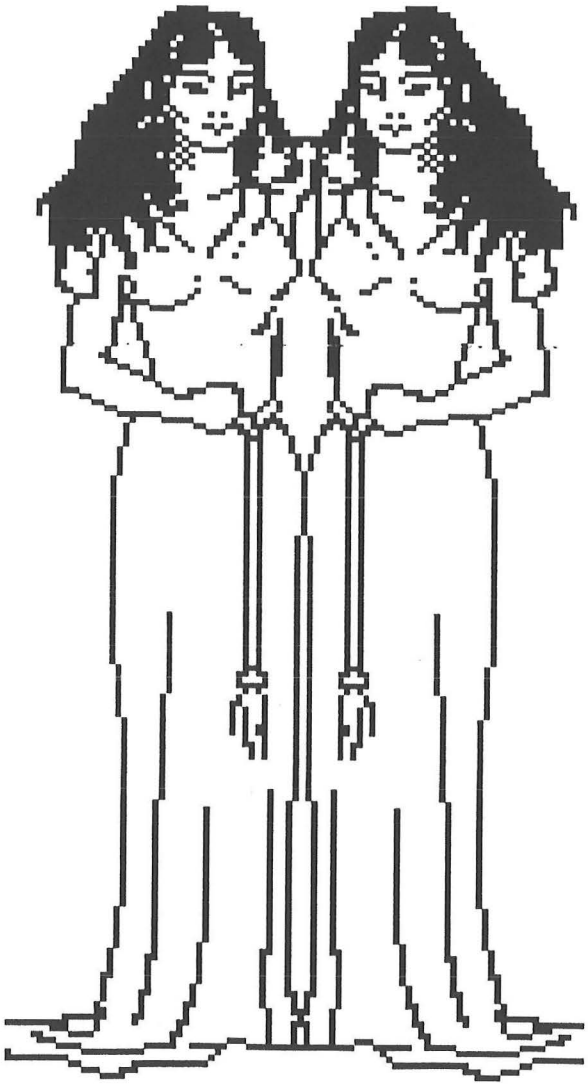
Estrogen Susceptibility: Are All Women Created Equal?



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INTRODUCTION

Loss of Ovarian Function. Menopause is defined as the permanent cessation of menstruation due to loss of ovarian function. The average age at which most women experience their final menses is about 51 years (1). Although the final menses retrospectively occurs at a discrete point in time, the transition to post-menopause can occur over months to years and is characterized by irregular menstrual cycles and the beginning of vasomotor symptoms. As a consequence of menopause, there is a marked reduction of estradiol, the principle product of the ovarian follicle from the 70-100 pg/dL in the reproductive years to levels below 20-pg/dl (2). The primary circulating estrogen after menopause is estrone, which is produced both in the ovary and by peripheral conversion of androstenedione primarily in adipose tissue. Testosterone, which is also produced by the ovary, declines to a more variable degree after menopause.

As women transition from the perimenopause into post-menopause, they may experience a number of symptoms including hot flashes, vaginal dryness, urinary frequency and incontinence, decreased sexual desire and changes in mood. It can be difficult to assess the frequency of these symptoms. A number of studies reveal the low number of women who seek care for one or more menopause symptoms and suggest therefore those who do not seek care are therefore asymptomatic or minimally symptomatic (3). This may not be the case. For example, in one study of 436 women ages 35 to 48 years, 41% reported hot flashes but only 5% of the women discussed menopause management with their physicians (4).

Hot flashes are the hallmark of perimenopause and menopause. Hot flashes are characterized by a sensation of warmth with flushing of the face, neck and upper body and perspiration followed by a chill. There is a large cross-cultural variability in the prevalence of hot flashes with reports ranging from 0 to 90% (5). Table I lists reported prevalence of hot flashes in studies from westernized countries (4, 6-9). In these 5122 women, 40% experienced hot flashes. Thus despite the fact that all women experience ovarian failure, not all will experience similar symptoms of estrogen withdrawal.

