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Stability and predictors of change in salivary cortisol measures over six years: MESA

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Summary

A major challenge in characterizing features of the daily cortisol curve is variability in features over time. Few studies have examined the stability of daily features of the cortisol curve over long periods or the predictors of long term changes. Repeated salivary cortisol measures on 580 adults from the MESA Stress study were used to examine the stability of various features of the daily cortisol curve (wakeup value, the cortisol awakening response (CAR), the early and late decline slope and the area under the curve (AUC)), over short periods (several days) and long periods (approximately 6-years) and to investigate the association of demographic factors with the changes. Intraclass correlation coefficients (ICCs) were used to estimate the short and long term stability. Piecewise linear mixed models were used to assess factors associated with changes in features over time. For most features, short term stability (ICCs: 0.17–0.74) was higher than long term stability (ICCs: 0.05–0.42), and long term stability was highest when several days were

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Conflict of interest
None declared.

Uncited references
Carvalhaes-Neto et al., 2003 and Van Cauter et al., 1996.

Appendix A. Supplementary data
Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2014.07.024.

averaged for each time point. The decline over the day showed the highest long term stability: when several days for each wave were averaged the stability of the daily decline slope across 6 years was similar (or higher) than the stability across short periods. AUC had high stability over short periods (ICCs: 0.65–0.74) but much lower stability across long periods (ICC: 0.05). All features of daily cortisol curve investigated changed significantly over the approximately 6 year follow-up period. The wakeup cortisol became higher; the CAR became smaller; both the early and late decline became flatter; and the AUC became larger. Hispanics experienced significantly larger increases in the wakeup value; and African-Americans and Hispanics showed less flattening over time of the early decline slope than Non-Hispanic Whites. Our findings have implications for characterization of features in studies linking cortisol to health outcomes. The presence of variability over time suggests opportunities for future investigation of the predictors of changes over time as well as the links between these changes and health outcomes.

Keywords

Salivary cortisol; Stress; Longitudinal study; Stability; Demographic; Multilevel model; Intraclass correlation coefficient

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Cortisol is a hormone produced by the hypothalamic-pituitary-adrenal (HPA) axis, a stress-responsive biological system that is responsible for mobilizing the body's resources when an individual encounters psychological or physical stressors (Sapolsky et al., 2000). The assessment of salivary cortisol has become increasingly popular in large population studies (e.g., Adam and Kumari, 2009; Steptoe et al., 2003) as a way to characterize the functioning of the HPA axis and study how the functioning of this axis may be related to both stressors and health outcomes. The measurement of cortisol in saliva is useful because of its simplicity and non-invasive nature (Aardal and Holm, 1995).

The secretion of cortisol follows a diurnal cycle with a sharp increase during the 30–40 min after awakening (known as the cortisol awakening response (CAR)) (Pruessner et al., 1997), followed by a gradual decline over the remainder of the day (Kirschbaum and Hellhammer, 1989). Features of the cortisol daily curve, including wake-up levels, the CAR and the diurnal cortisol slope, have been associated with socio-demographic factors, psychosocial factors, and measures of physical and mental health (Steptoe et al., 2003; Kunz-Ebrecht et al., 2004; Bennett et al., 2004; Steptoe et al., 2005; Wright and Steptoe, 2005; Cohen et al., 2006; Eller et al., 2006; DeSantis et al., 2007; Garcia et al., 2008; Champaneri et al., 2012, 2013). However, findings have not always been consistent and there is still substantial uncertainty regarding what features of the cortisol daily curve are most affected by social or psychosocial antecedents and most predictive of future health outcomes.

A major challenge in characterizing features of the daily cortisol curve pertains to variability in the measures over time. Many studies collect samples over a single day (Cohen et al., 2006; Eller et al., 2006) or sometimes across a small number of days (Kunz-Ebrecht et al., 2004; DeSantis et al., 2007). Yet studies investigating predictors or consequences of the daily cortisol profile implicitly assume stability over relatively long periods. Few studies have had the data to examine the stability of daily features of the cortisol curve over time or the predictors of long term change in various features of the daily cortisol profile. Ross et al.

(2014) recently analyzed the stability of cortisol features (including CAR, diurnal slope and total daily output) over periods spanning 8–24 months using data from 177 children and adolescents and 47 middle-aged adults and found generally low stability over long periods and proportionately large short-term variability. However, to the best of our knowledge there is no comparable data on older samples over longer periods, or on predictors of changes in cortisol features over time.

We used unique longitudinal data from a diverse population-based study of adults aged 48–87 participating in the Multi-Ethnic Study of Atherosclerosis (MESA) to investigate: (1) the stability of various daily cortisol curve features over six years and (2) the extent to which social and demographic factors including age, sex, race/ethnicity and income/wealth are associated with features of the daily cortisol curve over time.

1. Methods

1.1. Data

The Multi-Ethnic Study of Atherosclerosis (MESA) is a longitudinal study, designed to investigate risk factors for subclinical cardiovascular diseases and its progression to clinical disease. Ancillary study to MESA, the MESA Stress Studies (I and II) collected detailed measures of stress hormones, including salivary cortisol samples, at two time periods approximately 6 years apart. All procedures were carried out with the adequate understanding and written consent of the subjects.

