Published in final edited form as:

Psychoneuroendocrinology. 2013 July ; 38(7): 1036–1046. doi:10.1016/j.psyneuen.2012.10.007.

Examining the association between salivary cortisol levels and subclinical measures of atherosclerosis: the Multi-Ethnic Study of Atherosclerosis

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All authors declare they have no conflicts of interest.

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Abstract

Objective—To investigate the association between salivary cortisol and two markers of subclinical cardiovascular disease (CVD), coronary calcification (CAC), and ankle-brachial index (ABI).

Methods—Data from an ancillary study to the Multi-Ethnic Study of Atherosclerosis (MESA), the MESA Stress Study, were used to analyze associations of salivary cortisol data collected six times per day over three days with CAC and ABI. The authors used mixed models with repeat cortisol measures nested within persons to determine if specific features of the cortisol profile were associated with CAC and ABI.

Results—total of 464 participants were included in the CAC analysis and 610 in the ABI analysis. The mean age of participants was 65.6 years. A 1-unit increase in log coronary calcium was associated with a 1.77% flatter early decline in cortisol (95% CI: 0.23, 3.34) among men and women combined. Among women low ABI was associated with a steeper early decline (−13.95% CI: −25.58, −3.39) and a marginally statistically significant flatter late decline (1.39% CI: −0.009, 2.81). The cortisol area under the curve and wake to bedtime slope were not associated with subclinical CVD.

Conclusions—This study provides weak support for the link between cortisol and measures of subclinical atherosclerosis. We found an association between some features of the diurnal cortisol profile and coronary calcification and ABI but associations were not consistent across subclinical measures. There are methodological challenges in detecting associations of cortisol measures at a point in time with health outcomes that develop over a lifetime. Studies of short-term mechanisms linking stress to physiological processes related to the development of early atherosclerosis may be more informative.

Keywords

salivary cortisol; ankle brachial index; coronary calcification; atherosclerosis; stress; cortisol awakening response; cortisol diurnal pattern; Multi-Ethnic Study of Atherosclerosis

INTRODUCTION

Exposure to chronic stress has been linked to cardiovascular disease (CVD) (Hemingway and Marmot, 1999; Rosengren et al., 2004). Stress may impact the risk of CVD through the alteration of the functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Cohen et al., 1997). This alteration may result in differential exposure to the stress hormone cortisol. In addition to its metabolic, immunologic and homeostatic functions, cortisol has numerous physiologic effects relevant to the development of cardiovascular disease. For example, cortisol affects the development of insulin resistance (Phillips et al., 1998; Reynolds and Walker, 2003) and central adiposity (Björntorp and Rosmond, 2000), influences blood pressure regulation (Whitworth et al., 1995) and inflammatory processes (Petrovsky et al., 1998). In population studies levels of salivary cortisol within the first 30 minutes of awakening have been found to be positively associated with central obesity (Björntorp, 1997; Björntorp and Rosmond, 2000; Epel et al., 2000; Ranjit et al., 2005; Strike and Steptoe, 2004); flatter cortisol slopes throughout the day have been linked to inflammation

(DeSantis, 2012; Nijm and Jonasson, 2009; Petrovsky et al., 1998), and to absence of nocturnal blood pressure dipping (Holt-Lunstad and Steffen, 2007); and higher total cortisol output and flatter early declines have been associated with diabetes (Champaneri et al., 2012; Chiodini et al., 2005; Oltmanns et al., 2006).

Existing evidence of the association between cortisol and CVD risk factors has raised questions about the link between cortisol and the development of atherosclerosis. The investigation of subclinical atherosclerotic disease is of special relevance because it allows for examination of whether cortisol levels are related to early asymptomatic disease. Although there is not sufficient evidence to draw conclusions about the links between chronic stress and measured levels of cortisol, it has been suggested that chronic stress results in alteration of the daily cortisol pattern (Miller et al., 2007). Thus finding an association between alterations in the diurnal cortisol pattern and subclinical atherosclerosis would be consistent with the theory that chronic stress contributes to the development of early CVD. In addition, the use of subclinical measures avoids reverse causation biases that may occur if, for example, clinical events are themselves the cause of the cortisol alteration.

