# Cortisol, Testosterone, and Coronary Heart Disease: Prospective Evidence From the Caerphilly Study

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# Cortisol, Testosterone, and Coronary Heart Disease

## Prospective Evidence From the Caerphilly Study

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***Background***—There is a popular belief that chronic stress causes heart disease through psychoneuroendocrine mechanisms. We have examined whether an elevated circulating cortisol-to-testosterone ratio increases the risk of ischemic heart disease.

***Methods and Results***—We undertook a prospective cohort study of 2512 men aged 45 to 59 years between 1979 and 1983 from Caerphilly, South Wales, with a mean follow-up of 16.5 years. Subjects underwent a clinical examination, and morning fasting blood samples were taken for analysis of cortisol levels, testosterone levels, and other cardiovascular risk factors. The ratio of cortisol to testosterone showed weak associations with potential confounding factors but strong positive associations with components of the insulin resistance syndrome ($P<0.001$). A positive linear trend was seen across quintiles of cortisol:testosterone ratio for incident ischemic heart disease (age-adjusted OR per $z$ score change in ratio 1.22, 95% CI 1.07 to 1.38, $P=0.003$). This was markedly attenuated after adjustment for components of the insulin resistance syndrome (age-adjusted OR per $z$ score change in ratio 1.10, 95% CI 0.96 to 1.25, $P=0.18$). There was no association between the cortisol:testosterone ratio and other causes of death (age-adjusted hazard ratio 0.99, 95% CI 0.88 to 1.11, $P=0.81$).

***Conclusions***—This is the first population-based prospective study that has found a specific association between cortisol:testosterone ratio and incident ischemic heart disease, apparently mediated through the insulin resistance syndrome. Whether this reflects the effects of chronic stress, behavioral factors, or genetic influences remains to be determined. (***Circulation*. 2005;112:332-340.**)

**Key Words:** heart diseases ■ hormones ■ stress

The contribution of stress to coronary heart disease (CHD) risk has been investigated for many years, but considerable disagreement remains about whether stress influences CHD and, if so, the relative importance of this compared with other CHD risk factors. Although we now have a new animal model of stress-induced acceleration of atherosclerosis that provides scope for future studies into its etiology/pathogenesis,[1] the mechanisms through which stress can increase disease risk are currently poorly understood. One approach to this issue has been to investigate the association of questionnaire measures of stress and CHD risk[2]; however, such studies are seriously limited by the problems of reporting bias, reverse causation, and confounding.[3,4] A second approach is to use biomarkers of stress and relate these to CHD risk. There have been few methodologically sound prospective studies in this area, principally because most potential biomarkers of stress cannot be practically applied to large population samples. One exception is change in blood pressure in response to either psychological or physiological stressors. These have been used in prospective studies, with mixed findings. The largest prospective studies to date with measures of either hypertension or CHD incidence as the outcome have failed to detect important associations using this research paradigm.[5–7]

Neuroendocrine changes have been viewed as a central component of the stress response ever since Selye advanced his model of general adaptation.[8] Short-term increases in adrenaline and noradrenaline in response to acute stressors are well documented. Integrated measures over a long period of time would be required for these to be used in epidemiological studies, but such measurements could not plausibly be made on large populations given current technology. Speculation about the biological effects of stress on disease processes has particularly focused on glucocorticoids, which can show long-term elevation in response to chronic stressors.[9] Sapolsky's naturalistic studies among male olive baboons demonstrate the influence of external stressors and individual influences on early-morning basal blood cortisol levels.[10] These studies also demonstrate stress-related suppression of testosterone levels, which appears to be a consequence of

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cortisol elevation.[11] Glucocorticoids similarly suppress testosterone in men,[12,13] and low testosterone levels are a central component of the chronic physical and psychological stress response in men.[14,15] A high ratio of cortisol to testosterone is indicative of chronic stress. Increased cortisol, decreased testosterone, and the ratio between cortisol and testosterone levels have been widely used as endocrinological indicators of stress in small-scale human and animal studies.[14–21] Despite much speculation that the endocrinological response to chronic stress increases the risk of cardiovascular disease[22,23] there have been no prospective studies of hypothalamic-pituitary-adrenocortical system function and incident cardiovascular disease, probably because of the difficulties inherent in obtaining suitable measures on a large cohort. Here, we report the first study demonstrating an association between cortisol:testosterone ratio (C/T ratio) and incident ischemic heart disease (IHD) using data from a large representative sample of men from the town of Caerphilly, South Wales.

## Methods

The Caerphilly study is based on 100% of men in the appropriate age range selected from the town of Caerphilly and 5 adjacent villages. The men were chosen by date of birth so that they were aged 45 to 59 years when examined between 1979 and 1983. A total of 2512 men were seen (89% of the 2818 who were found to be eligible). At recruitment, the men were invited to a clinic at which a standard medical history was obtained and a questionnaire administered. Details of father's and own occupational social class, employment status at the time of the survey, alcohol consumption, smoking behavior, and symptoms of cardiovascular disease were obtained. Some personality questions were included. Five questions from the Framingham type A personality questionnaire were included,[24] for example, "Feeling at the end of an average day's work: felt very pressed for time? Often, occasionally, never." There were also 8 questions from the Cornell Medical Index (CMI) that covered anxiety,[25] for example, "Do you ever become nervous or agitated? Are you ever keyed up and jittery?" The response to each question was scored as never=0, occasionally=1, or often=2, so that a total score of 0 to 10 was produced for the Framingham questions and 0 to 16 for the CMI, where a high score indicates greater type A behavior or anxiety.

