Endogenous Sex Hormones and Ischemic Heart Disease in Men
The Caerphilly Prospective Study

J.W.G. Yarnell, A.D. Beswick, P.M. Sweetnam, and Diane Riad-Fahmy

Numerous case–control studies have suggested that elevated levels of endogenous estrogen and low levels of testosterone are associated with ischemic heart disease (IHD) in men. These findings were tested in the Caerphilly study of 2,512 men from the general population who were aged 45–59 years at baseline and were followed for 5 years. Some 153 men experienced a new episode of IHD (fatal and nonfatal) during the period of follow-up. Baseline values of estradiol were marginally higher in subjects who developed IHD than in those who did not, but the difference was not statistically significant. Plasma values of testosterone were similar in the two groups. Among quintiles of the distribution of the hormone values, the incidence of IHD was similar in the case of estradiol; there was also no clear trend in the case of testosterone. These findings provide no support for the suggestion that plasma estradiol or testosterone are primary risk factors for IHD, although the associations between plasma testosterone and other probable risk markers (triglycerides, insulin, body mass index, and high density lipoprotein cholesterol) indicate the possibility that testosterone may play an indirect role in the pathogenesis of IHD. (Arteriosclerosis and Thrombosis 1993;13:517–520)

KEY WORDS • testosterone • estradiol • ischemic heart disease • prospective studies

The lower incidence of ischemic heart disease (IHD) in women compared with that in men suggests that sex hormones may have some role in the development of the disease. Early investigations showed that, far from being protective, exogenous estrogens promoted IHD in both men and premenopausal women. Similarly, several studies of endogenous hormones in men found raised estrogen levels and others showed lowered testosterone levels in survivors of myocardial infarction compared with control subjects. These studies have been reviewed elsewhere. A prospective study of endogenous sex hormones found some evidence of low testosterone levels associated with other risk markers for IHD—blood pressure, triglycerides, fasting blood glucose, obesity, and high density lipoprotein (HDL) cholesterol—but a sample size of 1,009 failed to achieve statistical significance in the prediction of IHD at a 12-year follow-up.

Other prospective studies, based on matched case and control subjects, assayed for sex hormone levels after prolonged storage have also been reported. However, deterioration of plasma samples after the storage of samples has been noted and may hinder the interpretation of the results.

One of the objectives of the Caerphilly heart disease study was to examine the possible predictive power of plasma sex hormones in the subsequent risk of IHD, and plasma samples were collected from a cohort of middle-aged men for this purpose. Subjects were followed for evidence of new IHD events for 5 years. Baseline findings have been reported previously.

Methods

Study Population

A 100% sample of men was selected from within a defined area (total population, 40,000) of Caerphilly and several surrounding villages. They were 45–59 years of age when first examined. A total of 2,512 men were seen—89% of the 2,818 found to be eligible.

Survey Methods and Follow-up Procedure

At recruitment, the men attended an afternoon or evening clinic. A standard medical and smoking history was obtained; the London School of Hygiene and Tropical Medicine chest pain questionnaire was administered; height, weight, and blood pressure were measured; and a 12-lead electrocardiogram (ECG) was recorded. They then returned, after an overnight fast, to an early-morning clinic where a blood sample was taken with minimal venous stasis. For the plasma sex hormone assays, lithium heparin was used as the anticoagulant. Fasting samples were obtained from 2,368 men. Full details of the population sample and clinic procedures have been reported previously.

The present article reports the first follow-up of the men at a nearly constant interval of 61±5 (mean±SD) months. At follow-up, the chest pain questionnaire was administered again, and a second ECG was recorded. The chest pain questionnaire was extended to include questions about hospitalization for severe chest pain.
Those, together with Hospital Activity Analysis notifications of admissions coded International Classification of Diseases (ICD) 410–414, were used as the basis for a search of hospital notes for events that satisfied the World Health Organization (WHO) criteria for definite acute myocardial infarction. For men who had died before the follow-up, a copy of the death certificate was automatically received from the National Health Service Central Registry. In this follow-up, 83% of IHD deaths occurred in hospital or had been certified after a coroner’s postmortem examination. In the United Kingdom, general practitioners, who certified the remaining IHD deaths, are required to notify the coroner if they have not seen a patient in the 2 weeks before death. From the information obtained, three categories of incident IHD events were defined: 1) IHD death (cause of death coded to ICD 410–414), 2) clinical nonfatal myocardial infarction (an event satisfying the WHO criteria), and 3) ECG myocardial infarction (appearance of major or moderate Q-QS waves [Minnesota codes 1-1-1 through 1-2-5 or 1-2-7] on the follow-up ECG when there were no Q-QS waves [Minnesota codes 1-1-any, 1-2-any, or 1-3-any] on the recruitment ECG).