Research to date has investigated the association between cortisol and several CVD related outcomes. Data from the Whitehall II study found flatter cortisol slopes across the day were associated with CVD mortality (Kumari et al., 2011). Another prospective study reported the association between higher levels of urinary cortisol and CVD mortality (Vogelzangs et al., 2010). Others have found that high levels of serum cortisol were independent predictors of cardiac events and mortality among patients with chronic heart failure (Guder et al., 2007; Yamaji et al., 2009). In terms of subclinical atherosclerosis, a few studies have found an association between cortisol and coronary calcification (CAC) (Hamer et al., 2012; Hamer et al., 2010; Matthews et al., 2006), while others have found some evidence for an association of cortisol with intima media thickness (IMT), a measure of early atherosclerosis in the carotid artery (Eller et al., 2001). The number of lesions in either coronary or carotid arteries, and stenosis of these arteries have also been linked to cortisol (Alevizaki et al., 2007; Dekker et al., 2008; Koertge et al., 2002). In addition, studies have documented a link between cortisol and endothelial dysfunction (Broadley et al., 2005; Broadley et al., 2006; Fantidis, 2010; Violanti et al., 2009). Although the literature in general suggests an association between subclinical atherosclerosis and cortisol, only two studies were based on population based samples (Dekker et al., 2008; Matthews et al., 2006), many had very small sample sizes (Alevizaki et al., 2007; Broadley et al., 2005; Eller et al., 2001; Eller et al., 2005; Peppas-Patrikiou et al., 1998; Troxler et al., 1977; Violanti et al., 2009) and several collected only one serum or urine cortisol sample thus making it impossible to assess diurnal cortisol profiles (Alevizaki et al., 2007; Koertge et al., 2002; Peppas-Patrikiou et al., 1998; Reynolds et al., 2009).

The Multi-Ethnic Study of Atherosclerosis (MESA) provides a unique opportunity to improve upon previous studies in the examination of the association between salivary cortisol and well-established measures of subclinical cardiovascular disease. Detailed cortisol data allows for characterization of cortisol profiles over several days, while the availability of measures of subclinical disease allows for an examination with measures of atherosclerosis in different vascular beds. The nature of the data also allows for an analytic

approach that quantifies the association of various features of the daily cortisol curve with subclinical atherosclerosis. The goal of this study is to investigate the association between salivary cortisol and subclinical CVD in two vascular beds: CAC (a marker of atherosclerosis in coronaries) and ankle brachial index (ABI) (a marker of atherosclerosis in the lower extremities). Our apriori hypothesis was that there would be evidence of alterations of the daily cortisol profile (such as flatter slopes) among those with more advanced subclinical atherosclerosis.

METHODS

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. At baseline MESA included 6814 men and women aged 44 to 84 years without clinical cardiovascular disease recruited from six sites across the US. Eligibility for the study was ascertained through self-reported information (Bild et al., 2002).

The MESA Stress Study is an ancillary study which collected data on several stress hormones in conjunction with the third and fourth follow-up exams of the full MESA cohort (between 2004 and 2006). Participants were enrolled at the New York and Los Angeles MESA sites in the order in which they attended the follow-up exam. Enrollment continued until approximately 500 participants were enrolled at each site (total n=1002). This procedure resulted in an approximately random sample of white, black and Hispanic participants from each site. Compared to other eligible participants at the two sites, the MESA Stress Study was similar to the parent study, with few exceptions. There were fewer persons in the 75 – 84 year age range (12.1% compared to 18.2% in the overall MESA study), slightly more men (47.6% compared to 44.7%) and more participants with some college education (29.7% compared to 23.9%). The study was approved by the University of California Los Angeles, Columbia University and University of Michigan institutional review boards.

Measures

Cortisol was collected through six saliva samples over three days from 1002 participants. The first sample was taken immediately after waking (and before getting out of bed), the second sample 30 minutes later, the third sample around 1000h, the fourth sample around noon (or before lunch if lunch occurred before noon), the fifth sample around 1800h (or before dinner if dinner occurred before 1800h), and the sixth sample directly before bed. Saliva samples were collected using Salivette collection tubes. A time tracking device (Track Caps) automatically registered the time at which cotton swabs were extracted to collect each sample. Samples were excluded from analysis if they were missing cortisol values or did not have Track Caps data. Figure 1 provides an explanation of the exclusions used in this study. Samples were stored at -20°C until analysis. Before biochemical analysis, samples were thawed and centrifuged at 3000 rpm for three minutes 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 and was log transformed for analyses.

Two measures of subclinical atherosclerosis were investigated, CAC and ABI, each reflecting atherosclerosis in different vascular beds. Given the data collection schedule of the parent study, CAC and ABI were not collected simultaneously with cortisol for all MESA stress participants, thus associations with CAC and ABI were examined in subsets of the study populations (see Figure 1). Coronary calcium was assessed by chest CT using either a cardiac-gated electron-beam CT scanner (Breen et al., 1992) or a multidetector CT system (Carr et al., 2000; Carr et al., 2005). All participants were scanned twice over phantoms of known physical calcium concentration and agreement between the two scans was high (kappa statistic =0.92) (Carr et al., 2005). The use of a calibration phantom for each participant reduces the amount of variability produced by the different devices used (Nelson et al., 2005). All scans were read by a cardiologist to identify and quantify coronary calcification, calibrated according to the readings of the calcium phantom. Scans were read blindly with respect to scan pairs and to other participant data using a computer interactive scoring system similar to that described by Yaghoubi et al (Yaghoubi et al., 1995). The average Agatston score (Agatston et al., 1990) for the two scans was used in all analyses.