Table 1. Frequency of hot flashes.

Country	No. Women	%H.F
USA	436	41
USA	1178	49
Sweden	1474	43
Australia	1606	30
Netherlands	428	39

H.F.= hot flashes, From (4,6-9 respectively).

THE WORKING HYPOTHESIS

Why would the frequency of hot flashes vary? Why do some women sail through menopause with seemingly few symptoms while others are quite symptomatic? One possibility is that those women who are more estrogen "dependent," or susceptible to the effects of endogenous estrogen are more symptomatic from the withdrawal of estrogen

whereas those women who are less estrogen less susceptible, are less symptomatic following the withdrawal of estrogen. Table II lists a number of diseases and clinical conditions that are associated either positively or negatively with estrogen exposure. If in fact, this concept of global susceptibility to estrogen is true, we might expect two population extremes: an estrogen sensitive or susceptible group with a greater incidence of breast and endometrial cancer, fibroids and hot flashes while an estrogen resistant group with a higher incidence of osteoporosis, coronary heart disease and Alzheimer's with a relatively asymptomatic menopause (Figure 1).

Table II. Conditions positively and negatively associated with estrogen exposure.

Condition	E +/–
Breast Cancer	+
Endometrial Cancer	+
Osteoporosis	–
Coronary Heart Disease	–
Fibroids	+
Alzheimer's	–
Vasomotor Symptoms	+
Periodontal Disease	+
Skin Atrophy	–

It would be enormously helpful to be able to identify these population extremes so we can more effectively target prevention and disease screening in women and we alleviate the risk of potential morbidity of therapy that is of little value in a low risk population. For example, in an estrogen susceptive woman, breast cancer screening might start at 30 instead of 40. In estrogen resistant women, therapy aimed at raising HDL or lowering LDL would begin early even if other cardiac risk factors were not manifest.

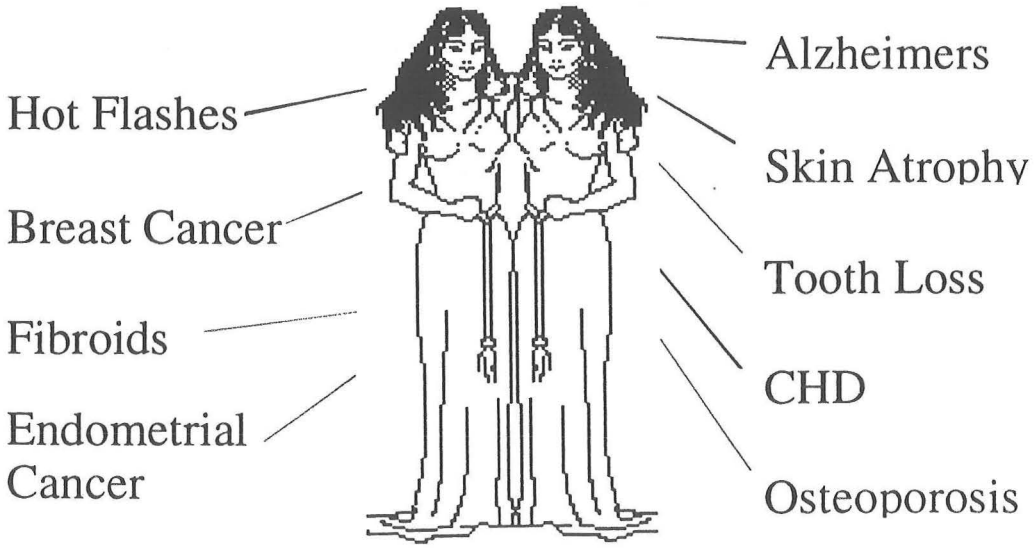

Figure 1. Clinical conditions affecting two hypothetical population extremes of estrogen susceptible and estrogen insensitive women