Laboratory Methods

All samples for hormone analysis were frozen at −20°C and assayed within 3 months of collection. Testosterone and estradiol were measured by radioimmunoassay. The method for testosterone used a testosterone-3,125I radioligand and solid-phase antiserum after ether extraction. Cross-reactivity was 0.41% with androstenedione and <0.01% with estradiol. The only steroid showing high cross-reactivity was 5α-dihydrotestosterone, which is rarely found at levels in male plasma that are likely to interfere with testosterone determinations.

Estradiol was measured, after ether extraction, with an estradiol-6-3H radioligand. Separation of bound and free radioligand was by dextran-coated charcoal. Cross-reactivities were 4.2% with estrone, 0.05% with estriol, and <0.01% with testosterone.

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=140 pairs) for testosterone and 22% (n=110 pairs) for estradiol. The intraclass correlation coefficients calculated from these duplicate pairs were 0.73 and 0.43 for testosterone and estradiol, respectively.

Table 1. Estradiol and Testosterone at Recruitment in Men With and Without Incident Ischemic Heart Disease

<table>
<thead>
<tr>
<th></th>
<th>No major IHD</th>
<th>Any major IHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pmol/L)</td>
<td>2,161±61</td>
<td>134±69</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>2,192±7.4</td>
<td>134±7.6</td>
</tr>
</tbody>
</table>

Analytical Methods

Testosterone was measured for 2,326 men, and estradiol was measured for 2,295 men. Univariate analyses are based on these numbers. Multivariate analyses (multiple logistic regression) involving smoking, blood pressure, body mass index, and total triglycerides were based on 40–89 fewer men, depending on which variables were included.

Results

Incidence of IHD

A total of 153 IHD events occurred during the period of follow-up (average, 61 months). The average annual incidence was 1.2%; 50% of the events were fatal, 39% were clinical nonfatal myocardial infarction, and 11% were ECG-defined myocardial infarction.

Univariate Analyses

Among the men for whom plasma estradiol and testosterone results were available, there were 134 IHD events at follow-up. Table 1 shows the mean values of plasma estradiol and testosterone at baseline by the IHD status at follow-up. Estradiol levels are slightly, but not significantly, raised in subjects who had an incident IHD event. Testosterone levels are similar in those who did and those who did not have an incident IHD event.

Table 2 shows the incidence of IHD by quintiles of the distribution of estradiol and testosterone and the age-standardized relative odds of subsequent risk of IHD. No consistent trends are apparent for either estradiol or testosterone.

Both estradiol and testosterone showed some associations with other possible risk factors for IHD. Table 3 shows the correlation coefficients between the hormones and possible risk factors. Estradiol shows no strong associations apart from that with testosterone (r=0.37), but testosterone shows correlations with body mass index, triglycerides, and insulin in particular. Neither of the hormones was significantly correlated with age in this group of men aged 45–59 years.

Multivariate Analyses

Multivariate analyses were carried out by multiple logistic regression. In the case of estradiol, the age-adjusted standardized relative odds (i.e., the proportionate change in odds for a 1 SD increase in the variable) are 1.15. Inclusion of the variables smoking habit, diastolic blood pressure, preexisting IHD, body mass index, and time of blood sample does not change the standardized relative odds (95% confidence interval, 0.97–1.37).

*Estradiol was measured using a standard assay for the first 75% of the men. This assay then failed, and the remaining samples were measured with a new assay (Steranti). Samples from 39 subjects were assayed by both methods. The correlation between the two estimates was 0.83, and the relation was linear. Overall, the differences between the two assays are small, and they are no more than differences between the same assay over different periods of time. Associations with other variables (testosterone, smoking, age, blood pressure, and body mass index) are similar for the two sets of measurements. Hence, the results from the two assays have been combined to produce a single estradiol estimate for 2,295 men.

†In a wider age group of men aged 30–69 years, there was a clear decrease in plasma testosterone with age.
For testosterone, the standardized relative odds are 1.01. Adjustment for all confounders tested in the case of estradiol increased the odds to 1.11 (95% confidence interval, 0.92–1.34). Further adjustment for insulin and triglyceride had little additional effect.