Main analyses examined the amount of calcification as a continuous variable. CAC was log transformed because of its skewed distribution and one was added to all values before transformation in order to incorporate those with no calcification into the analysis. CAC was examined using a few alternate specifications as well: dichotomized as the absence or presence of CAC (an Agatston score > 0), dichotomized as the top quintile of CAC versus not in the top quintile and the log of calcium among those with non-zero calcification. Stress participants were included in analyses of CAC if they had a calcium measure that was either simultaneous with cortisol assessment (n=347) or within 18 months after the cortisol assessment (n=153).

ABI was determined by measuring blood pressure with a Doppler probe in the bilateral brachial, dorsalis pedis, and posterior tibial arteries (McDermott et al., 2000). ABI was calculated as the ratio of systolic blood pressure from dorsalis pedis or posterior tibial (whichever was higher) to the average of the brachial pressures. Ratios were calculated separately for the left and right side and the minimum ratio was used for analyses. In clinical practice, ABI has been used to diagnose and assess the severity of peripheral artery disease (PAD) in the legs (Fowkes et al., 2008). ABI has been used as a measure of atherosclerosis of the lower extremities in prior work (McKenna et al., 1991; Vogt et al., 1993). Typically ABI values of 0.9 are considered high risk, but several recent studies have found that values between 0.9 and 1.1 are also linked to elevated CVD risk (Fowkes et al., 2008; McDermott et al., 2005). In our study the prevalence of ABI 0.9 was low (3.7%), thus using this cut point would have resulted in a lack of power. Therefore ABI was dichotomized as <1.1 versus 1.1. In addition to the dichotomous specification of ABI, we also examined it as a continuous variable. ABI was measured simultaneously with cortisol for 656 Stress study participants.

We conducted statistical tests for effect modification by sex, and examined sex-stratified models when effect modification by sex was present. All models were adjusted for sex (unless sex-stratified), age, race/ethnicity, income-wealth index and wake-up time. The income-wealth index ranges from zero to eight, where zero represents the poorest participants and eight the wealthiest. More detail on how the income-wealth index was derived is available elsewhere (Hajat et al., 2010). In addition diabetes, hypertension, smoking, body mass index (BMI) and dyslipidemia were considered potential confounders of the cortisol subclinical CVD association. Diabetes was defined by the 2003 ADA criteria (fasting glucose ≥ 126 mg/dL or use of hypoglycemic medication). Hypertension was based on the JNC VI criteria: systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or use of anti-hypertensive medication. Self-reported smoking was classified as current, former or never smokers. Body mass index was calculated as weight (kg)/height (m)². Dyslipidemia was defined as total cholesterol to high density lipoprotein (HDL) cholesterol ratio of greater than five or use of cholesterol reducing medications. Psychosocial measures such as depression, anxiety and chronic burden, may also confound the association between cortisol and subclinical disease. Depression was measured by the 20-item Center for Epidemiologic Studies Depression scale (Radloff, 1977). Anxiety was measured using the 10-item Spielberger trait anxiety inventory (Spielberger, 1980) and chronic burden was derived from a 5-item scale regarding difficulties in five separate domains of life (Bromberger and Matthews, 1996). We also adjusted for daily measures of acute stressors that could potentially affect cortisol on a given day. On the day of cortisol sampling participants were asked to rate their day in terms of how busy, stressed or pressured it was; responses included lower than normal, normal or higher than normal stress levels. We also adjusted for whether or not the participant attended work on the day of sampling.

Statistical analysis

We used mixed models with repeat log-transformed cortisol measures nested within persons as the dependent variable and the subclinical disease markers as predictors (in addition to relevant covariates) to estimate cross-sectional associations between features of the cortisol curve and subclinical atherosclerosis. Because the analyses are cross-sectional causality cannot be established. However the presence of a cross-sectional association would suggest that further research to establish causation may be warranted. The use of repeat cortisol measures as the dependent variable has the advantage of allowing us to directly examine associations of subclinical measures with various features of the cortisol curve simultaneously, using mixed models with splines as described below. This approach is more statistically efficient than approaches that create summary measures and then include those measures as predictors in models with health outcomes as dependent variables (Adam, 2006; Hruschka et al., 2005).