Height and weight were measured. Respiratory function tests were conducted with a McDermott spirometer, and forced expiratory volume in 1 second ($FEV_1$) was ascertained. Blood pressure was measured, and a 12-lead ECG was recorded. The subjects were then asked to return, after an overnight fast, to an early morning clinic examination at which a blood sample was taken with minimal venous stasis. Total cholesterol, HDL cholesterol, triglyceride, fibrinogen, insulin, glucose, testosterone, and cortisol were assayed on these samples. For the plasma sex hormone assays, lithium heparin was used as the anticoagulant.

Of the 2512 men seen in the study, 2482 had a fasting blood sample taken, and of these, 2368 samples were taken between 3 AM and 11 AM (the majority between 7 and 8 AM), of which 2323 had both testosterone and cortisol measured. The present report is concerned with these 2323 men, who compose 92% of the original sample. Full details of the population sample, clinic procedures, and laboratory methods have been reported previously.[26–29]

Insulin resistance was estimated according to the homeostasis model assessment[30] as the product of fasting glucose and insulin, divided by the constant 22.5. The higher the value, the greater the level of insulin resistance. Insulin or glucose measurements were missing for 267 of the 2323 men included in the main analyses, mainly owing to missing insulin results (254 subjects). The main reason for missing insulin results was that from around halfway through the screening, there were short runs of sequential subjects (usually 25 subjects but in 1 case 50 subjects) whose samples were never assayed. We believe that this was a simple laboratory handling error rather than due to any characteristics of the subjects. Insulin resistance scores are not calculated for diabetic subjects (38 men with a self-reported history of diabetes and 23 with a fasting blood glucose concentration of $\geq$7 mmol/L). Thus, insulin resistance scores are available for only 1995 of the men.

### Laboratory Methods

All samples for hormone analysis were frozen at −20°C and assayed within 3 months of collection. Testosterone and cortisol were measured by radioimmunoassay.[31,32] For the testosterone assay, cross-reactivity was 0.41% with androstenedione and <0.01% with estradiol. The only steroid that showed high cross-reactivity was 5 ∝-dihydrotestosterone, which is rarely found at levels in male plasma that are likely to interfere with testosterone determinations.

Pilot studies among a group of volunteers had indicated that between-subject variation was greater than within-subject variation (day-to-day variation). In the main study, split-sample duplicates were used to estimate the precision of the assays. The overall coefficient of variation was 17% (n=133 pairs) for testosterone, 16% (n=135 pairs) for cortisol and 28% for the crude unadjusted C/T ratio. The intraclass correlation coefficients calculated from these duplicate pairs were 0.75, 0.70, and 0.57 for testosterone, cortisol, and the C/T ratio, respectively.

### Classification of Outcome Variables

The records of all men at the National Health Service Central Registry were flagged so that notification of death was automatic and a copy of the death certificate was received. Deaths up to July 1, 1998 have been used in the present report (an average of 16.5 years after initial examination). All death certificates were coded according to the ninth revision of the International Classification of Diseases (ICD-9). Fatal IHD events were classified as deaths with ICD-9 codes 410 to 414. Nonfatal IHD events were ascertained through the following methods: (1) Men who attended follow-up clinics were asked about a doctor diagnosis of myocardial infarction. (2) Discharges from local hospitals with a diagnosis coded as ICD-9 410 to 414 (IHD) from hospital activity analysis were reviewed, and both self-reported IHD and a hospital discharge code of IHD were used as the basis for a detailed search of hospital notes to identify events that satisfied the World Health Organization criteria for definite acute myocardial infarction. (3) A new ECG was recorded at follow-up clinic examinations so that ECG changes indicative of new IHD could be recognized. ECG-defined IHD was as follows: no Q-QS wave (Minnesota codes 1-1, 1-2, and 1-3) on the recruitment-phase ECG but major or moderate Q-QS waves (Minnesota codes in the range 1-1-1 through 1-2-5 plus 1-2-7) on the follow-up ECG. A new history of angina or any revascularization procedure was not taken as evidence of an IHD event.

The results reported in the present study refer to IHD identified up to the last complete follow-up examination, which was performed between October 25, 1993 and February 23, 1997, an average of 13.7 years after initial examination. IHD at baseline was defined as a combination of either the latter ECG abnormalities or grade 2 angina on the basis of the Rose angina questionnaire.

