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Estrogens and women's health: interrelation of coronary heart disease, breast cancer and osteoporosis☆

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Abstract

The determinants of blood levels of estrogen, estrogen metabolites, and relation to receptors and post-transitional effects are the likely primary cause of breast cancer. Very high risk women for breast cancer can now be identified by measuring bone mineral density and hormone levels. These high risk women have rates of breast cancer similar to risk of myocardial infarction. They are candidates for SERM therapies to reduce risk of breast cancer. The completion of the Women's Health Initiative and other such trials will likely provide a definite association of risk and benefit of both estrogen alone and estrogen-progesterone therapy, coronary heart disease, osteoporotic fracture, and breast cancer. The potential intervention of hormone replacement therapy, obesity, or weight gain and increased atherogenic lipoproteinemia may be of concern and confound the results of clinical trials. Estrogens, clearly, are important in the risk of bone loss and osteoporotic fracture. Obesity is the primary determinant of postmenopausal estrogen levels and reduced risk of fracture. Weight reduction may increase rates of bone loss and fracture. Clinical trials that evaluate weight loss should monitor effects on bone. The beneficial addition of increased physical activity, higher dose of calcium or vitamin D, or use of bone reabsorption drugs in coordination with weight loss should be evaluated. Any therapy that raises blood estrogen or metabolite activity and decreases bone loss may increase risk of breast cancer. Future clinical trials must evaluate multiple endpoints such as CHD, osteoporosis, and breast cancer within the study. The use of surrogate markers such as bone mineral density, coronary calcium, carotid intimal medial thickness and plaque, endothelial function, breast density, hormone levels and metabolites could enhance the evaluation of risk factors, genetic-environmental intervention, and new therapies.

Keywords: Breast cancer; Osteoporosis; Estrogen metabolites

1. Introduction

In this paper we evaluate the interrelationships between endogenous and exogenous sex steroid hormones, energy metabolism, lipoproteins, coronary heart disease, osteoporosis, and breast cancer among women.

Estrogens interact with their intracellular receptors resulting in expression of genes that encode proteins. Estrogens have mitogenic effects in many tissues, including the breast. Metabolites of estrogen may also be potent mutagens, especially for the breast. Estrogens are eliminated from the body by their changes toward soluble metabolites and are then excreted in the urine or feces. The major metabolites include oxidation and conjugation with glucouronides, sulfates, or *O*-methylation. The cytochrome P-450 enzymes catalyze the oxidative metabolism of estrogen, primarily, in the liver [1,2].

The functional role of the specific estrogen metabolites is still not determined [3]. There are animal experimental and some human studies suggesting important and unique biological properties of the specific estrogen metabolites [4]. Estrogen metabolism, especially in target tissue (such as the breast), can substantially increase the concentrations of estrogens in these target tissues [5]. For example, sulfatase enzymes can remove sulfate from estrogen and aromatization of androgens in target tissues can substantially increase the local concentrations of estrogen such as in the breast.

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The levels of estrogen in breast tissue have been reported to be much higher than those measured in peripheral blood [6]. The measurement of estrogen levels in tissues, except from biopsy specimens, is difficult in human epidemiological studies. The measurements of estrogen in breast secretions, especially in premenopausal women, has been used to estimate estrogen levels in the breast tissue [6]. Whether the levels in the secretions are consistent with the levels in the tissues has not been determined. There is little information regarding the effects of local tissue estrogen levels and metabolites in coronary heart disease or osteoporosis [7].

The effects of sex steroid hormones is a likely explanation for the lower CHD rates for women [8]. The risk of myocardial infarction (MI) is lower for women than men, even at old ages, i.e. women never catch up to men [9]. The incidence of stroke, however, is similar among older men and women [10].

The risk factors for coronary heart disease are similar for men and women and including elevated serum LDLC, triglycerides, lower HDLC, increased waist circumference, decreased physical activity, cigarette smoking, higher blood pressure, and especially diabetes [11].

2. Results

2.1. Premenopausal women

The incidence of coronary heart disease is very low among premenopausal women. Cigarette smoking, obesity, hypertension, diabetes, hypercholesterolemia, and low HDLC are major risk factors for coronary heart disease in cross-sectional studies of premenopausal women [12].

There are several important diseases that are associated with a probable substantial increase in the risk of premenopausal coronary heart disease (Fig. 1and Fig. 2). This includes lupus erythematosus, insulin dependent diabetes mellitus, artificial menopause, i.e.

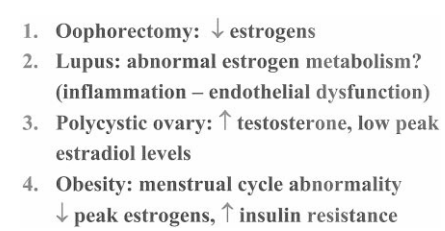

Fig. 1. Determinants of very high risk of premenopausal coronary heart disease (1) Oophorectomy:↓ estrogens (2) Lupus: abnormal estrogen metabolism? (inflammation — endothelial dysfunction) (3) Polycystic ovary: ↑ testosterone, low peak estradiol levels (4) Obesity: menstrual cycle abnormality ↓ peak estrogens, ↑ insulin resistance.

