Coronary heart disease (CHD) is the leading cause of death in women and accounts for a large proportion of illness and disability in women. There are approximately 250,000 deaths in women due to heart disease each year in the United States. The observations of a predominance of CHD among men, the diminishing differences in CHD rates in males versus females with increasing age, comparison of the risk of CHD across menopause, data on the use of oral contraceptives and noncontraceptive estrogens, and the risk of CHD strongly suggest that hormonal factors, specifically sex hormones, may play a major role in the development of CHD. The relation of serum sex hormones to both fatal and nonfatal CHD and to the extent of atherosclerosis has been examined in men. In contrast, little is known about the relation of serum sex hormones to CHD or to the development of atherosclerosis in women.

There is a very substantial increase in the risk of both CHD and atherosclerosis among women after surgical menopause. For natural menopause, mortality data from the United Kingdom and the United States do not show an abrupt change in mortality rates near the age of menopause. However, data from the Framingham Study suggest that female CHD morbidity rates accelerate more quickly than do those of males after age 45.

5.2). Examination of mean estrone levels on the basis of the number of occluded vessels was also not significant. The primary predictors of coronary artery disease in this population were a history of diabetes (OR, 8.8; CI, 1.5, 51.4) and age (5-year increments; OR, 2.1; CI, 1.2, 3.8). There was also some suggestion that women who reported higher lifetime physical activity levels were at a reduced risk for developing coronary artery disease (OR, 0.18; CI, 0.05, 0.65). These preliminary results do not support the hypothesis that serum estrogens are related to coronary artery disease in older women, but these findings need to be replicated in larger populations of older women. (Arterioscler Thromb. 1994;14:14-18.)

Key Words • estrone • estrogen • coronary artery disease • postmenopausal women

There has also been considerable research on the relation of exogenous estrogens to CHD in women. On the basis of a critical review and meta-analysis of all epidemiological data, Bush has estimated that long-term use of postmenopausal estrogen decreases the risk of cardiovascular disease in women by 50%. Estrogen therapy also reduces the risk of angiographically documented CHD.

In contrast, little is known about the relation of endogenous estrogen to the risk of CHD. Oral exogenous estrogens do not represent physiological doses and once ingested, they undergo metabolism by passage through the liver. This hepatic metabolism may determine the biological effects of exogenous estrogens. Endogenous estrogens in postmenopausal women, on the other hand, are primarily derived from the aromatization of androstenedione. Endogenous estrogens first affect target organs before their passage through the liver. Hence, the biological effects of oral exogenous estrogens cannot be extrapolated to endogenous estrogens.

Coronary artery disease (CAD) is clearly polyfactorial, and data on endogenous hormones may improve our prediction of CAD. The lack of information on the relation of endogenous estrogen to CAD in women represents a serious deficit in explaining the polyfactorial nature of CAD in women.

In the current study, we examined the relation of serum concentrations of estrone to CAD as determined by coronary angiography in 87 postmenopausal women.

Methods

Study Population

Eighty-nine women who were consecutively admitted to Allegheny General Hospital, Pittsburgh, Pa, for diagnostic cardiac catheterization were recruited. Serum for estrone analysis was available for 87 of these women. All of the women

Serum Estrone Concentrations and Coronary Artery Disease in Postmenopausal Women

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reported having experienced symptoms of coronary disease (ie, anginal chest pain) in the past year. Inclusion criteria included being female, white, and postmenopausal, defined as being amenorrheic for at least 12 months or having had surgically induced menopause and not taking estrogen or other hormonal therapy. If a woman reported that a hysterectomy had been performed before the natural cessation of menses (menopause), the onset of menopause was designated as unknown. To ensure that such women were clearly menopausal, these women were eligible for inclusion into the study if they were at least 58 years of age. Patients who were catheterized primarily for symptoms of valvular disease, cardiomyopathy, or other severe illnesses or who were in critical medical condition were excluded.

**Angiography Reports**

All angiography reports were reviewed by two board-certified cardiologists who were blinded to the patient’s sex hormone levels but not to the presence or absence of other cardiovascular risk factors. The degree of coronary artery occlusion was generated by computer and then confirmed by the two cardiologists. Left ventricular functional status was defined on the angiography report as any abnormal wall motion. The abstractor of the angiography reports (M.B.) was blinded to the medical history and sex hormone concentrations.

**Case Definition**

Women who were angiographically diagnosed with ≥50% occlusion of ≥1 coronary artery were considered to be cases (n=62). These patients were considered to have hemodynamically significant atherosclerosis. This group constituted a broad definition of cases with CAD as used in most angiography case-control studies.

**Control Definition**

The remaining individuals had 0% to 24% occlusion of all coronary arteries and were considered as control subjects. This represents a somewhat stringent control group. Previous research has indicated that inclusion of individuals in an angiography study who have 25% to 49% occlusion tends to include patients with subclinical disease.