BONE MINERAL DENSITY

Osteoporosis. Osteoporosis has been defined as "a systemic skeletal disease characterized by a low bone mass and microarchitectural deterioration with a consequent increase in bone fragility and susceptibility to fracture" (10). A working group of the World Health Organization has operationally defined osteoporosis as a bone mineral density (T score) that is 2.5 standard deviations below the mean peak value in adults). Bone mineral density can also be compared with a mean value in normal subjects of the same age and sex (z score). A number of risk factors for osteoporosis have been identified including a familial history of osteoporosis, cigarette smoking, excessive alcohol use, physical inactivity, thin habitus, low dietary intake of calcium, and a number of endocrinological diseases. Bone is also highly sensitive to circulating estrogen levels. Bone mineral density is positively correlated with menarche, length of reproductive life and parity and negatively correlated with oophorectomy, prolonged amenorrhea and natural menopause.

Given the fact that estrogens are an important determination of bone mineral density, bone mass density may then serve as a marker of cumulative exposure to estrogen. Bone mineral density might then positively or negatively correlate with the risk of the estrogen associated diseases listed above. We will discuss studies which examine the association of bone mineral density or fracture with single conditions and then look at the few studies that examine the relationships among three or more estrogen associated diseases.

Bone Mineral Density and Breast Cancer Risk. Two recent studies, using participants of the Study of Osteoporotic Fractures (11) or women from the Framingham Study database (12), have examined the association between bone mineral density and the risk of breast cancer among over 8,000, primarily Caucasian, postmenopausal women.

Cauley et al. evaluated a total of 6,854 women (no African Americans) who were 65 years of age or older and enrolled in the Study of Osteoporotic Fractures (11). Women had radius and calcaneus bone mineral density examined at baseline and approximately two years later and the risk of developing breast cancer over a follow-up of 3.2 years was then assessed. A total of 97 of these women developed breast cancer.

Table III. Relative risk and 95% CI of breast cancer by bone mineral density (BMD).

BMD Site	Age- adjusted	Multivariate-adjusted*
Proximal radius	1.34 (1.09-1.62)	1.30 (1.02-1.67)
Distal radius	1.37 (1.11-1.69)	1.50 (1.16-1.95
Calcaneus	1.20 (0.97-1.49)	1.15 (0.87-1.52)
Total hip	1.48 (1.17-1.88)	1.39 (1.01-1.90)
Total spine	1.37 (1.09-1.72)	1.28 (0.95-1.71)

*adjusted for age, education, modified BMI, walking for exercise, alcohol consumption, smoking, parity, age at first birth, age at menopause, family history of breast cancer and history of benign breast disease. From (11).

Table III lists the age adjusted and multi-variate relative risk of developing breast cancer by bone mineral density in terms of one standard deviation increase in bone mineral density at the proximal radius, distal radius, calcaneus, total hip and total spine. As indicated in the table, increased bone mineral density of the radius, hip or spine is significantly associated with the increased risk of breast cancer. The magnitude of the relative risk was more than twofold greater among women with the highest bone mineral density. These results are consistent with the hypothesis that increasing bone mineral density is associated with increasing risk of breast cancer

Zhang et al evaluated incident cases of breast cancer in 1373 women who underwent posterior anterior hand radiography to measure cortical width of the woman's second metacarpal in the Framingham Study. These women were enrolled between 1967 and 1970 and followed until the end of 1993 (12). Table IV shows the relation of each specific quartile of relative metacarpal cortical area to the risk of breast cancer. These data show that women in the highest age specific quartile of cortical bone mass had the greatest increase of risk of breast cancer. In this study, the magnitude of the relative risk was more than three fold greater among women with the highest bone mineral density.

Table IV. Relation of each specific quartile of relative metacarpal cortical area to the risk of breast cancer.