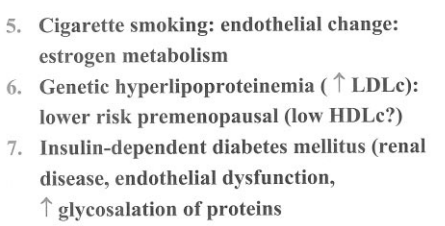

Fig. 2. Determinants of very high risk of premenopausal coronary heart disease (5) Cigarette smoking: endothelial change: estrogen metabolism (6) Genetic hyperlipoproteinemia (↑ LDLc): lower risk premenopausal (low HDLc?) 7. Insulin-dependent diabetes mellitus (renal disease, endothelial dysfunction, ↑ glycosalation of proteins.

oophorectomy, polycystic ovary syndrome and pre-eclampsia. The excess risk of cardiovascular disease associated with these diseases are all related in one way or another to hormone metabolism. Abnormal hormone metabolism, for example, is considered to be a probable cause of or a contributor to lupus. Women with this disease, given hormone replacement therapy, or during pregnancy with high estrogen levels, often do poorly. In insulin dependent diabetes higher insulin levels may be associated with increased testosterone levels, aberrant menstrual cycling, as well as glycosylation of proteins. Women with polycystic ovary are characterized by amenorrheic, low peak estradiol levels, high testosterone levels, obesity, and increased intra-abdominal fat, hyperinsulinemia, hypertriglyceridemia, hypertension, and diabetes. Oophorectomy is obviously associated with decreased estrogen levels. Toxemia or pre-eclampsia is more common in overweight women and is associated with many of the same biochemical abnormalities as in polycystic ovary. Abnormal endothelial function is the most likely common denominator to account for the higher risk of cardiovascular disease in these rather unique diseases of premenopausal women. Clinical and animal experimental studies have documented a rapid positive effect of estrogens on endothelial function with stimulation of nitric oxide synthase and greater nitric oxide production.

There is a much smaller sex difference for the aortic as compared to coronary atherosclerosis especially for premenopausal women. The reasons for this difference between coronary and aortic, atherosclerosis is likely to be due to unique effects of estrogens on coronary arteries [13]. There is a positive correlation, however, between extent of coronary and aortic calcification among women [14]. The extent of carotid intimal medial thickness is greater in men than women [15]. Men also have high femoral artery intimal medial thickness [16]. The sex difference is smaller than for coronary calcification. Carotid intimal medial thickness is a mix of increased medial and intimal thickness and not just a measure of atherosclerosis.

Studies in Iowa and recent data from the Pittsburgh Women's Healthy Lifestyle Project have shown that premenopausal obesity and waist circumference are major determinants of the extent of coronary calcium among pre- to very early postmenopausal women [17]. The frequency of abnormal menstrual cycling and lower peak estradiol levels are directly related to the extent of obesity among premenopausal women. Obese premenopausal women have a lower risk of breast cancer [18].

2.2. Postmenopausal women

The menopause transition is characterized by a marked decrease in ovarian function; estradiol levels, weight gain, about one pound per year, between age 45–54, an increase in percent body fat and intra-abdominal fat.

The determinants of sex steroid hormone levels change dramatically at the postmenopause. In premenopause, obesity is related to lower peak estradiol levels. In postmenopause, the primary hormone is estrone derived from the adrenal, androgen, androstenedione and the conversion to estrone by the enzyme aromatase in fat tissue. The greater degree of obesity among postmenopausal women, the higher the estrogen blood levels. The production of androstenedione by the adrenal decreases with age, but the aromatase enzyme activity increases with aging. Insulin stimulates aromatase enzyme activity.

Genetic variations (polymorphism) in the P450 aromatase enzymes have been identified as well as variations in the estrogen and androgen receptors [1,2]. There is growing evidence that genetic polymorphisms of receptors may be related to prostate cancer in men and breast cancer and osteoporosis in women.

In the Healthy Women Study in Pittsburgh, Zmuda et al. [19], reported that women on HRT (estrogen and progesterone) positive for the Xbal gene polymorphism of the estrogen receptor α and for the Pvull genotype had higher bone mineral density at several sites in the hip. There was no relationship of genotypes and bone density for women not on hormone replacement therapy. The relationship of specific polymorphisms to hormone levels, coronary atherosclerosis and heart attacks has not been determined.

An increase in LDLC during the peri- to postmenopause occurs in practically all populations. It occurs in primates following artificial menopause (oophorectomy). The increased activation of LDL receptors by estrogen accounts, to a large degree, for the lower LDLC levels among premenopausal women as compared to men or postmenopausal women. The increase in LDL is also greater among women who gain weight during the peri- to postmenopause [20,21].

The increase in LDLC is probably the most important effect of the menopause [20]. The rise in LDLC occurs before the time of cessation of menses. The amount of chain-length specific saturated fat and dietary cholesterol are major determinants of LDLC levels [22]. Greater intake of polyunsaturated fat reduced LDLC levels. In the Women's Healthy Lifestyle Project (WHLP) clinical trial, a decrease in intake of saturated fat and cholesterol and weight gain reduced the rise in LDLC between pre- to peri- to postmenopause [23]. There is no consistent evidence of an association of these specific fatty acids and hormone metabolism.

The use of hormone replacement therapy after the menopause, cessation of menses, reduced the levels of LDLC but not to premenopausal levels. Substantial bone loss also occurs during the peri to postmenopause [24]. Weight loss pre- to postmenopause results in increase in bone loss [25]. Hormone replacement therapy will reduce the risk of bone loss, but does not increase the amount of new bone formation [26].

There is little effect of menopause, i.e. hormonal change, on blood pressure, glucose, or insulin levels. The increase in body weight from pre- to postmenopause results in a substantially greater increase in triglycerides and ApoB and, later, rise in systolic blood pressure [27].

There is only a small effect of total HDLC at the time of the menopause, a substantial decline in HDL2 and increase in HDL3. The degree of obesity, waist circumference, physical activity, and alcohol consumption are major determinants of HDLC [34]. Levels of HDLC are inversely related to risk of cardiovascular disease (CVD). There is, however, no human clinical trial evidence in women that raising HDLC levels by drugs or nonpharmacological interventions reduces the risk of coronary artery disease [28].

Weight gain, especially pre- to postmenopause, is also associated with a substantial increase in waist circumference, the amount of visceral fat, and elevations in glucose and insulin, ultimately leading in higher risk women to diabetes mellitus. The elevated insulin levels and obesity result in a decrease in production of sex hormone binding globulin in the liver, and increase in unbound estradiol and testosterone. The higher central obesity in women resulted in greater androgen production in both pre- and postmenopausal women [29]. Weight gain from pre- to postmenopause is also a risk factor for breast cancer [18,30].

