Published in final edited form as:

Soc Sci Med. 2015 February ; 127: 143–149. doi:10.1016/j.socscimed.2014.09.050.

A blunted diurnal cortisol response in the lower educated does not explain educational differences in coronary heart disease: Findings from the AGES-Reykjavik Study

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Abstract

Lower educational attainment generally is a strong predictor of coronary heart disease (CHD). The underlying mechanisms of this effect are, however, less clear. One hypothesis is that stress related to limitations imposed by lower socioeconomic status elicits changes in hypothalamic-pituitary-adrenal axis functioning, which, in turn, increases risk of CHD. In a large cohort study, we examined whether educational attainment was related to risk of fatal and non-fatal CHD and the extent to which salivary cortisol mediated this relation independent of potential confounders, including lifestyles. Data came from 3,723 participants aged 66 thru 96 from the Age, Gene/Environment Susceptibility (AGES) – Reykjavik Study. Between 2002 and 2006, data were collected using questionnaires and examinations including morning and evening salivary samples. Hospital admission records and cause of death registries (ICD-9 and ICD-10 codes) were available until December 2009. Linear regression and Cox proportional hazards analyses were performed. Even after adjustment for potential confounders, including lifestyle, persons with lower educational attainment showed a blunted cortisol response and also greater risk of incident CHD.

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Conflict of interest: None declared.

However, our data did not support the role of cortisol as a mediator in the association between education and CHD in an older sample.

Keywords

Iceland; educational attainment; salivary cortisol; coronary heart disease; old age; stress response

Background

Coronary heart disease (CHD) is still one of the leading causes of death in higher income countries all over the world (World Health Organization, 2012). In many of these countries, lower educational level has been related to CHD (Kaplan & Keil, 1993). Although unhealthy lifestyles in the lower educated explain part of the relation between education and CHD, the underlying mechanism remains, to a large extent, unclear (Lynch et al., 2006; Marmot et al., 1997; Marmot et al., 2008; Singh-Manoux et al., 2008).

One hypothesis is that stress related to lower socioeconomic status (SES) elicits changes in hypothalamic-pituitary-adrenal (HPA) axis functioning (Steptoe & Marmot, 2002). In case of acute stress exposure, adaptive stress response patterns can be beneficial to health and functioning. However, in case of chronic stress, an accumulation of biological dysregulation, often denoted as ‘allostatic load’, may contribute to the pathogenesis of health problems, including CHD (McEwen, 2003; Rosmond, 2005; Seeman et al., 2004). Cortisol is one of the markers of allostatic load that has received most research attention (Dowd et al., 2009).

Salivary cortisol levels have been known to show long-term elevation in response to chronic stressors. Normally, cortisol levels are highest during the early morning hours (30–45 minutes after waking up) as part of the cortisol awakening response (Pruessner et al., 1997). These levels drop during the day and are lowest in the evening (Kirschbaum & Hellhammer, 1989). Research suggests that cortisol levels remain higher in stressful situations (McEwen, 1998). Accordingly, in a subsample of Whitehall II men (but not women), those in lower employment grades had higher salivary cortisol levels during the day (Kumari et al., 2010; Steptoe et al., 2003). This effect was, however, to a large extent explained by health and sleep behaviors. Similarly, in the Coronary Artery Risk Development in young adults (CARDIA) study, elevated evening levels of cortisol in the lower SES groups were mostly explained by health behaviors and psychosocial factors (Cohen et al., 2006). Others (Decker, 2000; Vreeburg et al., 2009) did not find evidence for a relation between SES and cortisol levels (Dowd et al., 2009).

Via stimulation of neuroendocrine, autonomic, and immune processes, elevated cortisol levels may affect health and functioning (Steptoe & Marmot, 2002). Higher cortisol levels during the day are, specifically, suggested to relate to the incidence of CHD (Brotman et al., 2007). However, while elevated cortisol levels have, indeed, been linked to a variety of processes, including insulin resistance, the metabolic syndrome (Rosmond, 2005) and coronary calcification (Seldenrijk et al., 2012), empirical evidence with regard to the relation between elevated cortisol and CHD outcomes is, however, still inconsistent (Kumari et al., 2011; Vogelzangs et al., 2010).