Quartile*	No. Cases	Incid. Rate#	Rate Ratio** (95% CI)
1	12	2.02	1.0
2	17	2.63	1.3 (0.6-2.8)
3	18	2.69	1.3 (0.6-32.7)
4	44	7.03	3.5 (1.8-6.8)

*numbered lowest (1) to highest (4); # cases/1000 person-years; **adjusted for height, body mass index, education, parity, age at first pregnancy, age at menopause, number of cigarettes smoked, alcohol consumption, physical activity and the use or nonuse of HRT. From (12).

Bone Mineral Density and Coronary Heart Disease Risk. Surprisingly, given the large databases of women in the Nurses Health Study and the Framingham Study, there is little data describing bone mineral density and coronary heart disease risk. Barengolts evaluated bone mineral density and coronary atherosclerosis measured by electron beam CT in 45 asymptomatic postmenopausal women with both normal and low bone mineral density (13). These women were categorized according to their levels of bone mineral density, either being normal (control), osteopenic or osteoporotic. Table V shows the characteristics of the study participants. There were no significant differences among the groups in terms of age, year since menopause, weight, height, body mass index, calcium intake, averages of hypertension, prevalence of risk factors for osteoporosis or coronary heart disease including cholesterol, family history of fractures or premature coronary heart disease or cigarette smoking. There was a negative correlation between total coronary calcium scoring and bone mineral density of the hip ($R = -0.34$, $p = 0.22$ and a trend toward a negative correlation between total coronary calcium and bone mineral

density of the spine (R = -0.28, p = 0.56). This is clearly a small study; however, it does support the hypothesis that higher bone mineral density, as a marker of lifelong estrogen exposure is associated with a lower risk of coronary heart disease.

Table V. Characteristics of the study participants.

Characteristic	Control	Osteopenia	Osteoporosis
No.	11	20	14
Age, years	65.3 ± 5.2	67.9 ± 7.3	64.8 ± 5.8
Cal intake, mg/day	673 ± 228	704 ± 206	690 ± 250
BMI, kg/m2	28.2 ± 3.4	28.7 ± 3.5	28.3 ± 3.2
Chol, mg/dL	200.6 ± 32.4	251.1 ± 62.4	235.7 ± 54.3
BMD,L1-4, g/cm2	0.96 ± 0.11	0.83 ± 0.03	0.73 ± 0.05
Cor Ca Score	41.9 ± 83.1	115.1± 181.9	221.7 ± 355.4

From (13).

Bone Mineral Density and Alzheimer's Disease Risk. It is beyond the scope of these Grand Rounds to fully evaluate the relationship between estrogen, neuroprotection, cognition, and the risk of Alzheimer's disease. However, there are good biological reasons to expect that estrogen could effect cognition and the development of Alzheimer's. Estrogen receptors are expressed in brain tissue and increasing evidence supports the role of estrogen as a neuroprotectant that can act independently or dependently on estrogen receptor activation (14). The epidemiological evidence for an association between estrogen and cognition function among postmenopausal women has yielded equivocal findings (15). A recent meta-analysis of 10 of these investigations reported a 29% decreased risk of developing dementia with estrogen use (16). Several positive relationships come from randomized clinical trials evaluating recent verbal memory and tasks incorporating concept formation and reasoning.

Again, testing our model here today, we might predict that patients with Alzheimer's might have an increased risk of osteoporosis compared to patients without Alzheimer's disease. These kind of studies are inherently difficult because elderly Alzheimer's victims are likely at greater risk for decreased physical activity, nutritional deficiencies, low body mass and sunlight deprivation. Fato et al. evaluated bone mass and vitamin D insufficiency in elderly women with Alzheimer's disease (17). Forty-six Alzheimer's patients of an average age of 81 were compared to 140 controls. While the Alzheimer's patients had significantly reduced bone mineral, they also had significantly reduced body mass index, Vitamin D and calcium intake, and sunlight exposure. No effort was made in this study to do a multivariate analysis to try to take these confounding factors into account to establish the independent effect of having Alzheimer's disease itself. The more relevant study would be to prospectively follow women with normal and low bone mineral density and determine the relative incidence rates of developing Alzheimers.

