High Long-Term Cortisol Levels, Measured in Scalp Hair, Are Associated With a History of Cardiovascular Disease

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Background: Stress is associated with an increased incidence of cardiovascular disease. The impact of chronic stress on cardiovascular risk has been studied by measuring cortisol in serum and saliva, which are measurements of only 1 time point. These studies yielded inconclusive results. The measurement of cortisol in scalp hair is a novel method that provides the opportunity to measure long-term cortisol exposure. Our aim was to study whether long-term cortisol levels, measured in scalp hair, are associated with cardiovascular diseases.

Methods: A group of 283 community-dwelling elderly participants were randomly selected from a large population-based cohort study (median age, 75 y; range, 65–85 y). Cortisol was measured in 3-cm hair segments, corresponding roughly with a period of 3 months. Self-reported data concerning coronary heart disease, stroke, peripheral arterial disease, diabetes mellitus, and other chronic noncardiovascular diseases were collected.

Results: Hair cortisol levels were significantly lower in women than in men (21.0 vs 26.3 pg/mg hair; $P < .001$). High hair cortisol levels were associated with an increased cardiovascular risk (odds ratio, 2.7; $P = .01$) and an increased risk of type 2 diabetes mellitus (odds ratio, 3.2; $P = .04$). There were no associations between hair cortisol levels and noncardiovascular diseases.

Conclusions: Elevated long-term cortisol levels are associated with a history of cardiovascular disease. The increased cardiovascular risk we found is equivalent to the effect of traditional cardiovascular risk factors, suggesting that long-term elevated cortisol may be an important cardiovascular risk factor. **(*J Clin Endocrinol Metab* 98: 2078–2083, 2013)**

Stress is thought to be one of the main factors negatively impacting health, resulting in an increased incidence of obesity, type 2 diabetes mellitus, and cardiovascular disease (1–3). The effects of stress are mediated by the stress hormone cortisol. Cortisol is involved in the regulation of glucose and lipid metabolism, body composition, and the immune system (4). Long-term pathologically elevated cortisol levels, as extensively described in patients with extreme endogenous cortisol production (Cushing's syndrome) and in patients using glucocorticoids (5–7), are associated with increased visceral fat mass, atrophy of the proximal muscles, hypertension, insulin resistance, and dyslipidemia, which all result in an increased cardiovascular risk (8, 9). The impact of slightly elevated cortisol levels, eg, due to chronic stress, is less clear. Several studies reported that higher cortisol levels were associated with a

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Abbreviations: BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

higher incidence of cardiovascular disease or cardiovascular risk factors (10–12). However, other studies have not shown associations between cortisol and cardiovascular risk factors (13, 14) or reported that low cortisol levels were associated with cardiovascular risk factors (15). In most of these studies, cortisol was measured in serum or saliva. Because cortisol is secreted in a circadian rhythm and with pulses, the timing of sample collection is crucial when measuring cortisol in serum or saliva. In addition, cortisol is a stress hormone, and acute stress, eg, caused by the research setting or venipuncture, will influence the measurement. A single measurement of cortisol in serum or saliva therefore poorly reflects long-term cortisol levels. An alternative method to measure cortisol is in scalp hair. This method offers long-term measurements of cortisol levels, with 1 cm of hair representing cortisol levels of 1 month. In the last few years, the measurement of cortisol in scalp hair has been well validated (16–19). Previous studies have already shown that chronic stress is associated with elevated hair cortisol levels (20–23). Therefore, the measurement of long-term cortisol levels in scalp hair may be a more accurate tool to study the association between cortisol and cardiovascular disease. The aim of our study was to investigate whether there is an association between long-term cortisol levels and cardiovascular disease in community-dwelling older persons. We hypothesized that high long-term cortisol levels, measured in scalp hair, are associated with an increased cardiovascular risk.

Subjects and Methods

Subjects

A randomly selected group of 574 individuals participating in the Longitudinal Aging Study Amsterdam (LASA) were asked to participate in this study. LASA is a large population-based cohort study on predictors and consequences of changes in physical, cognitive, emotional, and social function in older persons. Detailed information concerning data collection and sampling have been described previously (24, 25). The current study was performed as a substudy of LASA and was performed in the period October 2010 to June 2011. Of the 574 individuals asked to participate in this substudy, 244 refused to participate or did not have sufficient hair growth at the posterior vertex. Of the 330 individuals who were willing to participate, 47 were excluded because of glucocorticoid use in the 6 months prior to hair sample collection. This study was approved by the local ethics committee, and all participants gave written informed consent.