More recently, increased attention has been given to variations of cortisol over the day, rather than using absolute cortisol measurements, as a marker of biological dysregulation. It is postulated that long term chronic activation of the HPA-axis might ultimately lead to a blunted under-active HPA response. This is indicated by a less pronounced cortisol awakening response (Clow et al., 2010) (CAR) and a less steep decline over the day (i.e. the diurnal slope or area under the curve). This lower cortisol reactivity may have serious adverse consequences for health and functioning (Phillips et al., 2013). Evidence indeed shows relations between SES and blunted cortisol levels (Cohen et al., 2006; Kumari et al., 2010; Li et al., 2007) and blunted cortisol and health outcomes (Kumari et al., 2011). For the purpose of modeling diurnal variation, multiple measurements of cortisol over several days are necessary (Hellhammer et al., 2007). In large population-based cohort studies, however, these data are often difficult to ascertain.

Using data from a large population-based cohort in Reykjavik, Iceland: The Age, Gene/Environment Susceptibility (AGES) – Reykjavik Study, we formally tested whether salivary cortisol is a mediator in the SES-CHD relation. We studied the effect of educational attainment on salivary cortisol secretion and the incidence of CHD, independent of potential confounders including lifestyle factors. We hypothesized that morning and evening cortisol levels are higher in the lower educated and that these elevated cortisol levels are associated with a higher incidence of CHD. In congruence with the more recent studies on diurnal variation, we tested whether it was possible to find an effect of diurnal variation, calculated by using only two measures of cortisol over 24 hours. We hypothesize that the lack of reliability of individual measures of salivary cortisol (Hellhammer et al., 2007) (due to having only two measures of cortisol available) is likely to be compensated by our large sample size.

Methods

Study population

The Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) Study is drawn from the Reykjavik Study, a population-based cohort established in 1967. This cohort originally comprised a random sample of 30,795 men and women born between 1907 and 1935, residing in Reykjavik, the capital of Iceland. A random sample of 27,281 persons was invited to participate and 19,381 individuals entered the study. As part of the Reykjavik study, participants completed questionnaires and participated in a standardized medical examination. Baseline measurements took place over a period of more than 20 years, from 1967 until 1992 (Harris et al., 2007).

In 2002, recruitment for the AGES-Reykjavik Study began by randomly selecting 8,030 individuals from the people who were still alive from the original Reykjavik-cohort ($n = 11,459$). A total of 5,764 individuals entered the AGES-Reykjavik Study as participants (response rate=72%, 58% women). Study participants underwent a comprehensive evaluation that included questions on medical history, lifestyle, and family history and had a series of standardized health examinations and laboratory testing, including analyses of morning and evening salivary samples. In addition, data from hospitalization records and cause of death registries were linked to the participant data (Harris et al., 2007).

Selection of our analytical sample (Figure 1) was based on the method as described by Gerritsen et al. (Gerritsen et al., 2010; Gerritsen et al., 2009). From 5,228 participants, at least one valid cortisol measurement was available. Participants who used corticosteroid medication (n=325) or when the evening cortisol values were >5nmol/L higher than the morning values (n=124) were excluded. When either morning or evening cortisol levels were below the detection limit (0.43 nmol/l (Kirschbaum & Hellhammer, 1989)) (6 morning samples and 63 evening samples) this value was truncated at 0.43. Cortisol levels that were considered improbable (>100 nmol/L; 28 morning and 4 evening samples) were recoded to missing values (Peeters et al., 2008). Missing values existed also in 9.4% of relevant continuous and categorical covariates and these were imputed using multiple imputation techniques (IBM, 2010; Van Buuren et al., 2006).

In order to be able to study the relationship between cortisol and incident CHD, participants having prevalent CHD (n=1,056, based on self-reports, ECG changes, or previous hospitalizations because of CHD) were excluded, leaving 3,723 persons (47.3% men, mean age: 76.2, SD=5.6, range: 66–96) for the analyses.

Compared to the analytical sample, excluded persons (n=1,505) were on average older, had lower morning cortisol levels and higher evening cortisol levels (all $p<0.001$). Moreover, excluded persons were more likely to be of male gender ($p<0.001$), to have lower levels of education ($p=0.042$), and to report depressive symptoms ($p<0.001$).