Bone Mineral Density and Uterine Leiomyoma. Leiomyomas are fibroids or benign tumors of the uterine body that affect about 25% of women. Because leiomyomas appear during the reproductive years and regress after menopause, their growth is though to be

related reproductive hormone exposure. For example, the risk of uterine leiomyoma among premenopausal women is increased with increasing body mass index and is decreased in the presence of cigarette smoking (18). Roszenberg et al. evaluated 59 women suffering fibroids and measured bone mineral content in both spine and mid-radius (19). They found that bone mineral density was significantly higher at the lumbar site in women with fibroids than in the reference population. These differences were not observed for bone mineral density at the mid radius, however, they attributed this to the fact that trabecular bone is more sensitive to estrogen than cortical bone.

Bone Mineral Density and Tooth Loss. Two large prospective studies have found that the risk of tooth loss was significantly lower in women who used hormone replacement therapy than in those who did not (20, 21). Bone health is likely an important contributor to the tooth stability in the jaw. Payne et al. evaluated longitudinal alveolar bone loss in women who are osteoporotic or osteopenic (22). They found that the osteoporotic and osteopenic women exhibited a higher frequency of alveolar bone loss, crestal density loss and subcrestal density loss compared to women with normal bone mineral densities.

CORONARY HEART DISEASE

The Impact of Gender on Coronary Heart Disease Mortality. In general, women have their myocardial infarctions 10 years later than men do and it is thought that this delay is due to the protective effects of endogenous estrogen. The concept that pre-menopausal women may be more or less protected by these endogenous reproductive hormones came to mind approximately a year ago when a study was published by Vaccarino et al. in The New England Journal of Medicine entitled "Sex-based differences in early mortality after myocardial infarction" (14). The authors analyzed data on 384,878 patients, (approximately 156,000 women and 209,000 men, ages 30-89) who were enrolled in the National Registry of Myocardial Infarction between June of 94 and January of 98.

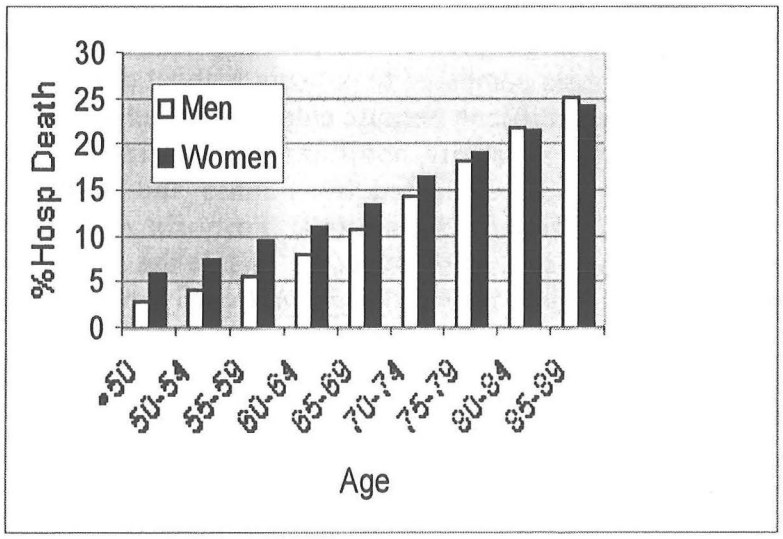

Figure 2. Rates of death during hospitalization for MI (14).

Figure 2 shows the rates of in-hospital death from MI among women and men according to age. In the group of patients who were less than 50 years of age, the mortality rate during hos-pitalization for MI was more than twice as high among women. This difference de-creased with increasing ageand was no longer significant at age 74.

Younger women were more likely than younger men to have diabetes, a history of congestive heart failure and a history of stroke were. At all ages, men were more likely to have had a history of MI, coronary bypass, grafting or angioplasty and be smokers. At younger ages, women were also more likely than men to be given a diagnosis other than myocardial infarction or unstable angina at admission were. Whereas the diagnosis at admission were similar among older men and women.