The specific causes of the weight gain during the peri- to the postmenopause have been very difficult to determine. It is extremely difficult to quantify total caloric intake except by such techniques as double label water, not available in epidemiological studies. Greater leisure time physical activity is inversely related to the measure of both BMI and waist circumference. There is an increase in percent of body fat pre- to postmenopause

and decreases in both BMD and lean body mass leading to osteoporosis, and sarcopenia [31,32]. There is also a gradual decline in growth hormone, IGF-I, which may also contribute to increases in body fat, intra-abdominal fat and decreased lean mass [33]. Resting metabolic rate may be declining because of the loss of lean body mass. A direct effect of estrogens on energy metabolism is undetermined from available data [32].

Lower physical activity levels, cigarette smoking, higher caloric intake were related to greater waist circumference in the Healthy Women Study [34]. Weight gain from the pre- to the postmenopause was much reduced among women who increased their physical activity levels in the Healthy Women Study. There also was an interesting positive association between selected behavioral attributes and waist circumference. Higher cortisol levels and insulin resistance are generally associated with an increase in intra-abdominal fat while higher growth hormones are associated with lower amounts of visceral or intra-abdominal fat [35]. Elevated levels of cortisol are associated with certain behavioral characteristics. Dr Karen Matthews and colleagues in the Healthy Women's Study have determined that baseline hostility and other behavioral measures are an independent predictor of the increase in waist circumference during the early postmenopausal period [36]. This was true even after adjusting for weight gain. These same behavioral attributes have also been linked to increased risk of coronary artery disease among both men and women. Whether the behavioral attributes are related to the cortisol levels and intra-abdominal fat, or behavioral changes result in modification of diet and exercise that lead to weight gain and increased intra-abdominal fat, are not determined. There are likely important genetic determinant of obesity and, probably, visceral fat.

Atherosclerosis is a systemic disease. Previous pathology studies, noted the strong correlation of extent of disease across vascular beds within the same individual. The extent of atherosclerosis was also highly correlated with coronary disease mortality rates. Women who die due to coronary heart disease usually have extensive coronary atherosclerosis and stenosis [37].

The current use of noninvasive methods of measuring atherosclerosis are an in vivo extension of these pathology studies. The high correlation of disease in different vascular beds provided an opportunity to utilize more easily accessible vascular beds, such as the carotid arteries, in studies of subclinical atherosclerosis.

The extent of subclinical atherosclerosis increases with age from the pre- to the postmenopausal women. The extent of subclinical coronary disease is much lower in women than men even to older age groups consistent with the lower incidence of myocardial infarction [38].

The levels of estrone, the primary postmenopausal estrogen, are highly correlated in the same woman over time, i.e. from first to second postmenopausal year up to eight to ten years postmenopausal. Body mass index is strongly associated with levels of postmenopausal estrone (Fig. 3).

The blood levels of postmenopausal estrogens are not related to the extent of coronary artery or aortic calcium or to carotid intimal medial thickness (Fig. 4). The higher blood levels of estrogens are strongly related to the woman's risk of breast cancer (Fig. 5) [39] and to levels of bone mineral density and risk of hip fracture (Fig. 6) [40].

There are no consistent longitudinal studies that show any relationship between postmenopausal blood estrogen levels in nonhormone users and the risk of heart attack or progression of subclinical cardiovascular disease. A major limitation of these studies has been the prior inability to quantify the relatively low levels of

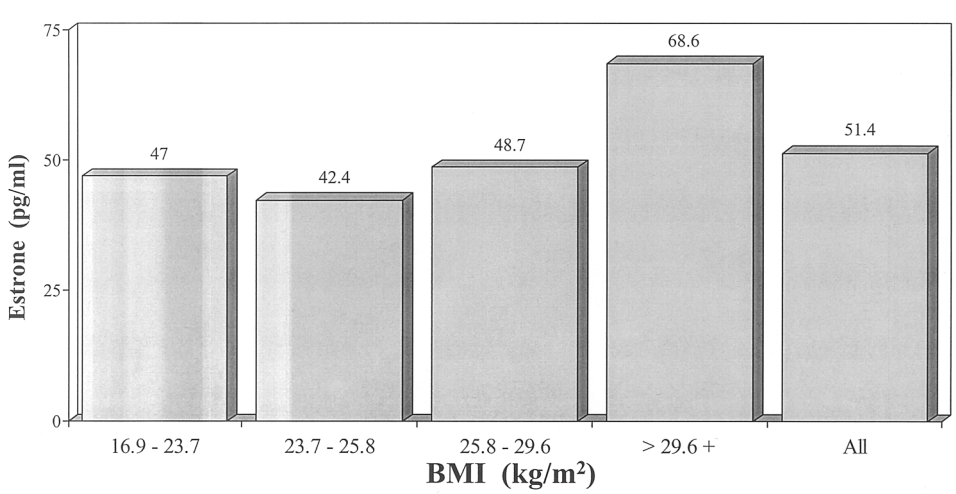

Fig. 3. Relationship of BMI to estrone level in NonHRT users at 8th postmenopausal exam.

