Meeting Summary

Endogenous Insulin and Sex Hormones in Atherosclerosis and Coronary Heart Disease

Richard P. Donahue, Elizabeth Barrett-Connor, Trevor J. Orchard, and James P. Gutai

Cardiovascular epidemiology has made tremendous advances over the last forty years in identifying risk factors for coronary heart disease (CHD). There is a wealth of data on lipoproteins, blood pressure, cigarette smoking, and obesity. In contrast, surprisingly little attention has been paid to the risk factor that is probably the strongest predictor of coronary disease, namely gender. Coronary heart disease in young, premenopausal women is an extremely uncommon occurrence. Women under the age of 44 in Framingham experienced a risk of CHD one-sixth of that of their male counterparts.

The protective factor(s) in women (and the causative factors in men) remain to be elucidated. Studies to date have indicated that the sex difference in biological, psychological, and socioeconomic factors do not completely explain the female "advantage" in coronary disease. One condition where women appear to lose much of their protection, however, is diabetes. A corresponding observation notes that raised insulin concentrations appear to predict coronary disease in men but not in women.

Interest in a role for endogenous sex hormones in the pathogenesis of coronary disease has been limited largely to studies of the effect of oophorectomy on heart disease in women and to case-control studies of sex hormones in men with coronary disease. These studies have suffered from a variety of methodologic problems. Most, but not all, studies suggest that male survivors of a myocardial infarction have higher estradiol concentrations compared to healthy men.

This workshop sought to address the role of endogenous insulin and sex steroid hormones and the sex differential in coronary disease risk, as well as a number of other related concerns including: 1) the laboratory assessment of endogenous hormones, 2) the cross-sectional relation between hormone concentrations and established coronary risk factors, and 3) the longitudinal association of hormones with clinically evident coronary heart disease.

Measurement of Hormones in Epidemiologic Studies

Assessment of Sex Hormones

The initial section of the workshop concerned the assessment of insulin and sex hormones with an emphasis on the laboratory and biologic variation of these measures. James P. Gutai emphasized the need for endocrine laboratories to recognize the particular needs of an epidemiologic study as compared to a clinically-based investigation. These needs include recognizing the benefits and limitations of using a "specific" antibody in the assay, establishing a broad range of values which are likely to be encountered in the general population, and performing quality assurance throughout the range of values. As an example, Dr. Gutai noted that the measurement of estradiol in men with clinical kits is not likely to be sensitive at the low end of the estradiol distribution.

Several case-control studies have used frozen samples to quantitate hormone concentrations years after the initial collection was performed. Dr. Gutai stated that the effect of prolonged freezing or repeated cycles of freezing and thawing on the hormones is not well known.

The adequacy of a single measurement to characterize an individual man has been assessed by Dr. Gutai and his colleagues and, at least for total testosterone, appears reasonable. Factors such as age, weight, and cigarette use are known to affect an individual's hormone profile. The situation in premenopausal women is more complex due to the cyclical variation in hormone concentrations. Studies that have examined the repeatability of hormone concentrations over time in premenopausal women are virtually nonexistent. The study of estrone in postmenopausal women appears more favorable, with a 2-year within-person correlation coefficient of 0.56 in 174 women. However, levels of estradiol may often be too low to be reliably quantitated.

Questions about the quantitation of sex hormone binding globulin in calculating the percent of unbound hormone were raised. There was no general agreement about the utility of sex hormone binding globulin since, as a liver-inducible enzyme, it is subject to several sources of variability itself. A direct (rather than calculated) measure of unbound hormone levels may, however, be useful.
Assessment of Insulin Sensitivity

The measurement of insulin concentration and insulin sensitivity was addressed by Richard N. Bergman. Glucose intolerance and insulin resistance have been linked with obesity, diabetes, hypertension, and a number of other clinical states. Although an oral glucose tolerance test is often used to assess insulin resistance, this measurement is confounded by the normal negative feedback loop between hyperglycemia and insulin action.

Dr. Bergman and his colleagues have developed the "minimal model" approach to estimating insulin sensitivity. In this approach, glucose, followed by tolbutamide, is injected intravenously. Blood is sampled over the next 3 hours and the resultant plasma glucose and insulin measures are used to identify the parameters of the simplest mathematical model of glucose disappearance. A minimum of seven blood samples is necessary to adequately characterize an individual.

Questions about the epidemiologic utility of this test (because of its invasive nature) and whether fasting or post-challenge insulin concentrations could be substituted were raised. Dr. Bergman has used this model approach in several clinical and population studies in adults and felt that insulin resistance, which led to fasting or postchallenge hyperinsulinemia, was already moderate and therefore much information concerning the early phase of insulin resistance is lost without this modelling approach.