Data collection

Body height was measured using a stadiometer, body weight was measured using a calibrated balance beam scale (without wearing upper clothing and shoes), and body mass index (BMI) was calculated. Waist circumference was measured twice, and the mean waist circumference was calculated. Smoking status (current, former, and never) and alcohol consumption were assessed with a questionnaire. Alcohol consumption was categorized as no alcohol consumption, light, moderate, excessive, and very excessive alcohol consumption, according to the alcohol consumption index adapted from the Garretsen alcohol index (26). The prevalence of chronic nonspecific lung disease (including asthma and chronic obstructive pulmonary disease), cancer, osteoporosis, diabetes mellitus, coronary heart disease (CHD), peripheral arterial disease, and stroke were based on self-report. The presence of cardiovascular disease was scored positive if 1 of the following diseases was present: CHD, stroke, or peripheral arterial disease. The presence of other chronic diseases was scored positive if patients were suffering from nonspecific lung disease, osteoporosis, or cancer.

Hair collection, preparation, and cortisol measurement

Hair samples of approximately 150 hair strands were cut from the posterior vertex, as close to the scalp as possible. The proximal 3 cm of hair, reflecting roughly the 3 months before hair sample collection, were used for the measurement of cortisol. Hair sample preparation has been described in detail (18, 19). In brief, a minimum of 15 mg of hair was weighed and cut into small pieces in a glass vial. Methanol was added to extract cortisol from the hair samples during an overnight incubation (16 h) at 52°C. Afterward, the methanol was transferred into a clean glass vial and was evaporated under a nitrogen stream until completely dry. The samples were dissolved in PBS (pH 8.0) and, before analysis, vortexed until thoroughly mixed. Cortisol levels in the hair extracts were measured using a commercial ELISA kit for salivary cortisol (DRG Instruments GmbH, Marburg, Germany). Cross-reactivity of the kit's antibodies with other steroids was reported as follows: corticosterone (29.00%), cortisone (3.00%), 11-deoxycortisol (<1.00%), 17-hydroxyprogesterone (<0.50%), other hormones (<0.10%). Intra-assay variation was below 5%, and interassay variation was below 8% as stated by the manufacturer. The low-end detection limit for this assay is 1.5 nmol/L. Recovery of the assay was tested and described elsewhere (18).

Statistical analyses

All statistical analyses were conducted using SPSS version 16.0 (SPSS Inc, Chicago, Illinois). Because hair cortisol levels were not normally distributed, hair cortisol levels were divided in quartiles, and logistic regression was used to investigate the relationship between hair cortisol levels and individual chronic diseases as well as total cardiovascular and total other chronic diseases. All analyses were adjusted for age, gender, smoking status, alcohol consumption, and physical activity. Correlations between hair cortisol, age, BMI, and waist circumference were tested using Spearman's correlation test. Differences in cortisol levels between men and women were tested using the Mann-Whitney *U* test. A *P*-value smaller than 0.05 was considered to indicate statistically significant differences.

Results

Characteristics of the study group are shown in Table 1. The age ranged from 65 to 85 years, with a median age of 75 years, and 66.1% was female. There were significant

Table 1. Group Characteristics

	Total Group	Women	Men
n (%)	283	187 (66.1)	96 (33.9)
Age, median (IQR), y	74.8 (70.1–79.6)	75.3 (70.8–79.7)	72.7 (69.1–79.4)
BMI, median (IQR), kg/m^2	27.1 (24.9–30.3)	27.8 (24.9–30.7)	26.8 (25.0–29.4)
Waist circumference, median (IQR), cm	99.0 (92.8–105.8)	96.9 (90.5–103.8)	102.5 (97.2–110.5)
Alcohol, n (%)			
Never	49 (17.4)	40 (21.4)	9 (9.5)
Light	149 (52.8)	106 (57.0)	43 (44.8)
Moderate	74 (26.2)	38 (20.4)	36 (37.5)
(very) Excessive	10 (3.6)	3 (1.6)	7 (7.3)
Smoking, n (%)			
Never	108 (38.2)	88 (47.1)	20 (21.1)
In the past	149 (52.7)	88 (47.1)	61 (64.2)
Current smoker	25 (8.8)	11 (5.9)	14 (14.7)
Chronic diseases, n (%)			
Cardiovascular disease			
CHD	75 (26.5)	44 (23.5)	31 (32.3)
Stroke	16 (5.7)	9 (4.8)	7 (7.3)
Peripheral arterial disease	57 (20.1)	38 (20.5)	19 (19.8)
Diabetes mellitus	43 (15.2)	22 (11.8)	21 (21.9)
Noncardiovascular disease			
Nonspecific chronic lung disease	22 (7.8)	16 (8.6)	6 (6.3)
Cancer	40 (14.1)	29 (15.5)	11 (11.5)
Osteoporosis	53 (18.7)	48 (25.7)	5 (5.2)
Cortisol, median (IQR), pg/mg hair	22.1 (16.9–30.5)	21.0 (16.0–27.0)	26.3 (20.6–35.5)