The MESA Stress I Study was conducted in New York and Los Angeles between 2004 and 2006 during MESA Exams 3 and 4. The study collected saliva samples and measured salivary cortisol on 1002 participants over 3 days with 6 time points measured per day. The first sample was to be taken immediately after waking (and before getting out of bed), the second sample 30 min later, the third sample at around 1000 h the fourth sample at around 1200 h (or before lunch if lunch occurred before noon), the fifth sample at around 1800 h (or before dinner if dinner occurred before 1800 h), and the sixth sample right before bed.

The MESA Stress II Study was conducted in New York, Los Angeles and Baltimore between 2010 and 2012 during MESA Exam 5. The study collected salivary samples and measured salivary cortisol over 2 days with 8 time points measured per day on 1082 participants, 56.4% (N = 610) of which were participants previously enrolled in MESA Stress I. Samples were taken immediately after waking (and before getting out of bed), 30 min after wakeup, 1 h after breakfast, around 1000 h, at noon, around 1600 h, around 1800 h before dinner and right before bed.

Participants were instructed not to eat or drink or brush their teeth 15 min before collecting the salivary samples. They were also instructed to leave the cotton swab in their mouths for less than 2 min until soaked, moving it around inside their mouth. Participants were instructed to record the exact time of sample collection on a special card. In MESA Stress I, a time tracking device was used to automatically register the time at which cotton swabs were extracted to collect each sample. Due to budget constraints this device was not used in

MESA Stress II, but participants were provided with an alarm clock to facilitate timely data collection.

Because of the focus on long term change analyses, only the 1002 enrolled in MESA Stress I Study (i.e., baseline) are eligible for this analysis. The analyses were then restricted to the 610 participants who participated in both MESA Stress waves. Invalid daily cortisol samples including samples with missing cortisol value or unreliable cortisol values (0 or >100 nmol/L) or missing time of sample collection were excluded, which lead to a further exclusion of $N = 30$ participants who had no valid samples on any exam day for at least one wave. The final analyses therefore included 580 participants who provided 2888 days of cortisol data (and a total of 18,597 valid cortisol samples) over the two waves. Compared to the 580 individuals who were included, the 422 excluded individuals were older (mean age: 63.7 versus 67.5 for included versus excluded; $p = 0.016$) and had a lower annual family income (less than $25,000: 36.3% versus 44.9%, $25,000 to $50,000: 32.5% versus 33.3% and more than $50,000: 31.1% versus 21.9% for included versus excluded; $p = 0.002$). There were no statistically significance differences in sex or race/ethnicity.

1.2. Measurement of salivary cortisol

Saliva samples were collected using Salivette collection tubes and stored at −20 °C until analysis. Before biochemical analysis, samples were thawed and centrifuged at 3000 rpm for 3 min to obtain clear saliva with low viscosity. Salivary cortisol levels were determined employing a commercially available chemi-luminescence assay (CLIA) with high sensitivity of 0.16 ng/mL (IBL-Hamburg, Germany). Intra- and inter-assay coefficients of variation were below eight percent. Cortisol was measured in nmol per liter.

1.3. Cortisol features

We investigated five features of the daily cortisol curve: wake up cortisol levels, CAR, standardized total area under the curve (AUC), early decline slope, and late decline slope. Due to its skewed distribution, cortisol was log-transformed before the cortisol features were calculated (Adam et al., 2006; Hajat et al., 2010; Champaneri et al., 2012). The CAR was calculated as the difference between the wake up cortisol levels and the levels at 30 min post-awakening (CAR was missing if the 1st or the 2nd sample was missing or the 2nd sample was collected later than 1 h post-awakening). The early decline slope (between 30 min and 2 h post-awakening) and late decline slope (between 2 h post-awakening and bedtime) were calculated as the average hourly rate of decline for the given time period (the early decline slope was missing if less than 2 samples were collected during 0.4–2.5 h post-awakening; the late decline slope was missing if less than 2 samples were collected after 2 h post-awakening). To calculate the AUC, we used linear splines to connect the values from each of the sample times and then calculated the area under the linear spline based on the trapezoid rule (Yeh and Kwan, 1978), using all available data and restricting estimates to a 16-h day duration for all participants (AUC was missing if less than 3 samples were collected for the exam day or the last 2 samples were missing). The area under the curve was then standardized by the length of duration (which is 16 h in our analysis). Each of the features was computed on a daily basis for each individual per wave. Of the 580 participants, 99% ($N = 573$), 92% ($N = 535$), 97% ($N = 561$), 99% ($N = 576$) and 97% ($N =$

562) had measures of wake up cortisol, CAR, early decline slope, late decline slope and AUC measures, respectively, from at least 1 day in each study wave (i.e., at least 1 out of 3 days for MESA Stress I and at least 1 out of 2 days for MESA Stress II).

1.4. Covariates

Demographic and socioeconomic factors previously shown to be associated with features of the cortisol curve in MESA and other studies (Clow et al., 2004; Ranjit et al., 2005; Cohen et al., 2006; Hansen et al., 2008; Hajat et al., 2010) were investigated as covariates. Covariates included age at baseline, sex, race/ethnicity (categorized as African-American, Hispanic, and non-Hispanic White), and income-wealth status (assessed at MESA Stress I and assumed time invariant). Participants were asked to report on both their income and their wealth at MESA Exam 3 prior to the Stress I study. The measures of wealth reflected a count of the number of specific assets that participants owned among the following: one or more cars, a home or paying mortgage on a home, land, or an investment (e.g., stocks, bonds, mutual funds, or retirement investments). The wealth information was combined into a wealth index ranging from 0 to 4 which participants received one point for ownership of each of the assets. Total family income was reported as one of the 13 income categories ranging from less than $5000 to greater than $100,000. The wealth index and the total family income were combined into an income-wealth index with a total of nine points ranging from 0 to 8 (Hajat et al., 2010). Those with an annual per capita family income in the lowest quin-tile and no assets received a score of zero and those with income in the highest quantile and all four assets received a score of eight. This scored variable was specified as continuous in regression models. Since wake up time has been associated with features of the curve (Karlamangla et al., 2013), in sensitivity analyses we further adjusted for wake up time.