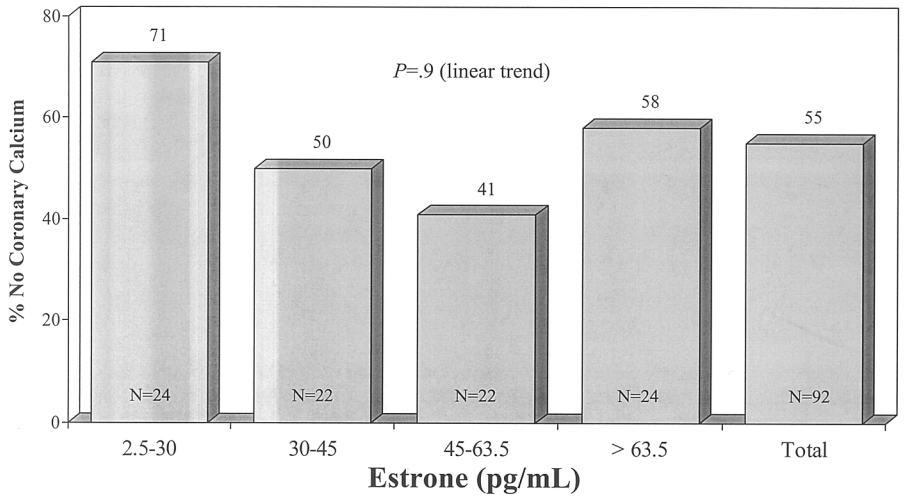

Fig. 4. Percentage of HWS participants with no coronary calcium by 8th postestrone levels (NonHRT users).

postmenopausal sex steroid hormones, especially estradiol. Substantial improvement in laboratory methods offers an opportunity to determine the relationship between endogenous sex steroid hormone levels in blood and the risk of heart attack, breast cancer, or osteoporosis.

There is a relatively weak association of postmenopausal hormone replacement therapy (i.e. estrogen or estrogen/progesterone) and extent of subclinical atherosclerosis. There is continued strong evidence of the effect of sex steroid hormone therapy especially estrogens on endothelial function, even among older women [41].

The absence of a strong effect of HRT therapy in postmenopausal women on the extent of subclinical atherosclerosis is worrisome, especially in the context of the negative results from the HER Study in secondary prevention [42] and the Tamoxifen study, a primary prevention breast cancer trial [43]. Neither study showed any benefit in reducing coronary heart disease.

The cardiovascular risk factor levels among pre- to postmenopausal women track extremely well, in spite of the effects of the menopause, even in women placed on hormone replacement therapy. Women with high premenopausal risk factor levels tend to have high levels as compared to other women 8–10 years postmenopausal, both for HRT users and nonusers.

The levels of cardiovascular risk factors measured premenopause 8–10 years prior, are very powerful predictors of the risk of coronary or aortic calcification or carotid intimal medial wall thickness among women at age 60, 10- years postmenopausal. The premenopausal risk factor levels are a better predictor of extent of subclinical disease than measurements done proximate in time to subclinical laboratory testing.

The extent of obesity and intra-abdominal fat is a major determinant of the levels or pro-inflammatory cytokines such as TNF alpha and IL6 [44]. The increase in IL6 results in a greater production of acute phase proteins such as C-reactive proteins by the liver. The Nurses Study has reported a very strong relationship between elevated CRP levels within the normal range and the risk of coronary heart disease among postmenopausal women [45].

Estrogen and progesterone therapy resulted in a decrease in fibrinogen, PAI-I and TPA antigen and increased fibrinolytic activity [46], but a marked increase in CRP. The highest CRP levels were found among women on HRT and higher levels of total or visceral fat. These differences in CRP could be associated with a very substantial increase in the risk of coronary artery disease, based on the prior observational studies of risk of CHD and higher CRP levels in women [45,47]. Estrogen therapy increases the production rate of both light and dense LDL. Estrogen therapy results in a decrease in LDL size (possibly atherogenic, LDL particles) [48].

Estrogen therapy increases the production of VLDL particles. Estrogen may increase the fractional catabolic rate of VLDL. Triglyceride levels are higher for postmenopausal women on hormone replacement therapy. The triglyceride levels also increase with weight gain. It has been previously suggested that the accelerated fractional catabolic rate of both LDL and VLDL may reduce their atherogenic potential [48].

In the Healthy Women Study we evaluated the relationship between blood triglycerides, characteristics of lipoproteins and coronary or aortic calcium using EBCT. We used NMR spectroscopy of the lipoprotein laboratory methods developed by Dr Otvos [49].

Nuclear magnetic resonance spectroscopy gathers lipoprotein subclass information in a manner that is completely unique and inherently far more efficient than existing methods. The technique relies on the

spectroscopic distinctness of lipoprotein particles of a particular size and requires no physical separation of one group of particles from the others. Refined over a 10-year period, the NMR process measures 15 subclasses of VLDL, LDL, and HDL simultaneously. The measurement requires only about 1 min, uses no chemical reagents, and is completely automated. By simply adding up the concentrations of the various subclasses, one obtains all of the information provided in a standard lipid panel (total, cholesterol, triglycerides, LDL and HDL cholesterol) [49].

The estimated blood levels, LDLC, HDLC and triglycerides using NMR techniques and standard methods of measurements are very highly correlated. The LDLC is higher, however, by NMR spectroscopy.

The level of LDL and number of LDL particles as measured by NMR spectroscopy was similar among the HRT users and nonusers. The large VLDL particles were higher among HRT users. The number of LDLC particles, triglyceride levels, and large VLDL were all related to the extent of both coronary and aortic calcification for both HRT users and nonusers. One reason for the lack of an effect of HRT on coronary and aortic calcification is related to the increase in VLDL particles, dense LDLC and in LDL particles for women taking HRT as compared to not taking HRT. The use of some progestins blunts the rise in HDLC associated with estrogen therapy and may further reduce the benefits of combined HRT therapy [50].

We further evaluated whether there was a relationship between the elevated LDLC and triglyceride levels and endothelial function among women on HRT using flow mediated vasodilation in a subsample of postmenopausal women in the Healthy Women's Study. The higher LDLC and triglyceride levels, the lower the extent of vasodilation, suggesting that these high LDLC or triglyceride levels or large VLDL particles may also be blunting the benefits of estrogens on endothelial function.

The higher levels of VLDL and lower large HDL, triglycerides, number of LDL particles, and dense LDLC were all positively correlated with measures of waist circumference or intra-abdominal fat, especially for HRT users.