differences between men and women in alcohol consumption, smoking status, and prevalence of osteoporosis and type 2 diabetes (Table 1). Hair cortisol levels were significantly higher in men than in women (median, 26.3 pg/mg hair [interquartile range (IQR), 20.6–35.5 pg/mg hair] vs 21.0 pg/mg hair [IQR, 16.0–27.0 pg/mg hair]; P value $< .001$).

Hair cortisol levels were divided in quartiles, and the lowest quartile was considered the reference quartile. Cutoff points for hair cortisol quartiles were: 16.9, 22.1, and 30.6 pg/mg hair. Compared with participants from the lowest quartile, the odds ratios (OR) for cardiovascular disease (including CHD, stroke, and peripheral arterial disease) were 1.9 ($P = .09$) for the second, 2.0 ($P = .08$) for the third, and 2.7 ($P = .01$) for the highest hair cortisol quartiles (Figure 1). The ORs and 95% confidence intervals (CIs) of the individual cardiovascular diseases are shown in Figure 2. Higher hair cortisol levels were associated with an increased risk of CHD ($P = .07$, $P = .14$, and $P = .03$ for the second, third, and fourth quartiles, respectively). In addition, there was a higher risk of peripheral arterial disease in participants with higher hair cortisol levels (OR, 1.4 [$P = .47$]; 1.5 [$P = .36$]; and 2.5 [$P = .05$] for the second, third, and fourth quartiles, respectively). Furthermore, participants in the third and fourth hair cortisol quartiles had an increased risk of stroke, although this was not statistically significant (OR = 3.6, $P = .16$ for the third quartile; and OR = 3.3, $P = .17$ for the fourth quartile). However, combining the third and the fourth quartiles resulted in a significantly increased OR of the upper half of the group compared to the lower part of the group (first and second quartiles) (OR, 3.5; 95% CI = 1.0–12.1; $P = .05$). Participants in the highest quartile also had an increased risk of type 2 diabetes mellitus (OR, 3.2; $P = .04$). We found similar results after correction for BMI in all analyses. There were no significant associations between hair cortisol levels and the total group of noncardiovascular diseases (Figure 1) and the individual noncardiovascular diseases such as

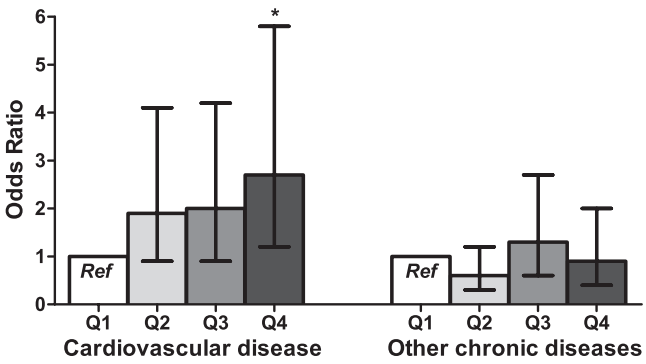

Figure 1. ORs for cardiovascular and other chronic diseases according to quartiles of hair cortisol. OR and 95% CI are adjusted for age, gender, smoking status, alcohol consumption, and physical activity. Cardiovascular disease includes CHD, stroke, and peripheral arterial disease. Other chronic diseases include nonspecific lung disease, osteoporosis, and cancer. Ref, reference quartile; Q, quartile. *$P < .05$.