Measures

Salivary cortisol—Between 2002 and 2006, saliva samples were collected using the ‘Salivette’ device (Sarstedt, Rommelsdorf, Germany). Participants were asked to bring two saliva samples with them on the day of clinic visit. They were instructed to take the first sample the evening before the clinic visit, prior to going to sleep. The second sample was to be taken in the morning, about 45 minutes after waking up (i.e. the samples were thus taken on two consecutive days. However, the time between the samples was not more than 12 hours.) Participants were instructed not to brush teeth before the samples were taken, to allow at least 30 minutes to pass after eating or drinking before taking the evening sample, and to refrain from eating and drinking until after the morning sample was taken.

Salivary cortisol was analyzed with a time-resolved immunoassay with fluorescence detection (DELFI) as described in detail elsewhere (Dressendorfer et al., 1992). Based on lab averages, the intra- and inter-assay variability was less than 10 and 12%, respectively (Dressendorfer et al., 1992). The lower detection limit was 0.43 nmol/l for a 50 microliters salivary sample. Diurnal variation was calculated as $([\text{morning cortisol} - \text{evening cortisol}] / \text{morning cortisol} \times 100)$, to represent the relative decrease of evening cortisol level compared with morning cortisol level (Gerritsen et al., 2009). Lower diurnal variation indicates a less steep decline of cortisol during the day. The distribution of cortisol measures was skewed; therefore natural logarithmic transformations were applied to normalize the distributions.

Coronary heart disease—Incident CHD events included fatal CHD or incident non-fatal CHD (International Classification of Diseases (ICD) 9th edition, codes 410, 411, 414, 429, and ICD-10th edition, codes I21–I25), obtained from cause of death registries and

hospitalization records (Conroy et al., 2003; Hardarson et al., 2001). Starting from the day of cortisol measurement until December 31, 2009, 484 CHD cases (fatal and non-fatal) were recorded. The average length of follow-up was 4.97 years.

Educational level—Educational level – as a proxy for socioeconomic status – was extracted from the Reykjavik Study questionnaire and was defined as the highest level of completed education in midlife. Four educational levels were identified from the answer to the following open question in the questionnaire: “Have you passed any examination since finishing elementary school? If yes, please specify (courses lasting less than 6 months not included)”. Level 1 included elementary school or less. Level 2 included high school education. Level 3 included Junior College education (i.e. upper secondary education, similar to higher vocational education). Level 4 included University education (e.g. bachelor’s degrees, master’s degree, PhD’s and MD’s). The usual ages for completion of these levels were 13, 16, 20, and 26 or higher, respectively. Because of small numbers in the third and fourth categories, college and university levels were combined so that three categories (i.e. lower, middle, higher) remained for the analyses (Hardarson et al., 2001). A linear term was added in the analyses based on the ordinal categories of education.

Covariates—Covariates were chosen on the basis of previous literature on salivary cortisol and its association with sociodemographic, sampling and health factors (Holsboer, 2000; Liu et al., 2005; Vreeburg et al., 2009). Covariates related to cortisol secretion or CHD or both included sex (e.g. diurnal slope is reported to be steeper in men (Vreebrug et al., 2009)), age (e.g. a higher evening cortisol level was associated with older age (Vreeburg et al., 2009)), season of sampling (i.e. months with a lot of daylight versus months with less daylight, e.g. morning cortisol levels were found to be higher in months with less daylight (Vreeburg et al., 2009)), depression (i.e. based on the Geriatric Depression Scale-15 (Valdimarsdottir et al., 2000), with a cut-off score of 5 or use of antidepressant medication, e.g. depression is associated with HPA-axis dysregulation (Holsboer, 2000)), sleeping problems (i.e. difficulty initiating or maintaining sleep or early morning awakening, e.g. diurnal slope is less steep in early awakeners (Vreeburg et al., 2009)), employment status (i.e. currently in a paid job versus retired or not in a paid job, e.g. diurnal slope is reported to be steeper in working persons (Vreeburg et al., 2009)), hypertension (i.e. diastolic >90 mmHg or systolic >140 mmHg, e.g. late night salivary cortisol levels are reported to be higher in participants with high bloodpressure (Liu et al., 2005)), and diabetes mellitus (i.e. according to self-reports, use of medication or fasting glucose levels of >7mmol/l, e.g. late night cortisol levels are reported to be elevated in male diabetes patients (Liu et al., 2005)). Lifestyle factors, including smoking (i.e. current/non-smoker), BMI (kg/m²), self-reported level of physical activity (i.e. categorized as moderate or high level vs. occasionally physically active at most), and self-reported alcohol use (i.e. never, former, current), have been shown to be strongly associated with cortisol levels (Vreeburg et al, 2009), as well as with socioeconomic status measures and CHD (Singh-Manoux, 2008). Because lifestyle factors could also play a mediating role in pathways between SES, cortisol and CHD, they were adjusted for in separate models of our analyses.