1.5. Statistical analyses

1.5.1. Aim 1: stability of cortisol features—We calculated the intra-class correlation coefficient (ICC) to assess the stability of cortisol features across the two waves of MESA Stress studies using a three-level multilevel model as follows:

$$\text{Level}\quad 1{:}y_{ijd}=\alpha_{ij}+\varepsilon_{ijd'}$$

where y_{ijd} is value of a cortisol feature of subject i at MESA Stress study j (j = 1, 2) on day d (3 days for MESA Stress I and 2 days for MESA Stress II); α_{ij} is the mean level of the cortisol feature for the subject i at study j; $\in_{ijd}$ is a normally distributed day-level deviation from the mean α_{ij} and has mean zero and variance τ^2 representing the variability of the cortisol feature between days conditional on individual i and study j.

In the level 2 model (study within individual), the mean level of cortisol features for subject i at study j is modeled as a function of an individual-level mean (β_{i0}) and a study-specific deviation ($\gamma_{j(i)}$:

$$\text{Level}\quad 2{:}\alpha_{ij}=\beta_{i0}+\gamma_{j(i)}$$

where $\gamma_{j(i)}$ is study level random effect for subject *i* at study *j* and is assumed to be normally distributed, conditional on individual *i*, with mean zero and variance δ^2 which represents the variability of the cortisol feature between studies.

In the level 3 model (individual level), the individual-level mean is modeled as a function of an overall mean (β_0) and an individual-specific deviation ($_i$):

$$\text{Level} \quad 3: \beta_{i0} = \beta_0 + \eta_i$$

where η_i is assumed to be normally distributed with mean zero and variance σ^2, representing between-subject variability of the cortisol feature.

The ICC representing stability of a cortisol feature across the two waves can be defined in two ways. ICC_1 represents the correlation between individual days across waves for the same individual (Ross et al., 2014):

$$ICC_1 = \text{Correlation}\left(y_{i1d'} y_{i2d'}\right) = \frac{\sigma^2}{\sigma^2 + \delta^2 + \tau^2}$$

In contrast ICC_2 represents the correlations between the wave–specific means (averaged across days) for the same individual (Mujahid et al., 2007):

$$ICC_2 = \text{Correlation}\left(\alpha_{i1}\alpha_{i2}\right) = \frac{\sigma^2}{\sigma^2 + \delta^2}.$$

ICC_2 ignores between-day (level 1) variability and purely considers between-study (level 2) and between-individual (level 3) variability, and as a result gives a larger value than ICC_1. Because we are interested in reporting correlation across long periods of time, ICC_2 is the most desirable quantity. However, ICC_1 may give a more realistic ICC in the sense that studies with only day per visit would not be able to partition variance into three components and thus account for the three levels of variability in the analyses, and in the sense that the states at given visits are not actually observed. By definition ICC_2 will always be smaller than ICC_1 (when multiple days are available at each time point) since in calculating ICC_2 between-day variability within waves is not included in the denominator because we are assuming that the mean α_{ij} at each wave is the best reflection of the state at that wave (see formulas above). In both types of ICCs, a higher value indicates stronger agreement on the values of a cortisol feature across studies (MESA Stress I and II) for a participant.

The ICCs calculated by the three-level model above are referred to as the unadjusted ICC. When the level 3 model is adjusted for individual level sociodemographic variables including age at MESA Stress I Study, sex, race/ethnicity, and income-wealth score, the resulting ICCs are referred to as the adjusted ICC. We calculated both adjusted and unadjusted ICCs for both versions of ICCs.

In addition to estimating the long-term stability across both waves, we used a similar approach to estimate the short-term stability across days within a wave, separately for each

wave of the study. We fitted a two-level multilevel model only including the individual level random effect and residual error. The ICC across days (ICC_3) was then calculated as the proportion of the between-subject variability over the total (between-individual and between-day) (Golden et al., 2014). Although the ICC across days within the same study could be calculated from analyses pooling across both visits as Correlation($y_{ijd}y_{ijd}$) = $\sigma^2 + \delta^2/(\sigma^2 + \delta + \tau^2)$, conducting the analyses stratified by visit allows us+to compare the between day ICC_3 across studies with less model assumptions. For the early and late decline slopes, the bottom 1% and top 1% of the distributions were omitted from all types of ICCs' calculation, owing to the presence of extreme outliers.

1.5.2. Aim 2: Sociodemographic predictors of change—A piecewise linear mixed effects model was used to estimate the associations of sociodemographic factors, including age at MESA Stress I, sex, race/ethnicity and income-wealth status with the change in features over time. Instead of modeling the change over time separately for each of the cortisol features, we include all cortisol samples in a longitudinal model adjusted for all sociodemographic factors, to maximize statistical efficiency. The change in cortisol features associated with each demographic factor was then derived from the relevant coefficients. In order to capture the nonlinearity of the cortisol daily profile, piecewise splines were used with two knots selected at 0.5 h and 2 h after wakeup (Hajat et al., 2010). The model also included time between Stress Studies and interactions of covariates with time to estimate how covariates were associated with changes over time. The model included a random intercept for each person and random person slopes only for the first and the third pieces of spline in order to avoid convergence problems. Robust standard errors are reported. Details of the statistical model fitted are provided in the Supplementary materials.