### Discussion

These data provide little support for the suggestion that plasma estradiol and testosterone are independent risk factors for IHD. Plasma estradiol shows a weak negative association with body mass index and none with smoking habit, which is in contrast to findings of other studies. Plasma testosterone is associated with several factors that may be associated with increased risk of incident IHD: preexisting IHD, blood pressure, HDL cholesterol, triglycerides, and insulin. Decreased levels of plasma testosterone in subjects with preexisting IHD may be consequent to symptomatic IHD, not a cause of subsequent IHD. However, it remains a possibility that plasma testosterone may play some role in the development of IHD by mechanisms associated with these factors.

A previous population-based prospective study showed a weak nonsignificant relation between plasma testosterone and subsequent risk of IHD. Other prospective studies based on cases and control subjects showed no relation. All studies used plasma samples stored for several years. The present study was planned specifically to evaluate the role of the plasma sex hormones among other objectives. Plasma samples were stored for a short period only. Pilot studies carried out among volunteers indicated that the biological variability of these hormones within individuals was considerably less than the between-person variability. Hence, single-point estimations were adequate as a basis for epidemiological investigation. However, the weakness of the majority of epidemiological investigations, which usually involve single-point estimations, is well recognized. This factor and the modest number of incidence cases of IHD (n=134) may have resulted in a null association in this study. A 10-year follow-up will be completed in due course.

Minimum significant differences between IHD cases and control subjects were calculated to be 1.30 mmol/L in the case of testosterone and 11.0 pmol/L in the case of estradiol. These differences were calculated by covariance analysis, adjusting for age, time of blood sample, preexisting IHD, diastolic blood pressure, body mass index, and smoking habit.

Testosterone and its analogues given therapeutically are reported to lower serum cholesterol and to increase fibrinolytic activity and have been used in Europe to treat cardiovascular disease. Consistent associations have been reported between endogenous plasma testosterone levels and plasma lipids, notably HDL cholesterol. Markedly reduced lipoprotein lipase activity has been described in men with severe coronary artery disease and low HDL cholesterol concentrations, and this has been attributed to low testosterone levels. However, decreased HDL cholesterol levels have been postulated as a result of a testosterone-stimulated increase in hepatic lipase activity.

These contradictory studies may serve to stimulate further research into the associations and the physiopathological significance of endogenous sex hormones in cardiovascular disease. However, the present findings indicate that endogenous sex hormones are not directly predictive of IHD, although plasma testosterone, in

### Table 2. Incidence of Major Ischemic Heart Disease by Quintiles of the Distributions of Estradiol and Testosterone

<table>
<thead>
<tr>
<th>Quintiles of distribution</th>
<th>Men (N)</th>
<th>Men with IHD</th>
<th>Relative odds*</th>
<th>Men (N)</th>
<th>Men with IHD</th>
<th>Relative odds*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (lowest)</td>
<td>456</td>
<td>25</td>
<td>5.5</td>
<td>1.0</td>
<td>462</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>463</td>
<td>26</td>
<td>5.6</td>
<td>1.02</td>
<td>453</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>456</td>
<td>31</td>
<td>6.8</td>
<td>1.31</td>
<td>471</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>458</td>
<td>25</td>
<td>5.5</td>
<td>1.04</td>
<td>476</td>
<td>26</td>
</tr>
<tr>
<td>5 (highest)</td>
<td>462</td>
<td>27</td>
<td>5.8</td>
<td>1.12</td>
<td>464</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>2,295</td>
<td>134</td>
<td>5.8</td>
<td>. . .</td>
<td>2,326</td>
<td>134</td>
</tr>
</tbody>
</table>

*Relative odds are age standardized.

### Table 3. Correlation Coefficients Between Estradiol, Testosterone, and Other Risk Factors for Ischemic Heart Disease

<table>
<thead>
<tr>
<th>Correlation coefficients</th>
<th>Body mass index</th>
<th>Diastolic BP</th>
<th>HDL cholesterol</th>
<th>Total triglycerides*</th>
<th>Fibrinogen</th>
<th>Insulin*</th>
<th>Testosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>-0.05†</td>
<td>0.02</td>
<td>0.08†</td>
<td>-0.02</td>
<td>-0.07‡</td>
<td>-0.02</td>
<td>0.37‡</td>
</tr>
<tr>
<td>Testosterone</td>
<td>-0.34‡</td>
<td>-0.18‡</td>
<td>0.13‡</td>
<td>-0.21‡</td>
<td>-0.07‡</td>
<td>-0.24‡</td>
<td></td>
</tr>
</tbody>
</table>

†p<0.05, ‡p<0.001.
particular, shows associations with several risk factors, and this would appear to warrant further study.

References

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