Evidence for an Association Between Dehydroepiandrosterone Sulfate and Nonfatal, Premature Myocardial Infarction in Males

Laura E. Mitchell, PhD; Dennis L. Sprecher, MD; Ingrid B. Borecki, PhD; Treva Rice, PhD; Peter M. Laskarzewski, PhD; D.C. Rao, PhD

Background Several studies indicate that endogenous hormones play a role in the etiology of coronary artery disease, either as independent risk factors or indirectly, via an effect on lipids, lipoproteins, or other heart disease risk factors.

Methods and Results The relation between endogenous hormone levels and premature (<56-year-old patients) myocardial infarction was assessed in a retrospective study involving 49 male survivors of premature myocardial infarction and 49 age-matched, volunteer male controls. Serum samples were obtained for each subject the morning after a ≥12-hour fast and frozen at −70°C for subsequent hormonal analysis. Among the male patients, the average duration between the most recent myocardial infarction and blood sampling was 3.4 years (range, 0.7 to 19.2 years). Individuals reporting the use of any medications with the potential to alter lipid, lipoprotein, or hormone levels were excluded from these analyses. Dehydroepiandrosterone sulfate levels were significantly lower in the patients than in the control subjects. This association remained statistically significant even after accounting for the effects of total cholesterol, triglycerides, the ratio of total to high-density lipoprotein (HDL) cholesterol, HDL, apolipoprotein A-I, apolipoprotein A-II, apolipoprotein B, and body mass index. There were no significant differences in the levels of estradiol, testosterone, or free testosterone or the ratio of estradiol to testosterone between patients and control subjects.

Conclusions Our conclusions are limited by the retrospective nature of this study. However, these data indicate that serum dehydroepiandrosterone sulfate levels are inversely related to premature myocardial infarction in males and that this association is independent of the effects of several known risk factors for premature myocardial infarction. (Circulation. 1994;89:89-93.)

Key Words • testosterone • estradiol • lipoproteins • dehydroepiandrosterone sulfate • coronary artery disease

Several studies indicate that endogenous hormones play a role in the etiology of coronary artery disease, either as independent risk factors or indirectly, via an effect on lipids, lipoproteins, or other heart disease risk factors.1-16 In general, these studies suggest that coronary artery disease is inversely related to serum androgen levels and positively related to serum estrogen levels. The existence of a causal relation between either estradiol or testosterone and coronary artery disease has not been supported by prospective investigations.17-20 A statistically significant, inverse relation between serum dehydroepiandrosterone sulfate (DHEAS) and coronary artery disease in males has, however, been documented in at least one prospective investigation.12

This retrospective, matched case-control study investigates the association between endogenous hormones, including DHEAS, and premature myocardial infarction in males. This study is unique in that the effects of a large number of heart disease risk factors, which may confound the relation between endogenous hormones and myocardial infarction, were evaluated. In addition, individuals reporting the use of medications with the potential to alter lipid, lipoprotein, or endogenous hormone levels were excluded from this study.

Methods

The Cincinnati Myocardial Infarction and Hormone (CIMIH) family study was designed to investigate the role of endogenous hormones in the etiology of myocardial infarction. Details of the study design and ascertainment of case and control families are described elsewhere.21 Briefly, case families were ascertained through living Caucasian males who had survived at least one myocardial infarction before the age of 56 years. These families were identified through private cardiologists, media advertisements, and fathers of adolescent boys who had recently participated in a study of male maturation.22 Study eligibility was restricted to male survivors of myocardial infarction with a spouse and at least one biological child willing to participate in the study protocol.

Control families were also ascertained through media advertisements, a local blood center, area food stores, and the records of the previously mentioned maturation study. The controls were restricted to Caucasian families consisting of at least one parent and one biological child who were willing to complete the study protocol.

During the period from June 1988 to June 1990, each study family visited the Cincinnati Lipid Research Clinic. Case and control families were seen randomly throughout the study period. Family members were instructed to fast for at least 12 hours before the clinic visit. Each study participant was interviewed to obtain family history information and information on the use of medications. Height and weight were...
recorded for each subject, and blood was drawn for subsequent biochemical analysis. The study protocol was approved by the University of Cincinnati's committee on human research. Informed consent was obtained from each study participant. Three hundred seventy-eight families (252 control and 126 case families) completed the study protocol. The myocardial infarction survivors had suffered from one to five myocardial infarctions (mean, 1.4). The average duration from the first myocardial infarction to the time of assessment was 5.3 years (range, 0.4 to 24.2 years). The average interval between the most recent myocardial infarction and the time of assessment was 3.6 years (range, 0.4 to 19.2 years).

