



# Salivary cortisol responses to mental stress are associated with coronary artery calcification in healthy men and women

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## Aims

Psychosocial stress is a risk factor for coronary heart disease (CHD), although the mechanisms are incompletely understood. We examined the cross-sectional association between the cortisol response to laboratory-induced mental stress and a marker of sub-clinical coronary atherosclerosis.

## Methods and results

Participants were 514 healthy men and women (mean age =  $62.9 \pm 5.7$  years), without history or objective signs of CHD, drawn from the Whitehall II epidemiological cohort. Salivary cortisol was measured in response to mental stressors, consisting of a 5 min Stroop task and a 5 min mirror tracing task. Coronary artery calcification (CAC) was measured using electron beam computed tomography. Approximately 40% of the sample responded to the stress tasks with a notable ( $\geq 1$  nmol/L) increase in cortisol. Significant CAC (Agatston score  $\geq 100$ ) was recorded in 23.9% of the sample. The cortisol response group demonstrated a higher risk of significant CAC (odds ratio = 2.20, 95% CI, 1.39–3.47) after adjustments for age, gender, baseline cortisol, employment grade, and conventional risk factors, although cortisol was unrelated to the presence of detectable CAC. Among participants with detectable CAC, the cortisol response group also demonstrated higher log Agatston scores compared with non-responders (age and sex adjusted scores;  $4.51 \pm 0.15$  vs.  $3.94 \pm 0.13$ ,  $P = 0.004$ ).

## Conclusion

In healthy, older participants without history or objective signs of CHD, heightened cortisol reactivity is associated with a greater extent of CAC. These data support the notion that heightened hypothalamic pituitary adrenal activity is a risk factor for CHD.

## Keywords

Mental stress • Psychophysiology • HPA axis • Sub-clinical atherosclerosis • Electron beam computed tomography

## Introduction

Evidence from population cohort studies suggests that psychosocial stress is a risk factor for coronary heart disease (CHD).<sup>1–3</sup> An important way of investigating the mechanisms underlying these associations is acute psychophysiological stress testing, involving measurement of biological responses to laboratory-induced stress.<sup>4,5</sup> Psychophysiological stress testing allows individual differences in biological responses to standardized stress to be evaluated and related to psychosocial risk factors.<sup>6</sup> Heightened biological stress reactions predict future hypertension,<sup>7</sup> increases in lipid levels and adiposity,<sup>8,9</sup> and the progression of sub-clinical cardiovascular disease.<sup>10</sup>

Much of the focus of psychophysiological stress testing has been on cardiovascular responses, but the hypothalamic pituitary adrenal (HPA) axis plays an important role in the stress response by releasing cortisol into the circulation. Abnormalities in HPA function have been described in several chronic inflammatory disorders and are thought to be one of the possible mechanisms through which psychosocial stress may influence the risk of CHD, although there is presently little direct evidence to support this assertion.<sup>11,12</sup> Two population studies have demonstrated associations between cortisol and sub-clinical cardiovascular disease; Dekker *et al.*<sup>13</sup> observed an association between total cortisol exposure while awake and higher carotid plaque scores in a sample of older adults, whereas Matthews *et al.*<sup>14</sup> showed a

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greater presence of coronary artery calcification (CAC) in younger participants with a flatter diurnal cortisol decline. No studies have yet examined the association of cortisol responses to laboratory-induced mental stress with a marker of sub-clinical atherosclerosis. The aim of the present study was therefore to examine the association between cortisol responses and CAC in a sample of older adults without history or objective signs of CHD.

## Methods

### Participants

A sample of participants was drawn from the Whitehall II epidemiological cohort<sup>15</sup> for psychophysiological testing during 2006 to 2008. The criteria for entry into the study included no history or objective signs of CHD, no previous diagnosis or treatment for hypertension, inflammatory diseases, or allergies. Volunteers were of white European origin, aged 53–76 years, and 56.5% were in full-time employment. Selection was stratified by grade of employment (current or most recent) to include higher and lower socioeconomic status participants. From the initially invited participants ( $n = 1169$ ), 27.6% were not eligible (mainly because of prescribed medications) and 25.9% declined to take part. A higher proportion of participants from lower work grades declined to take part compared with higher grades, (38.6% vs. 20.3%, respectively). Participants were prohibited from using any anti-histamine or anti-inflammatory medication 7 days before testing and were rescheduled if they reported colds or other infections on the day of testing. Participants gave full informed consent to participate in the study and ethical approval was obtained from the UCLH committee on the Ethics of Human Research.

