HHS Public Access

Author manuscript

Psychoneuroendocrinology. Author manuscript; available in PMC 2015 May 01.

Published in final edited form as:

Psychoneuroendocrinology. 2014 May ; 43: 30–40. doi:10.1016/j.psyneuen.2014.01.025.

Salivary Cortisol Protocol Adherence and Reliability by Sociodemographic Features: the Multi-Ethnic Study of Atherosclerosis

Sherita Hill Golden[1], **Brisa N. Sánchez**[2], **Amy S. DeSantis**[3], **Meihua Wu**[2], **Cecilia Castro**[4], **Teresa E. Seeman**[5], **Sameh Tadros**[5], **Sandi Shrager**[6], and **Ana V. Diez Roux**[2]

[1]Departments of Medicine and Epidemiology, Johns Hopkins University Schools of Medicine and Public Health, Baltimore, MD, 21287, USA

[2]Department of Biostatistics, University of Michigan – Ann Arbor, MI, 48109, USA

[3]Center for Social Epidemiology and Population Health, Department of Epidemiology, University of Michigan – Ann Arbor, MI, 48109

[4]Department of Department of Epidemiology, Department of Medicine, Columbia University, New York, NY, 10032

[5]Department of Epidemiology, Department of Medicine, University of California-Los Angeles, CA 10945

[6]Department of Biostatistics, University of Washington, Seattle, WA 98115

Abstract

Collection of salivary cortisol has become increasingly popular in large population-based studies. However, the impact of protocol compliance on day-to-day reliabilities of measures, and the extent to which reliabilities differ systematically according to socio-demographic characteristics, has not been well characterized in large-scale population-based studies to date. Using data on 935 men and women from the Multi-ethnic Study of Atherosclerosis, we investigated whether sampling protocol compliance differs systematically according to socio-demographic factors and

*Corresponding author: Address correspondence and requests for reprints to: Dr. Sherita Hill Golden, Johns Hopkins University School of Medicine, Division of Endocrinology and Metabolism, 1830 E. Monument Street, Suite 333, Baltimore, MD 21287 Tel: (410) 502-0993, Fax (410) 955-8172, sahill@jhmi.edu.

AUTHOR DISCLOSURES

S. Golden, A. DeSantis, B. Sanchez, M. Wu, C. Castro, T. Seeman, S. Tadros, and S. Shrager have nothing to disclose. A. Diez Roux is funded by a research grant from NIH-NHLBI that supports MESA Stress.

CONTRIBUTORS

S. Golden and A. DeSantis wrote the first draft of the manuscript and made critical revisions and interpreted the analyses. A. DeSantis and B. Sanchez designed the statistical analyses and M. Wu conducted the analyses. A. Diez Roux obtained funding, designed and wrote the protocol for the MESA Stress Study, and critically reviewed the manuscript. A. Diez Roux, T. Seeman, C. Castro, S. Tadros, and S. Shrager were involved in data collection for MESA Stress. All authors contributed to critical manuscript revisions and have approved the final manuscript.

whether compliance was associated with cortisol estimates, as well as whether associations of cortisol with both compliance and socio-demographic characteristics were robust to adjustments for one another. We further assessed the day-to-day reliability for cortisol features and the extent to which reliabilities vary according to socio-demographic factors and sampling protocol compliance. Overall, we found higher compliance among persons with higher levels of income and education. Lower compliance was significantly associated with a less pronounced cortisol awakening response (CAR) but was not associated with any other cortisol features, and adjustment for compliance did not affect associations of socio-demographic characteristics with cortisol. Reliability was higher for area under the curve (AUC) and wake up values than for other features, but generally did not vary according to socio-demographic characteristics, with few exceptions. Our findings regarding intra-class correlation coefficients (ICCs) support prior research indicating that multiple day collection is preferable to single day collection, particularly for CAR and slopes, more so than wakeup and AUC. There were few differences in reliability by socio-demographic characteristics. Thus, it is unlikely that group-specific sampling protocols are warranted.

Keywords

Salivary cortisol; Cortisol awakening response (CAR); Diurnal cortisol; Reliability; Compliance; Population-based study

---

Collection of salivary cortisol has become increasingly popular in large population studies (Steptoe et al., 2003; Cohen et al., 2006; Adam & Kumari, 2009). Although valid measures of cortisol levels can be easily obtained from saliva samples providing valuable information on the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, the impact of differences in compliance on estimates of various features of the daily cortisol curve is not well characterized. An additional measurement challenge pertains to the reliability of measures across days, which has important implications for the number of days over which samples need to be collected (Kraemer et al., 2006; reviewed in Adam & Kumari, 2009).

Cortisol levels typically follow a strong circadian rhythm across the day, in which levels are typically high upon waking, increase by 50 to 75% during the 30 to 40 minutes post-awakening (referred to as the cortisol awakening response or CAR; Pruessner et al., 1997), and decline across the remainder of the day, reaching a nadir in the late evening some 18+ hours after awakening (Kirschbaum & Hellhammer, 1989; Karlamangla et al., 2013). Both the size of the CAR and the rate of the cortisol decline across the day represent important aspects of HPA axis functioning and have been associated with socio-demographic factors, psychosocial wellbeing, and physical and mental health (Sephton et al., 2000; Adam & Gunnar, 2001; Gunnar & Vazquez, 2001; Kunz-Ebrecht et al., 2004; Wright & Steptoe, 2005; Cohen et al., 2006; Saxbe et al., 2008; Hajat et al., 2010; Kumari et al., 2011).

Because differences in the size of the CAR, rates of decline, and absolute levels at various points during the day (e.g., upon awakening) are often used to characterize HPA axis functioning, it is important that participants adhere to the requested sampling protocols in order to avoid biased estimates. Poor adherence to the sampling protocol may influence interpretations of a person's diurnal cortisol profile and has been found to produce unreliable estimates, with the cortisol profiles of non-compliant individuals differing significantly from

those of compliant persons (Kudielka et al., 2003; Broderick et al., 2004; Kudielka et al., 2007), although research is equivocal (Jacobs et al., 2005). Moreover, systematic differences according to socio-demographic or other characteristics may lead researchers to believe that there are meaningful differences according to these factors; however, they may simply reflect biases in estimates due to differences in compliance across groups. Conversely, significant group-level differences in cortisol activity may be obscured by poor compliance. Thus, systematic assessment of whether compliance differs significantly according to socio-demographic factors may help improve interpretation of estimates of group-level differences in cortisol profiles, and contribute to the development of protocols that result in better estimates.