Relation of Endogenous Hormone Concentrations to Established Coronary Risk Factors

Hormones and Body Fat Distribution

Increased adiposity in the upper or central part of the body has been noted as a risk factor for diabetes, hypertension, hyperlipidemia, and coronary heart disease. Steven M. Haftner reviewed the evidence from the San Antonio Heart Study linking alterations in the distribution of body fat to an atherogenic hormone profile independent of body mass index.

An index of central fat disposition was calculated by dividing the subscapular skinfold thickness by the tricep skinfold. Among both sexes, this centrality index was positively correlated with the sum of the insulin values obtained at 0, 30 minutes, 60 minutes, and 120 minutes after a 75 g glucose load. No relation was seen between the centrality index and either total testosterone or total estradiol. Sex hormone binding globulin was inversely related to the insulin sum independent of all measures of body fat distribution. These data suggest that the increased risk of cardiovascular disease among subjects with upper body obesity may be due to two mechanisms: 1) hyperinsulinemia or insulin resistance, and 2) increased androgenicity as reflected by lower concentrations of sex hormone binding globulin.

Insulin and Hypertension

Michaela Modan presented data from nondiabetic subjects in the Israeli Heart Study on the triad of hypertension, hyperinsulinemia, and glucose intolerance. Elevated insulin levels were positively related to the frequency of glucose intolerance, hypertension, obesity, and elevated triglyceride concentrations. These findings were independent of age, sex, ethnicity, or use of antihypertensive medication. A substudy of cation imbalance showed a twofold excess of at least one cation (red blood cell sodium ≥7.0 mEq/l, red blood cell potassium <92.5 mEq/l or plasma potassium ≥4.5 mEq/l) in the insulin-resistant group compared to a euglycemic control group. Dr. Modan stated that insulin and blood pressure may be etiologically related by: 1) a direct effect on sodium reabsorption by the kidney, 2) an effect at the cellular level involving cation cotransport mechanisms, or 3) insulin's stimulatory effect on the sympathetic nervous system.

The second potential mechanism was explored in detail by Clare Bunker. Dr. Bunker and her colleagues sought to determine if the association between obesity and blood pressure is mediated through a relation between insulin and red blood cell sodium-lithium countertransport. Data on 144 white and 15 black normotensive premenopausal women were presented. Among white women, diastolic blood pressure was positively related to sodium-lithium countertransport, fasting insulin, and body mass index. Multivariate analyses revealed that the magnitude of the relation between blood pressure and obesity was reduced after controlling for countertransport activity, although body mass index remained a significant predictor of blood pressure. Black women displayed lower countertransport activity than whites, which was not explained by racial differences in obesity or insulin concentrations.

Although many studies have found a direct association between hyperinsulinemia and hypertension, it is less clear if elevated insulin levels precede the development of hypertension. If insulin concentration were related to blood pressure in normotensive subjects, this finding would support a role for elevated insulin in the etiology of hypertension.

Richard Donahue presented data on this question from two separate studies of normotensive young adults (ages 18 to 30 years): one, the Beaver County, Pennsylvania cohort (white men and women ages 21 to 25 years) and the other, a biracial cohort from the CARDIA study (ages 18 to 30 years). Fasting serum insulin was positively associated with systolic and diastolic blood pressure in both studies. This relation was independent of body mass index and pulse rate, indicating that, in normotensive young adults, insulin correlates positively with blood pressure over its entire range of values.

Hormones, Lipoproteins, and Atherogenesis

Trevor Orchard reviewed several problems that have complicated the study of insulin and atherogenesis. These include the fact that a given level of circulating insulin may have different consequences on different tissues (e.g., target cells like adipose tissue vs. atheromatous plaques). Also, the association of insulin with glucose appears linear only in the normoglycemic range. Thus, a low serum insulin concentration may represent enhanced insulin sensitivity among euglycemic subjects, but insulin deficiency among those with elevated blood sugar (i.e., impaired glucose tolerance/diabetes).
Measurement variability is especially problematic in this area. For example, Dr. Orchard indicated that the within-person variance of total cholesterol is much less than that for insulin. This imprecision in the measurement of serum insulin will tend to reduce the relative strength of relationships between insulin and heart disease compared to other coronary risk factors. Dr. Orchard then presented several studies performed at the University of Pittsburgh which substantiated a role for insulin in relation to the sex, body mass index, and age differences in the cardiovascular risk profile. Dr. Orchard suggested that nondiabetic women secrete more insulin than do men to overcome the insulin-antagonist affects of estrogen. Under this hypothesis, premenopausal women have a greater degree of insulin action, which results in a more favorable lipoprotein profile compared to men. However, with the development of diabetes, this insulin advantage of women is lost, with a consequential equalization of cardiovascular risk.