### Psychophysiological testing

Testing was performed in either the morning or afternoon in a light temperature-controlled laboratory and was based on a protocol previously used in this laboratory.<sup>16</sup> Participants were instructed to refrain from drinking caffeinated beverages or smoking for at least 2 h before the study and not to have performed vigorous physical activity or consumed alcohol the previous evening. After a 30 min rest period, baseline blood pressure (using an automated UA-779 digital monitor) and a saliva sample were taken. Two behavioural tasks, designed to induce mental stress, were then administered in a random order. The tasks were a computerized version of the Stroop task and mirror tracing, both of which have been used extensively in psychophysiological research.<sup>17</sup> The tasks each lasted for 5 min. Cardiovascular measurements were continuously assessed during tasks and will be presented elsewhere. Participants then rested for 75 min. Saliva samples were collected immediately following the tasks and then at 20, 45, and 75 min post-stress for the assessment of salivary cortisol. The samples were collected using Salivettes (Sarsted, Leicester, UK), which were stored at  $-30^{\circ}\text{C}$  until analysis. Levels of cortisol were assessed using a time resolved immunoassay with fluorescence detection, at the University of Dresden. The intra- and inter-assay coefficients of variation were less than 8%.

### Coronary artery calcification

The assessment of CAC was performed using electron beam computed tomography (GE Imatron C-150, San Francisco, CA, USA) as previously described.<sup>18</sup> In brief, 40 contiguous 3 mm slices were obtained during a single breath-hold starting at the carina and proceeding to the level of the diaphragm. Scan time was 100 ms/slice, synchronized to 40% of the R–R interval. Agatston and volumetric calcium scores were calculated to

quantify the extent of CAC by a single experienced investigator blinded to the psychophysiological and clinical data on an Aquarius workstation (TeraRecon Inc., San Mateo, CA, USA). Since calcified volume was very highly correlated with Agatston score (Spearman's  $r = 0.99$ ), we present data for Agatston score only.

### Covariates

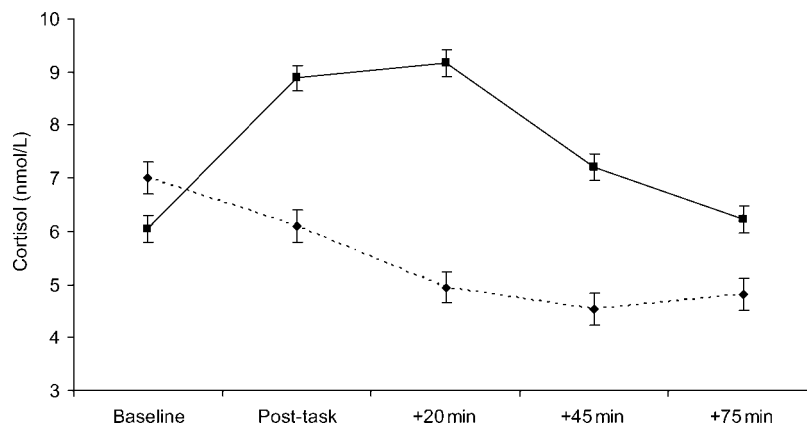
Participants reported current smoking levels and weekly alcohol intake (units per week). Height, weight, waist, and hip measures were recorded in light clothing for the calculation of body mass index (BMI) and waist to hip ratio. Fasting blood samples were taken during a separate clinical assessment. Analysis of the inflammatory marker, C-reactive protein, was performed using high-sensitivity ELISA (R&D Systems, Oxford, UK). Total and high-density lipoprotein (HDL) cholesterol and triglycerides were measured within 72 h in serum stored at  $4^{\circ}\text{C}$  using enzymatic colorimetric methods.<sup>19</sup> Low-density lipoprotein (LDL) cholesterol was derived using the Friedewald equation.<sup>20</sup> Glucose homeostasis was assessed from glycated haemoglobin ( $\text{HbA}_{1\text{C}}$ ) concentration, assayed using boronate affinity chromatography, a combination of boronate affinity and liquid chromatography.