Another major challenge in the characterization of cortisol patterns using repeat salivary samples pertains to the number of days over which samples need to be collected as well as the number of samples needed per day in order to characterize the features believed to be relevant (Kraemer et al., 2006; Adam & Kumari, 2009). For example, it is important to determine whether the intensive sampling protocols that have been most commonly implemented in salivary cortisol research (e.g., 3 to 9 samples per day over multiple days (reviewed in Adam & Kumari, 2009) are necessary and/or optimal for assessing HPA axis functioning. Thus, investigation of levels of day-to-day reliability of each feature of the cortisol profile, and the extent to which reliability differs systematically across groups who are likely to be the focus of analyses, is warranted.

Making use of unique data available from a sample of participants in the Multi-Ethnic Study of Atherosclerosis this study investigates: (1) whether sampling compliance differs systematically according to socio-demographic characteristics; (2) whether sampling compliance is associated with features of the cortisol curve (e.g., CAR, decline); (3) whether associations of socio-demographic characteristics with cortisol parameters are robust to adjustments for compliance, as well as whether associations of sampling compliance with cortisol parameters are robust to adjustments for socio-demographic factors; and (4) the day-to-day reliability for various features of the cortisol profile and the extent to which reliability varies according to socio-demographic characteristics and level of compliance with the sampling protocol.

METHODS

Study Population

MESA is a multi-center, longitudinal cohort study of the prevalence and correlates of subclinical cardiovascular disease and the factors that influence its progression (Bild et al, 2002). Between July 2000 and August 2002, 6814 men and women without clinical cardiovascular disease who identified themselves as white, black, Hispanic, or Chinese, and were 45 to 84 years of age were recruited from six U.S. communities: Baltimore City and Baltimore County, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; Northern Manhattan and the Bronx, New York; and St. Paul, Minnesota. Details on the sampling frames and the cohort examination procedures have been published elsewhere (Bild et al, 2002).

An ancillary study to MESA, the MESA Stress study collected detailed measures of stress hormones, including salivary cortisol measures, on a subsample of 1002 participants enrolled at the New York and Los Angeles MESA sites, as previously reported (Hajat et al., 2010). Participants using steroids (n=35) were excluded since steroid use interferes with HPA axis assessment and an additional 32 (3.3%) were excluded because insufficient data were collected to compute the cortisol features (e.g. missing track cap time, saliva sample not provided). Compared to individuals who were included, excluded individuals were less likely to have a bachelor's degree or higher (23.6% for included versus 15.6% for excluded; p=0.029) and were less likely to be white (19.6% for included versus 3.1% for excluded; p=0.003). There were no differences in age, sex, or income. The final analyses include 935 participants who provided 2774 days of cortisol data, and represent an ethnically and socio-economically diverse group of participants: African-American (27.6%), Hispanic (52.8%), Non-Hispanic White (19.6%) and an approximately even distribution of sexes: 51.6% female and 48.4% male. The mean age was 65±9.8 years (median 65 years; interquartile range 57 to 73 years).Annual household income was divided into three categories: less than $25,000(38.7%), $25,000 to $50,000 (31.1%) and $50,000 or more (26.8%). Education was also summarized into three categories: completed high school or GED or less (38.3%), some college (30.9%), and associates degree and Bachelors degree or more (25.8%). Informed consent was obtained from each participant, and the study was approved by the Institutional Review Boards of each institution.

Hormonal Measures

Each MESA Stress participant was instructed to collect six saliva samples per day over 3 weekdays, resulting in a maximum of 18 samples per person. 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 noon (or before lunch if lunch occurred before 1200 h), the fifth sample at around 1800 h (or before dinner if dinner occurred before 1800 h), and the sixth sample right before bed.

A trackcap device, with time-stamp bottle openings, was used to register the times that the bottle was opened for sample collection. Participants were informed that compliance with sampling protocols was being monitored using these devices (trackcaps, MEMS 36, San Diego, CA). The participants were also instructed to report their wake-up time and bedtime for each day, which allowed us to derive the requested time for the 1^{st}, 2^{nd}, and 6^{th} samples for each participant on each day. The requested times for the 3^{rd}, 4^{th} and 5^{th}samples were uniform across the study: 1000 h, 1200 h, and 1800 h. The difference between the requested time and the track-caps time (which was the actual collection time) was divided into four categories (0 to 5 minutes, 5 to 10 minutes, 10 to 15 minutes, > 15 minutes), and was used to characterize the level of compliance of participants.

Assessment of Protocol Compliance

We developed a compliance score coding scheme to minimize the impact of larger differences between track cap times and requested times in the samples later in the day. Evening times for salivary cortisol collection were more variable because they were contingent upon when participants ate dinner, compared to collections earlier in the day.

Using raw differences in minutes between the track caps time and the requested time resulted in a highly skewed distribution, such that evening difference might have driven the overall differences in compliance scores. Thus, we created a point system so that any sample with a difference in track caps and requested time larger than 15 minutes got the worse score, as summarized below.

Compliance scores for each sample were constructed as follows: participants received 3 points if the difference between the requested sample time and the trackcap time was 0 to 5 minutes, 2 points for gaps of 5 to 10 minutes, 1 point for gaps of 10 to 15 minutes, and 0 points for gaps of more than 15 minutes. A score for each person was computed by aggregating points across all samples. Higher scores indicate higher compliance with the protocol. Each participant's compliance score reflects their mean compliance score across all samples. We then classified participants into three compliance categories: low, moderate, and high compliance, based on the tertiles of the compliance score distribution. In order to characterize the association of socio-demographic factors with compliance, we compared the distributions of race/ethnicity, sex, age, income and education across compliance categories. Data on age, race/ethnicity, sex, years of education, highest level of education achieved, and annual income were self-reported using standard protocols (Bild et al, 2002).

Statistical Analysis

We summarized salivary cortisol data into five features of the cortisol curve: wake up cortisol levels, cortisol awakening response (CAR), area under the curve (AUC), early decline, and late decline. Each of the features was computed on a daily basis. The cortisol awakening response (CAR) was calculated as the difference between the wake up cortisol levels and the levels at 30 minutes post-awakening. The early decline (between 30 minutes and 2 hours post-awakening) and late decline (between 2 hours post-awakening and bedtime) were calculated as the hourly rate of decline for the given time period, estimated separately for each person on each day as the slope of the regression line fitted through the samples that fell within the specified time period. 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. AUC estimates were restricted to a 16-hour day for all participants. As previously reported, 85% of participants collected at least 5 samples per day for all days on which they collected samples and 97% of participants collected samples on all 3 days (Hajat et al., 2010). Of the participants, 97%, 83%, 91%, 98% and 94% had sufficient data from at least two days to calculate wake up cortisol, CAR, early decline slope, late decline slope and AUC measures, respectively. Cortisol values were log-transformed prior to calculating the features.