The daily pattern of cortisol levels includes a sharp rise during the first 30 – 45 minutes immediately after awakening (known as the cortisol awakening response (CAR)), followed by a gradual decline over the rest of the day. In order to capture this non-linear pattern and based on previous analyses (Hajat et al., 2010), we used a piecewise linear regression model with two fixed knots placed at 30 and 120 minutes after wake-up. The location of the knots

indicates where there are changes in the diurnal cortisol profile. Knot locations were chosen based on exploratory analysis using LOESS curves¹. The main effects of subclinical disease measures as well as interactions between the subclinical measures and various slopes were used to estimate associations of subclinical measures with various features of the cortisol profile (e.g. wake up, cortisol awakening response, early decline and late decline).

Main effects of covariates as well as their interactions with different pieces of the daily slope were included to estimate adjusted associations of subclinical disease with cortisol before and after adjustment for the potential confounders listed above. Coefficients from the models were exponentiated (as were confidence intervals) and are interpreted as percent differences.

Within-person correlations and person-to-person variation in slopes were accounted for by random components for the person specific intercept and person specific slopes. Day level variability was addressed through fixed effects for days. The first and third slopes were modeled as random; however, results were invariant regardless of which of the two slopes were modeled as random. An unstructured covariance matrix was used for the random components.

In addition, we investigated two summary measures of the daily cortisol profile: area under the curve (AUC) for a 16 hour day and wake-up to bed-time slope. Both measures were investigated using variants of the mixed models described above.

Alternative analytic approaches were also explored. We used a mixture model to identify three distinct cortisol profiles (or trajectories) over the course of the day (Jones et al., 2001): a consistently high, consistently low and a relatively normal cortisol profiles. We then modeled these profiles as a function of subclinical measures and covariates using repeated measures multinomial models. Results were highly imprecise (i.e. confidence intervals were wide) and are therefore not reported.

RESULTS

Table 1 provides summary characteristic for all participants included in either the ABI or CAC analysis (n=664), overall and by gender. A total of 410 participants were in both the ABI and CAC analyses. About 6% of the samples were missing (n=746) across the three days, but most participants collected at least some samples on each of the three days. Only 1% (n=20) of days were missing completely. Specifically 85% of participants had five or more samples collected on all 3 days of data collection. The mean age of the sample was 65 years old with more than half the participants identifying as Hispanic (57.0%), 27.5% as African-American and 15.5% as white. The overall smoking prevalence was 9.9% while hypertension, dyslipidemia and diabetes prevalence were 50.9%, 40.7% and 16.3% respectively. There were significant differences between men and women for several CVD risk factors. Women had a higher obesity and hypertension prevalence than men, and men had a higher smoking prevalence than women. There were also statistically significant

¹LOESS stands for locally-estimated scatter plot smoothing. It is a nonparametric technique which allows for greater flexibility because no assumptions about the global form of the regression surface are needed.

differences in ABI and CAC by gender. Men had more CAC (measured by log calcium or prevalence of some calcification) and women had lower values of ABI (indicating a higher risk for PAD). Cortisol was similar for men and women at wake-up, 30 minutes later and at bedtime, but greater for men for the three other samples.

After excluding those with no track-cap time, insufficient sample for assay, unreliable cortisol values, steroid users and those with missing covariate data we were left with 463 participants for the CAC analysis and 599 participants for the ABI analysis. Tables 2 shows percent differences in different aspects of the daily cortisol profile associated with CAC and ABI. Separate estimates are shown for cortisol at wake-up and for three different portions of change over the day: (1) CAR or the morning rise (the increase between wakeup and 30 min), (2) the decline between 30 and 120 min after wake-up (henceforth referred to as “early decline”) and (3) the decline between 120 min after wake-up and bedtime (henceforth referred to as “late decline”). Positive percent differences in wake-up levels indicate higher cortisol levels. Positive percent differences in the CAR indicate a more pronounced or steeper increase and positive percent differences in the early or late decline indicate a less pronounced or flatter decline. Model 1 adjusts for age, race/ethnicity, income-wealth index, day and wake-up time. In addition to the covariates in model 1, model 2 adjusts for BMI, dyslipidemia, hypertension, diabetes and smoking, model 3 further adjusts for depression, anxiety and chronic burden and model 4 adds measures of daily stress such as if the participant went to work and a self-reported assessment of how stressful their day was relative to a normal day.