Analyses

All analyses were performed using IBM SPSS version 19.0 (IBM, 2010). Differences in characteristics of the study sample by educational attainment were determined using t-tests or F-test statistics for continuous data and chi-square tests for categorical data. We assessed the extent to which cortisol levels mediated the education-CHD relation by using Baron and Kenny's (1986) procedure for testing mediational hypotheses (Baron & Kenny, 1986). This procedure implies to first test the causal relation between education and CHD, the relation between education and cortisol, and between cortisol and CHD before calculating the reduction of the effect size due to adding cortisol.

Accordingly, Cox proportional hazard models were fitted to determine if there were educational differences in (time to) incidence of CHD, adjusted for age, sex, and lifestyle factors. If a person developed CHD, based on a hospital admission (n=399), or died because of CHD with no previous hospital admission due to CHD (n=85), person time (in days since cortisol measurement) was set at the date at which the CHD event was reported. Persons surviving with no evidence of CHD (n=2,453) were censored at the last date at which death registration was available (December 31, 2009). Those who died with no evidence of CHD (n=786) were censored at the date of death.

Secondly, linear regression analyses were fitted to estimate the association between educational attainment and log-transformed measures of cortisol secretion. Analyses were adjusted for sex, age (model 1), sleeping problems, season and day of sampling, depressive symptoms, hypertension, employment status, diabetes mellitus (model 2) and - in a separate model because of a possible mediating role - lifestyle factors (model 3).

Thirdly, Cox proportional hazard models were fitted to determine if there were cortisol differences in (time to) incidence of CHD. Similar models of adjustment were used as described above.

Only if these three relations reached statistical significance, attenuation of the HR of CHD by education due to adding cortisol was calculated. A percentage reduction in HR was computed using: $(HR_{\text{model without cortisol}} - HR_{\text{model with cortisol}}) / (HR_{\text{model without cortisol}} - 1) * 100\%$. (Groffen et al., 2012)

The proportional hazard assumption was investigated by testing the constancy of the log hazard ratio over time by means of log-minus-log survival plots and interactions with time (log transformed). According to the tests, the proportional hazard assumption was not violated ($p > 0.05$). After log-transformation of cortisol measures, assumptions of the linear regression model were also met. Moreover, interactions between age, sex and measures of cortisol and educational level were not significant (all $p > 0.10$).

Results

Table 1 shows how covariates, cortisol and coronary heart disease outcomes were distributed among participants from lower, middle, and higher educational levels. Compared to the higher educated, people from lower educational level were on average older and more likely

to be women. Furthermore, they were more likely to lead unhealthy lifestyles (i.e. being physically inactive, and having a higher BMI)), to report sleeping problems, and to suffer from hypertension and depressed mood. Moreover, they showed lower morning cortisol levels, higher evening cortisol and a less steep decline of cortisol levels during the day (i.e. lower diurnal variation). Univariate analyses showed, however, no differences in the incidence of fatal and non-fatal CHD between educational groups.

Table 2 shows that people from lower educational levels had about 46% increased risk of developing CHD during follow-up compared to people from higher educational levels. The linear term confirmed a lower risk of CHD in higher educated. This effect was not explained by lifestyle factors.

Linear regression analyses (Table 3) showed that educational level was associated with lower morning cortisol levels and a less steep decline of cortisol during the day, even after adjustment for all relevant covariates, including lifestyle factors. Educational level was not associated with evening cortisol levels. Supplementary table 1 shows how covariates, including lifestyle factors, are associated with cortisol levels.

Log-transformed measures of morning cortisol, evening cortisol, and diurnal variation were not related to the incidence of CHD (Table 4).

Due to the absence of an independent cortisol – CHD relation, cortisol could not be a mediator in the relation between education and CHD. Adding cortisol variables to the model explained about 2% of the relation between education and CHD (Supplementary table 2).