**Data Collection**

Potential study participants were identified by the nurse practitioners for each of three groups of physicians who use the cardiac catheterization laboratories at Allegheny General Hospital, Pittsburgh, Pa. On the basis of these referrals, patients were screened for eligibility. Eligibility status was determined in part by available inpatient information. When patients agreed to participate and signed the informed consent form, a nonfasting sample of serum was obtained for lipid and hormonal therapy. If a woman reported that a hysterectomy had been performed before the natural cessation of menses (ie, anginal chest pain) in the past year. Inclusion criteria included being female, white, and postmenopausal, defined as being amenorrheic for at least 12 months or having had surgically induced menopause and not taking estrogen or other hormonal therapy. If a woman reported that a hysterectomy had been performed before the natural cessation of menses (menopause), the onset of menopause was designated as unknown. To ensure that such women were clearly menopausal, these women were eligible for inclusion into the study if they were at least 58 years of age. Patients who were catheterized primarily for symptoms of valvular disease, cardiomyopathy, or other severe illnesses or who were in critical medical condition were excluded.

**Lipid Analyses**

The total high-density lipoprotein cholesterol (HDL-C) was precipitated with heparin and manganese chloride. The coefficients of variation were 2.4% for total cholesterol and 4% to 5% for total HDL-C.

**Sex Hormones**

Estrone levels were measured by highly specific methods that were published previously by our laboratory. Each of these methods involves an extraction step, Sephadex LH-20 column chromatography to improve specificity, and a radioimmunoassay with a specific antibody. These methods were necessary in view of the very low concentrations of estrogen in postmenopausal women. Caution must be used in the interpretation of results that are obtained from studies that are solely dependent on the specificity of the antibody. The within-assay and between-assay variations were 10% and 15%, respectively. For subjects whose hormone levels were found to be below the level of sensitivity (2.5 pg/mL), it was used in the analysis.

**Analytical Methods**

The STATISTICAL ANALYSIS SYSTEM (SAS) package was used. The distribution of estrone was skewed; logarithmic transformation was therefore performed and geometric means reported. Tests were performed to test differences between cases and control subjects for the mean levels of the continuous variables (age, lipid levels, serum estrone [log normal], BMI, age at last period, age at first pregnancy, and number of all births). The equality of the proportions of women with each risk factor by case-control status was performed for the dichotomous variables (total cholesterol ≥240 mg/dL; history of myocardial infarction; diabetic status; history of elevated triglycerides; history of hypertension; any use of estrogen; prior surgical menopause; history of hysterec- tomy; any pregnancy; any cigarette use; and physical activity in the past 12 months, at age 50, at age 30, and during the teenage years). To adjust for confounding, a multivariate logistic analysis was performed. We included as confounding variables those selected factors that have been shown to be related to coronary atherosclerosis in previous research. Odds ratios and their 95% confidence intervals were calculated.

**Results**

The frequency distribution of the number of coronary arteries with ≥50% occlusion is shown in the Figure. Forty-two percent of those with hemodynamically significant CAD (≥50% occlusion) had ≥3 diseased vessels. Of those, 92% had clinically significant left anterior descending CAD. Furthermore, the case group included 49 subjects (79%) with ≥80% occlusion of at
number of vessels with ≥50% occlusion (x axis) and the number of subjects (y axis).

Frequency distribution of the number of vessels with ≥50% occlusion (x axis) and the number of subjects (y axis).

least 1 coronary artery. Of those, 8 (16%) had ≥3 diseased vessels. Thirty-five percent of the case group had abnormal left ventricular function compared with 4% in the control group.

Cases were significantly older than the control subjects, 66.9 years versus 62.7 years, respectively, and more likely to have reported diabetes (37% versus 7%) and prior myocardial infarction (42% versus 15%) (all P<.01; Table 1). There was no difference between cases and control subjects for BMI, lipid concentrations, age at last period, age at first pregnancy, number of live births, history of cigarette smoking, or hypertension. Approximately 31% of cases and 26% of control subjects reported a surgical menopause (P=.65). A high proportion of both the cases and control subjects reported a previous hysterectomy. There was no difference in the proportion of cases and control subjects who reported a high level of physical activity during the previous 12 months. At age 50, 50% of the control subjects reported a high level of physical activity compared with 30% of the cases, a difference that approached statistical significance. Significant differences were found for reported high physical activity levels at age 30 and during the teenage years. The proportion of identified cases with a high lifetime physical activity summary score was significantly lower, 31%, compared with 63% for the control group.

Estrone concentrations were similar in both cases and control subjects (Table 1). There was no suggestion that estrogen levels varied according to the number of atherosclerotic lesions present (Table 2).