Another possible reason for the weak association of estrogens or estrogen and progesterone, and extent of subclinical or clinical cardiovascular disease may be related to variations in estrogen metabolism that could either be due to lifestyle risk factors, such as obesity or specific dietary factors, i.e. dietary fat, exercise, or to the genetic polymorphisms of estrogen metabolism or interaction with estrogen receptors and post-translational effects.

Endogenous estrogen (i.e. estradiol and estrone) can be hydroxylated by multiple pathways [1]. The 2-hydroxylation of estrone is the major metabolic pathway in the liver [1]. The 4-hydroxylation is a relatively minor pathway, at least in the liver, but may be important in other tissues. The 2-hydroxy metabolite levels are very low in blood because of their rapid conversion to *O*-methylation or other metabolic products. The 2-hydroxylation can bind to the estrogen receptor with very limited hormonal effect.

The 16-hydroxylation pathway includes about 10% of the metabolic pool of estrone and the 4-hydroxylation pathways both retain potent hormonal activities by activating the estrogen receptor. Bradlow, Fishman et al., have hypothesized that the extent of 16-hydroxylation may increase the risk of breast cancer. The 16-α-estrone binds to the estrogen receptor with about 3% of the infinity of estradiol. Once bound, however, it does not down-regulate the receptor. The 16-α-hydroxylation also may form addicts with DNA. In animal

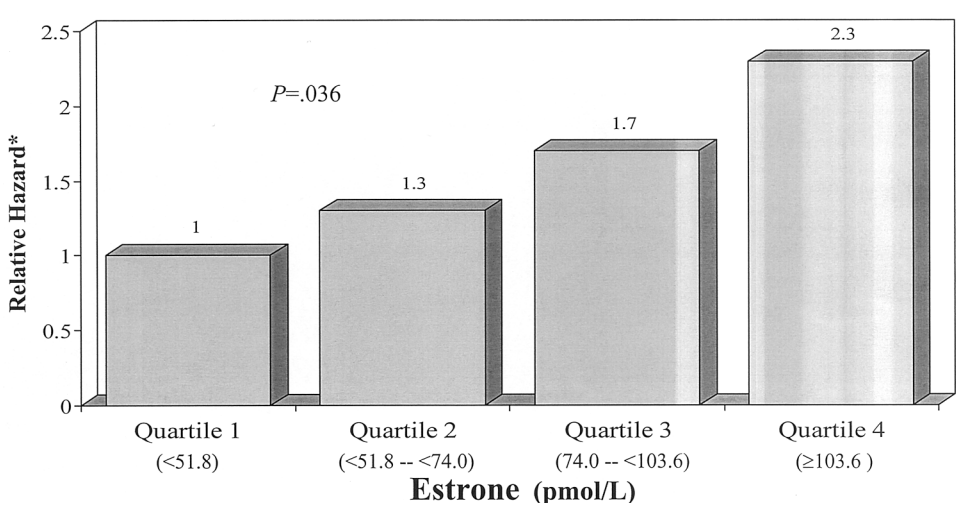

Fig. 5. Relative hazard for breast cancer by concentration of sex-steroid hormones.

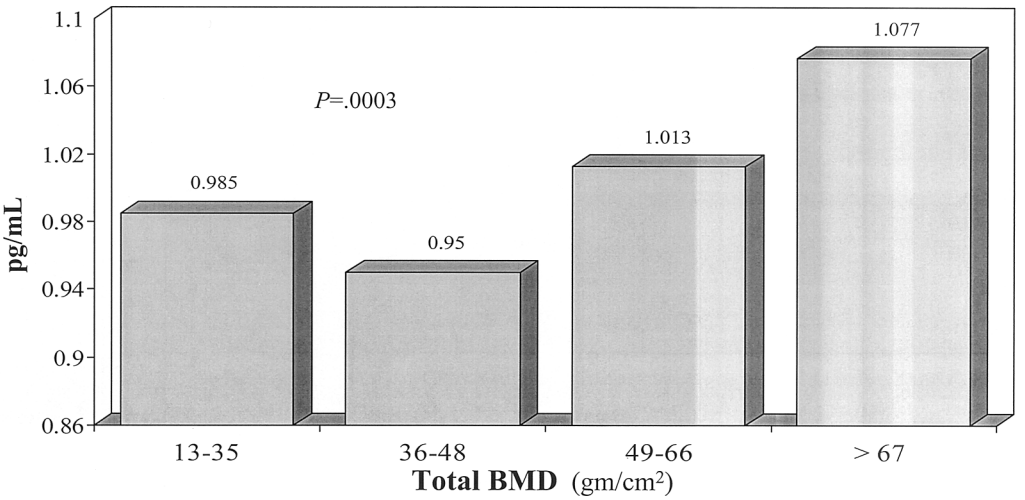

Fig. 6. Blood estrone level at 5th postmenopausal exam and total bone mineral density in the *healthy women study* — NonHRT users.

experimental studies the 16-α-hydroxylation has stimulated the growth of breast cancer cell lines [51,52].

Other investigators have suggested that higher levels of the 4-hydroxy metabolite of estrone is a cause of breast cancer. The 4-hydroxy pathway may be important to extra hepatic tissues. The P-450 B1 catalyzes the formation of 4-hydroxy estrone in breast cancer cell lines [53]. In human uterine myoma 4-hydroxylation predominates over the 2-hydroxylation pathway.

We previously evaluated a competitive solid phase enzyme immunoassay for 2- and 16-hydroxy estrone in the urine in premenopausal women enrolled in our Women's Healthy Lifestyle Project (WHLP). The ratio of 2–16 hydroxy estrone in the urine was 2.30 at baseline and 2.55 during 6 months' follow-up. Intraclass correlations of duplicate urine specimens collected at baseline was 0.95 and intraclass correlations measured 6 months later was 0.81 [54].

Ziegler et al., also evaluated estrogen metabolites, 2- and 16-hydroxy estrone in the urine. Reproducibility over a 4 month period was good — both metabolites and premenopausal women and satisfactory in postmenopausal women with coefficients of variation of about 8–14% in premenopausal women and about 19% for postmenopausal women [64]. Reproducibility was not very good in postmenopausal women over time. Correlation was between the enzyme immunoassay and GC-MS measurement was excellent for both metabolites with correlations greater than 0.9 except for 2-hydroxy in postmenopausal women (about 0.6) [55,56].