Sensitivity analyses, analyzing fatal and non-fatal CHD cases separately (Supplementary table 3–6), or using only the cases with complete data ($n=3,334$) (i.e. not imputed data) (Supplementary table 7–9) yielded similar results. While analyses using only fatal CHD cases (Supplementary table 5) showed evening cortisol to be a significant predictor of fatal CHD (Fully adjusted HR: 1.47, 95% CI: 1.02–2.11), its contribution to the relation between education and CHD was, however, minimal (1.7%).

Discussion

In this group of older men and women, lower education was associated with a blunted cortisol response, indicated by lower levels of morning cortisol and a less steep decline of cortisol during the day. This association remained significant, even after adjusting for lifestyle factors. Lower education also predicted a higher incidence of CHD. However, this relationship could not be explained by differences in cortisol secretion.

Despite having only two measurements of salivary cortisol available, and adjusting for possible confounding lifestyle factors including smoking, we were still able to find consistent evidence for a ‘chronic stress - less responsiveness’ hypothesis. In this hypothesis, cumulative lifelong stress is thought to blunt the biological stress response: the HPA axis adapts to chronic stress by downregulation (Elzinga et al., 2008; Phillips et al., 2013; Sanchez, 2006). Similar results have been reported in previous studies. For example, Hajat et al. (2010) found morning cortisol levels to be lower in blacks and people with lower

incomes, as compared with whites and people with higher incomes, respectively (Hajat et al., 2010). Agbedia et al (2011) also found a blunted decline of cortisol during the day among older adults (Agbedia et al., 2011). In our study, we found lower educational level to be related to a blunted stress response almost three decades later. Mostly attained in adolescence and young adulthood, educational level may either represent socioeconomic conditions during a critical period in young adulthood (having a lifelong influence on cortisol) or it may represent an early proxy for later socioeconomic attainment processes in which occupation and income are the genuine risk indicators for cortisol. Without further information on such socioeconomic life-course processes, we cannot exclude the possibility that educational attainment constitutes the critical period during which important biological patterns are established.

It is unclear whether or not our effect sizes for cortisol differences between low and high education are clinically or meaningfully significant. Additional analysis (Supplementary table 1) shows that effect sizes for cortisol differences are similar to those of persons using antidepressant medication, smokers and having higher BMI. Additional research on this issue is, however, recommended.

There is considerable literature on stress as a potential cause of CHD, much of which postulates a role for neuro-endocrine mediators (Brotman et al., 2007; Eller et al., 2009; Kuper et al., 2002; Steptoe & Marmot, 2002). Empirically, Vogelzangs and colleagues (2010) showed that high urinary cortisol levels strongly predict cardiovascular death (Vogelzangs et al., 2010). Kumari and colleagues (2011) were the first to show that flatter slopes in cortisol decline across the day were associated with cardiovascular disease mortality (Kumari et al., 2011). In our study, however, we did not find evidence for such a relation, possibly because only relatively healthy older people were included in our analyses or because of the unreliability of our rather limited measurement of cortisol (see the limitations section below). Sensitivity analyses, however, did show evening cortisol levels to be related to fatal CHD. These results – despite relying on a small number of fatal cases – indicate the need for more attention to the relation between cortisol and mortality outcomes, preferably using longer follow-up.

An increasing number of studies present the idea that stress mediates the relationship between SES and health outcomes. Current empirical evidence for the mediating role of cortisol, however, is weak (Dowd et al., 2009). Because we did not find a relation between cortisol and CHD, we were not able to find evidence for cortisol being a mediator in the relation between education and CHD either. Given these findings, future studies need to search for possible other psychobiological mediators of the SES-health effect. Taking into account our older population, it is recommended to also study the effect of cortisol – or other indicators of biological dysregulation – on health outcomes in different age groups.

Strengths and limitations

The strengths of this study include the use of hospitalization and cause of death registries and the ability to exclude cases with prevalent CHD. These features allowed us to study the associations between education, cortisol secretion, and CHD incidence in a relatively healthy population. By excluding prevalent CHD cases from our analyses, we have ruled out a

potential selection effect of poor health affecting educational attainment. Moreover, our findings regarding education and CHD are consistent with findings of the original Reykjavik Study (Hardarson et al., 2001). There are, however, some limitations that need to be considered.