For CAC, a 1 unit increase in log coronary calcium was associated with a 1.94% flatter early decline (95% confidence intervals (CI): 0.49, 3.41) among the total population. This association was slightly attenuated after adding additional covariates to the model: model 2 (1.77% CI: 0.21, 3.36), model 3 (1.74% CI: 0.17, 3.34) and model 4 (1.77% CI: 0.23, 3.34). Associations of CAC with a slightly steeper late decline were found in models 3 and 4 (−0.24% CI: −0.48, 0.01). Effect modification by sex was not statistically significant in the minimally adjusted model (p-value = 0.15), so sex-stratified analyses were not conducted. Other specifications of CAC, such as comparing those with and without CAC and looking only at those with some calcification showed no association with salivary cortisol. As seen in table 3, the AUC and wake-to-bed slope models revealed no association between cortisol and CAC.

Participants with low ABI had a steeper early decline compared to those with normal ABI (model 1: −8.44% CI: −16.18, −1.21). This association was attenuated in model 4 (−7.01 CI: −15.09, 0.50) and did not reach statistical significance (p-value = 0.07). For ABI, the interaction term for sex was statistically significant in the minimally adjusted model (p-value = 0.02), so sex-stratified analyses were conducted. Stratification by sex revealed that the pattern was only present among women. Model 1 showed a 15.4% steeper early decline among women with low ABI compared to those with normal ABI (CI: −26.78, −5.03). This association was slightly attenuated in model 2 (13.98% steeper decline CI: −26.01, −3.10) and similar in models 3 and 4, respectively (14.11% steeper decline in those with low vs normal ABI (CI: −26.01, −3.34) in model 3 and 13.95% steeper decline (CI: −25.58, −3.39) in model 4). Also, the late decline was flatter among women with low ABI versus those with

normal ABI in models 1 – 3 (model 1: 1.39% flatter decline (CI: 0.11, 2.70); model 2: 1.50% flatter decline (CI: 0.11, 2.90); and model 3: 1.41% flatter decline (CI: 0.03, 2.81)). In model 4 the magnitude of the estimate was similar to previous models (1.39% flatter decline) but the CI was slightly wider and not significant at the 0.05 level (–0.009, 2.81). The data also suggested that women with low ABI had 13.96% higher wake-up cortisol levels after controlling for all potential confounders (model 4), but the results were not statistically significant at the 0.05 level (CI: –0.28, 30.22, p -value=0.055). There was no association between ABI and cortisol among men. ABI specified as a continuous variable was not associated with cortisol, nor did we find any evidence of an association between ABI and AUC or wake-to-bed slope (table 3).

DISCUSSION

In this population-based sample we observed few associations between selected features of the cortisol curve and markers of subclinical atherosclerosis after controlling for CVD risk factors and other possible confounders. Associations were generally weak, were present for only some specifications of the subclinical measures, and were not always consistent across men and women. Furthermore, summary measures such as AUC and wake-to-bed slope showed no association with subclinical CVD. Among the total population the early decline was flatter for those with more coronary calcification. Women with low ABI had steeper early declines and flatter late declines compared to women with normal ABI. Population-based studies exploring the association between cortisol and subclinical disease are sparse. An important strength of our study compared to prior work is the investigation of associations of daily cortisol patterns with measures of subclinical atherosclerosis in different vascular beds.

To our knowledge, only one existing study has examined CAC and cortisol in a population-based cohort. In a younger cohort ($n=718$) with a low prevalence of CAC, Matthews et al found that persons with flatter cortisol slopes over the day were more likely to have CAC (Matthews et al., 2006). Our study is consistent with Matthews et al in that we found some evidence of a flatter decline in the early part of the day associated with higher levels of continuous measures of CAC. However, in contrast to Matthews et al we found no associations with the dichotomized measure of CAC (presence vs. absence). The differential effect of various specifications of CAC may have to do with the age of the participants and with differences in the distribution of CAC. Younger participants such as those studied by Matthews et al have lower prevalence of CAC than older ones like ours (CAC prevalence was 8.1% in Matthews et al vs 54.4% in our sample). When prevalence is higher measures that take advantage of the full distribution (such as our continuous measure) may be better suited to detect associations especially in the context of small samples. In contrast in younger samples the prevalence of CAC may be a better marker of those with substantial disease early in life and it may be these more extreme cases that are linked to cortisol. It is also possible that cortisol is more relevant to the development of atherosclerosis very early in the process and therefore primarily in younger populations rather than in older samples with already substantial subclinical disease.