Blood was collected into EDTA (0.1%) and sterile tubes for analysis of lipids and lipoproteins and of endogenous hormones, respectively. All samples were refrigerated and centrifuged (for 20 minutes at 2500 rpm and 4°C) within 1 hour of collection. Serum for the endogenous hormone assays was stored at −70°C. During the spring of 1991, these samples were assayed in consecutive batches, which were random with respect to case-control status. Lipid and lipoprotein analyses were performed in a clinical laboratory within 2 hours of blood collections. The technicians performing these assays were blind to the case-control status of the samples.

Serum testosterone was measured by double antibody radioimmunoassay (RIA) after extraction using a 3:2 solution of ethylacetate:hexane. The sensitivity of the testosterone assay is 2 ng/mL, and the intra-assay and interassay coefficients of variation (CV) are 5% and 9.3%, respectively. Free (ie, unbound or biologically available) testosterone levels were calculated23 from serum levels of testosterone and testosterone-estrogen-binding globulin (TEBG), the primary carrier of testosterone in serum. TEBG was measured by the charcoal absorption method.23

Serum DHEAS and serum estradiol-17β were measured by double antibody RIAs without prior extraction. The sensitivity of the DHEAS assay is 15 ng/mL, and the intra-assay and interassay CVs are 5.2% and 10.5%, respectively. The sensitivity of the estradiol assay is 5 pg/mL, and the intra-assay and interassay CVs are 8.3% and 13%, respectively.

Total plasma cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were measured enzymatically using a Hitachi 705 analyzer (BMD, Indianapolis, Ind) after precipitation with heparin-manganese. These assays were standardized in accord with the LRC-Core Laboratory manual24 using serum calibrators obtained from the Centers for Disease Control (Atlanta, Ga).

Plasma concentrations of apolipoprotein (apo) A-I, apo A-II, and apo B were determined by an ELISA system.25 The interassay CVs for apo A-I, apo A-II, and apo B, at average plasma concentrations, are 5%, 7%, and 7%, respectively. Additional details regarding the determination of hormone and lipid concentrations can be found in the report by Sprecher et al.21

The males through whom case families were ascertained and the fathers in the control families form the study population for these analyses and are referred to as case and control probands, respectively. Ten of the randomly ascertained families did not contain information on the father and therefore were excluded from these analyses. This resulted in a total sample consisting of 242 control probands, two of whom had a history of myocardial infarction, and 126 case probands.

Probands were excluded from these analyses if they were diabetic, had not fasted for at least 12 hours before blood collection, or reported the use of any medication(s) that might affect endogenous hormone, lipid, or lipoprotein levels. Probands were also excluded if they had missing information for any of the study variables. The two control probands with a history of myocardial infarction were both excluded on the basis of one or more of the criteria described above. In total, 35 control probands (14.5%) and 59 case probands (46.8%) were excluded on the basis of these criteria (Table 1).

After the above exclusions were made, the mean age of case males (51.02 years) was significantly greater than the mean age of controls (43.4 years, P=.0001 by Wilcoxon rank-sum test). Because of the difference in the age distributions of cases and controls and the association between age and both myocardial infarction and endogenous hormone levels, each case was age matched to a single control. Cases were matched to the control with the closest age (to a 10th of a year at the time of blood sampling). If more than one control met this criterion, the control whose blood was drawn closest to that of the case was selected. A suitable control was not available for 18 of the cases. Thus, only 49 matched pairs were available for analysis.