In order to estimate associations of compliance with these features, we modeled each feature (up to three measures for each participant per day) as a function of compliance (in categories) using linear mixed models with a person-level random effect to account for within-subject correlation. Associations of compliance with cortisol features were examined before and after adjustment for socio-demographic characteristics. The impact of adjustment for sociodemographic characteristics on the associations of compliance with the outcomes was quantified by estimating the percent change in the coefficients associated with

compliance categories when socio-demographic characteristics were added. These analyses provide information regarding whether there are differences in the estimates of features of the cortisol curve based on compliance level, as well as whether these differences are robust to adjustment for socio-demographic characteristics, which may influence both compliance and cortisol activity.

Using similar linear mixed models, we estimated differences in the features of the cortisol profile across various socio-demographic factors before and after adjustment for compliance. We also estimated the percent change in the coefficients associated with socio-demographic characteristics after compliance was added to the model. These analyses allow us to examine whether observed socio-demographic differences in cortisol features are partially attributable to systematic differences in levels of protocol compliance across different socio-demographic groups.

We used the intraclass correlation coefficient (ICCs) to assess the reliability of the cortisol features across days, overall and according to various socio-demographic factors and compliance. For each cortisol feature and socio-demographic factor, we fitted a single linear mixed model that allows both between-subject variability and between-day within subject variability to be dependent on the levels of the socio-demographic factor. Then, the ICC for each category of the socio-demographic variable was computed as the proportion of the between-subject variability relative to the total variability, i.e., for the k^{th} group of a given socio demographic factor,

$$ICC_k = \frac{\sigma^2_{bk}}{\sigma^2_{bk} + \sigma^2_{dk}}$$

where σ^2_{bk} is the estimated between-individual variance for a given feature for the kth group of the demographic factor, and σ^2_{dk} is the between-day variance for the feature. This quantity reflects the similarity or correlation of the features within individuals, and ranges from 0 (no similarity in the feature values across days) to 1 (values are the same for all days) (Park and Lake, 2005).

Note that when estimating the ICCs, the single model approach that we employed is equivalent to fitting multiple models separately for each category of the socio-demographic factors. However, the single model approach provides the benefit of allowing us to compute the *p*-values reflecting whether the ICCs differ significantly across socio-demographic categories or compliance. For the early and late decline slopes, the bottom 1% and top 1% of the distribution were omitted from the ICC calculation, owing to the presence of extreme outliers.

All models were estimated using maximum likelihood (ML); separate models for each cortisol feature were fitted. By using ML, available observations from a given participant can be included for parameter estimation (Allison, 2012). For instance, if a participant had only 1 or 2 measures of CAR, the participant's 1 or 2 CAR measures were used toward estimating model parameters. This approach produces valid model estimates under the

assumption that the unavailable measure(s) are missing at random (Little and Rubin, 2002). All analyses were carried out using SAS version 9.2 software.

RESULTS

Table 1 shows compliance with the requested collection times for various samples. Overall, 54% of wake up samples and 62% of bed time samples were collected within 5 minutes of waking up and going to bed, respectively. Participants were least likely to comply with the requested protocol for the afternoon samples, as 43.8% and 44.6% of participants failed to provide samples within 15 minutes of the requested times for the noon and 6 PM samples, respectively. Levels of compliance did not differ greatly across the three days of the study: 44%, 44%, and 42.5% of participants collected all samples within 5 minutes of the requested times on days 1, 2, and 3, respectively. On the other hand, between 30.5% and 32.2% of participants were non-compliant by an average of more than 15 minutes on each of the collection days. Overall, nearly 57% of samples were provided within 10 minutes of the requested times.

Table 2 shows the percentage of individuals within categories of selected socio-demographic characteristics of the sample by tertiles of the overall compliance score. Individuals in the higher compliance score categories were more likely to be of higher income and higher education. Those in the highest tertile of sampling compliance were marginally more likely to be non-Hispanic Whites ($p = 0.07$).

Table 3 presents the crude and adjusted associations of compliance with each of the five cortisol features investigated. The coefficients reflect log relative differences in cortisol features according to levels of compliance (top), since cortisol levels were log transformed prior to constructing the features. Standardized coefficients (bottom) allow direct comparisons of the association with compliance across different features. People in the lowest tertile of compliance scores had CARs that were 9% lower (−0.09 in log-scale; 95% C.I.: −0.18, −0.01) than those in the highest tertile of compliance scores on average, prior to adjustment for covariates. This association is attenuated by 15% after adjustment for socio-demographic covariates (0.08 log-scale units lower in the low versus high compliance group (95% C.I.: −0.16, 0.00). The 0.09 difference is equivalent to 0.15 of a standard deviation of the (log) CAR distribution. Compliance was not significantly related to wake up, early decline (30 minutes to 2 hours post-awakening), late decline (2 to 16 hours post-awakening), or total cortisol output (AUC). The magnitude of the associations between compliance and these four additional features was less than 0.09 standard deviations of the distributions of the features.

Table 4 shows associations of socio-demographic characteristics with each cortisol feature, before and after adjustment for compliance scores, with significant associations in bold face. Among those significant associations, adjustments for compliance never resulted in more than 10% change in the coefficients. Among the remaining non-significant associations, eleven of them changed by more than 10% after adjustment for compliance. There were no changes in direction of associations or in the levels of significance of *p*-values for pairs of

associations among any of the variables, implying that adjustment for compliance does not have a large impact on the associations between demographic variables and cortisol features.

Table 5 presents intraclass correlations (ICCs) in the overall sample and across socio-demographic groups and tertiles of compliance levels. In general, reliabilities were higher for the AUC (0.66 for the full sample) followed by the wake up value (0.48). Reliabilities ranging from 0.28 to 0.37 were observed for the other samples. Adjustment for covariates (age, sex, race/ethnicity, education, income) did not materially change these estimates. There were no significant differences in ICCs for wake up cortisol levels or CARs according to socio-demographic characteristics, although there was a marginal trend for people in the highest tertile of compliance scores to have higher ICC for CARs (p= 0.06). Older people also had lower CAR ICC although differences with age were not statistically significant (p =0.08). The ICC for early decline differed significantly according to compliance scores and age. People who were more compliant with the sampling protocol and people ages 55 to 64 had significantly higher ICCs than people who were less compliant with the protocol and people under 55 or over 65 years of age, respectively. People ages 75 and older had the lowest early decline ICCs among all of the age groups. Persons with high compliance also had higher reliabilities for the early (p< 0.01) and late decline slope (p<0.05). Hispanic participants showed higher reliabilities for AUC than other race/ethnic groups but no other statistically significant differences were observed. There were no sex by age interactions in the observed associations (data not shown).