Another cross-sectional laboratory-based study found no association between cortisol response following a mentally challenging task and the presence of detectable CAC (Agaston score > 0), but did report an association between cortisol response and the presence of CAC (Agaston score >100) (Hamer et al., 2010). A recent follow-up study in the same population found no association between the progression of CAC and baseline levels of cortisol or AUC (Hamer et al., 2012). Our findings are consistent with the null association between diurnal cortisol and the presence or absence of CAC in the cross-sectional study reported by Hamer et al. The authors attribute the null findings in the progression study to the diurnal nature of cortisol and advocate for more rigorous characterization of the cortisol profile, as is done in our study.

Our CAC analyses included persons with cortisol and CAC measured simultaneously as well as persons with CAC measured at most 18 months after cortisol. We examined the correlations coefficients for those Stress Study participants with at least two CAC measurements taken at different times and found a high overall correlation between the two measures (0.94); therefore, we felt using CAC measured after cortisol was an acceptable approach as it helped increase our sample size. In addition, having CAC measures after cortisol is consistent with a conceptual model in which cortisol profiles are related to the subsequent development of atherosclerosis.

We are aware of only one study which investigated plasma cortisol levels in relation to low ABI (defined as < 0.9) and found no association (Reynolds et al., 2009). Some authors have argued that the use of plasma cortisol is not as useful as salivary cortisol because it does not provide information about the diurnal rhythm of cortisol which is seen as an essential indicator of the HPA axis functioning (Adam and Kumari, 2009). We found associations of low ABI with early and late decline in cortisol only among women. Low ABI was significantly more common in women than in men in our sample which could affect our ability to detect associations of cortisol with ABI. However, associations of ABI with daily cortisol patterns were inconsistent and unexpected in that low ABI was associated with a steeper early decline but a flatter late decline. Further studies on ABI and cortisol need to be conducted before any conclusions can be drawn as these findings may have resulted from chance fluctuations because of small sample size.

CAC and ABI are measures of atherosclerosis in different vascular beds. It appears that CAC is a stronger predictor of incident CVD events compared to other measures such as carotid intima media thickness (Folsom et al., 2008). ABI also predicts future CVD events, even after controlling for CAC and carotid intima media thickness (Criqui et al., 2010). Both subclinical measures are predictive of future CVD events, but each appears to capture different information regarding the presence of atherosclerosis. A limitation of CAC is that by definition it measures only calcified atherosclerosis; in our analysis we did not have a measure of noncalcified or vulnerable plaques. The presence of vulnerable plaques among those with the absence of calcium has been documented in the MESA cohort (Rosen et al., 2009). Additional work is needed to better understand whether cortisol might be differentially related to atherosclerosis in various vascular beds or to calcified versus noncalcified lesions.

In our study cortisol was measured over a three day period, while subclinical atherosclerosis develops over a lifetime. The stability of cortisol measurements over time is unknown, thus it is unclear if the cortisol measures obtained during our study are representative of the participant's long-term cortisol levels. Furthermore, we do not know how long one must be exposed to alterations of cortisol in order for subclinical atherosclerosis to be adversely affected. As with other studies, variation in adherence to the cortisol sampling protocol could be another potential weakness of our study. However, our use of track caps to capture the exact time the sample was taken has been shown to improve compliance with cortisol measurements (Kudielka et al., 2003). An additional limitation in our study is the fact that the sample was restricted to persons without clinical CVD at baseline. It is plausible that cortisol levels are less strongly associated with subclinical disease in these individuals.

Furthermore, the cross-sectional nature of this study makes causality impossible to determine. Future work is needed to assess longitudinal associations using multiple measures of cortisol and subclinical disease over longer time periods (Adam, 2006; Hruschka et al., 2005).

There are several strengths to our study. First, ours is one of the few population based studies to incorporate a large multi-ethnic sample. Second, our measurement of cortisol was more rigorous than previous studies (six samples over three days). Having three days of cortisol samples provides additional power to our study; analyzes based only on the first day of samples suggest roughly similar estimates (in the same direction as the pooled sample) with less precision. Our track-caps data also indicates excellent compliance (overall 86% of the samples were collected within 15 minutes of the requested time) (Hajat et al., 2010). In addition, we took advantage of state-of-the art measures of subclinical atherosclerosis. Lastly, much of the literature on cortisol has used summary measures such as the overall daily slope or the AUC to describe the impact of cortisol on health outcomes. Our study used both these summary measures and the piecewise linear model which allowed for detection of an association at specific and potentially important times of the day.

In summary our results provide weak evidence of a link between cortisol levels and subclinical atherosclerosis in an older sample. We found an association between some features of the diurnal cortisol profile and coronary calcification in the total population and ABI among women. These associations were not consistent across gender or different specifications of the subclinical measures and there was no association between summary salivary cortisol measures (AUC or wake-to-bed slope) and subclinical factors. There are methodological challenges in detecting the association between cortisol and measures of subclinical atherosclerosis which develop over a lifetime. Studies of short-term mechanisms through which stress and physiological processes relate to the development of early atherosclerosis may be more informative.