All analyses for Aim 1 and Aim 2 were carried out using SAS version 9.2 software.

2. Results

Participants had a mean age of 63.7 (SD: 9.1, range: 48–87) at MESA Stress I. They were race/ethnically diverse: African-American (27.6%), Hispanic (54.1%), and Non-Hispanic White (18.3%). Among the Hispanic participants, 32.5% were US-born and 41.4% had English as their primary language spoken at home. They were approximately evenly distributed across sexes (52.8% women and 47.2% men) and study site (55.7% New York site and 44.3% Los Angeles site). The median time between the two waves of MESA Stress studies was 6 years. At both waves, samples were collected over the entire year, spanning all seasons.

The daily cortisol curves for the two study waves were similar in that they both showed a sharp increase after wakeup, a rapid decrease after the peak, and a more gentle decline until bedtime (Fig. 1). However, consistent with the patterns previously described for aging, cortisol levels were higher at MESA Stress II than MESA Stress I and the curve for MESA Stress II was generally flatter than the curve for MESA Stress I. Between the two waves the wakeup value increased by 11%; the CAR value decreased substantially (−43%); the early decline slope and late decline slope increased by 6% and 18%, respectively (indicating flattening of the slopes over time); and the AUC increased by 17%. In unadjusted analyses

there were no statistically significant differences in changes over time by sociodemographic factors (Table 1).

Table 2 shows the ICCs for the five cortisol features over long periods (across the two waves, median time between waves 6 years) and across days within waves. The long term stability is reported using ICC_1 and ICC_2. Across all cortisol features, the long terms stability between days from different waves of the study (adjusted ICC_1 ranging from 0.02 to 0.12) was much lower than the short term stability across days within each wave of the study (adjusted ICC_3 ranging from 0.17 to 0.74 across features). ICC_2 representing correlations between wave-specific means across both waves was generally larger than ICC_1, representing correlations between days from separate waves (adjusted ICC_2: 0.05–0.42) Within studies, stability over days was highest for area under the curve, followed by wake up, and decline slopes, and lowest for the CAR. Across studies (ICC_2) stability has higher for the early and late declines than for the other features.

Table 3 shows adjusted associations of sociodemographic factors with cortisol features at baseline and with changes in features across the two waves. Because coefficients in the statistical model of Aim 2 are mean differences in log-transformed cortisol values, they can be exponentiated to obtain an estimate of the percent difference in the feature associated with the covariate (except in the case of the AUC where this mathematical transformation does not apply) (see footnote to Table 3). At baseline, older age was associated with a less pronounced early and late decline (1.9%, 95% confidence interval (CI): 0.1–3.7 and 0.3%, CI: 0.1–0.5 respectively) and higher AUC (mean difference (MD) in logcortisol area unit: 0.05, CI: 0.03–0.07). Men showed a less pronounced early decline (15.4%, CI: 7.4–21.4) and a larger AUC (MD: 0.13, CI: 0.05–0.20) than women. Compared to Non-Hispanic Whites, African-Americans had a lower wakeup value (−21.6%, CI: −38.2 to −10.5), a flatter early and late decline slope (11%, CI: 0.3–20.5 and 2.6%, CI: 1.3–3.9), and Hispanics had lower wakeup value (−16.8%, CI: 31.1 to −5.7), a flatter early decline (14.7%, CI: 5.4–22.1), a more pronounced late decline slope (−1.5%, CI: −2.7 to −0.3) and a smaller AUC (MD: − 0.14, CI: −0.24 to −0.04) at baseline. Higher income wealth index at baseline was also associated with a higher wakeup value (2.8%, CI: 0.4–5.1) and more pronounced late decline (−0.2%, CI: −0.4 to −0.01).

All cortisol features changed significantly over the two waves. After adjusting for age, sex, race/ethnicity and income-wealth, the wakeup value increased by 2.9% per year (CI: 1.5–4.1); the CAR dropped by 3.7% per year (CI: −5.1 to −2.5); the early and late decline slope became flatter by 1% per year (CI: 0.1–2.0) and 0.4% per year (CI: 0.3–0.5), respectively; and the AUC increased by 0.03 per year (CI: 0.02–0.04) in log-cortisol area unit (log(nmol/L) h). Neither age nor sex was significantly associated with changes over time. However, changes over time did differ by race/ethnicity; comparing with Non-Hispanic Whites, Hispanics experienced significantly larger increases in the wakeup value (5.3%, CI: 1.4–9.0); and both African-American and Hispanics showed less flattening over time of the early decline slope (−4.3%, CI: −7.5 to −1.3 and − 3.6, CI: −6.5 to −0.7, respectively). No statistically significant differences in the change over time were observed by income/wealth. Wakeup time has previously been found to be associated with cortisol features (Karlamangla et al., 2013). In sensitivity analyses, additional adjustment for wake up time did not

meaningfully alter any of the results reported. Adjustment for season of sample collection did not substantially affect the results either.

