37	0.15	0.02	0.22	0.13	0.09
80+	0.20	0.15	0.17	0.34	0.16	0.04	0.27	0.15	0.07
Gender									
Male	ref			ref			ref		
Female	-0.07	0.11	0.51	-0.13	0.12	0.28	-0.09	0.12	0.45
Age X Gender									
50-59 Male	ref			ref			ref		
60-64 Female	0.41	0.15	0.01	0.43	0.15	0.01	0.21	0.14	0.12
65-69 Female	-0.11	0.15	0.47	-0.09	0.17	0.58	-0.06	0.14	0.66
70-74 Female	-0.07	0.16	0.67	0.02	0.16	0.88	-0.03	0.15	0.87
75-79 Female	-0.23	0.17	0.17	-0.26	0.18	0.14	-0.13	0.16	0.41
80+ Female	-0.05	0.18	0.78	-0.06	0.20	0.75	-0.04	0.17	0.83
Ethnicity									
Whites	ref			ref			ref		
Ethnic minorities	-0.11	0.15	0.45	-0.41	0.20	0.04	-0.15	0.20	0.45
Marital Status									
Married/Cohabiting	ref			ref			ref		
Single	0.06	0.10	0.58	0.16	0.14	0.28	0.11	0.13	0.40
Widowed	0.05	0.07	0.47	-0.01	0.08	0.94	0.02	0.08	0.79
Divorced/Separated	0.08	0.07	0.24	0.12	0.11	0.27	0.07	0.09	0.46
Hair Treatment									
Yes	ref			ref			ref		
No	0.10	0.06	0.09	0.09	0.07	0.17	0.09	0.07	0.21
Hair colour									
Blonde/Ginger	ref			ref			ref		
Brunette/Black	0.15	0.07	0.04	0.22	0.08	0.01	0.19	0.08	0.02
Grey/White/MixedGrey/Other	0.07	0.07	0.35	0.08	0.08	0.30	0.09	0.08	0.30
Season of hair collection									
Winter (Dec-Feb)	ref			ref			ref		
Spring (Mar-May)	0.07	0.09	0.48	0.06	0.11	0.57	0.03	0.11	0.80
Summer (Jun-Aug)	0.09	0.06	0.15	0.17	0.08	0.04	0.07	0.08	0.37
Autumn (Sep-Nov)	0.06	0.05	0.24	0.08	0.07	0.28	0.05	0.06	0.39
Phase of hair analysis									
1(2015)	ref			ref			ref		
2(2018)	-0.20	0.05	<0.01	-0.17	0.06	0.01	-0.19	0.05	<0.01
Intercept	1.95	0.12	<0.01	1.85	0.13	<0.01	1.92	0.14	<0.01

Notes: 1. Sample size for CCA and IPW is 4,573, for MI is 8,145
2. Cortisol and cortisone were log transformed.
3. Linear regression model with Coefficients, Standard Errors (SEs), and p-values

Table D6: Log cortisone regressed on social class adjusted for covariates using three methods to compensate for missing data									
	Complete Case Analysis (CCA)			Inverse Probability Weighting (IPW)			Multiple Imputation (MI)		
	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values	Coef (b)	SEs	p-values
Variables									
Social class									
Managerial & Professional	ref			ref			ref		
Intermediate	0.00	0.03	0.90	0.00	0.04	1.00	0.02	0.03	0.52
Routine & Manual Occupations	0.10	0.03	<0.01	0.10	0.03	<0.01	0.13	0.03	<0.01
Age categories									
50-59	ref			ref			ref		
60-64	-0.03	0.06	0.67	0.03	0.07	0.66	0.00	0.06	0.98
65-69	-0.03	0.06	0.59	0.02	0.07	0.79	-0.03	0.06	0.61
70-74	-0.03	0.06	0.68	0.02	0.07	0.78	-0.03	0.06	0.57
75-79	-0.15	0.07	0.02	-0.06	0.08	0.40	-0.11	0.06	0.09
80+	-0.22	0.07	<0.01	-0.17	0.07	0.02	-0.09	0.07	0.19
Gender									
Male	ref			ref			ref		
Female	-0.30	0.06	<0.01	-0.26	0.07	<0.01	-0.28	0.06	<0.01
Age X Gender									
50-59 Male	ref			ref			ref		
60-64 Female	0.00	0.07	0.97	-0.04	0.08	0.65	-0.02	0.07	0.72
65-69 Female	0.00	0.07	0.96	-0.05	0.09	0.54	-0.01	0.07	0.84
70-74 Female	-0.02	0.08	0.84	-0.08	0.09	0.34	-0.02	0.08	0.79
75-79 Female	0.12	0.08	0.14	0.04	0.09	0.69	0.04	0.07	0.56
80+ Female	0.32	0.09	<0.01	0.29	0.08	<0.01	0.15	0.08	0.07
Ethnicity									
Whites	ref			ref			ref		
Ethnic minorities	-0.17	0.07	0.02	-0.28	0.12	0.02	-0.13	0.10	0.20
Marital Status									
Married/Cohabiting	ref			ref			ref		
Single	0.08	0.05	0.09	0.15	0.08	0.05	0.12	0.06	0.04
Widowed	0.04	0.03	0.17	0.04	0.04	0.35	0.05	0.04	0.19
Divorced/Separated	0.11	0.04	<0.01	0.14	0.05	0.01	0.11	0.04	0.01
Hair Treatment									
Yes	ref			ref			ref		
No	0.24	0.03	<0.01	0.22	0.03	<0.01	0.22	0.04	<0.01
Hair colour									
Blonde/Ginger	ref			ref			ref		
Brunette/Black	0.23	0.03	<0.01	0.23	0.04	<0.01	0.23	0.04	<0.01
Grey/White/Mixed Grey/Other	0.15	0.04	<0.01	0.12	0.04	0.01	0.15	0.04	<0.01
Season of hair collection									
Winter (Dec-Feb)	ref			ref			ref		
Spring (Mar-May)	0.02	0.05	0.65	0.04	0.05	0.51	0.01	0.06	0.82
Summer (Jun-Aug)	0.01	0.03	0.66	0.07	0.04	0.08	0.02	0.04	0.63
Autumn (Sep-Nov)	0.03	0.03	0.25	0.06	0.03	0.06	0.03	0.03	0.31
Phase of hair analysis									
1(2015)	ref			ref			ref		
2(2018)	0.41	0.02	<0.01	0.40	0.03	<0.01	0.41	0.03	<0.01
Intercept	1.68	0.06	<0.01	1.65	0.07	<0.01	1.67	0.06	<0.01