The associations between premature myocardial infarction and five endogenous hormone measures—DHEAS, testosterone, free testosterone, estradiol, and the ratio of estradiol to testosterone—were evaluated. The odds ratio was used as the measure of association between case-control status and each of the endogenous hormone measurements. The odds ratio associated with a particular variable was obtained by conditional logistic regression analysis, where the predictor (or explanatory) variable is the difference between the corre-

Table 1. Exclusion Criteria for Case and Control Probands in the Cincinnati Family Study of Endogenous Hormones and Myocardial Infarction

<table>
<thead>
<tr>
<th>Reason for Exclusion</th>
<th>Cases (n=126)</th>
<th>Controls (n=242)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Diabetic</td>
<td>2</td>
<td>1.59</td>
</tr>
<tr>
<td>Nonfasting</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>Missing data*</td>
<td>6</td>
<td>4.76</td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>33</td>
<td>26.19</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>31</td>
<td>24.60</td>
</tr>
<tr>
<td>Lipid-altering drugs</td>
<td>22</td>
<td>17.48</td>
</tr>
<tr>
<td>Diuretics</td>
<td>8</td>
<td>6.35</td>
</tr>
<tr>
<td>Hormone supplements</td>
<td>3</td>
<td>2.38</td>
</tr>
<tr>
<td>Steroids</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>No match</td>
<td>18</td>
<td>14.28</td>
</tr>
<tr>
<td>Total number of exclusions</td>
<td>77</td>
<td>61.11</td>
</tr>
</tbody>
</table>

*Probands with a missing value for any independent variable were excluded from all analyses.
TABLE 2. Mean Values (±SE) of the Independent Variables in Case and Control Probands and Mean Difference Between Matched Pairs in the Cincinnati Family Study of Endogenous Hormones and Myocardial Infarction

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n=49)</th>
<th>Controls (n=49)</th>
<th>Difference (Case-Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>49.12 ± 1.02</td>
<td>49.13 ± 1.03</td>
<td>-0.01 ± 0.03</td>
</tr>
<tr>
<td>DHEAS, ng/mL</td>
<td>1473.59 ± 116.51</td>
<td>1867.92 ± 118.85</td>
<td>-394.33 ± 148.57</td>
</tr>
<tr>
<td>Testosterone, ng/dL</td>
<td>423.00 ± 24.88</td>
<td>502.51 ± 31.88</td>
<td>-79.51 ± 37.66</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>12.13 ± 0.54</td>
<td>13.77 ± 0.79</td>
<td>-1.63 ± 1.00</td>
</tr>
<tr>
<td>Estradiol, pg/mL</td>
<td>33.18 ± 2.54</td>
<td>32.02 ± 2.53</td>
<td>1.16 ± 3.56</td>
</tr>
<tr>
<td>Estradiol-to-testosterone ratio</td>
<td>0.09 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>220.33 ± 5.50</td>
<td>211.94 ± 5.57</td>
<td>8.39 ± 9.55</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>173.65 ± 13.23</td>
<td>135.18 ± 10.92</td>
<td>38.47 ± 17.89</td>
</tr>
<tr>
<td>TC/HDL</td>
<td>5.70 ± 0.20</td>
<td>4.66 ± 0.23</td>
<td>1.05 ± 0.35</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>40.35 ± 1.49</td>
<td>49.33 ± 2.00</td>
<td>-8.98 ± 2.70</td>
</tr>
<tr>
<td>Apo A-I, mg/dL</td>
<td>111.92 ± 2.52</td>
<td>129.31 ± 3.40</td>
<td>-17.39 ± 4.56</td>
</tr>
<tr>
<td>Apo A-II, mg/dL</td>
<td>44.04 ± 1.24</td>
<td>47.88 ± 1.15</td>
<td>-3.84 ± 1.90</td>
</tr>
<tr>
<td>Apo B, mg/dL</td>
<td>129.69 ± 4.51</td>
<td>118.18 ± 4.07</td>
<td>11.51 ± 6.87</td>
</tr>
<tr>
<td>Body mass index, (weight [kg]/height²[cm]) × 1000</td>
<td>2.87 ± 0.07</td>
<td>2.73 ± 0.06</td>
<td>0.15 ± 0.08</td>
</tr>
</tbody>
</table>

DHEAS indicates dehydroepiandrosterone sulfate; TC, total cholesterol; HDL, high-density lipoprotein; and apo, apolipoprotein.

The cases used in these analyses had suffered between one and five myocardial infarctions (mean, 1.4). The average duration from the first myocardial infarction to the time of assessment was 4.6 years (range, 0.7 to 20.2 years), and the average interval from the most recent myocardial infarction to the time of assessment was 3.4 years (range, 0.7 to 19.2 years). None of the corresponding values of the case and his matched control (ie, case-control).