Because prior work in Hispanic populations has suggested variation in health behaviors and participation by nativity status (Jurkowski, 2006), we conducted analyses to examine the association between nativity (foreign born status) and compliance, and the ICC of the cortisol curve features by nativity. Of the 493 Hispanic participants, 378 (76.5%) were foreign born. We found a marginal association between foreign born status and compliance (p=0.064). The percentage of foreign born individuals in the high compliance category (82.6%) was greater than in the low (75.3%) or medium (71.9%) compliance categories(Table 6). Except for the ICC of the early decline slope (p<0.001), we found no statistically significant difference in the reliability of the features by foreign born status(Table 7). For the early decline slope, the reliability among US born was much smaller (ICC=0.11) compared to foreign born (ICC=0.39). This difference was primarily due to a larger between-subject variance among foreign born compared to US born. The next largest difference in ICC was for CAR (p=0.34), where the ICCs among US and foreign born was 0.28 and 0.19, respectively.

DISCUSSION

This study reports on levels of sampling protocol compliance and reliability of cortisol features on a large population–based diverse sample, and the extent to which compliance and reliability vary by socio-demographic characteristics. A higher compliance score was associated with higher income and higher education but not with age or sex. Compliance was associated with higher CAR values, but was not associated with any of the other features of the cortisol curve. Compliance likely most impacts the CAR because its determination is most impacted by timing of sample collection and the accuracy of the

wake-up time. If the first sample is not taken right at awakening and is delayed, it will be higher and there will be less difference in the awakening and 30 minute sample, resulting in a lower CAR. Similarly, if the wake-up sample is accurately collected but the timing of the 30 minute sample is delayed, the CAR will be underestimated because the second sample will have been collected after the actual 30 minute peak. In our analyses, adjustment for compliance did not affect associations of socio-demographic factors with various features of the daily cortisol profile. Reliability was higher for wake up values and AUC than for other features. Reliability did not vary substantially by socio-demographic characteristics although there was a suggestion of lower reliability at older ages and at lower levels of compliance for some features.

Knowing whether and how participants deviate from the requested sampling protocol is widely believed to be important for calculating reliable estimates of cortisol parameters. We found that the majority of saliva samples were provided within ten minutes of the requested time, although there were also a fair number of samples provided outside of a 15-minute window. Larger deviations from the requested protocol were most likely to occur in the middle of the day, rather than at wake up and bed time, which are particularly important samples for calculating the CAR, AUC, and late decline. We speculate that there were more protocol deviations in the afternoon because individuals were told to collect samples at noon (or before lunch if lunch occurred before noon) or at 6 pm (or before dinner if dinner happened before 6 pm), thus introducing greater flexibility in the protocol for these times. In addition they may have had difficulty remembering to collect these samples as scheduled if they were working during the day. Also, it may have been easier for participants to incorporate collection of waking and bed time samples into their established morning and night time routines whereas afternoons likely have less predictable routines. This has implications for estimating the diurnal slope. If samples are collected later than scheduled, but the time specified in the protocol is assumed as the time of collection, the diurnal slope estimate will likely be steeper than the actual slope as cortisol levels continue to fall throughout the afternoon. Failure to comply with protocols has previously been shown to produce unreliable and possibly biased estimates of cortisol features, with the cortisol profiles of non-compliant individuals differing significantly from those of compliant persons (Kudielka et al., 2003; Broderick et al., 2004), although one study found no differences in profiles between compliant and non-compliant persons (Jacobs et al., 2005). We found evidence that variations in compliance affected the CAR estimate but not other features of the cortisol profile.

To our knowledge, this is the first study to systematically investigate socio-demographic differences in compliance. We found that higher income and education were associated with slightly higher compliance. The extent to which compliance to sampling protocols differs across various demographic groups may have implications for the interpretation of findings regarding group level differences in some cortisol features. We found lower rates of compliance among persons of lower socioeconomic status, who have been previously shown to have flatter rates of decline across the day (Kudielka et al., 2003; Cohen et al., 2006; Hajat et al., 2010). Other work has reported that lower compliance is associated with more negative estimates of the daily decline (i.e., steeper slopes) (Kudielka et al., 2003) (although there was no evidence of this in our analyses). Taken together this suggests that prior studies

may have underestimated the associations of low socioeconomic status with a flatter decline. In contrast, prior research on the size of the CAR has been mixed, with persons of lower socioeconomic status having higher (Kunz-Ebrecht et al., 2004; Wright and Steptoe, 2005), lower (Bennett et al., 2004), or similar CARs across studies (Steptoe et al., 2003; Cohen et al., 2006; Hajat et al., 2010; Kumari et al., 2010). Although it is difficult to predict to what extent lack of compliance contributes to conflicting findings regarding associations of socioeconomic status and CARs, findings of lower CARs among persons of lower socioeconomic status could be partially attributable to socioeconomic status differences in compliance. In our analyses, however, compliance did not substantially affect estimates of associations of socio-demographic factors with cortisol. Nonetheless the socio-demographic differences in compliance that we did observe suggest that population variation in compliance could affect estimates of associations of various characteristics with cortisol in other samples.

The overall reliability for the AUC and wake up cortisol was moderate-to-high. The reliability of the CAR, and early and late decline slopes was lower and varied significantly by compliance to the protocol, with higher reliability for declines in particular among more compliant persons. Prior research has indicated that adherence to the protocol may be especially important when calculating estimates of the CAR, as cortisol levels are known to change most rapidly within the 30 to 45 minutes of waking (Pruessner, 1989; Kudielka, 2007). Our data support these prior findings. As previously discussed, delay in collection of the wake-up and/or 30 minute sample can underestimate the CAR. Prior studies of cortisol have analyzed rates of cortisol decline differently than the current study (typically measuring hourly rates of decline between wakeup to bed time, excluding the CAR sample), whereas we analyzed the decline in two segments (between 30 minutes and 2 hours post-awakening and between 2 hours post-awakening and bed time). Hence, it is unclear if the significant differences in reliability in early and late decline would be applicable to prior studies. Although differences were not large, there was some evidence that reliability was greater among persons who were more compliant.