### Statistical Analysis

Cortisol is known to have a circadian rhythm such that the time of day at which the blood is taken has a large effect on cortisol concentration.[33] Adjustment of cortisol level for the time of day is therefore of importance. This was undertaken by use of fractional polynomials.[34] This tests a family of fractional polynomial functions with 2 sets of powers (eg, $\beta_0+\beta_1 x^p+\beta_2 x^q \log x$), where p and q are chosen from the following family of powers: −2, −1, −0.5, 0, 0.5, 1, 2, 3. The best power or combination of powers (44 variations) is ascertained by the lowest deviance, defined as minus twice the log likelihood. The mean value of the observed cortisol distribution was then added to the residuals from the best-fitting model to produce a time-adjusted cortisol variable.

**TABLE 1. Mean Values of Cortisol and Testosterone by Quintiles of C/T Ratio**

| | C/T Ratio Quintiles (Adjusted for Time) | | | | | | |
|---|---|---|---|---|---|---|---|
| Variable | Lowest Quintile | 2nd | 3rd | 4th | Highest Quintile | *P* for Heterogeneity | $P_{trend}$ |
| Cortisol, nmol/L | 306.6 | 401.7 | 448.4 | 491.7 | 553.6 | <0.001 | <0.001 |
| Testosterone, nmol/L | 29.4 | 25.6 | 23.0 | 20.3 | 16.1 | <0.001 | <0.001 |

Cortisol values have been adjusted for time of sampling. *P* values are for both heterogeneity and trend across quintiles, controlling for age.

The association between C/T ratio and other possible risk factors for IHD was investigated using quintiles of the ratio. The mean value of each risk factor was calculated for each quintile, and the probability value for trend across quintiles was computed after age adjustment (except in the case of age itself) with linear regression. We also calculated the probability value for heterogeneity, which simply calculates the probability that the value observed for any specific quintile differs by chance from what would be expected if the null hypothesis were true. Risk factors that were log-normal were analyzed with the log values, and the means were then converted back to the original units to produce the geometric means. For risk factors that were binary, logistic regression was used to produce the probability values for association, controlling for age.

We undertook Cox proportional hazards survival analysis for all-cause mortality, IHD mortality, and deaths due to other causes and hence calculated hazard ratios, 95% CIs, and probability values. Because age is a strong determinant of mortality risk, and individuals entered the study at different ages, we controlled for current age in all models using age as the follow-up time scale. The proportional hazards assumption was investigated by testing that the log-hazard ratio was constant over time for each model. For IHD events, we undertook logistic regression analysis, because this outcome included major ECG changes that could not be dated. For this outcome, we present ORs. For each regression model, we present the effect estimate for each quintile of C/T ratio, time-adjusted cortisol quintiles, and testosterone quintiles. We also present the test for trend using these measures as ordinal variables. Finally, we show the effect estimate for a 1-SD change (*z* score) in the distribution of each of the above. Multivariable analysis was undertaken in 4 stages: (1) adjustment for age alone; (2) age and the following potential confounders: smoking status (never, ex-smoker, or 1 to 14, 15 to 24, or ≥25 cigarettes per day), adult social class (nonmanual versus manual labor), alcohol consumption (4 groups), height, $FEV_1$/height$^2$, fibrinogen (log transformed), and white cell count (log transformed); (3) age and the following potential intermediaries: systolic blood pressure, triglycerides (log transformed), cholesterol (log transformed), HDL cholesterol (log transformed), body mass index, fasting glucose (log transformed), and fasting insulin (log transformed); and (4) all of the above. In order for the models to be directly comparable, we kept the number of observations constant across models (1936 subjects). To preserve power, we imputed missing insulin levels, because this was the variable with the most missing data and the pattern of missingness suggested that it may be missing at random or completely at random (see above). We also compared risk factor results for men with and without missing insulin levels. We used multiple imputation by chained equations and created 5 copies of the data set with imputed values. Parameter estimates (hazard ratios and ORs) were then averaged across data sets to give a single value, and Rubin rules were used to allow for the between- and within-imputation components of variation.[35] Our results are therefore now based on these imputed data sets, but we also provide comparable parameter estimates using the complete case analysis for comparison. The simple age-adjusted models were also repeated with the maximum number of observations as a sensitivity analysis. To exclude the possibility of reverse causality, the mean log C/T levels were calculated for subjects with and without IHD at baseline. In addition, simple age-adjusted models were run that both excluded such subjects and adjusted for them to determine whether the effect estimates were altered substantially.

## Results

Most men had their blood sample taken between 7 and 8 in the morning (before 6 AM 5.2%, 6 to 7 AM 17.8%, 7 to 8 AM 39.3%, 8 to 9 AM 25%, and 9 AM or later 12.2%). The mean cortisol value was 440 nmol/L, with an SD of 134.5 nmol/L. The best polynomial expressing cortisol as a function of time involved fitting a 2-parameter model with both time and log time as explanatory variables. This model alone, however, only explained ≈3% of the variance. The mean value of the unadjusted cortisol levels was then added to the residuals of the above model to rescale the data back to the original units (time-adjusted mean cortisol 440 nmol/L with SD of 132.5 nmol/L). The Pearson correlation coefficient between the crude and time-adjusted cortisol levels was 0.99. Testosterone did not show any clear circadian pattern over the morning measurements in the present study, as in other studies,[36] and thus, uncorrected values were used. The time-adjusted C/T ratio is used in the remainder of this report.