The major predictor of CAD in this population was a positive history of diabetes: diabetic women were almost nine times more likely to have ≥50% occlusion (Table 3). Age was also a significant predictor of CAD: a 5-year difference in age resulted in a doubling of the risk. Individuals who reported high physical activity levels were less likely to develop CAD. Estrone was not significantly related to CAD in these women. A difference of 6 pg/mL in serum estrone was associated with an increased risk of CAD, but the confidence intervals were wide and the results not significant. Total cholesterol, history of smoking or MI, and the degree of obesity were not significant predictors of CAD. Inclusion of other risk factors in the model, such as total HDL-C, any use of estrogen, history of hypertension, or surgical menopause, had no effect on the results.

Discussion

To our knowledge, this is the first report of a study on the relation of endogenous estrogens and CAD in women. We were unable to find a difference in serum estrone levels between women with significant CAD and those without. These results are consistent with our observation that endogenous estrogens are not related to lipoprotein levels among postmenopausal women.22 These data suggest that changes in serum estrone levels may not contribute to the rise in CHD in women. Despite strong relations that have previously been observed with exogenous estrogens,9,10 endogenous estrogens may not be important determinants of CAD in women.

It is possible that serum estrogen levels in postmenopausal women fall below the threshold levels that are required to prevent atherosclerosis. The latency period for CAD is long, and it is possible that endogenous estrogen concentrations are critical during the development phase of atherosclerosis that occurs years before the manifestation of symptoms.

We adjusted for potentially confounding variables, including the degree of obesity, age, and history of diabetes mellitus. Obese women have higher serum estrogen levels, a finding consistent with the fact that the aromatization of estrone occurs primarily in fatty tissue23 and that obese women have a higher conversion rate of androstenedione to estrone.23 Obesity may also be a risk factor for CHD in women,24 and hence, in any study designed to assess whether a relation exists between serum estrogens and coronary disease, it is critical that the degree of obesity be considered as a confounder. Cases were significantly older than control subjects, and as in previous reports, older age was a significant predictor of disease.24 The majority of studies have not shown a significant decline in estrogens with age.25,26 Major changes in estrogen occur in the immediate postmenopausal period, with little subsequent change with age.

A positive history of diabetes is an established risk factor for CHD in women.24,27 Our results are consistent with this observation. Obesity, in particular abdominal obesity, is a risk factor for the development of non–insulin-dependent diabetes.28 Given the fact that obese women have higher serum estrogen levels,12,22 there may be an association among estrogens, distribution of body fat, and diabetes. Hyperandrogenicity has been shown to be related to non–insulin-dependent diabetes mellitus, which may be secondary to the hyperandrogenicity of abdominally obese women.28 Little is known about serum estrogens in women with non–insulin-dependent diabetes mellitus. Johnston et al29 have shown that serum estrone concentrations are significantly higher and androstenedione and testosterone levels significantly lower in diabetic compared with nondiabetic women even after controlling for the degree of obesity. At each level of obesity, estrone was higher in diabetic than in nondiabetic (control) women. Estradiol was higher only in diabetic women who were <63 years old.39 Rader et al30 have not found differences in estrone and estradiol in diabetics and hypertensives compared with control subjects. These authors, however, have not distinguished between diabetic subjects and those with hypertension. In the current study, serum estrone concentrations were similar in women.
with a history of diabetes and those without. Nevertheless, there may be an interaction of endogenous estrogens and androgens with insulin concentrations, insulin resistance, the degree of obesity, distribution of body fat, diabetic status, and CAD, which needs to be explored in future research.

In the current report, lifetime physical activity was inversely associated with CAD in women. Women who reported levels of high physical activity during the teenage years, at age 30, and at age 50 were 80% less likely to have at least 1 coronary artery with ≥50% occlusion. This observation was independent of the traditional cardiovascular risk factors. These data suggest that a lifetime of a high level of physical activity could retard the development of atherosclerosis.

Total serum cholesterol level, total HDL-C, obesity, and cigarette smoking have all been found to be risk factors for CHD.\(^24\) We were unable to replicate these findings, which may reflect the relatively low variability in these selected risk factors in our sample.

This study is limited by its small sample size. We previously reported the minimum detectable difference in sex hormones for different sample sizes.\(^19\) These estimates were based on 174 white postmenopausal women and suggested that we had sufficient power to detect a 6.1-pg/mL difference in serum estrone. It is unlikely that a difference <6 pg/mL would in fact be biologically significant.
We were unable to evaluate estradiol concentrations in this study. Estradiol is clearly more biologically potent than estrone, but estrone is the primary estrogen in postmenopausal women. Practically all of the estradiol in postmenopausal women is derived from estrone. Finally, estrone concentrations can be measured more reliably. The intraindividual variation in estradiol was almost as great as the interindividual variation, and more measurements are needed for estradiol. Angiography studies may be biased, since all subjects present with clinical symptoms of heart disease. In the current study, all of the women reported a history of angina. On the other hand, angiography studies will result in less misclassification of disease. To our knowledge, this is the first attempt to analyze the relation between serum estrogens and CAD in women. Our results do not support such an association, but these results need to be confirmed in other populations of women. Prospective cohort studies are needed to examine whether endogenous estrogens predict coronary disease in women who are free of symptoms at entry into the study.

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