Acknowledgments

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>.

Role of funding sources

Psychoneuroendocrinology. Author manuscript; available in PMC 2014 July 01.

This work was supported in part by R01 HL101161 (Diez Roux PI) and R21 DA024273 (Diez Roux PI). MESA was supported by contracts N01-HC-95159 through N01-HC-95169 from the National Heart, Lung, and Blood Institute (NHLBI). NHLBI had no further role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. This research was also supported in part by the Michigan Center for Integrative Approaches to Health Disparities (P60MD002249) funded by the National Center on Minority Health and Health Disparities.

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p = persons
d = days
s = samples

Figure 1.
Exclusion criteria and participation for MESA Stress study

p = persons

d = days

s = samples

Table 1Characteristics of selected participants overall and by sex, MESA Stress Study, 2004 – 2006^a

	Total population (n=664)	Men (n=327)	Women (n=337)	p-value
Demographics				
Mean age (SD)	65.60 (9.73)	65.54 (9.70)	65.66 (9.76)	0.87
% white	15.21	14.68	15.73	0.92
% black	25.60	25.99	25.22	
% Hispanic	59.19	59.33	59.05	
Mean income-wealth index (SD)	3.54 (2.33)	3.67 (2.30)	3.41 (2.36)	0.16
CVD risk factors				
% obese	30.42	24.46	36.20	<0.001
% overweight	43.22	50.76	35.91	
% current smoker	9.68	12.31	7.14	<0.001
% hypertensive	51.21	46.77	55.52	0.02
% dyslipidemia	41.72	43.43	40.06	0.38
% diabetic	16.72	17.13	16.32	0.78
CVD subclinical measures				
Mean log coronary calcium ^b (SD)	4.37 (1.88)	4.58 (1.97)	4.11 (1.74)	0.04
Mean log coronary calcium plus 1 ^c (SD)	2.66 (2.58)	3.19 (2.62)	2.18 (2.40)	<0.001
% presence of CAC	60.13	68.78	52.26	0.0003
Mean ABI (SD)	1.13 (0.13)	1.14 (0.14)	1.11 (0.12)	0.002
% with low ABI	34.74	26.27	42.94	<0.001
Cortisol (mean and SD) ^d				
Wake-up	13.57 (9.43)	13.62 (9.18)	13.52 (9.68)	0.81
30 minutes later	18.90 (11.28)	18.45 (10.55)	19.34 (11.93)	0.09
1000h	9.15 (6.76)	9.56 (6.51)	8.75 (6.97)	0.01
1200h	7.27 (6.34)	8.00 (6.10)	6.56 (6.49)	<0.0001
1800h	4.14 (5.17)	4.43 (5.41)	3.86 (4.92)	0.02
Bed time	3.29 (5.72)	3.14 (4.97)	3.43 (6.36)	0.29

Abbreviations: ABI, ankle brachial index; CAC, coronary calcification; CVD, cardiovascular disease; MESA, Multi-Ethnic Study of Atherosclerosis; SD, standard deviation

^a Characteristics are for participants in either ABI or CAC analysis. The overlap between the two analyses is 410 participants. All numbers are expressed as percentage unless otherwise indicated.

^b Mean log coronary calcium among those with calcification (n =279)

^c Mean log coronary calcium after adding 1 to all values (n=464)

^d Cortisol measured in nmol/L. Values were averaged over the three days of data collection. N = 11098 samples.

Table 2

Percent differences (95% confidence intervals) in selected features of the cortisol profile associated with coronary artery calcification (CAC) and ankle-brachial index (ABI)