Multivariate analyses, also using conditional logistic regression, were used to estimate the odds ratio associated with a particular hormone while controlling for the effects of other myocardial infarction risk factors. The other risk factors that were considered are body mass index, total cholesterol, triglycerides, the ratio of total cholesterol to HDL (TC/HDL), HDL, apo A-I, apo A-II, and apo B. Stepwise, conditional logistic regression was used to select a group of variables those variables that are independently related to premature myocardial infarction. Variables were entered and retained in a model only if they were significantly related to case-control status (ie, P<.05), given the other variables in the model.

**Results**

The cases used in these analyses had suffered between one and five myocardial infarctions (mean, 1.4). The average duration from the first myocardial infarction to the time of assessment was 4.6 years (range, 0.7 to 20.2 years), and the average interval from the most recent myocardial infarction to the time of assessment was 3.4 years (range, 0.7 to 19.2 years). None of the matched controls had reported a history of myocardial infarction.

Mean values among cases and controls and mean matched-pair differences for each of the endogenous hormone measures, lipids, lipoproteins, and body mass index are summarized in Table 2. The case and control probands are well matched for age, with a mean difference of only 0.01 year. Cases and controls are also similar with respect to body mass index; serum levels of free testosterone, estradiol, and the estradiol-to-testosterone ratio; and plasma total cholesterol and apo B concentrations. However, DHEAS, testosterone, HDL, apo A-I, and apo A-II concentrations tend to be lower and triglycerides and TC/HDL tend to be higher in cases than in controls.

The unadjusted, or crude, odds ratio for the association between myocardial infarction and each of the endogenous hormone measures is provided in Table 3. The association between testosterone and premature myocardial infarction is of borderline significance (P=.05). DHEAS is, however, the only endogenous hormone that is significantly related to case-control

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Clarifying the Table:

**Table 3.** Crude and Adjusted Odds Ratios and 95% Confidence Intervals for the Association Between Endogenous Hormones and Case-Control Status in the Cincinnati Family Study of Endogenous Hormones and Myocardial Infarction

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Unit</th>
<th>Crude Odds Ratio (95% CI)</th>
<th>Adjusted Odds Ratio* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEAS</td>
<td>100 ng/mL</td>
<td>0.92 (0.87-0.99)</td>
<td>0.80 (0.66-0.96)</td>
</tr>
<tr>
<td>Free testosterone</td>
<td>1</td>
<td>0.93 (0.85-1.02)</td>
<td>0.95 (0.84-1.07)</td>
</tr>
<tr>
<td>Testosterone</td>
<td>100 ng/dL</td>
<td>0.79 (0.62-1.00)</td>
<td>0.81 (0.59-1.10)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>10 pg/mL</td>
<td>1.04 (0.83-1.30)</td>
<td>1.06 (0.80-1.41)</td>
</tr>
<tr>
<td>Estradiol-to-testosterone ratio</td>
<td>0.1</td>
<td>2.32 (1.86-6.24)</td>
<td>1.99 (0.53-7.46)</td>
</tr>
</tbody>
</table>

Cl indicates confidence interval; DHEAS, dehydroepiandrosterone sulfate.

*Each odds ratio is adjusted for the effects of total cholesterol, triglycerides, TC/HDL, HDL, apo A-I, apo A-II, apo B, and body mass index.
status (ie, the 95% confidence interval for the odds ratio associated with DHEAS excludes 1). The odds of having a premature myocardial infarction are inversely associated with DHEAS concentrations; for each 100-ng/mL decrease in DHEAS, the odds of being a case increases by approximately 8%.

Table 3 also provides the odds ratio for the association between myocardial infarction and each endogenous hormone measure after adjusting for the effects of eight additional risk factors: cholesterol, triglycerides, TC/HDL, HDL, apo A-I, apo A-II, apo B, and body mass index. Adjusting for the effects of these eight variables does not significantly influence the odds ratio associated with any of the endogenous hormone measures. DHEAS remains a significant predictor of case-control status after adjusting for the effects of these eight risk factors. However, the association between testosterone and case-control status is no longer even of borderline significance (P=.18) after adjusting for these factors, and the associations between case-control status and free testosterone, estradiol, and the ratio of estradiol to testosterone remain nonsignificant (ie, the associated 95% confidence intervals include unity).

Using stepwise, conditional logistic regression analysis, DHEAS and apo A-I were identified as the only two study variables that are significantly and independently related to a history of premature myocardial infarction. After adjusting for the effects of apo A-I, the odds ratio for the association between premature myocardial infarction and DHEAS is 0.91 (95% confidence interval, 0.84-0.99). The odds ratio for the association between premature myocardial infarction and apo A-I is 0.96 (95% confidence interval, 0.94-0.99), after adjusting for the effects of DHEAS.