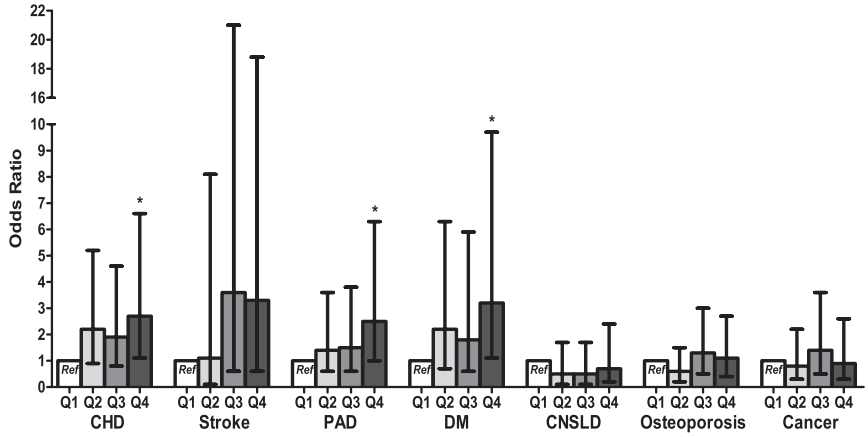

Figure 2. ORs for chronic diseases according to quartiles of hair cortisol. OR and 95% CI are adjusted for age, gender, smoking status, alcohol consumption, and physical activity. PAD, peripheral arterial disease; DM, type 2 diabetes mellitus; CNSLD, chronic nonspecific lung disease; Ref, reference quartile; Q, quartile. *$P < .05$.

cancer, osteoporosis, and chronic nonspecific lung diseases (Figure 2).

In addition, we found no correlation between hair cortisol levels and BMI (r = 0.06; $P = .36$), waist circumference (r = 0.11; $P = .08$), or age (r = −0.02; $P = .76$) in the total group, and also not when studied in men and women separately. There were also no differences in hair cortisol levels between participants based on their smoking status. Hair cortisol levels were 21.2 pg/mg hair (IQR, 16.6–26.9) in participants who never smoked, 22.1 pg/mg hair (IQR, 17.4–33.6) in former smokers, and 26.3 pg/mg hair (IQR, 18.7–32.3) in current smokers ($P = .22$). We found significantly higher hair cortisol levels in the group of participants with higher alcohol consumption. Hair cortisol levels were 21.2 pg/mg hair (IQR, 15.2–29.4) in participants who did not drink alcohol at all, 21.7 pg/mg hair (IQR, 16.9–28.9) in participants with light alcohol consumption, 24.5 pg/mg hair (IQR, 17.1–36.3) in moderate alcohol consumers, and 30.4 pg/mg hair (IQR, 25.0–45.0) in participants with (very) excessive alcohol consumption ($P = .05$).

Discussion

We investigated the association between long-term cortisol levels, measured in scalp hair, and history of cardiovascular diseases in a community-dwelling older population. The major finding of our study is the increased cardiovascular risk in participants with high long-term cortisol levels, whereas no associations were found between long-term cortisol levels and chronic noncardiovascular diseases.

The relationship between cortisol and cardiovascular diseases has been extensively studied, but with conflicting results. Some studies have shown that high cortisol levels are associated with an increased cardiovascular risk, or increased cardiovascular mortality, whereas others cannot confirm this association (10–15, 27). These inconclusive results might be due to the methods used to measure cortisol levels. Our study is the first study that used the measurement of cortisol in scalp hair to investigate the association between cortisol and cardiovascular disease in a population-based group of elderly individuals. Over the past few years, several studies have documented that the measurement of cortisol in scalp hair is a reliable method to measure cortisol exposure over prolonged periods of time and that it is a potential biomarker for chronic stress (20, 22, 23). Furthermore, it has been shown that hair cortisol levels correlate with typical cortisol tissue effects (18, 28). The measurement of cortisol in scalp hair is the reflection of the mean cortisol level over a prolonged period (in our study, 3 mo) and is therefore not influenced by the time of sample collection and acute stress. For this reason, the measurement of cortisol in scalp hair is a very suitable method to study the effects of long-term cortisol levels, and therefore chronic stress, in population-based studies.

We found a 2.7-fold increased risk of cardiovascular disease in our participants in the highest hair cortisol quartile compared to participants in the lowest quartile. This OR is in the same range as previously described ORs of traditional cardiovascular risk factors such as hypertension (OR, 2.5) and abdominal obesity (OR, 2.2) and is only slightly lower than the increased risk of cardiovascular disease caused by diabetes mellitus (OR, 3.1) and dyslipidemia (OR, 3.3) (29). This suggests that high long-term cortisol levels might be an important risk factor for cardiovascular disease as well, which is also supported by a study from Pereg et al (30), who measured hair cortisol levels in male patients admitted to the emergency department with and without acute myocardial infarction. They showed that hair cortisol levels in the 3 months before admission to the emergency department were significantly higher in the patients with an acute myocardial infarction than in patients who were admitted for other reasons such as nonmyocardial chest pain, infections, and syncope. In contrast to the study of Pereg et al (30), our study is cross-sectional and can therefore only show an associ-

ation between hair cortisol levels and a history of cardiovascular disease. However, because Pereg et al (30) showed that high cortisol levels are present before the onset of a cardiovascular event, the association we found may reflect a causative association. This is also supported by the well-known increased cardiovascular disease risk in patients suffering from Cushing's syndrome, which is characterized by pathologically elevated endogenous cortisol (31). Because our study and the study by Pereg et al (30) are the only 2 studies that have investigated the association between long-term cortisol levels and cardiovascular disease, and currently no studies have evaluated changes in cortisol after cardiovascular events, replication in larger studies is necessary to reveal the true significance of high long-term cortisol levels in the development of cardiovascular disease.