Coronary artery calcification ^a	Ankle-brachial index ^b		
	Total population	Men	Women
% difference at wake-up			
Model 1 ^c	-0.02 (-2.48, 2.39)	-0.61 (-15.07, 12.03)	14.31 (-0.08, 30.77)
Model 2 ^d	0.55 (-1.86, 3.02)	-1.87 (-17.81, 11.91)	13.91 (-0.31, 30.16)
Model 3 ^e	0.63 (-1.78, 3.11)	-3.19 (-19.53, 10.92)	14.69 (0.38, 31.04)*
Model 4 ^f	0.44 (-1.97, 2.91)	-4.48 (-21.27, 10.00)	13.96 (-0.28, 30.22)
% difference in cortisol awakening response			
Model 1	-2.05 (-6.66, 2.35)	14.52 (-10.61, 46.70)	5.66 (-18.12, 36.34)
Model 2	-2.06 (-6.66, 2.35)	11.75 (-15.31, 47.45)	3.89 (-19.06, 33.35)
Model 3	-2.25 (-6.87, 2.16)	12.35 (-15.19, 48.84)	2.77 (-20.03, 32.09)
Model 4	-2.07 (-6.63, 2.29)	13.91 (-14.46, 51.70)	4.40 (-18.30, 33.40)
% difference at early decline			
Model 1	1.94 (0.49, 3.41)*	-8.44 (-16.18, -1.21)*	0.67 (-8.42, 10.67)
Model 2	1.77 (0.21, 3.36)*	-7.40 (-15.48, 0.11)	2.68 (-6.72, 13.02)
Model 3	1.74 (0.17, 3.34)*	-6.96 (-15.04, 0.56)	3.46 (-6.25, 14.18)
Model 4	1.77 (0.23, 3.34)*	-7.01 (-15.09, 0.50)	3.43 (-6.60, 14.52)
% difference at late decline			
Model 1	-0.18 (-0.41, 0.05)	0.63 (-0.33, 1.60)	-0.52 (-2.00, 0.94)
Model 2	-0.23 (-0.47, 0.01)	0.59 (-0.46, 1.66)	-0.62 (-2.21, 0.95)
Model 3	-0.24 (-0.48, -0.01)*	0.46 (-0.60, 1.53)	-0.63 (-2.26, 0.97)
Model 4	-0.24 (-0.48, -0.01)*	0.49 (-0.58, 1.57)	-0.74 (-2.39, 0.89)

^aOne was added to all CAC values and then the log was taken. Estimates correspond to a one unit increase in log Agaston score.

^bEstimates correspond to low versus normal ABI

^cModel 1 was adjusted for age, race, income-wealth index, wake-up time and day of data collection.

^dModel 2 was adjusted for covariates in model 1 plus body mass index, dyslipidemia, hypertension, diabetes and smoking.

^eModel 3 was adjusted for all covariates in model 2 plus depression, anxiety and chronic burden.

^fModel 4 was adjusted for all covariates in model 3 plus acute daily stressor variables, if participant worked the day of sample collection and stress rating of that day (low, normal or high).

* Statistically significant at < 0.05 level.

Table 3

Percent differences (95% confidence intervals) in area under the curve (AUC) and wake-to-bed slope associated with coronary artery calcification (CAC) and ankle-brachial index (ABI)

	Coronary artery calcification ^a		Ankle-brachial index ^b	
	Total population	Total population	Men	Women
AUC				
Model 1 ^c	0.15 (−0.15, 0.44)	0.43 (−0.79, 1.66)	0.25 (−1.54, 2.04)	0.55 (−1.16, 2.26)
Model 2 ^d	0.16 (−0.15, 0.47)	0.22 (−1.01, 1.45)	0.22 (−1.60, 2.04)	0.76 (−0.91, 2.43)
Model 3 ^e	0.13 (−0.17, 0.44)	0.13 (−1.10, 1.37)	0.09 (−1.74, 1.92)	0.69 (−1.00, 2.37)
Model 4 ^f	0.13 (−0.18, 0.43)	0.27 (−0.95, 1.49)	0.04 (−1.77, 1.85)	0.79 (−0.87, 2.45)
Wake to bed slope				
Model 1	0.0001 (−0.002, 0.002)	−0.0006 (−0.007, 0.008)	−0.002 (−0.01, 0.008)	0.002 (−0.009, 0.01)
Model 2	−0.0003 (−0.002, 0.002)	−0.001 (−0.007, 0.009)	−0.002 (−0.01, 0.009)	0.004 (−0.007, 0.02)
Model 3	−0.0005 (−0.002, 0.001)	0.0003 (−0.008, 0.009)	−0.003 (−0.01, 0.009)	0.003 (−0.008, 0.14)
Model 4	−0.0005 (−0.002, 0.001)	0.0005 (−0.008, 0.009)	−0.002 (−0.01, 0.009)	0.003 (−0.009, 0.02)

^aOne was added to all CAC values and then the log was taken. Estimates correspond to a one unit increase in log Agaston score.

^bEstimates correspond to low versus normal ABI

^cModel 1 was adjusted for age, race, income-wealth index, wake-up time and day of data collection.

^dModel 2 was adjusted for covariates in model 1 plus body mass index, dyslipidemia, hypertension, diabetes and smoking.

^eModel 3 was adjusted for all covariates in model 2 plus chronic burden, depression and anxiety.

^fModel 4 was adjusted for all covariates in model 3 plus acute daily stressor variables, if participant worked the day of sample collection and stress rating of that day (low, normal or high).