### Statistical analysis

Since the time course of the cortisol response to mental stress can vary between individuals, participants were categorized as responders if an increase in cortisol was detected ( $\geq 1$  nmol/L representing on average a 20% increase relative to baseline) either immediately or at 20 min post-stress, relative to baseline. Multivariate logistic regression analyses were employed to examine the association of cortisol responses with both the presence of detectable CAC (Agatston score  $> 0$ ) and significant CAC (Agatston score  $\geq 100$ ). This threshold was based on the St Francis Heart Study that demonstrated maximum sensitivity and specificity for detecting cardiovascular events at a threshold calcium score of  $\geq 100$ .<sup>21</sup> We calculated odds ratios (OR) and 95% confidence intervals (CI) for the risk of CAC according to the cortisol response group, adjusting for age, gender, time of testing (am or pm), baseline cortisol, employment grade (as a marker of social position), smoking, alcohol, BMI, systolic blood pressure, LDL and HDL cholesterol, triglycerides, C-reactive protein, and  $\text{HbA}_{1\text{C}}$ . Independent  $t$ -tests (two-sided) were conducted to examine differences in demographic, cardiovascular risk factors, and extent of CAC (using  $\log [\text{CAC} + 1]$ ) between cortisol response groups. A repeated measures analysis of variance was conducted (with five trials) to examine the change in cortisol across time. All analyses were conducted using SPSS version 15.

## Results

A total of 543 participants attended the testing session, although 29 of them were excluded from the present analyses because of missing data, leaving a final sample size of 514 (mean age =  $62.9 \pm 5.7$  years). Excluded participants did not differ significantly from the main sample. The main reasons for exclusion included missing saliva samples ( $n = 17$ ) and bloods ( $n = 12$ ). There was a significant main effect over trials for cortisol [ $F(4, 2044) = 39.5$ ,  $P < 0.001$ ], although considerable variation in the response was observed, with  $\sim 40\%$  of the sample responding to the stress tasks with a notable increase in cortisol (Figure 1). There were no notable differences between the cortisol stress response group and non-response group in relation to demographic or cardiovascular risk factors (Table 1). Participants had moderately



**Figure 1** Laboratory cortisol profiles in responders and non-responders. Solid line represents cortisol responders. Values are mean  $\pm$  SEM.

**Table 1** Characteristics of the study population according to cortisol stress response

Variable	Non-responder (n = 308)	Responder (n = 206)	P-value
Age (years)	62.8 $\pm$ 5.7	63.2 $\pm$ 5.6	0.45
Men (%)	53.5	56.1	0.78
Work grade (% lower grades)	24.2	20.3	0.80
Marital status (% married)	63.5	67.3	0.52
Current smokers (%)	6.2	4.3	0.37
Alcohol (U/week)	9.0 $\pm$ 8.6	9.5 $\pm$ 9.3	0.58
Baseline systolic BP (mmHg)	123.2 $\pm$ 16.6	125.2 $\pm$ 16.4	0.18
Body mass index (kg/m <sup>2</sup> )	25.6 $\pm$ 4.1	26.1 $\pm$ 3.7	0.21
Waist to hip ratio	0.88 $\pm$ 0.10	0.88 $\pm$ 0.09	0.85
Total cholesterol (mmol/L)	5.89 $\pm$ 0.94	5.81 $\pm$ 1.05	0.35
HDL cholesterol (mmol/L)	1.72 $\pm$ 0.48	1.67 $\pm$ 0.47	0.16
LDL cholesterol (mmol/L)	3.93 $\pm$ 0.91	3.88 $\pm$ 1.00	0.65
Triglycerides (g/L) <sup>a</sup>	1.21 $\pm$ 0.73	1.28 $\pm$ 0.88	0.33
Glycated haemoglobin (%)	5.46 $\pm$ 0.38	5.43 $\pm$ 0.39	0.46
C-reactive protein (mg/L) <sup>a</sup>	1.61 $\pm$ 2.18	1.67 $\pm$ 2.21	0.73

Values are means  $\pm$  SD and P-values are from two-sided tests. Differences in categorical variables were examined using  $\chi^2$  tests. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

<sup>a</sup>t-Tests were performed on log-transformed values and data presented as geometric mean.

**Table 2** Logistic regression of cardiovascular risk markers on risk of coronary artery calcification

Independent variable	OR (95% CI)	P-value
5 years age increment	1.88 (1.48–2.37)	<0.001
Female gender	0.21 (0.11–0.40)	<0.001
Systolic blood pressure	1.01 (0.99–1.02)	0.44
LDL cholesterol	1.08 (0.85–1.39)	0.51
HDL cholesterol	1.05 (0.58–1.90)	0.86
Triglycerides	0.94 (0.68–1.29)	0.69
C-reactive protein	0.97 (0.87–1.07)	0.55
Glycated haemoglobin	2.26 (1.25–4.10)	0.007
Body mass index	1.04 (0.97–1.11)	0.25

Odds ratios (OR) of CAC  $\geq$  100 per unit increase in the independent variable, adjusted for age, sex, and mutually for all risk factors (n = 514).

raised cholesterol, but HbA<sub>1c</sub> levels were in the non-diabetic range, and BMIs were not markedly elevated.