Table 1 shows how the mean cortisol and testosterone levels vary according to quintiles of the C/T ratio. Tables 2 and 3 present data on either potential confounding variables or variables that we had conceptualized a priori as potential metabolic intermediaries (associated with the metabolic syndrome) between C/T ratio and IHD.

Few of the potential confounding variables were associated with the C/T ratio. A higher C/T ratio was associated with younger age, higher fibrinogen levels, and being a nonsmoker, although the trends were not consistent across quintiles. More favorable socioeconomic position was associated with higher C/T ratios, but this was not seen for parental social class. Although drinking status itself was not associated with the quintiles of C/T ratio, among drinkers, the mean C/T ratio increased with amount of alcohol consumed. As expected, all the potential intermediary variables were strongly associated with C/T ratio, except for HDL levels. There was no association between total scores for either the subset of Framingham type A questions ($P_{trend}$=0.77) or the anxiety questions from the CMI ($P_{trend}$=0.63). Men with missing insulin levels had similar risk factor profiles as those for whom we had insulin levels for all risk factors except lower HDL cholesterol levels ($P$=0.003), and they were more likely to be manual workers ($P$=0.04).

Over the follow-up period, 482 men died; 192 of these deaths were due to IHD and 290 to other causes. The total number of IHD cases, including fatal and nonfatal cases, was 320. Table 4 presents the hazard ratios and ORs for all-cause mortality, IHD cumulative incidence and mortality, and

**TABLE 2. Association Between Potential Confounding Variables and Quintiles of C/T Ratio Adjusted for Age and Time of Sampling**

| | C/T Ratio Quintiles (Adjusted for Time) | | | | | | |
|---|---|---|---|---|---|---|---|
| Variable | Lowest Quintile | 2nd | 3rd | 4th | Highest Quintile | *P* for Heterogeneity | $P_{trend}$ |
| Age, y | 52.4 | 52.4 | 51.9 | 51.7 | 52.0 | 0.09 | 0.02 |
| Height, m | 1.71 | 1.71 | 1.71 | 1.71 | 1.71 | 0.93 | 0.98 |
| Fibrinogen,* g/L | 3.65 | 3.61 | 3.71 | 3.67 | 3.76 | 0.07 | 0.02 |
| White cell count,*($\times 10^9$/L | 6.69 | 6.77 | 6.84 | 6.95 | 6.89 | 0.27 | 0.04 |
| $FEV_1$/height$^2$ | 90.4 | 91.6 | 91.1 | 90.1 | 90.8 | 0.88 | 0.82 |
| Manual social class, % | 75.1 | 65.1 | 65.4 | 67.0 | 67.5 | 0.008 | 0.06 |
| Father in manual social class, % | 87.4 | 86.6 | 89.4 | 88.0 | 87.8 | 0.78 | 0.64 |
| Smoking status | | | | | | | |
| Current smoker, % | 55.5 | 57.3 | 60.1 | 52.6 | 50.0 | 0.02 | 0.03 |
| No. of cigarettes per day | 15.7 | 15.4 | 16.1 | 15.6 | 15.9 | 0.24 | 0.80 |
| Alcohol status | | | | | | | |
| Current drinker, % | 85.6 | 85.3 | 83.3 | 86.0 | 87.8 | 0.42 | 0.27 |
| Alcohol, mL/wk | 187 | 209 | 207 | 234 | 226 | 0.04 | 0.005 |

*P* values are for both heterogeneity and trend across quintiles, controlling for age.
*Geometric mean (variable is log normal).

non-IHD causes of death by quintiles of C/T ratio and for a 1-SD increase in the log C/T ratio.

Risk of all-cause mortality, IHD mortality, and IHD incidence was positively associated with C/T ratio. There was no association of C/T ratio with non-IHD mortality. This indicates that the all-cause mortality association is simply due to the IHD contribution. The associations for IHD incidence showed a strong dose-response effect. These positive associations were only weakly attenuated after adjustment for potential confounders but were markedly attenuated after adjustment for the intermediary variables.

We repeated the above analyses with cortisol and testosterone quintiles (Tables 5 and 6). Although there was a weaker positive association between cortisol and IHD when analyzed as a continuous variable, it was clear from the risk pattern with the quintile measure that this was not particularly dose-responsive, with highest risk seen in quintile 4. This association showed some attenuation for IHD deaths but far less attenuation for IHD events. Testosterone showed a more linear inverse association with IHD, but this was statistically weaker than that observed with the C/T ratio. This did show attenuation after adjustment for potential mediators. Neither cortisol or testosterone showed much of an association with other causes of death.