Since there is limited research on the day-to-day reliability of estimates of cortisol features, no clear consensus exists regarding the optimal number of days of sampling. The existing research indicates that multiple day collection is preferable to single day collection whenever feasible (Smyth et al., 1997; Kraemer et al., 2006; reviewed in Adam &Kumari, 2009). Our findings regarding ICCs also points to the need for multiple days of sampling, but more so for CAR and decline slopes than wakeup and AUC.

Our study is among the first to examine whether reliability of cortisol features varies systematically according to socio-demographic factors. With the exception of lower reliability of the early decline slope in older persons and higher reliability of the AUC in Hispanics compared to other groups there were no marked differences in reliability by socio-demographic characteristics. Thus, it is unlikely that use of group-specific sampling protocols would be warranted, particularly if implementing them would increase the challenges of working with large, diverse populations. Our study is also the first to examine variation in sampling protocol compliance by nativity status in Hispanic individuals, where foreign born status was marginally associated with slightly higher compliance compared to

US born status. This is in contrast to health screening preventive practices which are more common among US compared to foreign born Mexican-Americans (Jukowski, 2006). Thus, the reason for slight differences in compliance based on nativity status in our population is unclear and warrants further investigation.

It is worth noting that the current study is limited by several factors. First, although we utilized trackcaps to monitor the timing of samples, we did not objectively measure time of waking. However, prior research indicates that participants are generally accurate in their reports of wake up times (Kraemer et al., 2006; DeSantis et al., 2009). In addition, we weighted all samples equally when calculating compliance scores. It is conceivable that weighting analyses such that wake up, wake up + 30 minutes, and possibly bed time samples, received more emphasis in the calculation of compliance scores than mid-day samples might have produced different results. Estimates of the wake up levels, CAR, and late decline are directly influenced by adherence to the protocol for these three samples in particular. Furthermore, estimates of AUC are largely determined by wake up values, as well as the CAR sample, as cortisol levels are typically the highest in the morning. Although we utilized one of the more intensive protocols of any large-scale population-based study, there were relatively few samples in the afternoon. Because this is the time period with the highest degree of non-compliance, it is difficult to know how our findings might differ if the protocol included more afternoon samples. Finally, we assessed short-term ICCs in samples collected over 3 days for cortisol curve features and our findings may not apply to sample collections separated by longer term follow-up. A recent study found much lower cortisol curve ICCs (most ≤0.13) across three populations compared to our study; however, their ICCs were determined for samples collected 8 to 24 months apart where greater long-term variability is expected (Ross et al, 2014). Additional studies are needed to confirm ICCs for sample collections separated by months to years. Their study participants were also younger (mean age in the 3 populations were 12.9, 17, and 50 years) than ours (mean age 65 years). Because our study population was older, this may limit generalizability of our findings to other populations.

This study documents acceptable compliance with a complex collection protocol in a large and diverse population sample. Although compliance was associated with socio-demographic characteristics, differences were small and adjustment for compliance did not appreciably modify estimates of associations of socio-demographic characteristics with cortisol features. Reliability was acceptably high for AUC, moderate for wake up values and lower for other features. Reliability of estimates of CAR and early declines were greater when compliance was greater. However, reliability was generally not associated with socio-demographic characteristics. Overall results suggest that three days of sampling with the given 6-sample protocol yields acceptable compliance and acceptable reliability, but that additional sampled days (or possibly additional samples per day) may be necessary to achieve higher reliability for some features.

ACKNOWLEDGEMENTS

Grants: MESA was supported by contracts NO1-HC-95159 through NO1-HC-95165 and NO1-HC-95169 from the National Heart, Lung, and Blood Institute. MESA Stress Study was supported by RO1 HL10161-01A1 and R21 DA024273 (PI: Dr. Diez-Roux).

The authors thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

References

1. Adam EK, Gunnar MR. Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. Psychneuroendocrinology. 2001; 26(2):189–208.
2. Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology. 2009; 34(10):1423–1436. [PubMed: 19647372]
3. Allison PD. Handling missing data by maximum likelihood. 2012 SAS Global forum: http://support.sas.com/resources/papers/proceedings12/312-2012.pdf.
4. Bennett GG, Merritt MM, Wolin KY. Ethnicity, education, and the cortisol response to awakening: a preliminary investigation. Ethn Health. 2004; 9:337–347. [PubMed: 15570679]
5. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr, Kronmal R, Lui K, Nelson JC, O'Leary D, Saad MF, Shea S, Szklo M, Tracy RP. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol. 2002; 156(9):871–881. [PubMed: 12397006]
6. Broderick JE, Arnold D, Kudielka BM, Kirschbaum C. Salivary cortisol sampling compliance: comparison of patients and healthy volunteers. Psychoneuroendocrinology. 2004; 29:636–650. [PubMed: 15041086]
7. Cohen S, Schwartz JE, Epel E, Kirschbaum C, Sidney S, Seeman T. Socioeconomic status, race and diurnal cortisol decline in the coronary artery risk development in yo*ung ad*ults (CARDIA) study. Psychosom Med. 2006; 68:41–50. [PubMed: 16449410]
8. DeSantis AS, Adam EK, Mendelsohn K, Doane LD. Concordance between self-reported and actual wake-up times in ambulatory salivary cortisol research: Implications for the cortisol response to awakening. Int'l J Behav Med. 2009; 17(1):74–78.
9. Gunnar MR, Vazquez DM. Low cortisol and a flattening of expected daytime rhythm: potential indices of risk in human development. Dev. Psychopathol. 2001; 13(3):515–538. [PubMed: 11523846]
10. Hajat A, Diez Roux AV, Franklin TG, Seeman T, Shrager S, Ranjit N, Castro C, Watson K, Sanchez BN, Kirschbaum C. Socioeconomic and race/ethnic differences in daily salivary cortisol profiles: The Multiethnic Study of Atherosclerosis. Psychoneuroendocrinology. 2010; 35:932–943. [PubMed: 20116177]
11. Jacobs N, Nicolson NA, Derom C, Delespaul P, van Os J, Myin-Germeys I. Electronic monitoring of salivary cortisol sampling compliance in daily life. Life Sci. 2005; 76(21):2431–2443. [PubMed: 15763075]
12. Jurkowski JM. Nativity and cardiovascular disease screening practices. J Immigr Minor Health. 2006; 8(4):339–346. [PubMed: 16732435]
13. Karlamangla A, Friedman E, Almeida D, Stawksi R, Seeman T. Daytime trajectories of cortisol: demographic and socioeconomic differences. Findings from the National Study of Daily Experiences. Psychoneuroendocrinology. 2013; 38:2585–2597. [PubMed: 23831263]
14. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychobiological research: an overview. Neuropsychobiology. 1989; 22(3):150–169. [PubMed: 2485862]
15. Kraemer HC, Giese-Davis J, Yutsis M, O'Hara R, Neri E, Gallagher-Thompson D, Taylor CB, Spiegel D. Design decisions to optimize reliability of daytime cortisol slopes in an older population. Am J Geriat Psychiat. 2006; 14(4):325–333.
16. Kudielka BM, Broderick JE, Kirschbaum C. Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. Psychosom Med. 2003; 65:313–319. [PubMed: 12652000]
17. Kudielka BM, Hawkley LC, Adam EK, Cacioppo JT. Compliance with ambulatory saliva sampling in the Chicago health, aging, and social relations study and associations with social support. Ann Behav Med. 2007; 34:209–216. [PubMed: 17927559]