There was no detectable CAC in 44% of the sample, 32.1% recorded Agatston scores of 1–99, 14.9% scores of 100–399,

and 9% scores of 400 or more. The 50th and 75th percentile calcium scores were largely comparable to those of the Multi-Ethnic Study Atherosclerosis reference values,<sup>22</sup> although slightly higher in older men and lower in older women from the present study (see Supplementary material online, *Table S1*). Each 5-year increment in age was associated with an increased risk of significant CAC, (OR = 1.88, 95% CI, 1.48, 2.37), and women were at significantly lower risk (OR = 0.21, 95% CI, 0.11, 0.40). Glycated haemoglobin was significantly associated with CAC, and there were marginal associations of BMI (*Table 2*). There was a higher prevalence of significant CAC in the cortisol stress response group (*Figure 2*), although a cortisol stress response was not related to the presence of detectable CAC (age and sex adjusted OR = 1.17, 95% CI, 0.80, 1.72). Among participants with detectable CAC, the cortisol stress response group also demonstrated higher log CAC scores compared with the non-response group, after adjustment for age and gender (4.51  $\pm$  0.15 vs. 3.94  $\pm$  0.13, *P* = 0.004). The association of cortisol response and risk of CAC was unchanged after adjustment for potential confounders including smoking, alcohol, systolic blood pressure, fasting cholesterol,

C-reactive protein, BMI, and HbA<sub>1C</sub> (Table 3). Additional adjustment for waist to hip ratio instead of BMI did not alter the associations.

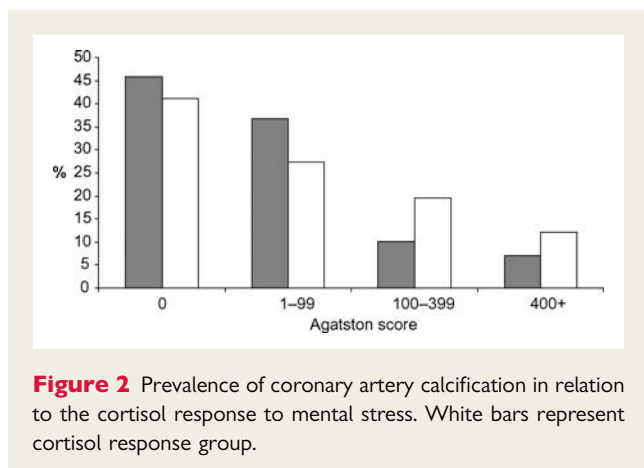
## Discussion

Coronary artery calcification, the extent of calcified plaque detected by computed tomography, is an indicator of subclinical coronary atherosclerosis, and is a predictor of future CHD events. Coronary artery calcification scores of 100–400, 400–999, and ≥1000 predict up to 4-, 7-, and 11-fold increases in risk, respectively, compared with patients with no detectable CAC.<sup>23</sup> In the present study, we observed a greater extent of CAC in participants responding with increases in cortisol to acute behavioural stress tasks, although cortisol was not associated with the presence of detectable CAC. There were no other noticeable differences in risk factors between the cortisol stress response groups, although it should be noted that participants in the present study were a healthy group of older men and women, without history or objective signs of CHD. Since participants demonstrated relatively normal lipid profiles and blood pressure, low levels of obesity and C-reactive protein, this may explain why traditional risk factors were not associated with CAC in this study. Previous evidence has generally demonstrated that traditional risk factors are associated with CAC,<sup>21</sup> although the relation between CAC and novel risk markers, such as C-reactive protein, is less consistent.<sup>24</sup> Also, participants with CAC are at a potentially higher risk than the Framingham risk score

might suggest.<sup>25</sup> Interestingly, glucose homeostasis as assessed by HbA<sub>1C</sub> concentration was strongly associated with CAC in this study, which confirms previous findings that demonstrated an important role of glycaemic control in the progression of CAC.<sup>18</sup> However, current evidence suggests that CAC is independently predictive of CHD risk over and above traditional cardiac risk factors,<sup>21,26–28</sup> thus may be important in the early detection of atherosclerosis.