3. Discussion

Using repeat measures from a large multi-ethnic population-based study, our study investigated long term stability of features and predictors of change in the cortisol daily rhythm over a 6-year period. We empirically estimated the stability of cortisol features over long periods (6 years) and compared it to stability over a short period (days). For wake up and CAR, short term stability (i.e., correlation between days within a study wave, ICC_3: 0.17–0.52) was higher than long term stability (correlation between days across two study waves, ICC_1: 0.02–0.06), although long term stability of the average feature for each wave was larger (ICC_2: 0.09–0.11). However, for the early and late decline over the day, the long term stability across 6 years for the average feature within wave was similar (or higher) than the stability across days within study. AUC had high stability over days within study wave (ICC_3: 0.65–0.74) but much lower stability across long periods (ICC_2: 0.05).

Our findings on the short term stability were generally consistent with previous studies showing that measures of total cortisol secretion like AUC are highly stable over short periods (Pruessner et al., 1997; Rotenberg et al., 2012) whereas other features like diurnal slopes or single sample measures like the wakeup levels are less stable (Rotenberg et al., 2012). Like others (Almeida et al., 2009) we found high day-to-day variability in the CAR. In addition, the long term stability over 6 years was highest for the daily decline or slope (adjusted ICC_2: 0.25 for the early decline and 0.42 for the late decline) but much lower for the other features. Our findings suggest that over long periods (years), the non-slope cortisol features can change significantly; in contrast the long term variability for the slope features was no greater than the short term variability (between days within waves). However, the use of a single day to characterize each wave resulted in much lower stability of the slope features (ICC_1: 0.08–0.12), which suggests a high day-to-day variability in the slope features.

A recent examination on the long term stability of cortisol curve features also reported a relatively low stability in cortisol curve features over 8–24 months, with ICCs for periods over 1 year less than 0.13 (Ross et al., 2014). The long term ICC estimate reported by Ross et al. (2014) is analogous to our ICC_1 estimates which were also low and similar in magnitude (ranging from 0.02 to 0.12 for a 6 year period). Like Ross et al. (2014) we also found lower stability for the CAR than for the slope or the AUC (although this was not consistent across the three studies reported by Ross et al., 2014). Our ICC_1 estimates of stability of the slopes are also comparable to that reported by Shirtcliff et al. (2012) (who reported a 6-year stability estimate for the diurnal slope of 0.13). Our estimates of stability of mean features averaged over days within waves (ICC_2) are substantially higher (ranging from 0.05 to 0.42). Estimates of long term stability based on ICC_2 effectively ignore day to day variability within waves because they are based on the average across days at each wave. By definition this results in higher estimates of stability than when ICC_1 is used (which does not ignore day to day variability within waves). However, the ICC_2 estimates are still meaningful as many researchers will pool data over several days when generating

estimates for a given individual and time point. This is justifiable given the relatively high short term stability across days within waves (ICC_3: 0.17–0.74). Using a different methodology, Platje et al. (2013) reports a 3-year tracking stability for CAR which is substantially higher than our results.

Our study also investigated the predictors of changes in cortisol features over approximately a 6-year period. At baseline, we found that age was associated with less pronounced early and late decline and higher AUC; male sex was associated with a less pronounced early decline and a higher AUC; higher income wealth was associated with a higher wake up value and a more pronounced late decline; and Hispanics and African-Americans had lower wake up values and less pronounced early declines than Non-Hispanic Whites. In addition, Hispanics had a more pronounced late decline and a lower AUC and African-American had a flatter late decline than Non-Hispanic Whites. Our results regarding associations of age, sex, race/ethnicity and income/wealth with cortisol features at baseline are generally consistent with previously reported cross-sectional analyses (Kirschbaum and Hellhammer, 1989; Clow et al., 2004; Ranjit et al., 2005; Cohen et al., 2006; DeSantis et al., 2007; Hansen et al., 2008; Hajat et al., 2010; Heaney et al., 2012) although our data allowed us to generate separate estimates for the early and late declines. However, our results regarding associations of covariates with the pieces of the decline are generally consistent with results for the overall decline.

Our study then extended this cross sectional work by examining the magnitude of the cortisol feature change and the association of the change over several years with socio-demographic variables. We found significant long term changes in cortisol features. Over time the wakeup cortisol became higher; the CAR became significantly smaller; both the early and the late decline became flatter; and the AUC became larger. These longitudinal findings are consistent with the patterns observed when differences in cortisol features by age are examined cross-sectionally and confirm that the aging process itself creates these changes in cortisol profiles (Heaney et al., 2012). In addition to differences by age we found some suggestive differences by race/ethnicity: wake up values increased more over time in African Americans and Hispanics than in Non-Hispanic Whites (although differences for African Americans were not statistically significant). Further replication of these findings would confirm whether the aging process of the HPA axis differs by race/ethnicity (or factors linked to race/ethnicity).

Our findings regarding changes over time in the cortisol diurnal rhythm are consistent with the known effects of aging on HPA axis activity. Previous studies using the dexamethasone (DEX) challenge tests (Golden et al., 2011) suggest that the increases in cortisol observed with aging can be attributed to impairment of feedback inhibition of HPA activity due to neuronal loss in the hippocampal area (Oxenkrug et al., 1983; Yen and Laughlin, 1998). The decreased HPA sensitivity to the cortisol feedback inhibition found in human aging (Wilkinson et al., 1997) may also explain the decreased CAR and the flattened slopes of cortisol rhythm that was observed as participants aged.