18. Kumari M, Shipley M, Stafford M, Kivimaki M. Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. J Clin Endocr Metab. 2011; 96(5):1478–1485. [PubMed: 21346074]
19. Kumari M, Badrick E, Chandola T, Adler NE, Epel E, Seeman T, Kirschbaum C, Marmot MG. Measures of social position and cortisol secretion in an aging population: findings from the Whitehall II study. Psychosom Med. 2010; 72(1):27–34. [PubMed: 19995885]
20. Kunz-Ebrecht SR, Kirschbaum C, Marmot M, Steptoe A. Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. Psychoneuroendocrinology. 2004; 29(4):516–528. [PubMed: 14749096]
21. Little, RJA.; Rubin, DB. Statistical analysis with missing data. 2nd ed.. Hoboken, NJ: Wiley; 2002.
22. Park S, Lake ET. Multilevel modeling of a clustered continuous outcome. Nurs Res. 2005; 54(6): 406–413. [PubMed: 16317362]
23. Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, von Auer K, Jobst S, Kaspers F, Kirschbaum C. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. Life Sci. 1997; 61(26):2539–2549. [PubMed: 9416776]
24. Ross KM, Murphy MLM, Adam EK, Chen E, Miller GE. How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. Psychoneuroendocrinology. 2014; 39:184–193. [PubMed: 24119668]
25. Saxbe DE, Repetti RL, Nishina A. Marital Satisfaction, Recovery from Work, and Diurnal Cortisol Among Men and Women. Health Psychol. 2008; 27:15–25. [PubMed: 18230009]
26. Sephton SE, Sapolsky RM, Kraemer HC, Spiegel D. Diurinal cortisol rhythm as a predictor of breast cancer survival. J Nat'l Cancer Inst. 2000; 92(12):994–1000. [PubMed: 10861311]
27. Smyth JM, Ockenfels MC, Gorin AA, Catley D, Porter LS, Kirschbaum C, Hellhammer DH, Stone AA. Individual differences in the diurnal cycle of cortisol. Psychoneuroendocrinology. 1997; 22(2):89–105. [PubMed: 9149331]
28. Steptoe A, Kunz-Ebrecht SR, Owen N, Feldman PJ, Willemsen G, Kirschbaum C, Marmot M. Socioeconomic status and stress-related responses over the working day. Psychosom. Med. 2003; 65:461–470. [PubMed: 12764220]
29. Wright CE, Steptoe A. Subjective socioeconomic position, gender and cortisol responses to waking in an elderly population. Psychoneuroendocrinology. 2005; 30:582–590. [PubMed: 15808928]

Table 1

Percent of the study sample within categories of absolute time differences between actual and requested sample collection time, by sample time and day of study. Actual sampling times were recorded by Track Caps.

	0 to 5 mins	>5 to10 mins	>10 to15 mins	>15 mins	Missing
Average Wake[1]	53.9% (51.0%, 56.7%)	14.1% (13.9%, 14.3%)	7.8% (6.8%, 8.9%)	21.0% (19.7%, 21.9%)	3.3% (2.9%, 3.9%)
Average Wake + 30 mins[1]	38.6% (36.8%, 40.5%)	14.4% (13.8%, 14.9%)	10.0% (9.9%, 10.3%)	31.2% (30.0%, 33.4%)	5.8% (5.2%, 6.1%)
Average 1000 h[1]	42.1% (39.7%, 43.4%)	14.7% (13.5%, 16.6%)	7.5% (6.6%, 8.1%)	31.7% (28.2%, 34.7%)	4.0% (4%, 4.1%)
Average 1200 h[1]	30.4% (28.9%, 33.3%)	12.6% (11.4%, 13.6%)	8.1% (7.0%, 8.7%)	43.8% (40.3%, 45.8%)	5.1% (4.7%, 5.6%)
Average 1800 h[1]	33.9% (31.7%, 37.8%)	9.7% (8.1%, 11.3%)	8.4% (7.7%, 9.7%)	44.6% (41.8%, 46.3%)	3.4% (2.9%, 3.9%)
Average Bedtime[1]	62.1% (61.7%, 62.8%)	12.5% (12.2%, 12.9%)	4.7% (4.1%, 5.3%)	16.5% (15.5%, 17.2%)	4.3% (3.7%, 5.3%)
Average Day 1[2]	44.0%	13.3%	8.0%	30.4%	4.3%
Average Day 2[2]	44.0%	13.2%	7.1%	31.8%	4.0%
Average Day 3[2]	42.5%	12.5%	8.2%	32.2%	4.7%
All Samples	43.5%	13.0%	7.7%	31.5%	4.3%

[1]"Average" in the top six rows for wake, wake + 30 minutes, 1000 h, 1200 h, 1800 h and bedtime refers to the percentage of people whose average time difference between requested and actual sampling time falls within a given time range across the three days.

[2]The "average" numbers for the rows labeled "Day 1, Day 2, and Day 3" refers to the average delay in minutes across the six samples for each individual day.

Note: The numbers in parentheses represent the range for the percentage of people who fall within a certain time range for each sample for the three days.

Table 2

Percent of individuals within each category of selected socio-demographic characteristics by categories of compliance score.