The results with C/T ratio were essentially unaltered when we used all available observations rather than just participants without missing values and imputed insulin levels. For example, the simple age-adjusted ORs for IHD incidence by C/T ratio quintiles was 1.00, 1.08, 1.29, 1.52, and 1.75. Subjects with IHD at baseline had slightly higher mean log

**TABLE 3. Association Between Potential Intermediary Variables and Quintiles of C/T Ratio Adjusted for Age and Time of Sampling**

| | Time-Corrected C/T Ratio Quintiles | | | | | | |
|---|---|---|---|---|---|---|---|
| Variable | Lowest Quintile | 2nd | 3rd | 4th | Highest Quintile | *P* for Heterogeneity | $P_{trend}$ |
| Systolic blood pressure, mm Hg | 137.6 | 139.4 | 140.2 | 142.4 | 145.3 | <0.001 | <0.001 |
| Diastolic blood pressure, mm Hg | 85.9 | 87.7 | 89.0 | 90.3 | 91.9 | <0.001 | <0.001 |
| Cholesterol,* mmol/L | 5.52 | 5.60 | 5.54 | 5.65 | 5.72 | 0.03 | 0.005 |
| HDL,* mmol/L | 1.08 | 1.08 | 1.07 | 1.09 | 1.05 | 0.22 | 0.27 |
| Cholesterol/HDL ratio* | 5.12 | 5.18 | 5.19 | 5.16 | 5.47 | 0.03 | 0.01 |
| Triglycerides,* mmol/L | 1.44 | 1.57 | 1.70 | 1.76 | 2.03 | <0.001 | <0.001 |
| BMI, kg/m$^2$ | 25.6 | 25.7 | 26.1 | 26.4 | 27.1 | <0.001 | <0.001 |
| Glucose,* mmol/L | 4.76 | 4.78 | 4.85 | 4.96 | 5.18 | <0.001 | <0.001 |
| Insulin,* mIU/L | 5.0 | 5.3 | 6.0 | 6.3 | 7.2 | <0.001 | <0.001 |
| HOMA index* | 1.04 | 1.12 | 1.20 | 1.32 | 1.52 | <0.001 | <0.001 |

*P* values are for both heterogeneity and trend across quintiles, controlling for age.
*Geometric mean (variable is log normal).

**TABLE 4. Hazard Ratios for All-Cause and Cause-Specific Mortality and ORs for Incident IHD Across Quintiles of C/T Ratio**

| | C/T Ratio Quintiles (Adjusted for Time) | | | | | | |
|---|---|---|---|---|---|---|---|
| Outcome | Lowest Quintile | 2nd | 3rd | 4th | Highest Quintile | $P_{trend}$ | Risk for 1-SD Rise in Log Ratio (95% CI) |
| All deaths, n | 88 | 89 | 107 | 95 | 103 | | |
| Controlling for age | 1.00 | 0.98 | 1.22 | 1.11 | 1.18 | 0.17 | 1.06 (0.97–1.17) |
| Age and confounding variables* | 1.00 | 0.99 | 1.20 | 1.08 | 1.16 | 0.25 | 1.06 (0.96–1.16) |
| Age and potential mediators† | 1.00 | 0.96 | 1.14 | 1.02 | 1.03 | 0.74 | 1.01 (0.92–1.11) |
| All of the above | 1.00 | 0.97 | 1.15 | 1.00 | 1.02 | 0.86 | 1.01 (0.92–1.11) |
| IHD deaths, n | 30 | 33 | 41 | 40 | 48 | | |
| Controlling for age | 1.00 | 1.07 | 1.37 | 1.38 | 1.61 | 0.02 | 1.19 (1.03–1.39) |
| Age and confounding variables | 1.00 | 1.06 | 1.30 | 1.28 | 1.49 | 0.06 | 1.16 (1.00–1.34) |
| Age and potential mediators | 1.00 | 1.02 | 1.20 | 1.13 | 1.18 | 0.44 | 1.06 (0.91–1.24) |
| All of the above | 1.00 | 0.99 | 1.18 | 1.06 | 1.10 | 0.66 | 1.05 (0.90–1.22) |
| Any IHD event, n‡ | 50 | 55 | 65 | 71 | 79 | | |
| Controlling for age | 1.00 | 1.10 | 1.35 | 1.55 | 1.73 | 0.001 | 1.22 (1.07–1.38) |
| Age and confounding variables | 1.00 | 1.09 | 1.30 | 1.49 | 1.66 | 0.004 | 1.20 (1.05–1.37) |
| Age and potential mediators | 1.00 | 1.02 | 1.22 | 1.32 | 1.29 | 0.10 | 1.10 (0.96–1.25) |
| All of the above | 1.00 | 1.00 | 1.19 | 1.28 | 1.26 | 0.14 | 1.10 (0.96–1.26) |
| Other causes of death, n | 58 | 56 | 66 | 55 | 55 | | |
| Controlling for age | 1.00 | 0.93 | 1.14 | 0.98 | 0.95 | 0.89 | 0.99 (0.88–1.11) |
| Age and confounding variables | 1.00 | 0.96 | 1.17 | 0.98 | 0.97 | 0.94 | 0.99 (0.88–1.12) |
| Age and potential mediators | 1.00 | 0.93 | 1.11 | 0.96 | 0.94 | 0.83 | 0.98 (0.87–1.11) |
| All of the above | 1.00 | 0.97 | 1.15 | 0.97 | 0.97 | 0.90 | 0.99 (0.87–1.12) |

n indicates number of events.