There are only a few studies that have attempted to evaluate the relationship of the estrogen metabolites to risk of cancer and even fewer to either cardiovascular disease or osteoporosis. The previous studies of 2- and 16-hydroxylation metabolites have measured them in the urine and evaluated the relationship of 2- and 16-hydroxy metabolites to risk of breast cancer in a prospective design or in case-control studies [57,58]. The results of these studies have been inconsistent; some showing a relationship and others, no relationship between 2- and 16-hydroxylation products in the urine and breast cancer risk.

Modification of the immunoassay method for measuring the metabolites in blood has now become available. We have evaluated the 2- and 16-hydroxy estrone glucouronide levels in blood samples in the Healthy Women Study. The present serum (EIA) are competitive, solid-phase enzyme immunoassays. In this assay format a biotinylated monoclonal antibody is captured on the proprietary avidin coded solid-phase and the antigen (estrogen metabolite) is covaliantly labeled with the enzyme. In the test, binding of the antigen enzyme conjugate by the immobilized antibody is inhibited by the addition of free antigen in assay standards or in the test sample.

The reproducibility of the assay method was evaluated in 34 postmenopausal women in the Healthy Women Study; half were on HRT and the other half not on HRT. The Spearman correlations of the 2-hydroxy estrone were 0.92 and for the 16-hydroxy estrone 0.95. The coefficient of variation was about 23% for the 2-hydroxy estrone and 12.5% for the 16-hydroxy estrone (i.e. coefficient of variation equals the square root of the sum of the differences squared divided by 2n, divided by the mean times 100). There are also very high Spearman correlations of both the 2- and the 16-measurements done over time (between 0.84 for 2- and 0.80 for the 16-hydroxy estrone). The coefficient of variation, however, was high, showing that women tracked over time compared with other women, but there is substantial within individual variation (both biological and laboratory variation over time).

Women on HRT, as expected, had substantially higher both 2- and 16- levels in their bloods. The ratio of 2 to 16 for women on HRT was about 2.5. For women not on HRT, the ratio was 0.77 (Table 1).

Table 1
Relationship of 2 and 16 hydroxy estrone levels to hormone replacement therapy in the healthy women study

Ng/mL	HRT	NonHRT
2	437	95
16	188	143
Ratio 2/16	2.5	0.77

The levels of 2 and 16 were both significantly correlated (0.26 for the 2 and 0.33 for the 16) with blood estrone levels measured using a sensitive radio immunoassay methods in a different laboratory. Among nonHRT users the levels of both the 2- and 16-hydroxy estrone metabolites increased significantly with increasing body mass index, waist circumference, or percent body fat. Among women on a hormone replacement therapy, on the other hand, greater body mass index, waist circumference, or percent body fat was inversely associated with both the 2- and 16-hydroxy estrone levels, especially the 16-hydroxy estrone (Fig. 7, Fig. 8).

These results suggest that the relationship of BMI to hormone metabolite levels are totally confounded (i.e. they go up in women who are not on HRT and decline in women on HRT). If, for example: (1) the hormone levels are an important determinant of the risk of breast cancer; (2) the measure of the metabolites in blood are a reflection of active hormone exposure at the breast; (3) the relative risk of breast cancer comparing hormone users to nonhormone users would be substantially greater for thinner as compared to more obese women. The glucouride metabolite is not recognized by the estrogen receptor. The levels measured in blood are a possible marker of risk.

There was a strong positive relationship between bone mineral density and both the 2- and 16-hydroxy estrone levels among nonHRT users but not HRT users. There was no evidence that the ratio of 2–16 was a better predictor than levels of bone mineral density.

There was no significant relationship of coronary or aortic calcification or carotid plaque and the levels of 2- or 16-hydroxy estrone in the blood in either HRT users or nonusers. We are currently evaluating the relationship between the 2- and 16-hydroxy estrone metabolites and risk of breast cancer in the Study of Osteoporotic Fracture (SOF). A blood measurement for the 4-hydroxy metabolite is not currently available.

A higher BMD is likely a surrogate marker for higher estrogen levels. There is a very striking association of the risk of breast cancer and BMD for both stage one and more advanced stage breast cancer among older women (Fig. 9) [59–61]. This relationship is independent of HRT use. Risk of breast cancer increases, as noted, in women who have higher postmenopausal BMI. However, most of this effect appears to be related to higher BMD, a marker of estrogen levels. Women with higher BMI are at very high risk of breast cancer, with rates similar to MI for older women (i.e. about 8/1000/year). Women with lower BMD are unfortunately at increased risk of fracture and for total mortality. We found no relationship between coronary calcium, and BMD among postmenopausal women. We also found no consistent relationship between BMD and the risk of myocardial infarction.

3. Discussion

There are very important interrelationships between breast cancer, osteoporosis, and cardiovascular disease among women. The levels and metabolism of sex-steroid hormones are related to all three of these diseases. We, and others, have shown (as noted) that blood estrogen levels are associated, among postmenopausal women, with a substantial increase in breast cancer and decreased risk of osteoporosis.