First, a significant limitation that could potentially explain the lack of mediation by cortisol includes our measurement of salivary cortisol: only two measurements of salivary cortisol secretion and no exact sampling time were available. Moreover, the evening and morning sample were collected on different days (i.e. the evening sample was taken on the evening before the exam visit and the morning sample the following morning). Ideally, information on cortisol secretion is obtained from multiple collections of saliva throughout a day, repeated over several days (Kraemer et al., 2006), which enables a more sensitive assessment of the daily rhythm. Also, the cortisol awakening response is preferably calculated by taking into account cortisol levels at awakening, time of awakening and exact sampling time (Clow et al., 2010). These data were not available. Moreover, instructions given to participants prior to cortisol sampling were rather limited. Participants were, for example, not instructed to not engage in strenuous exercise within 2 hours of collection and not to smoke within 1 hour. Our large sample size might, however, have compensated for the possible variation and unreliability in cortisol measurements.

Second, our study might be limited by our choice of covariates. These were chosen on the basis of previous literature on this topic. However, some of them might not be considered as classic confounders but rather as mediators. For example, hypertension and diabetes might be part of the pathway (i.e. mediators) between education and CHD and between cortisol and CHD. Others (e.g. season of sampling) are only chosen because they are likely to affect cortisol secretion. Moreover, adjusting for lifestyle factors – being mediators in the relation between education and SES and potentially affecting cortisol secretion – might be considered as overadjustment. However, by adjusting for these factors in separate models of our analyses, we believe that the negative impact of these covariates on our results is rather limited.

Third, the study sample consisted of the survivors of the original Reykjavik Study. Previous results from the Reykjavik Study (Hardarson et al., 2001) indicated strong educational differences in mortality, with the lower educated showing higher mortality rates. Consequently, survivors from the Reykjavik Study, now participating in the AGES-Reykjavik Study, are higher educated and have better health (i.e. healthy survivor effect). Moreover, excluded cases were in general lower educated and showed less favorable cortisol levels over the day (see Study Population section). This pattern of selective response and attrition may have led to an underestimation of the reported associations.

Finally, another limitation that should be considered is the lack of generalizability of our results to countries with more income inequality or without universal health care. Iceland, like other Nordic countries, is unique in having a low Gini index and universal healthcare, making educational level perhaps less of an important predictor for CHD in this country than it would be in other countries.

Conclusion

Even after adjustment for potential confounders, including lifestyle, persons with lower educational attainment showed a blunted cortisol response and also greater risk of incident CHD. However, our data did not support the role of cortisol as a mediator in the association between education and CHD in an older sample.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Age, Gene/Environment Susceptibility Reykjavik Study is funded by NIH contract N01-AG-12100, the NIA Intramural Research Program, Hjartavernd (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament).

The researchers are indebted to the participants for their willingness to participate in this study.

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Highlights

- We assessed whether salivary cortisol mediated the relation between education and heart disease.
- Lower education was associated with a blunted cortisol response and greater risk of heart disease.
- Educational differences in heart disease were not explained by differences in cortisol response.

Figure 1.
Flowchart response and analytical sample AGES-Reykjavik Study

Table 1Characteristics of the study sample by educational level (n=3,723)^a

	Education in midlife			
	Total n=3723	Low n=1303	Middle n=1744	High n=676
Covariates				
Men (%)	47.3	25.2	40.2	52.7
Age (Mean, SD)	76.2 (5.6)	77.2 (5.9)	75.5 (5.6)	76.2 (5.0)
Current Smoker (%)	12.3	14.0	12.2	9.5
Physically inactive (%)	68.9	76.3	66.7	60.3
Current drinker (%)	65.1	54.6	68.4	77.0
BMI (Mean, SD)	27.0 (4.4)	27.3 (4.7)	27.0 (4.3)	26.5 (4.3)
Sleeping problems (%)	30.2	34.1	29.2	25.3
Hypertension (%)	50.5	53.6	48.6	49.7
Diabetes (%)	10.8	10.7	10.4	12.0
Sampling season (% winter-fall)	56.7	56.5	57.1	56.1
Depressed mood (GDS>5) (%)	7.0	9.0	6.5	4.3
Employment status (currently working) (%)	10.5	6.0	11.4	16.9
Using antidepressant medication (%)	13.6	14.9	13.7	10.9
Cortisol^b				
Morning cortisol (mean, SD)	20.5 (13.4)	19.9 (13.9)	20.7 (13.1)	21.1 (13.3)
Evening cortisol (mean, SD)	3.5 (4.2)	3.6 (4.3)	3.4 (4.4)	3.3 (3.3)
Diurnal variation (mean, SD)	79.9 (19.8)	78.3 (19.8)	80.6 (18.2)	81.2 (18.1)
Outcomes				
Incident fatal and non-fatal CHD (%)	13.0	13.7	13.3	11.0