Stressful experiences have been shown to be important influences on HPA axis functioning (Gunnar et al., 2001; Halligan et al., 2004). We therefore expected to observe differences in

changes over time by race/ethnicity and wealth/income which have previously been linked to chronic stress. Although we observed some differences in changes over time by race/ethnicity no clear patterns emerged. Sample size limitations may have made it difficult to detect what are likely relatively small effects of these domains on changes in cortisol features.

Several limitations in this study need to be considered in the interpretation of the results. First, many studies have documented non-compliance with the study protocol, specifically with morning samples, resulting in misleading cortisol curves in the morning hours (Clow et al., 2004; Kunz-Ebrecht et al., 2004; Wright and Steptoe, 2005). In analyses of the MESA Stress I study, Golden et al. (2014) found that the compliance was likely to be higher in the morning and bedtime samples but lower in the afternoon samples and reported that 64.2% of all samples were collected within 15 min of the requested time. As part of MESA Stress I we used Track-caps devices to monitor collection times, which have been shown to increase compliance (Kudielka et al., 2003). Although Track-caps devices were not used at MESA Stress II due to budget constraints, a recent investigation on short term cortisol reliabilities reported no association between compliance with protocol and cortisol features but a strong association between a poor compliance with protocol and low within wave ICC (Golden et al., 2014). Given our finding of a similar level of short term ICC in the two waves of study, it seems that the impact of the usage of Track-caps devices on compliance may not be strong in this population of older individuals. However, this argument needs to be tested via studies with compliance with protocol measured in multiple time points. Variability in compliance across waves could have affected our estimates of change in features. While our finding that the CAR becomes less pronounced with age is consistent with previous cross-sectional findings (Hajat et al., 2010), it is possible that the less pronounced morning peak value or shorter CAR could be due to the less compliance with the timing of collection at MESA Stress II.

Measurement error resulting from self-reported collection times undoubtedly affected our results and may have resulted in underestimates of stability and reduced our ability to detect factors associated with changes over time. Although our study was unique in the availability of multiple daily measures approximately 6 years apart on a large sample, the time between visits may have been too short to detect significant associations of factors with change. Our sample population was relatively old at baseline age (ages ranging from 48 to 87 years), which may have limited generalizability to younger populations. Although follow up measures at MESA Stress II were only available for about 60% of the MESA Stress I participants, those included and excluded had similar levels of the cortisol features except for a lower CAR and a slightly flatter early decline slope for the excluded participants (but the differences were not statistically significant).

Our study is one of only a few population-based cohort studies with well-characterized data on diurnal cortisol curve characteristics collected over long periods in a diverse sample (other studies including Steptoe et al., 2003; Cohen et al., 2006; Karlamangla et al., 2013). Although our study benefitted from multiple measures on multiple days at each wave, additional data (more samples per day and more days) could potentially improve estimates

of both short and long term stability. In addition, the availability of more follow-up waves would allow us to broaden our understanding on the long term stability of cortisol rhythms.

To the best of our knowledge, our study is among the first investigations of the stability and predictors of cortisol change over long periods. Our results showed significant changes in cortisol features over long periods associated with aging. Our finding of high variability of cortisol features over long periods suggests that characterization of a stable trait (for at least some features) may be challenging. However changes associated with aging provide opportunities for future investigations on the predictors of these changes as well as the links between these changes and health outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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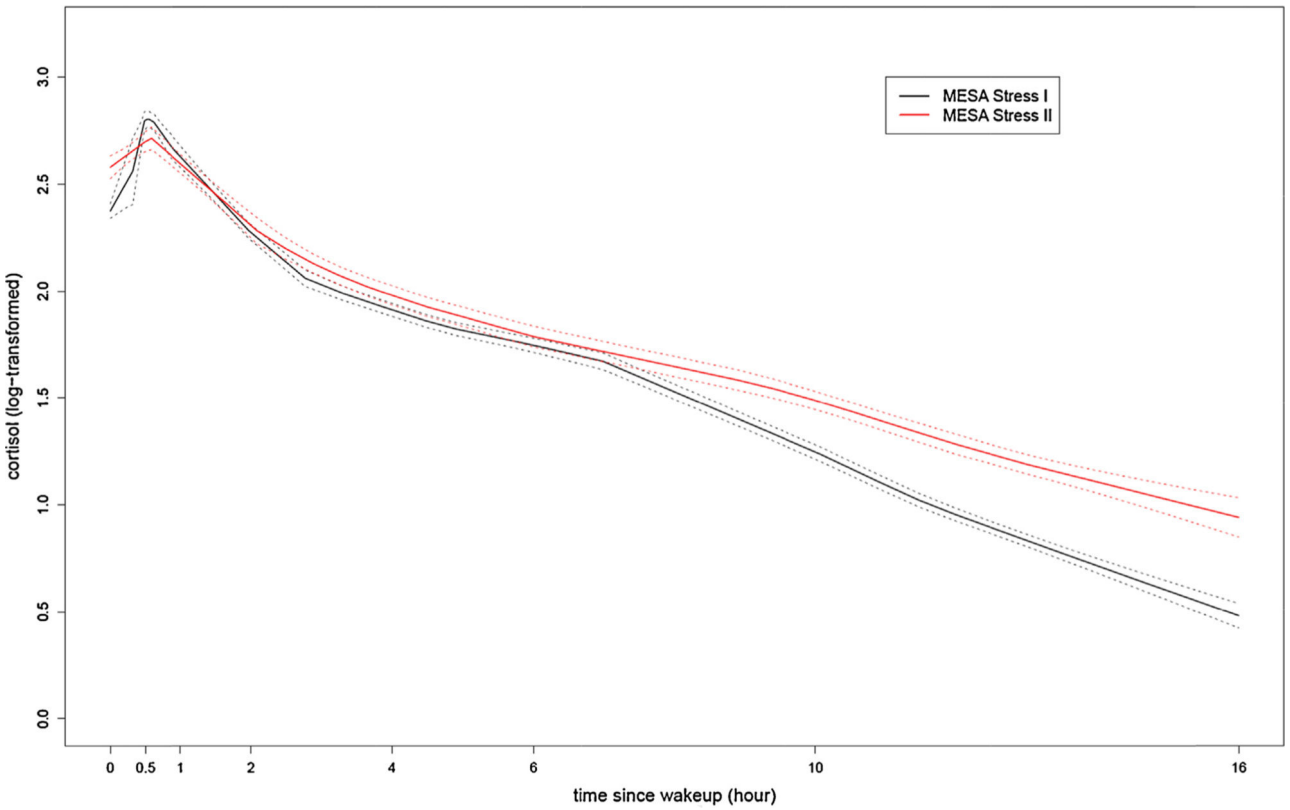