*Smoking status (never, past, 1–14, 15–24, ≥25 cigarettes/d), adult social class, alcohol consumption, height, $FEV_1/height^2$, fibrinogen (log transformed), and white cell count (log transformed).

†Systolic blood pressure, triglycerides (log transformed), cholesterol (log transformed), HDL cholesterol (log transformed), body mass index, fasting glucose (log transformed), and fasting insulin (log transformed).

‡ORs.

C/T ratios (difference in means 0.06, 95% CI −0.02 to 0.14, $P=0.15$). Exclusion of these subjects from the analysis barely altered the results (age-adjusted ORs for IHD incidence by C/T ratio quintiles 1.00, 1.03, 1.16, 1.40, and 1.65), nor did adjustment for existing IHD at baseline (age- and IHD-at-baseline–adjusted ORs for IHD incidence by C/T quintiles 1.00, 1.07, 1.28, 1.48, and 1.57). Fifty-two subjects were on night shift work. In some cases, they were seen at an early evening clinic, and hence their results were excluded. However, 36 of these subjects did have a morning sample taken. These subjects showed, as expected, much lower cortisol and C/T ratio levels than the rest of the group ($P<0.0001$). We repeated the analysis excluding these subjects; age-adjusted ORs for IHD incidence by C/T ratio quintiles were 1.00, 1.05, 1.18, 1.37, and 1.63. The results were little altered. We repeated the analysis for incident IHD stratifying by socioeconomic position but failed to find any evidence of an interaction ($P=0.19$).

Finally, we repeated the analysis of C/T quintiles with IHD mortality, adjusting for potential intermediaries, without the imputed insulin levels but only with the subset of subjects with complete data for comparison (1734 subjects). The hazard ratios for each quintile were similar but showed even less of an association than that observed with the imputed data set (age and potential mediator adjusted hazard ratios: 1.0, 0.92, 0.98, 1.01, and 1.03).

## Discussion

In this prospective study, C/T ratio was positively associated with IHD mortality and incidence. Adjustment for potential socioeconomic and behavioral confounding variables had little influence on these associations, but they appeared to be mediated by components of the insulin resistance syndrome (elevated blood pressure, triglyceride levels, body mass index, total cholesterol, HDL cholesterol, and impaired glucose tolerance). The strength of the association between C/T ratio and IHD risk may appear, at first, modest compared with other conventional risk factors such as systolic blood pressure and serum cholesterol. In the Caerphilly data set, the age-adjusted ORs for the top versus bottom quintile for systolic blood pressure and cholesterol were 2.3 and 2.2 respectively. However, both cortisol and testosterone are measured much less reliably than either blood pressure or cholesterol, given their marked biological variability. Adjustment for the intraclass correlation of the C/T ratio suggests the "true" OR for incident IHD should be ≈2.6 versus 2.3 for the adjusted cholesterol measure (intraclass correlation for cholesterol

**TABLE 5. Hazard Ratios for All-Cause and Cause-Specific Mortality and ORs for Incident IHD Across Quintiles of Cortisol**

| | Cortisol Quintiles (Adjusted for Time) | | | | | | |
|---|---|---|---|---|---|---|---|
| Outcome | Lowest Quintile | 2nd | 3rd | 4th | Highest Quintile | $P_{trend}$ | Risk for 1-SD Rise in Log Cortisol (95% CI) |
| All deaths | | | | | | | |
| Controlling for age | 1.00 | 1.11 | 1.02 | 1.25 | 1.07 | 0.77 | 1.06 (0.97–1.16) |
| Confounding variables* | 1.00 | 1.08 | 0.98 | 1.18 | 0.98 | 0.88 | 1.03 (0.94–1.13) |
| Potential mediators† | 1.00 | 1.07 | 0.97 | 1.17 | 0.96 | 0.98 | 1.02 (0.93–1.12) |
| All of the above | 1.00 | 1.07 | 0.96 | 1.14 | 0.91 | 0.69 | 1.00 (0.91–1.10) |
| IHD deaths | | | | | | | |
| Controlling for age | 1.00 | 1.05 | 0.85 | 1.25 | 0.93 | 0.94 | 1.07 (0.92–1.23) |
| Confounding variables | 1.00 | 1.09 | 0.86 | 1.22 | 0.92 | 0.91 | 1.04 (0.90–1.20) |
| Potential mediators | 1.00 | 1.00 | 0.80 | 1.12 | 0.77 | 0.43 | 1.00 (0.86–1.15) |
| All of the above | 1.00 | 1.05 | 0.84 | 1.12 | 0.78 | 0.41 | 0.99 (0.85–1.14) |
| Any IHD‡ | | | | | | | |
| Controlling for age | 1.00 | 1.03 | 1.12 | 1.36 | 1.15 | 0.21 | 1.12 (0.99–1.27) |
| Confounding variables | 1.00 | 1.03 | 1.12 | 1.30 | 1.11 | 0.35 | 1.10 (0.97–1.25) |
| Potential mediators | 1.00 | 1.03 | 1.10 | 1.33 | 1.02 | 0.53 | 1.08 (0.95–1.22) |
| All of the above | 1.00 | 1.05 | 1.12 | 1.31 | 1.00 | 0.66 | 1.06 (0.93–1.21) |
| Other causes of death | | | | | | | |
| Controlling for age | 1.00 | 1.16 | 1.14 | 1.25 | 1.18 | 0.93 | 1.06 (0.94–1.19) |
| Confounding variables | 1.00 | 1.08 | 1.06 | 1.16 | 1.03 | 0.78 | 1.02 (0.90–1.15) |
| Potential mediators | 1.00 | 1.11 | 1.09 | 1.18 | 1.10 | 0.56 | 1.04 (0.92–1.18) |
| All of the above | 1.00 | 1.06 | 1.04 | 1.13 | 1.01 | 0.87 | 1.01 (0.90–1.15) |