Dr. Orchard stated that treated insulin-dependent diabetic subjects often show similar or higher high density lipoprotein cholesterol (HDL-C) concentrations compared to healthy controls. Data were presented which indicated that the HDL₃ subtraction may be preferentially raised in these diabetic subjects and thus not necessarily lead to a reduced risk of heart disease, as might be expected by examining only total HDL-C levels. This area, however, remains controversial.

Barbara Howard presented results from a study of lean and obese Southwestern American Indians recruited from the Gila River Indian Community. This study examined the role of obesity, lipoprotein lipase activity, and sex hormones on HDL-C concentrations in men and women. Obesity (measured by percent fat determined by underwater weighing) was inversely related to HDL-C in both sexes, but more strongly so in women. Serum estradiol was lower in obese women as compared to lean women, while hepatic lipase activity showed the opposite relation. Dr. Howard and her coworkers hypothesized that the lower HDL-C and HDL₃ concentrations seen in obese women may be a consequence of the higher hepatic lipase and lower estradiol concentrations. Among men, there were only minimal affects of sex hormones and obesity on HDL-C levels.

In contrast, associations between HDL-C and measures of insulin-mediated glucose disposal were stronger in men. These findings were independent of body mass index and triglyceride concentration. In summary, this study suggested that sex hormones and insulin action are related to HDL-C, but that the extent of these associations differs somewhat by sex. Whether these sex differences in HDL-C metabolism are related to the sex difference in risk of coronary heart disease remains to be elucidated.

Dr. Donahue provided evidence from the same two populations discussed previously and showed that fasting serum insulin was inversely related to HDL-C concentrations in young adults. This relationship was strongest among white men and weakest among white women, with intermediate correlations among black men and women. Controlling for other covariates, such as body mass index, cigarette use, and alcohol intake, did not eliminate the inverse association. Dr. Donahue noted that interventions known to improve insulin sensitivity (or reduce insulin resistance), such as weight loss and increased physical activity, are also known to increase HDL-C levels. It is possible that the insulin/HDL-C link is mediated via lipoprotein lipase activity during the catabolism of triglycerides. In this report, however, insulin was related to HDL-C independent of the triglyceride concentration.

Before the onset of sexual maturation, boys and girls have similar levels of HDL-C. The well-known male pattern of lower HDL-C and higher cholesterol and triglyceride concentrations after puberty have focused attention on the potential importance of sex hormones as determinants of these changes, particularly in white men. Sathanur Srinivasan presented data from the Bogalusa Heart Study showing that despite similar mean concentrations of testosterone among black and white boys, testosterone was inversely related to HDL-C only in white adolescent boys. Black adolescent males had higher estradiol concentrations, however, which may act to attenuate the influence of testosterone on lipoproteins. These findings may relate to the higher HDL-C levels seen in black men compared to white men.

Subjects with noninsulin-dependent diabetes mellitus are more prone to develop coronary heart disease than nondiabetic persons. Much of this excess risk is due, no doubt, to an accelerated atherosclerotic process. Annick Fontbonne examined the potential role of sex hormones in diabetes in 38 type I diabetic men, 37 type II diabetic men, and 150 healthy controls. The strongest determinant of the estradiol/testosterone ratio was body mass index. When subjects were matched for body mass index, this ratio was not related to HDL-C, hemoglobin A₁C, or cardiovascular complications. Mean levels of estradiol and testosterone did not differ between diabetic cases and controls. Thus, in men matched for body mass index, estradiol and testosterone were unrelated to diabetic status or coronary risk factors.

**Hormones and Coronary Morbidity and Mortality**

Hyperestrogenemia has been implicated in etiology of coronary disease in a number of case-control studies. In these studies, however, the hormone measurement has been taken at variable points in time, ranging from early in the acute hospitalization phase to many years after a coronary event has occurred. Thus, raised estrogen concentrations could be a consequence of a heart attack, rather than a cause. A number of more recent studies have addressed this question through the prospective collection of serum before the occurrence of clinical events.

**Steroid Sex Hormones and Coronary Heart Disease**

Elizabeth Barrett-Connor utilized the 40- to 79-year-old male population from the Rancho Bernardo Study to examine the association of hormone levels with the 14-year risk of coronary disease. The assayed hormones included total concentrations of testosterone and estra-
diol, as well as estrone, androstenedione, and sex hormone-binding globulin. None of the sex hormones measured was significantly associated with all cause, cardiovascular, or ischemic heart disease mortality in univariate or multivariate analyses.

Another prospective study using “high-risk” men from the Multiple Risk Factor Intervention Trial was presented by Jane Cauley. Cases included 61 men who experienced a nonfatal myocardial infarction and 102 cases of fatal coronary disease. An equal number of controls were matched on age, serum cholesterol, randomization group, date, and clinic. There were no differences between cases and controls in total or free testosterone, total or free estradiol, androstenedione, or estrone.

Thus, in these two population-based studies of middle-aged men, no evidence of a link between sex hormones and heart disease was seen. Prospective studies of young men where the body mass index/risk factor associations are strongest may be worthwhile.