**Discussion**

In this retrospective, matched case-control study, premature myocardial infarction is significantly and inversely related to serum levels of DHEAS. Moreover, this association is independent of several traditionally recognized myocardial infarction risk factors. Apo A-I levels are also independently and inversely related to premature myocardial infarction in these data. The fact that apo A-I is significantly related to premature myocardial infarction (after adjusting for the effects of the endogenous hormones and other lipids and lipoproteins) and HDL is not suggests that the apolipoprotein composition of HDL is an important determinant in the development of cardiovascular disease.26,27

The basis for an association between DHEAS and coronary artery disease is unclear. It has been suggested that DHEAS may be indirectly related to coronary artery disease because of an inhibitory effect on the rate-limiting enzyme in the pentose cycle, ie, glucose-6-phosphate dehydrogenase.28 Because the pentose cycle produces NADPH, a coenzyme required in the synthesis of lipids, decreased DHEAS levels may promote NADPH production and, thus, NADPH-dependent lipogenesis. Based on the observation that dehydroepiandrosterone has an inhibitory effect on cell growth and proliferation, it has also been suggested that decreased DHEAS levels may promote proliferation of vascular intimal cells and the formation of atherosclerotic plaques.29

The strongest evidence for an association between serum levels of DHEAS and cardiovascular disease in males comes from the prospective investigation reported by Barrett-Connor et al.12 This study provides evidence of a statistically significant, inverse relation between DHEAS and subsequent cardiovascular disease mortality in males over the age of 50. Furthermore, although weaker, evidence for an inverse association between DHEAS and coronary artery disease is provided by two additional prospective investigations.20,29 Contoreggi et al20 reported lower DHEAS levels among males who subsequently developed coronary artery disease relative to those who did not. This association was, however, only of borderline significance (P=.059) after adjusting for the effects of age. In addition, LaCroix et al20 reported decreased DHEAS levels (odds ratio, 0.46; 95% confidence interval, 0.19-1.07, after adjusting for eight additional risk factors) among males of Japanese ancestry who subsequently suffered a fatal myocardial infarction, but not among those who had suffered nonfatal events, relative to age-matched controls. Although individually neither of these studies provides strong evidence for an association between DHEAS and cardiovascular disease, they are supportive of a trend toward decreased DHEAS levels among males who are at increased risk of cardiovascular disease.

Additional evidence of an inverse association between serum DHEAS levels and cardiovascular disease in males is provided by the current report and the reports of Herrington et al15 and Slowinska-Szrednicka et al.14 Herrington et al15 found a significant, inverse association between DHEAS and the extent of angiographically defined coronary atherosclerosis in males ≤50 years old. In addition, Slowinska-Szrednicka et al14 reported significantly lower DHEAS levels in male survivors of premature myocardial infarction (<40 years), relative to healthy male controls.

Despite differences in study design and case definitions, the majority of recent studies are suggestive of an inverse relation between serum DHEAS levels and cardiovascular disease in males. Differences in the strength of the reported associations across studies are likely to reflect, at least in part, differences in case definition. Variations in serum DHEAS levels across populations may also account for some interstudy heterogeneity. For example, mean DHEAS levels among males of Japanese ancestry have been shown to be markedly lower than those among Caucasian males.30 Therefore, mean differences in DHEAS levels of case and control males of Japanese ancestry may be less than those among Caucasians and, consequently, harder to detect. One potential explanation for the lack of a statistically significant association between DHEAS and nonfatal myocardial infarction is reported by LaCroix et al.30

In summary, this study provides further evidence for an inverse association between serum DHEAS and myocardial infarction and confirms previous reports of an inverse association between apo A-I and myocardial infarction. Firm conclusions regarding the temporal or causal patterns of these associations are, however, precluded by the retrospective nature of this study. Consequently, the relatively low levels of DHEAS and apo A-I among the case males in this study may be directly attributable to myocardial infarction or may reflect a survivorship effect. Prospective investigations have, however, provided evidence that low DHEAS12 and apo A-I levels31 are precursors of cardiovascular disease. Thus, it seems likely that the associations detected in
this study preceded the first myocardial infarction in the case males.

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References

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