*Smoking status (never, past, 1–14, 15–24, ≥25 cigarettes/d), adult social class, alcohol consumption, height, $FEV_1/height^2$, fibrinogen (log transformed), and white cell count (log transformed).

†Systolic blood pressure, triglycerides (log transformed), cholesterol (log transformed), HDL cholesterol (log transformed), body mass index, fasting glucose (log transformed), and fasting insulin (log transformed).

‡ORs.

0.96). The present data therefore are likely to underestimate the true effect estimates for C/T ratio.

The association of C/T ratio with mortality was specific for IHD: there was no association with mortality due to causes other than IHD. Because behavioral factors (such as smoking) and socioeconomic deprivation showed similar associations with IHD and overall non-IHD mortality, the lack of association between C/T ratio and non-IHD mortality provides further evidence that the association between C/T ratio and IHD mortality is not caused through confounding by such factors.

There is considerable literature on stress as a potential cause of CHD, much of which postulates a role for neuroendocrine mediators.[23,37–42] It is noticeable, however, that the evidence base to support this supposition is weak with respect to studies with disease end-point data. Cortisol is the potential mediator between stress and cardiovascular disease that has been most discussed in the literature. Four small cross-sectional studies suggested that early-morning plasma cortisol levels correlate with the degree of coronary artery disease detected on angiograms,[43–46] but 2 studies failed to find this association.[45,47,48] There is limited additional evidence from studies comparing poorly characterized groups of patients with various diseases (including cardiovascular disease) with subjects without these diseases, which suggests that blood cortisol levels may be related to cardiovascular disease.[49,50] Endogenous corticosteroid treatment has also been associated with elevated cardiovascular disease risk,[51,52] and evidence from animal studies suggests detrimental effects on cardiovascular health of elevated cortisol.[53] In the only prospective study to date, elevated urinary cortisol level was combined with other physiological indicators (blood pressure, waist-hip ratio, total cholesterol/HDL cholesterol ratio, glycosylated hemoglobin, urinary norepinephrine, and urinary epinephrine levels) to produce an index of "allostatic load." This index predicted incident cardiovascular disease, but it is impossible to ascertain any particular contribution of cortisol given the fact that well-established cardiovascular risk factors are included in the index.[54] With respect to the insulin resistance syndrome, several studies have found that early-morning plasma cortisol levels (ie, measures directly comparable to those in the present study) were correlated with various measures of the syndrome and its subcomponents,[55,56] and other studies with different indices of cortisol metabolism provide supportive evidence.[57,58]

With respect to circulating testosterone levels, the picture is mixed, with testosterone either having no association or an inverse association with CHD risk.[59–61] One randomized, placebo-controlled trial produced a reduction in angina symptoms in participants randomized to testosterone undecanoate,

**TABLE 6. Hazard Ratios for All-Cause and Cause-Specific Mortality and ORs for Incident IHD Across Quintiles of Testosterone**

| Outcome | Testosterone Quintiles | | | | | $P_{trend}$ | Risk for 1-SD Rise in Log Testosterone (95% CI) |
|---|---|---|---|---|---|---|---|
| | Lowest Quintile | 2nd | 3rd | 4th | Highest Quintile | | |
| All deaths | | | | | | | |
| Controlling for age | 1.00 | 0.81 | 0.92 | 0.93 | 0.96 | 0.85 | 1.01 (0.92–1.10) |
| Confounding variables* | 1.00 | 0.88 | 0.93 | 0.91 | 0.91 | 0.63 | 0.98 (0.89–1.07) |
| Potential mediators† | 1.00 | 0.87 | 0.99 | 1.02 | 1.09 | 0.36 | 1.04 (0.94–1.14) |
| All of the above | 1.00 | 0.94 | 1.00 | 1.00 | 1.04 | 0.70 | 1.02 (0.92–1.12) |
| IHD deaths | | | | | | | |
| Controlling for age | 1.00 | 0.91 | 0.65 | 0.78 | 0.69 | 0.07 | 0.85 (0.73–0.99) |
| Confounding variables | 1.00 | 1.02 | 0.68 | 0.83 | 0.70 | 0.08 | 0.86 (0.74–1.00) |
| Potential mediators | 1.00 | 1.03 | 0.74 | 0.97 | 0.92 | 0.61 | 0.94 (0.80–1.10) |
| All of the above | 1.00 | 1.15 | 0.78 | 1.01 | 0.93 | 0.61 | 0.94 (0.80–1.11) |
| Any IHD‡ | | | | | | | |
| Controlling for age | 1.00 | 0.96 | 0.77 | 0.75 | 0.77 | 0.07 | 0.89 (0.79–1.01) |
| Confounding variables | 1.00 | 1.03 | 0.79 | 0.75 | 0.76 | 0.06 | 0.88 (0.77–1.00) |
| Potential mediators | 1.00 | 1.06 | 0.88 | 0.92 | 1.06 | 0.91 | 0.99 (0.87–1.14) |
| All of the above | 1.00 | 1.13 | 0.89 | 0.91 | 1.02 | 0.68 | 0.97 (0.84–1.12) |
| Other causes of death | | | | | | | |
| Controlling for age | 1.00 | 0.72 | 1.15 | 1.06 | 1.20 | 0.08 | 1.11 (0.99–1.24) |
| Confounding variables | 1.00 | 0.77 | 1.13 | 0.98 | 1.07 | 0.39 | 1.05 (0.94–1.18) |
| Potential mediators | 1.00 | 0.76 | 1.17 | 1.07 | 1.21 | 0.12 | 1.10 (0.98–1.24) |
| All of the above | 1.00 | 0.80 | 1.15 | 1.01 | 1.10 | 0.37 | 1.06 (0.94–1.20) |