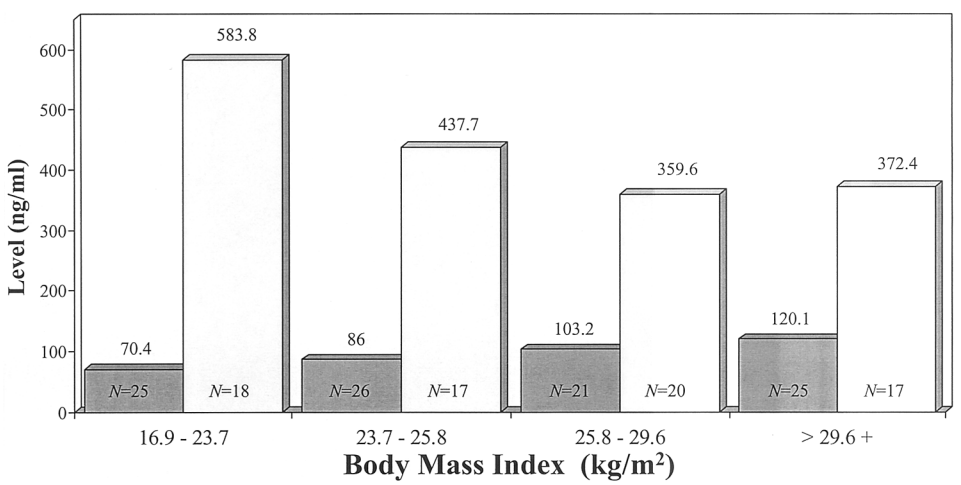

Fig. 7. Level of 2 hydroxy estrone: 8th post type of hormone.

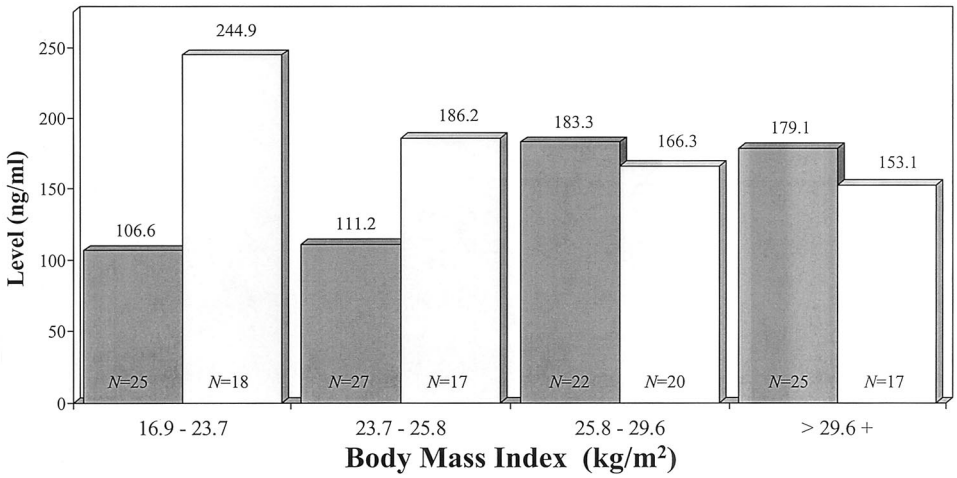

Fig. 8. Level of 16 hydroxy estrone: 8th post type of hormone.

Surprisingly, no studies have been able to consistently show a relationship between blood estrogen levels and risk of coronary heart disease. There is no consistent relationship between the extent of subclinical cardiovascular disease such as increased coronary calcium, angiography evidence of coronary artery disease, or carotid intimal medial wall thickness and blood estrogen levels.

Hormone replacement therapy has been shown to decrease the risk of osteoporotic fractures. Observational studies suggest that long-term use of hormone replacement therapy may increase the risk of breast cancer [62] and probably decrease the risk of coronary heart disease, at least in a subset of women [63,64]. Unfortunately, there is no clinical trial evidence to support the benefit of estrogen or estrogen/progesterone therapy to reduce the risk of coronary heart disease nor increased risk of breast cancer.

Higher bone mineral density is a probable marker of long-term greater estrogen levels or metabolism. It is a powerful predictor of the risks of breast cancer among postmenopausal women. Higher bone mineral density is related to reduced risk of osteoporotic fracture and apparently not associated with coronary heart disease. The traditional risk factor models (Gail model) [65] is not very good for predicting breast cancer among older women (i.e. 60–65+ years of age). Family history of breast cancer and history of 'benign breast disease' are still predictors of breast cancer among older women. Higher bone mineral density is a much more powerful predictor of breast cancer.

Hormone levels (both genetic and environmental lifestyles) are very important in determining the risks of these diseases. Obesity or body fatness is a major (if not the most important) determinant of postmenopausal estrogen levels, decreased sex-hormone binding globulin, and increasing free testosterone and estradiol [66]. Greater degrees of obesity, as noted, are a strong risk factor for breast cancer among postmenopausal women and, especially, weight gain from pre- to postmenopause. Risk of osteoporotic fracture is also substantially reduced among overweight or obese women [67]. Obesity is associated, however, with higher blood pressure, decreased HDLC, and higher triglyceride levels: all important risk factors for cardiovascular disease — both coronary heart disease and stroke [68]. However, there are very weak associations between obesity and cardiovascular disease, especially myocardial infarction, among older postmenopausal women [69]. The higher estrogen levels among older obese women may, in part, be protective for risk of cardiovascular disease.

There are no long-term clinical trials of the effects of weight loss on cardiovascular disease or breast cancer. Observational studies, however, have noted that weight loss is associated with decrease in bone mineral density and increased risks of osteoporotic fracture among postmenopausal women [70].

Intra-abdominal fat or more central obesity, as measured by increased waist circumference, remains an important risk factor for cardiovascular disease and, probably, breast cancer among older women. Women with higher intra-abdominal fat have greater insulin resistance, risk of diabetes, higher triglyceride levels, and elevated cytokines (such as TNFα, IL-6, and acute inflammatory markers such as c-reactive proteins). The higher insulin and cytokine levels may also stimulate aromatase activity, increasing estrogen levels and decreasing sex hormone binding globulin.

The higher triglyceride levels and, especially, large VLDL particles, small dense LDLC and number of LDLC particles among women with increased body fat or intra-abdominal fat on HRT, as well as the increase in inflammatory markers such as CRP, may reduce the benefits of HRT. This may especially be true in women

on combination estrogen and progesterone in which the progesterone blunts or reduces the effects of oral estrogens on raising HDLC. Thus, it would be important to determine whether prevention of the lipid metabolic changes associated with weight gain, central obesity, and intra-abdominal fat will enhance the benefits of hormone replacement therapy on reduction of risks of cardiovascular disease.