Notes: 1. Sample size for CCA and IPW is 4,573, for MI is 8,145
2. Cortisol and cortisone were log transformed.
3. Linear regression model with Coefficients, Standard Errors (SEs), and p-values

Table D7. Predicted levels of log cortisol and log cortisone by education, wealth tertiles, and social class in three statistical methods produced from models in Tables D1-D6.

Education	**Complete Case Analysis (CCA)**				**Inverse Probability Weighting (IPW)**				**Multiple Imputation (MI)**			
Cortisol	Pr(logcortisol)	SEs	95% Confidence Intervals		Pr(logcortisol)	SEs	95% Confidence Intervals		Pr(logcortisol)	SEs	95% Confidence Intervals	
Higher education	2.08	0.04	2.00	2.16	2.03	0.04	1.95	2.12	2.07	0.05	1.97	2.17
High school	2.08	0.04	2.01	2.16	2.11	0.05	2.01	2.21	2.10	0.04	2.02	2.19
Foreign or no qualification	2.16	0.04	2.09	2.23	2.16	0.05	2.07	2.26	2.17	0.05	2.08	2.27
Cortisone	Pr(logcortisone)	SEs	95% Confidence Intervals		Pr(logcortisone)	SEs	95% Confidence Intervals		Pr(logcortisone)	SEs	95% Confidence Intervals	
Higher education	1.96	0.02	1.93	2.00	2.06	0.03	2.01	2.11	2.08	0.02	2.03	2.13
High school	1.98	0.02	1.94	2.01	2.09	0.02	2.04	2.13	2.10	0.02	2.06	2.15
Foreign or no qualification	2.03	0.02	1.99	2.06	2.14	0.02	2.10	2.18	2.16	0.02	2.12	2.21
Wealth Tertlies	**CCA**				**IPW**				**MI**			
Cortisol	Pr(logcortisol)	SEs	95% Confidence Intervals		Pr(logcortisol)	SEs	95% Confidence Intervals		Pr(logcortisol)	SEs	95% Confidence Intervals	
Highest tertile	2.04	0.04	1.97	2.11	2.06	0.05	1.97	2.16	2.04	0.05	1.95	2.14
Middle tertile	2.04	0.04	1.97	2.11	2.04	0.04	1.95	2.12	2.03	0.04	1.94	2.11
Lowest tertile	2.26	0.04	2.18	2.34	2.21	0.05	2.11	2.31	2.25	0.05	2.16	2.34
Cortisone	Pr(logcortisone)	SEs	95% Confidence Intervals		Pr(logcortisone)	SEs	95% Confidence Intervals		Pr(logcortisone)	SEs	95% Confidence Intervals	
Highest tertile	1.94	0.02	1.90	1.97	2.04	0.03	1.99	2.09	2.05	0.02	2.01	2.10
Middle tertile	1.97	0.02	1.94	2.01	2.08	0.02	2.03	2.12	2.09	0.02	2.04	2.13
Lowest tertile	2.08	0.02	2.04	2.11	2.17	0.02	2.12	2.21	2.20	0.02	2.15	2.24
Social Class	**CCA**				**IPW**				**MI**			
Cortisol	Pr(logcortisol)	SEs	95% Confidence Intervals		Pr(logcortisol)	SEs	95% Confidence Intervals		Pr(logcortisol)	SEs	95% Confidence Intervals	
Managerial & Professional	2.07	0.04	1.99	2.14	2.03	0.04	1.95	2.11	2.06	0.05	1.97	2.15
Intermediate	2.05	0.04	1.97	2.13	2.07	0.06	1.95	2.18	2.05	0.05	1.95	2.15
Routine & Manual Occupations	2.19	0.04	2.12	2.26	2.20	0.05	2.11	2.29	2.20	0.04	2.12	2.29
Cortisone	Pr(logcortisone)	SEs	95% Confidence Intervals		Pr(logcortisone)	SEs	95% Confidence Intervals		Pr(logcortisone)	SEs	95% Confidence Intervals	
Managerial & Professional	1.96	0.02	1.92	1.99	2.06	0.02	2.01	2.10	2.06	0.02	2.02	2.10
Intermediate	1.96	0.02	1.92	2.00	2.06	0.03	2.00	2.11	2.08	0.03	2.03	2.13
Routine & Manual Occupations	2.05	0.02	2.02	2.08	2.15	0.02	2.11	2.20	2.18	0.02	2.14	2.22