Serum Insulin and Coronary Heart Disease

Michael Lichtenstein presented cross-sectional findings from 2512 men ages 45 to 59 years from the Caerphilly Study in Wales. Of the lipid fractions measured, triglyceride concentration was the strongest correlate of prevalent heart disease. Men with coronary disease had significantly lower mean values of testosterone and higher values of fasting serum insulin compared to disease-free men. In multivariate analyses, triglyceride and insulin concentrations were related to prevalent coronary disease independent of age, body mass index, and blood pressure. Estradiol levels were unrelated to heart disease in either univariate or multivariate analysis. Thus, these cross-sectional results support a role for insulin in the pathogenesis of heart disease, but do not support a similar role for sex hormones.

The role of insulin as predictor of clinical coronary events was explored in the last two presentations of the workshop. Eveline Eschwege presented her data gathered from the Paris Prospective Study and Dr. Fontbonne reported the results from the Helsinki Policemen’s Study on behalf of Kalevi Pyorala.

In the Paris study, 7434 middle-aged men were classified into one of three categories based upon World Health Organization criteria: normoglycemic, impaired glucose tolerance, or diabetic (new and known). Over a mean follow-up of 11 years, the impaired glucose tolerance group experienced 1.9 times the risk of heart disease mortality compared to the normoglycemic group. The risk was 2.5 times greater among the diabetic men. Among normoglycemic men, fasting insulin (but not 2-hour insulin) predicted coronary mortality independently of the standard coronary risk factors. When all men were considered in the analysis, glucose tolerance status did not predict coronary mortality independently of fasting insulin concentration.

The Helsinki Policemen’s Study followed for 9.5 years 982 men ages 35 to 59 years who were free of coronary disease and diabetes at the study entry. A total of 63 coronary events including 26 deaths occurred. Both 1-hour and 2-hour postchallenge insulin concentrations (but not fasting insulin) were positively related to fatal coronary heart disease. The 1-hour and 2-hour glucose levels were also elevated in men who suffered a fatal event. When the 1-hour measurements were considered in a multivariate model, 1-hour insulin retained its significance, while the corresponding glucose value did not. Similarly, when the 2-hour values were considered, insulin was retained and glucose dropped out of the predictive model.

Summary

A major unresolved question in cardiovascular disease epidemiology pertains to the largely unexplained male excess of coronary disease risk, particularly among young and middle-aged men. To what extent differences in hormone concentrations and function explain this puzzle is poorly understood.

Research at all levels is necessary. Adequate laboratory accuracy and precision in the measurement of hormones is a mandatory first step. Currently, there is no standardization program for assaying sex steroid hormones, nor is one planned.

The relationship between hormones and coronary risk factors and disease appears strongest for insulin. However, studies are inconsistent with regard to which measures of insulin activity are “best” (fasting or postchallenge). In addition, quantitating circulating insulin may not correlate well with its function, and other more complex techniques to measure insulin resistance may be helpful. In particular, we need to develop two indices: one of insulin action and one to examine elevated insulin concentrations.

The interrelationships among insulin, obesity, physical activity, blood pressure, and lipoproteins probably will not be completely disentangled through purely observational studies. Experimental investigations, including weight loss, dietary change, and increased physical activity, are required to isolate the specific effects of these alterations on the hormones and coronary risk profile. Included in such programs should be measures of body fat distribution. Upper body fat appears more closely related to increased risk for coronary disease than does lower body obesity. Concomitants of upper body fat include hyperinsulinemia and increased androgenization, which themselves may produce atherogenic changes in the risk factor profile. Little is known concerning the genetic and environmental determinants of body fat distribution. If there are easily identifiable environmental determinants, then fat distribution may be subject to favorable modifications, which could have a large impact on coronary disease. Genetic influences, if they exist, may aid in better identifying the high risk coronary candidate and lead to earlier preventive efforts.

A role for endogenous sex steroid hormones in relation to coronary disease risk appears to be absent in men. There are, however, several limitations in the methods used to assay the hormones and the populations studied. Low-order associations also may reflect our ability to quantitate only the concentration or composition of a particular hormone, rather than to examine its biological function.
The fact that gender is arguably the strongest risk factor for coronary heart disease has long been recognized, but unexplained. As gender itself is obviously an immutable risk factor, many researchers concerned with intervention and lifestyle modification may not have pursued it as rigorously as other risk factors. Furthermore, a clearer understanding of those factors that cannot be altered may provide insight into the fundamental aspects of atherosclerosis and lead to new therapeutic approaches to CHD. The study of endogenous hormones in relation to coronary risk factors and disease is one important approach to this problem.

Index Terms: endogenous insulin • sex hormones • coronary heart disease • atherosclerosis • estrogen
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