Women on HRT, as noted, will have much higher blood estrogen levels and estrogen metabolites than women not on HRT. They should have a much higher risk of breast cancer. The risks associated with endogenous estrogen levels and breast cancer are higher than those related to the use of HRT. The interaction between body weight and hormone therapy such that heavier women on HRT may have lower hormone metabolites than thinner women on such therapy may, in part, explain this observation. Many women on hormone replacement therapy, at least in the United States, are overweight or obese and their relative risks compared with women not on hormone therapy for breast cancer, may be blunted. We would hypothesize that the highest relative risks of breast cancer among women on HRT as compared to those not on HRT, will be found among thinner older women on long-term therapy.

Increased leisure time physical activity is associated with a possible decreased risk of both cardiovascular disease and breast cancer in postmenopausal women [30]. There is, however, no evidence that increased leisure time physical activity modifies hormone levels, the amount of subclinical vascular disease, or has any major effect on overall bone mineral density.

Alcohol intake is associated with an increased risk of breast cancer, even among older women, but lower rates of cardiovascular disease. Increased alcohol intake has been associated in some, but not all, studies with higher estrogen levels, even among postmenopausal women [71].

The higher intake of specific saturated fat and cholesterol in the diet raises the levels of LDLC. The higher LDLC are a primary determinant of the extent of subclinical atherosclerosis among postmenopausal women and risks of coronary heart disease [72]. There is little evidence, however, that the amount of saturated fat or cholesterol in the diet is related to blood estrogens or estradiol levels.

International comparisons have suggested that greater amounts of total fat in the diet increase risk of postmenopausal breast cancer. Decreased total dietary fat may reduce blood estrogen levels [73]. The Women's Health Initiative is currently testing the hypothesis that decreased fat intake will reduce the risk of breast cancer. The effects of other nutrients on risk of breast cancer, osteoporosis, and cardiovascular disease such as phytoestrogens and soy proteins, anti-oxidants, flavinoids, etc. is currently being evaluated. There is, however, no clinical trial evidence at the present time that any of these nutrients has a consistent effect on hormone metabolism, breast cancer, osteoporosis, and bone density or cardiovascular disease.

There are weak familial aggregations of coronary heart disease, osteoporosis, and breast cancer among older postmenopausal women. Familial hypercholesterolemia due to genetic polymorphisms of LDLC receptors are associated with substantial increased risk of coronary heart disease among women. The risk is not as great as for men. Genetic polymorphism of HDL and triglyceride metabolites likely also contribute to risk of coronary heart disease among men and women. Estrogens have important effects on hepatic triglyceride lipase and bile acid metabolism that may be related to risk of cardiovascular disease.

An increase in bone reabsorptiometry cytokines, IL-1, TNFα, IL-6 postmenopausal due to estrogen deficiency may be a major contributor to postmenopausal bone loss. Genetic polymorphism of the cytokines and/or receptors and, especially, interaction with estrogen, may account, in part, for variations in rate of bone loss.

The genetic variations in the polymorphism associated with production and metabolism of estrogens could have an important role in the risk of coronary

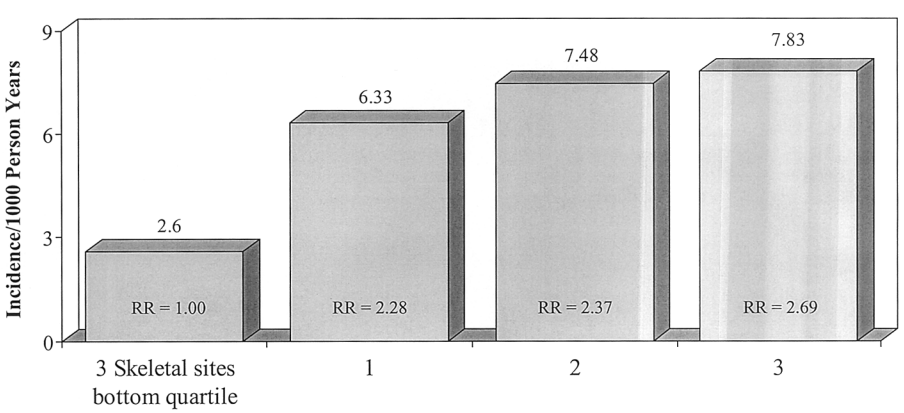

Fig. 9. Incidence rate and multivariate–adjusted relative risk* of breast cancer by number of skeletal sites with low or high BMD.

heart disease, breast cancer, and osteoporosis. There is no consistent evidence to date that any of the measured genetic polymorphism of estradiol production are related to risk of breast cancer or to blood estrogen levels. Few studies have evaluated these polymorphism in relation to coronary heart disease or osteoporosis.

The genetic polymorphism of the metabolites of estrogen, such as the 2,4,16-hydroxy estrone, estradiol has been associated with risk of breast cancer in animal experimental models and in a few case-control and longitudinal studies. The results of these studies to date have not been consistent for either benefit or lack of benefit [1,2].

Genetic polymorphism of the catechol-*O*-methyl transferase catalyze the *O*-methylation of estrogen catechols, 2,4-hydroxy estrone. The less active allele has been associated with increased risk of breast cancer in several studies [74,75].

Polymorphisms of the estrogen receptors, especially the α-receptor and the androgen receptors, have been associated with increased risk of prostate cancer in men [76] and with higher bone mineral density [77,78]. Little research has estimated the polymorphisms of estrogen levels, estrogen metabolites [79,80], or receptors and either osteoporosis or clinical or subclinical cardiovascular disease. Future studies will need to link the environmental determinants of hormone levels and metabolism, genetic polymorphisms and specific outcomes such as breast cancer, osteoporosis, atherosclerosis, and cardiovascular disease.

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