The present findings are consistent with another study of older adults, which demonstrated an association between total cortisol exposure while awake and higher carotid plaque scores.<sup>13</sup> In a small sample of healthy individuals, the cortisol response to awakening was positively related to progression of carotid intima-media thickness in women but not men.<sup>29</sup> In patients undergoing coronary angiography, there were also associations between elevated morning plasma cortisol levels and severity of coronary artery disease (CAD).<sup>30,31</sup> Another study from our group recently showed that the cortisol rise following waking in the morning was greater in patients with documented CAD than in controls.<sup>32</sup> In contrast, Nijm et al.<sup>33</sup> reported reduced cortisol stress responses in patients with stable CAD. The present study, however, is the first to demonstrate an association of cortisol responses to laboratory-induced stress with a marker of subclinical coronary atherosclerosis. A previous study demonstrated that each 10 mmHg change in systolic blood pressure during a video game stressor was associated with increased odds of having CAC after 11 years follow-up in a sample of young adults.<sup>34</sup> Thus, the evidence suggests that acute physiological responses to laboratory-induced stress may index variations in cardiovascular and neuroendocrine responses to behavioural challenges that are relevant to the development of cardiovascular disease. Factors such as low socioeconomic status, anger, and hostility are associated with heightened psychophysiological reactivity.<sup>6</sup> The associations of these psychosocial factors with cardiovascular disease risk may be mediated in part by heightened neuroendocrine stress reactivity.

The mechanisms by which HPA activity directly influences atherosclerosis remain poorly understood, although there is some evidence that increased circulating cortisol levels may promote perivascular inflammation,<sup>35</sup> and treatment with glucocorticoids has been shown to enhance calcification within arteriosclerotic lesions.<sup>36</sup> A previous study in healthy participants demonstrated that mental stress-induced endothelial dysfunction and baroreflex impairment was prevented by blocking cortisol production with



**Figure 2** Prevalence of coronary artery calcification in relation to the cortisol response to mental stress. White bars represent cortisol response group.

**Table 3** Logistic regression of cortisol response on risk of coronary artery calcification (CAC ≥ 100)

	Cases/total n 123/514	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Non-responder	57/308	1.00	1.00	1.00
Responder	66/206	2.10 (1.35–3.27)	2.12 (1.36–3.32)	2.20 (1.39–3.47)
P-value		0.001	0.001	0.001

Model 1 adjusted for age, gender, baseline cortisol, time of testing (am/pm).

Model 2 with further adjustment for employment grade, smoking, alcohol.

Model 3 with further adjustment for baseline systolic blood pressure, LDL and HDL cholesterol, C-reactive protein, HbA<sub>1C</sub>, body mass index.

metrapone.<sup>37</sup> Thus, heightened cortisol responses may to some extent drive changes in haemodynamic function. Glucocorticoids are known to suppress testosterone in men, and higher cortisol: testosterone ratio was associated with greater risk of incident CHD in the Caerphilly cohort study,<sup>38</sup> which was partly explained through insulin resistance. Data from the main Whitehall II cohort have also shown an association between 24 h cortisol secretion and the metabolic syndrome.<sup>39</sup> In the present analyses, adjustments for inflammatory markers, lipids, and glucose homeostasis did not appreciably alter the association between cortisol reactivity and risk of CAC, thus it is unlikely that these mechanisms can explain the relationship. We cannot, however, rule out the influence of unmeasured confounding risk factors or genetic influences that might account for cortisol response patterns<sup>40</sup> and CHD risk.<sup>41</sup> For example, recent evidence suggests that a common glucocorticoid receptor gene is related to higher pro-inflammatory activity and greater risk of CHD.<sup>41</sup>

The present study has a number of strengths and limitations. First, the study is cross-sectional, thus we cannot determine the directionality of the observed relationship. For example, sub-clinical atherosclerosis might alter HPA function or psychological factors relevant to stress responding. It should be noted that only 40% of participants in this study were defined as cortisol responders, which is consistent with our previous findings from another sample tested with the same behavioural tasks.<sup>42</sup> Cortisol responses to stress tend to be greater when participants are confronted by social-evaluative challenges, rather than psychomotor and problem-solving tasks of the type used here.<sup>43</sup> Participants were divided into two broad groups of cortisol responders and non-responders to stress, so it was not possible to analyse dose–response associations. Cortisol stress responses were measured on a single occasion, and there may be adaptation on repeated testing, although we have previously demonstrated strong reproducibility of these responses over two repeated stress sessions.<sup>44</sup> The strengths of this study include the careful selection of participants with no objective signs of CHD, no previous diagnosis or treatment for hypertension, inflammatory diseases, or allergies, and the use of a highly validated marker of sub-clinical coronary atherosclerosis.

In conclusion, we have demonstrated an association between cortisol responses to laboratory-induced mental stress and CAC. These findings provide support for the hypothesis that hyper-reactivity of the HPA axis is one of the mechanisms through which psychosocial stress may influence the risk of CHD.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

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**Conflict of interest:** none declared.

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