Figure 1.

LOESS plot of the cortisol daily curve for entire population at MESA Stress I and II. *Note*: Locally estimated scatter plot smoothing (LOESS) curves were used to examine the shape of the cortisol profile over the course of the day (Cleveland et al., 1988; Cleveland and Devlin, 1988). The daily cortisol curves for the two study waves were similar in that they both showed a sharp increase after wakeup, a rapid decrease after the peak, and a more gentle decline until bedtime while cortisol levels were higher at MESA Stress II than MESA Stress I and the curve for MESA Stress II was generally flatter than the curve for MESA Stress I. The plot was based on log-transformed cortisol value (unit: log(nmol/L)). Dotted lines refer to the 95% confidence region of LOESS plot for the daily cortisol curve at each MESA Stress study.

Table 1

Selected Cortisol features[c] (median) for both waves of the study and percent change in features between waves for the full sample and by categories of sociodemographic characteristics.

	Category	No. subj.	Study	Wakeup	CAR	Early decline slope	Late decline slope	AUC
Entire population								
		580	1	2.43	0.40	–0.47	–0.13	1.50
			2	2.70	0.23	–0.44	–0.10	1.75
			% change	11	–43	6	18	17
Sex								
	Female	306	1	2.43	0.42	–0.55	–0.12	1.43
			2	2.69	0.27	–0.48	–0.10	1.71
			% change	11	–36	13	16	20
	Male	274	1	2.43	0.38	–0.39	–0.13	1.56
			2	2.70	0.20	–0.39	–0.10	1.80
			% change	11	–47	0	20	15
			p-value[b]	0.992	0.729	0.893	0.254	0.130
Age								
	<55	112	1	2.36	0.40	–0.49	–0.13	1.32
			2	2.69	0.32	–0.49	–0.11	1.61
			% change	14	–18	0	13	21
	55–65	199	1	2.45	0.41	–0.52	–0.13	1.48
			2	2.69	0.24	–0.45	–0.10	1.71
			% change	10	–42	13	22	16
	65–75	199	1	2.42	0.40	–0.41	–0.12	1.55
			2	2.72	0.18	–0.41	–0.10	1.86
			% change	12	–55	–1	17	20
	75+	70	1	2.49	0.32	–0.50	–0.12	1.66
			2	2.71	0.17	–0.44	–0.08	1.82
			% change	9	–47	11	32	10
			p-value[b]	0.864	0.571	0.691	0.815	0.292
Race/ethnicity								
	White	106	1	2.60	0.44	–0.55	–0.14	1.60
			2	2.75	0.32	–0.44	–0.10	1.81
			% change	6	–28	19	23	13
	Black	160	1	2.37	0.34	–0.44	–0.11	1.54
			2	2.62	0.23	–0.50	–0.09	1.76
			% change	11	–32	–13	18	14
	Hispanic	314	1	2.40	0.42	–0.45	–0.13	1.43
			2	2.75	0.20	–0.41	–0.11	1.75
			% change	14	–52	9	17	22
			p-value[b]	0.151	0.077	0.172	0.863	0.494

Category	No. subj.	Study	Wakeup	CAR	Early decline slope	Late decline slope	AUC
Income wealth index [a]							
(0–1)	99	1	2.30	0.47	–0.45	–0.12	1.44
		2	2.65	0.11	–0.43	–0.10	1.77
		% change	15	–76	4	16	23
(2–3)	156	1	2.40	0.39	–0.46	–0.13	1.44
		2	2.71	0.18	–0.40	–0.10	1.74
		% change	13	–53	13	20	21
(4–6)	232	1	2.46	0.39	–0.46	–0.13	1.53
		2	2.69	0.28	–0.46	–0.10	1.73
		% change	9	–29	2	20	13
(7–8)	91	1	2.53	0.42	–0.55	–0.13	1.56
		2	2.77	0.32	–0.49	–0.11	1.82
		% change	9	–24	12	15	17
		p-value[b]	0.532	0.206	0.225	0.631	0.990

[a] 2 subjects have missing value in the income wealth index

[b] *p*-values were calculated based on individual samples of cortisol feature change since baseline using ANOVA (sex and race) and trend tests (age and income-wealth index).