*Smoking status (never, past, 1–14, 15–24, ≥25 cigarettes/d), adult social class, alcohol consumption, height, $FEV_1$/height$^2$, fibrinogen (log transformed), and white cell count (log transformed).

†Systolic blood pressure, triglycerides (log transformed), cholesterol (log transformed), HDL cholesterol (log transformed), body mass index, fasting glucose (log transformed), and fasting insulin (log transformed).

‡ORs.

with improvement in the degree of myocardial ischemia on ECG.[62] This small study needs replication before its findings can be considered reliable. Animal evidence suggests that testosterone produces coronary artery relaxation, which would improve coronary perfusion, although there are some contradictory findings in this regard.[63] Endogenous testosterone levels have also been related to fasting glucose and insulin concentrations and have predicted the onset of type 2 diabetes mellitus.[61] Intervention studies have also suggested that testosterone treatment improves insulin sensitivity.[61]

Cortisol and testosterone secretion are interrelated. Activation of the hypothalamo-pituitary-adrenal axis not only results in an elevation of adrenal corticosteroids but also inhibits gonadotrophin secretion,[64] which will result in a secondary reduction of estrogen in the female and testosterone in the male. The actions of cortisol and testosterone with respect to components of the insulin resistance syndrome are generally inverse, with cortisol being associated with adverse effects and testosterone with favorable effects. Some of the effects of cortisol may be magnified by the concurrent inhibition of testosterone secretion. Therefore, the ratio of cortisol to testosterone, as used in this and other studies,[9–11,14,15,17,18,33,65] is a useful proximal marker of processes that may lead to worsening insulin resistance and thus increased risk of CHD.

In the present study, the C/T ratio was not strongly associated with social class or the questionnaire measures of stress and personality. In olive baboons, high early-morning cortisol levels and low testosterone levels have been associated with low position in the social hierarchy, and this has been invoked as a potential mechanism linking adverse socioeconomic circumstances and CHD in humans. However, in primates, the association between position in the social hierarchy and cortisol levels varies dramatically, being positive in some species and negative in others,[66] and the primary investigator in the olive baboon studies has cautioned against direct analogies between his results and predictions for humans.[67] Conversely, other influences such as dietary factors and exercise patterns may influence C/T ratio.

There are several important limitations that need to be considered. First, this study is of a representative sample of middle-aged men from South Wales. Although they may be generalizable to other men from the United Kingdom, these observations need to be replicated among other populations, including studies of women. Second, we only had measures of cortisol and testosterone at baseline. It is therefore impossible in the present study to know whether elevations in the C/T ratio lead to insulin resistance and hence disease, or whether the reverse may be true. Such a question is better tested in experimental animal studies or, where ethical, with

human trials. Third, our measures of cortisol and testosterone were not very reliable. This is not simply because of assay difficulties but reflects the marked intrinsic biological variability of such hormones. As we have discussed, we believe that our results actually underestimate the true association. Fourth, our measures of psychological stress may have been inadequate. We cannot, therefore, exclude that the C/T ratio may be elevated secondary to stress, although our data do not support this notion.

C/T ratio in the present study was associated with a specific elevation in IHD risk that was robust to adjustment for potential confounding factors but appeared attributable to components of the insulin resistance syndrome, which were less favorable among men with higher C/T ratios. We found no strong association with social class, nor any evident behavioral factors related to C/T ratio, although diet and exercise were not studied. Evidence from various sources suggests that high cortisol and low testosterone levels are associated with a worse profile of insulin resistance syndrome components, and that modification (in particular, testosterone supplementation) improves this pattern. This suggests that methods of reducing the C/T ratio may improve insulin resistance and reduce the risk of CHD. If our apparently robust finding is replicated in other studies, then the identification of modifiable influences on the C/T ratio could facilitate prevention.

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