Compliance		**Overall Compliance Score**			
		Lowest 1/3 (n=311)	**Middle 1/3 (n=321)**	**Highest 1/3 (n=303)**	***p*-value**
	%	**%**	**%**	**%**	
Age category (Mean/Median)		66.3/68	65.0/65	64.6/64	
<55 years old	17.0	15.1	16.2	19.8	
55 to 64 years old	31.1	28.3	33.3	31.7	
65 to 74 years old	33.0	33.1	33.6	32.3	
75 or older	18.9	23.5	16.9	16.2	
p-value					*0.18*
Sex					
Male	48.4	50.5	49.5	45.2	
Female	51.6	49.5	50.5	54.8	
p-value					*0.38*
Race/Ethnicity					
African American	27.6	31.5	27.4	23.8	
Hispanic	52.8	53.4	52.0	53.1	
Non-Hispanic White	19.6	15.1	20.6	23.1	
p-value					*0.07*
Income[†]					
< $25,000	38.4	44.7	36.1	34.3	
$25,000 to < $50,000	31.7	29.6	31.1	34.3	
$50,000 and higher	27.2	21.9	29.9	29.7	
p-value					*0.03*
Education					
Completed HS/GED or less	48.1	55.3	42.4	46.5	
Some college to Associate Degree	28.3	26.4	32.4	26.1	
Bachelors degree or more	23.6	18.3	25.2	27.4	

Compliance		Overall Compliance Score			
		Lowest 1/3 (n=311)	Middle 1/3 (n=321)	Highest 1/3 (n=303)	*p*-value
	%	%	%	%	
p-value					*0.01*

Note: The *p*-values obtained from Chi-square statistics, testing whether the compliance scores are significantly associated with the demographic variables.

† The income categories do not add to 100% due to missing data.

Table 3

Mean differences in selected cortisol features associated with categories of compliance score (high compliance as the reference) before and after adjustment for socio-demographic characteristics. Estimates are shown for cortisol values log-transformed prior to com computing individual-level features (Log-cortisol units), and for standardized variables (standard deviation units) to enable comparison across features.

	Wakeup		CAR		Early Decline Slope		Late Decline Slope		AUC	
	Crude	**Adj***	**Crude**	**Adj***	**Crude**	**Adj***	**Crude**	**Adj***	**Crude**	**Adj***
Log-cortisol units†										
Middle vs High compliance	0.02 (−0.07, 0.1)	0.01 (−0.07, 0.09)	−0.06 (−0.13, 0.01)	−0.05 (−0.12, 0.02)	0.00 (−0.07, 0.07)	0.00 (−0.07, 0.06)	0.01 (0, 0.02)	0.01 (0, 0.01)	0.04 (−0.03, 0.11)	0.03 (−0.03, 0.10)
Low vs High compliance	−0.04 (−0.13, 0.06)	−0.04 (−0.13, 0.05)	**−0.09 (−0.18, −0.01)**	**−0.08 (−0.16, 0.0)**	0.03 (−0.06, 0.11)	0.01 (−0.07, 0.09)	0.01 (0, 0.02)	0.00 (0, 0.01)	−0.01 (−0.08, 0.07)	−0.03 (−0.1, 0.05)
Standard deviation units										
Middle vs. high compliance	0.03 (−0.1, 0.15)	0.02 (−0.11, 0.14)	−0.09 (−0.21, 0.02)	−0.08 (−0.2, 0.03)	0.00 (−0.11, 0.11)	0.00 (−0.11, 0.11)	0.09 (0.01, 0.17)	0.08 (0.00, 0.16)	0.08 (−0.06, 0.21)	0.06 (−0.06, 0.19)
Low vs high compliance	−0.06 (−0.19, 0.08)	−0.06 (−0.2, 0.08)	**−0.15 (−0.29, −0.02)**	**−0.13 (−0.27, 0.00)**	0.04 (−0.1, 0.19)	0.02 (−0.12, 0.16)	−0.01 (−0.13, 0.12)	−0.02 (−0.14, 0.11)	−0.01 (−0.16, 0.14)	−0.05 (−0.2, 0.09)

Note:

† Beta coefficients from log-cortisol unit models (log relative differences) can be exponentiated to obtain relative differences in cortisol features by compliance score category.

* Adjusted models control for race/ethnicity, sex, age, income, and education. Statistically significant associations are bolded.

Table 4

Mean differences in selected cortisol curve features associated with socio-demographic characteristics before and after adjustment for compliance. Estimates are shown for cortisol values log-transformed prior to computing individual-level features (Log-cortisol units).

	Wakeup		CAR		Early Decline Slope		Late Decline Slope		AUC	
	Model 1	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**	**Model 1**	**Model 2**
Race/ethnicity										
African American	**−0.23 (−0.4, −0.07)**	**−0.23 (−0.39, −0.06)**	−0.1 (−0.26, 0.06)	−0.09 (−0.25, 0.07)	0.12 (−0.03, 0.28)	0.12 (−0.04, 0.28)	**0.23 (0.08, 0.38)**	**0.22 (0.08, 0.37)**	−0.09 (−0.26, 0.09)	−0.08 (−0.26, 0.1)
Hispanic	**−0.23 (−0.39, −0.07)**	**−0.23 (−0.39, −0.07)**	−0.06 (−0.22, 0.09)	−0.06 (−0.22, 0.09)	**0.20 (0.05, 0.35)**	**0.20 (0.04, 0.35)**	**−0.21 (−0.36, −0.07)**	**−0.22 (−0.36, −0.07)**	**−0.35 (−0.52, −0.17)**	**−0.35 (−0.52, −0.17)**
Gender										
Male	0.01 (−0.09, 0.12)	0.02 (−0.09, 0.12)	−0.1 (−0.2, 0)	−0.1 (−0.2, 0.01)	**0.30 (0.2, 0.4)**	**0.30 (0.2, 0.4)**	**−0.11 (−0.2, −0.02)**	**−0.11 (−0.2, −0.02)**	**0.25 (0.14, 0.36)**	**0.25 (0.14, 0.36)**
Age										
55–64	**0.15 (0, 0.31)**	**0.15 (0, 0.31)**	0.04 (−0.11, 0.19)	0.04 (−0.11, 0.19)	−0.07 (−0.21, 0.08)	−0.07 (−0.21, 0.08)	0.04 (−0.1, 0.18)	0.03 (−0.11, 0.17)	**0.18 (0.01, 0.35)**	**0.18 (0.01, 0.34)**
65–74	**0.26 (0.11, 0.41)**	**0.26 (0.11, 0.41)**	−0.1 (−0.24, 0.05)	−0.09 (−0.24, 0.06)	0.02 (−0.13, 0.17)	0.02 (−0.13, 0.16)	**0.14 (0, 0.28)**	**0.14 (0, 0.28)**	**0.37 (0.2, 0.54)**	**0.37 (0.2, 0.53)**
75+	**0.44 (0.26, 0.61)**	**0.44 (0.27, 0.62)**	−0.13 (−0.3, 0.05)	−0.12 (−0.29, 0.05)	0.01 (−0.16, 0.18)	0 (−0.17, 0.17)	**0.21 (0.05, 0.37)**	**0.2 (0.05, 0.36)**	**0.57 (0.38, 0.76)**	**0.57 (0.38, 0.76)**
Income										
$25,000 to <$50,000	−0.1 (−0.23, 0.02)	−0.1 (−0.23, 0.02)	0.07 (−0.06, 0.19)	0.06 (−0.07, 0.18)	0.07 (−0.06, 0.19)	0.08 (−0.05, 0.2)	−0.07 (−0.18, 0.05)	−0.06 (−0.18, 0.05)	−0.06 (−0.2, 0.08)	−0.06 (−0.2, 0.08)
$50,000 and higher	0.07 (−0.08, 0.22)	0.07 (−0.08, 0.22)	0.05 (−0.09, 0.2)	0.05 (−0.1, 0.19)	0.02 (−0.12, 0.17)	0.03 (−0.11, 0.18)	−0.16 (−0.29, −0.02)	−0.15 (−0.29, −0.02)	−0.09 (−0.25, 0.08)	−0.09 (−0.25, 0.07)
Education										
Some college to Associate Degree	0.08 (−0.05, 0.21)	0.08 (−0.05, 0.21)	−0.03 (−0.16, 0.1)	−0.02 (−0.15, 0.11)	**−0.15 (−0.28, −0.02)**	**−0.14 (−0.27, −0.01)**	0.09 (−0.03, 0.21)	0.09 (−0.03, 0.2)	−0.07 (−0.21, 0.07)	−0.08 (−0.22, 0.06)
Bachelors degree or more	0.07 (−0.09, 0.23)	0.07 (−0.09, 0.22)	0.01 (−0.14, 0.16)	0.01 (−0.15, 0.16)	−0.14 (−0.29, 0.01)	−0.13 (−0.28, 0.02)	0.16 (0.02, 0.31)	0.16 (0.02, 0.31)	0 (−0.17, 0.17)	−0.01 (−0.18, 0.16)