[c] Due to its skewed distribution cortisol was log-transformed before the cortisol features were calculated (Adam and Kumari, 2009; Hajat et al., 2010; Champaneri et al., 2012); estimates reported are therefore in log(nmol/L) units; the % change of cortisol features are relative difference of log-cortisol unit features between the two waves of the Stress study.

Table 2

Intraclass correlation coefficients (ICC)[b] between and within waves for selected Cortisol features before and after adjustment for covariates.

Period	ICC	Wakeup	CAR	Early decline slope	Late decline slope	AUC
Across studies	ICC_1: unadjusted	0.07	0.03	0.09	0.14	0.07
	ICC_1: adjusted[a]	0.06	0.02	0.08	0.12	0.04
	ICC_2: unadjusted	0.12	0.09	0.28	0.46	0.09
	ICC_2: adjusted[a]	0.11	0.09	0.25	0.42	0.05
MESA stress I	ICC_3: unadjusted	0.52	0.31	0.33	0.29	0.67
	ICC_3: adjusted[a]	0.51	0.31	0.31	0.28	0.65
MESA stress II	ICC_3: unadjusted	0.52	0.18	0.27	0.27	0.74
	ICC_3: adjusted[a]	0.52	0.17	0.25	0.25	0.74

[a] Baseline age, sex, race/ethnicity and income-wealth score were adjusted in the multi-level models for the adjusted ICC.

[b] ICC_1 represents the long-terms stability of cortisol feature values calculated as the correlation between values at individual days across waves for the same individual; ICC_2 represents the long-term stability of cortisol feature values calculated as the correlation between the wave–specific means (averaged across days) for the same individual; ICC_3 represents the short-term stability of cortisol feature values across days within a wave for the same individual. For both ICC_1 and ICC_2, a higher value indicates stronger agreement on the values of a cortisol feature across studies (MESA Stress I and II) for a participant; for ICC_3, a higher value indicates stronger agreement on the values of a cortisol feature across days at certain wave of MESA Stress study for a participant.

Table 3

Percent differences[d] in features of the Cortisol curve at baseline and mean differences in percent changes[d] over time associated with sociodemographic characteristics.

	Wakeup		**CAR**		**Early decline slope**		**Late decline slope**		**AUC**	
	% difference	**95% CI**	**% difference**	**95% CI**	**% difference**	**95% CI**	**% difference**	**95% CI**	**Mean difference[d]**	**95% CI**
Mean differences at baseline										
Age (5-year increase)	1.8	(–0.6, 4.2)	–0.8	(–3.0, 1.3)	1.9	(0.1, 3.7)	0.3	(0.1, 0.5)	0.05	(0.03, 0.07)
Male[b]	1.9	(–7.7, 11.5)	–5.3	(–13.6, 2.8)	15.4	(7.4, 21.4)	–0.6	(–1.5, 0.2)	0.13	(0.05, 0.20)
African-American[a]	–21.6	(–38.2, –10.5)	–9.3	(–22.4, 2.8)	11.0	(0.3, 20.5)	2.6	(1.3, 3.9)	–0.04	(–0.14, 0.07)
Hispanic[a]	–16.8	(–31.1, –5.7)	–5.2	(–17.1, 6.3)	14.7	(5.4, 22.1)	–1.5	(–2.7, –0.3)	–0.14	(–0.24, –0.04)
income wealth index (1-score increase)	2.8	(0.4, 5.1)	–0.3	(–2.3, 1.7)	–1.0	(–2.7, 0.7)	–0.2	(–0.4, –0.01)	0.00	(–0.02, 0.02)
Annual changes over time and mean differences in the changes										
Time (years since baseline)[c]	2.9	(1.5, 4.1)	–3.7	(–5.1, –2.5)	1.0	(0.1, 2.0)	0.4	(0.3, 0.5)	0.03	(0.02, 0.04)
Male × time	–0.2	(–2.8, 2.4)	–1.4	(–3.9, 1.2)	–0.1	(–1.9, 1.8)	0.2	(0, 0.4)	–0.01	(–0.03, 0.02)
Age × time	–0.1	(–0.8, 0.6)	–0.2	(–0.9, 0.5)	0.1	(–0.4, 0.5)	0.0	(0, 0.1)	0.00	(–0.01, 0.00)
African-American × time	3.7	(–0.6, 7.8)	3.5	(–0.7, 7.6)	–4.3	(–7.5, –1.3)	–0.1	(–0.4, 0.2)	0.00	(–0.03, 0.03)
Hispanic × time	5.3	(1.4, 9.0)	0.7	(–3.4, 4.8)	–3.6	(–6.5, –0.7)	–0.1	(–0.4, 0.2)	0.00	(–0.02, 0.03)
Income wealth × time	–0.1	(–0.7, 0.5)	0.3	(–0.3, 0.9)	–0.2	(–0.6, 0.2)	0.0	(–0.1, 0)	0.00	(–0.01, 0.00)

[a] with Non-Hispanic White as the reference group.

[b] with female as reference group.

[c] Age (centered at 65), sex (dummy variable), race/ethnicity (dummy variables) and income wealth index (centered at 4) were centered before fitting adjusted in model; therefore, the change in cortisol features per year is the average change over the sample population.

[d] Because coefficients in the statistical model of Aim 2 are differences in log-transformed cortisol values, they can be exponentiated to obtain an estimate of the percent difference in the feature associated with the covariate except in the case of the AUC where this mathematical transformation does not apply and the mean differences/changes are shown in log-cortisol area units (log(nmol/L)h). The values highlighted in bold are statistically significant (*alpha* = 0.05).