Interestingly, we did not find a significant correlation between hair cortisol levels and BMI, and there was only a tendency toward a correlation between hair cortisol levels and waist circumference in our group of elderly subjects. This is in contrast with our previous reports in younger adults (18, 28). However, several studies have shown that at an older age, a change in body composition occurs, with an increase in body fat and a decrease in fat free mass, despite a stable BMI (32, 33). Therefore, a lower BMI might also be the result of lower muscle mass rather than fat mass. Due to these changes in body composition at an older age, BMI may not be a reliable marker of body fat and therefore not an accurate predictor of cardiovascular disease and mortality at an older age. Furthermore, it might be that at older ages, the effects of cortisol on BMI and abdominal obesity are not as clear as in younger adults and that other factors, such as activity level and health status, affect BMI more than slightly elevated cortisol levels. In addition, several studies have shown that BMI at an older age is not predictive of mortality risk (both all-cause mortality and cardiovascular mortality) and that higher BMI at an older age is even associated with an increased survival in elderly individuals (34–36).

The lower hair cortisol levels in women than in men may be explained by differences in age, number of participants that dyed and bleached their hair, and the number of participants that used hair products (18, 19). However, we did not find a correlation between hair cortisol levels and age, and after exclusion of women with dyed and bleached hair and all participants using hair products, hair cortisol levels remained significantly higher in men (data not shown). This suggests that the difference in hair cortisol levels is a true gender difference, not caused by gender differences in external influences. In our previous study in healthy adults (age, 18–63 y), we did not find any gender differences in hair cortisol levels (18). In addition, other hair cortisol studies have reported no gender differences in hair cortisol levels (37, 38). However, these studies have been performed in participants aged 20–51 (38) or have compared hair cortisol levels of women aged $>$ 40 years to hair cortisol levels of men aged $<$ 40 years (37). Furthermore, these studies had only small sample sizes. Interestingly, Dettenborn et al (39) found higher cortisol levels in men aged 18–49 compared to women of the same age, and no difference in hair cortisol levels between men and women at ages 50–90. A limitation of that study was that there were only 31 participants (19 men, 12 women) in the age group 50–90 years. Our study is the first study in which hair cortisol levels were measured in a large group of elderly subjects. Replication is needed to confirm our findings of gender differences in long-term cortisol levels at older ages. In our study, we did not find a correlation between hair cortisol levels and age. This is in contrast with the study performed by Dettenborn et al (39). Dettenborn et al described a quadratic relationship between hair cortisol levels and age, with higher hair cortisol levels in (very) young children and old adults. However, the studied group was rather small, with only 31 participants in the age group 50–90 years, and the association was weak. We studied a large group of elderly subjects, with an age range of 65–85 years. In this age range, we did not find a correlation between hair cortisol and age.

We studied the association between long-term cortisol levels, measured in scalp hair, and cardiovascular disease in a group of 283 elderly individuals. The population studied is representative for the community-dwelling older population in The Netherlands. However, a few limitations need to be discussed. First, the prevalence of chronic diseases was based on self-report. Nevertheless, self-report was found to be valid after comparing it to general practitioners' information (40). Second, the prevalence of some chronic diseases, such as stroke and nonspecific lung disease, was low ($<$10%), which resulted in limited statistical power. Third, we did not have any data concerning blood pressure or lipid status. These factors might be confounders in the association between hair cortisol levels and increased cardiovascular risk.

Fourth, data on other comorbidities that might influence cortisol levels, such as psychiatric illnesses or sleep disorders, and psychological stress levels were not available.

In conclusion, our results suggest that higher long-term cortisol levels are associated with an increased risk of cardiovascular disease. The odds of cardiovascular disease in

elderly individuals with increased hair cortisol levels were similar to the odds reported for established cardiovascular risk factors. This suggests that long-term elevated cortisol may be an important risk factor for cardiovascular disease.

Acknowledgments

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