Note: Beta coefficients represent the log relative difference in cortisol features by sociodemographic category, since cortisol levels were log-transformed prior to constructing the features. For example, African Americans have 100%*[1-exp (−0.23)]=20.5%lower wake-up cortisol than whites with adjustment for other socio-demographic features (model 1) and compliance score (model 2). Reference category for race is non-Hispanic White; for gender is female; for age is 45 to 54 years old; for income is less than or equal to $25,000/year; and for education is high school/GED or less. Model 1 is adjusted for all other socio-demographic characteristics (race/ethnicity, sex, age, income, and education). Model 2 is additionally adjusted for level of compliance. Statistically significant associations are bolded.

Table 5

Intraclass correlation coefficients for repeat measures across days for each feature of the cortisol diurnal profile by sociodemographic characteristics

	Wakeup	CAR	Early Decline Slope	Late Decline slope	AUC
	ICC	**ICC**	**ICC**	**ICC**	**ICC**
Full Sample, Crude ICC	0.48	0.28	0.37	0.32	0.66
Full Sample, ICC after adjusted for covariates (race/ethnicity, sex, age, income, education, compliance)	0.45	0.27	0.34	0.28	0.63
Race/Ethnicity					
African American	0.49	0.31	0.39	0.31	**0.60**
Hispanic	0.47	0.26	0.34	0.29	**0.69**
Non-Hispanic White	0.45	0.29	0.36	0.25	**0.61**
p-value	*0.80*	*0.73*	*0.66*	*0.57*	***0.03***
Sex					
Male	0.50	0.29	0.34	0.30	0.64
Female	0.46	0.27	0.36	0.33	0.66
p-value	*0.24*	*0.68*	*0.60*	*0.42*	*0.55*
Age category					
45–54	0.37	0.25	**0.33**	0.38	0.65
55–64	0.48	0.25	**0.46**	0.31	0.63
65–74	0.50	0.37	**0.34**	0.32	0.66
75+	0.53	0.19	**0.22**	0.24	0.62
p-value	*0.10*	*0.08*	***0.01***	*0.29*	*0.81*
Income Category					
< $25K	0.51	0.29	0.36	0.34	0.64
>=$25 <50K	0.48	0.28	0.38	0.35	0.70
>=50K	0.44	0.27	0.33	0.26	0.63
p-value	*0.37*	*0.97*	*0.74*	*0.22*	*0.20*
Education					
Completed HS/GED or less	0.46	0.25	0.35	0.28	0.67
Some college to Associate Degree	0.49	0.28	0.38	0.34	0.68

	Wakeup	CAR	Early Decline Slope	Late Decline slope	AUC
	ICC	**ICC**	**ICC**	**ICC**	**ICC**
Bachelors degree or more	0.50	0.33	0.36	0.34	0.58
p-value	*0.75*	*0.45*	*0.87*	*0.42*	*0.09*
Compliance Score					
Lowest tertile	0.46	0.25	**0.35**	**0.28**	0.63
Middle tertile	0.44	0.22	**0.26**	**0.28**	0.65
Highest tertile	0.54	0.35	**0.47**	**0.40**	0.70
p-value	*0.09*	*0.06*	***<0.01***	***0.03***	*0.20*

ICC=intraclass correlation coefficient

Statistically significant associations are bolded.

Table 6

Percent of Hispanic individuals (N=494) who were foreign or US born by categories of compliance score.

Compliance		Overall Compliance Score			
		Lowest 1/3 (n=166)	Middle 1/3 (n=167)	Highest 1/3 (n=161)	*p*-value
	%	%	%	%	
Nativity					
Foreign Born	76.5	75.3	71.9	82.6	
US Born	23.5	24.7	28.1	17.4	
p-value					*0.07*

Note: The *p*-value is derived from the Chi-square statistic, testing whether the compliance scores are significantly associated with nativity.

Table 7

Intraclass correlation coefficients for repeat measures across days for each feature of the cortisol diurnal profile by nativity among the Hispanic sample (N=494).

	Wakeup	CAR	Early Decline slope	Late Decline slope	AUC
	ICC	ICC	ICC	ICC	ICC
Nativity					
Foreign Born	0.47	0.28	**0.39**	0.30	0.69
US Born	0.45	0.20	**0.11**	0.28	0.69
p-value	0.83	0.34	<**0.001**	0.82	0.89

ICC=intraclass correlation coefficient
Statistically significant associations are bolded.