^aResults in bold indicate a significant difference^bUntransformed means and standard deviations are presented. ANOVA tests of significance were based upon log-transformed cortisol measures.

Table 2

Incident coronary heart disease by educational level (n=3,723)

Education	Model 1 ^a	Model 2 ^b
	HR (95% CI)	HR (95% CI)
Low	1.46 (1.11–1.93)	1.44 (1.08–1.90)
Middle	1.35 (1.04–1.76)	1.34 (1.03–1.75)
High	ref	ref
Linear ^c	0.84 (0.74–0.96)	0.85 (0.75–0.97)

^aModel 1: Age and sex adjusted^bModel 2: Additionally adjusted for physical activity, BMI, smoking status, and alcohol consumption.^cThe linear term is based on the ordinal variable of education (i.e. level 1 thru 4)

Table 3

Cortisol levels by educational level (n=3,723)

Model	Education	LN Morning cortisol	p	LN Evening cortisol	p	LN Diurnal variation	
		B (SE)		B (SE)		p	
1 ^a	Low	-0.106 (0.032)	0.001	0.037 (0.027)	0.168	-0.174 (0.247)	<0.001
	Middle	-0.038 (0.030)	0.208	0.012 (0.025)	0.632	-0.051 (0.050)	0.277
	High	Ref		ref		ref	
	<i>Lineard</i>	0.055 (0.016)	<0.001	-0.020 (0.013)	0.138	0.092 (0.024)	<0.001
2 ^b	Low	-0.097 (0.033)	0.003	0.033 (0.027)	0.226	-0.157 (0.050)	0.002
	Middle	-0.031 (0.030)	0.299	0.010 (0.025)	0.693	-0.040 (0.047)	0.385
	High	Ref		Ref		ref	
	<i>Lineard</i>	0.049 (0.016)	0.003	0.0001 (0.013)	0.997	0.084 (0.024)	0.001
3 ^c	Low	-0.086 (0.033)	0.009	0.021 (0.028)	0.438	-0.128 (0.051)	0.012
	Middle	-0.027 (0.030)	0.379	0.005 (0.025)	0.852	-0.027 (0.047)	0.560
	High	Ref		ref		ref	
	<i>Lineard</i>	0.048 (0.017)	0.004	0.006 (0.014)	0.654	0.069 (0.025)	0.005

^aModel 1: Age and sex adjusted^bModel 2: Additionally adjusted for sleeping problems, season of sampling, hypertension, diabetes, depressive symptoms (GDS >5 or use of antidepressant medication), and employment status.^cModel 3: Additionally adjusted for physical activity, BMI, smoking status, and alcohol consumption.^dThe linear term is based on the ordinal variable of education (i.e. level 1 thru 4)

Table 4

Incident coronary heart disease by cortisol (n=3,723)

Cortisol	Model 1 ^a	Model 2 ^b	Model 3 ^c
	HR (95% CI)	HR (95% CI)	HR (95% CI)
LN morning cortisol	0.96 (0.84–1.10)	0.97 (0.84–1.11)	0.97 (0.85–1.12)
LN evening cortisol	1.12 (0.96–1.32)	1.11 (0.95–1.30)	1.10 (0.94–1.29)
LN diurnal variation	0.94 (0.86–1.02)	0.95 (0.87–1.03)	0.95 (0.87–1.04)

^aModel 1: age and sex adjusted^bModel 2: Additionally adjusted for sleeping problems, season of sampling, hypertension, diabetes, depressive symptoms (GDS >5 or use of antidepressant medication), and employment status.^cModel 3: Additionally adjusted for physical activity, BMI, smoking status, and alcohol consumption.