Figure 3 shows the unadjusted and adjusted odds ratios for death during hospitalization following myocardial infarction in women as compared to men according to age. The unadjusted odds ratio shown in Panel A were derived from a model that included sex, age and the year of discharge. The adjusted odds ratio shown in Panel B was derived from a model that included race and insurance status, medical history, severity of clinical abnormalities on admission, type of regiment in the first 24 hours after admission and time of presentation.

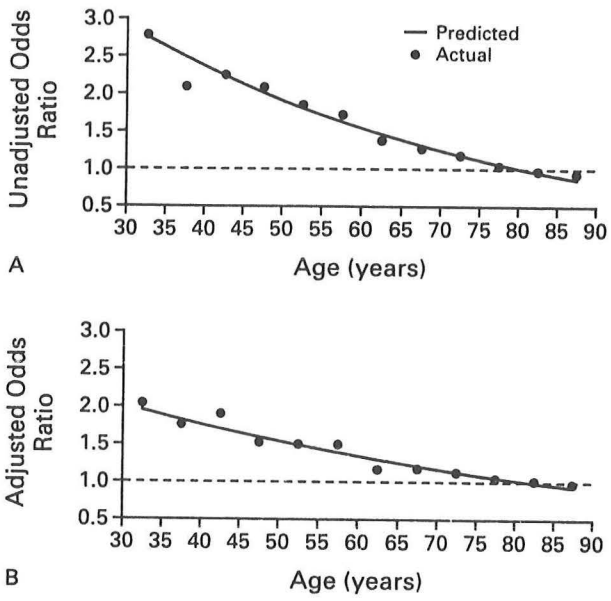

Figure 3. Odds ratio for death during hospitalization for MI in women compared with men, according to age (see text). From (14).

There are a number of reasons why younger women may have been more likely to die in the initial hospitalization. For example, although women, in general, present at an older age with their first myocardial infarction compared to men, the presence of diabetes in women abolishes this delay. In this study, younger women were more likely to have diabetes than their male counterparts. However, adjustments for diabetes and other co-existing conditions account for only about 10% difference in the risk and there is no significant interaction between sex and diabetes (14). Another possibility is that these young women had lower application of life saving treatments for myocardial infarction such as beta-blockers, thrombolytic therapy and aspirin. Again, after adjustment, treatment differences accounted for only about 10% of the effect of the interaction between sex and age in this study. Another possibility is simply that young men and women are just as likely to die from myocardial infarction however, the men die before they reach the hospital and the women die after they reach the hospital. Several registries addressing this issue in 29 populations and 18 countries fine considerable variation in the rates of death before hospitalization.

The authors hypothesized that younger but not older women had higher mortality rates during hospitalization than their male peers perhaps because these younger women who

had myocardial infarction may represent a distinct group in terms of risk factors and pathophysiology. Perhaps these younger women represent that population of women who are less estrogen susceptible and thus the lifelong loss of the presumably cardioprotective genomic and non-genomic effects of estrogen puts them at increased risk.

Cardiovascular Risk in Women with Alzheimer's Disease. Few studies have evaluated the prevalence of cardiac disease in patients with dementia or common psychiatric disorders. Tresch et al. evaluated the prevalence and significance of cardiovascular disease in hypertension in elderly patients with dementia of the Alzheimer's type, multi-infarct dementia and major depression (23). The patient population was comprised of 117 women and 66 men with a mean age of 73 years. Figure 4 shows the percent prevalence of cardiovascular disease in a number of diagnoses in patients with Alzheimer's, multi-infarct dementia and major depression. As shown, they found a very low prevalence of clinical cardiac disease in their Alzheimer's patients. Compared to epidemiological studies in various populations, most studies show that between 40 and 50 % of elderly patients will demonstrate some form of heart disease. Clearly the prevalence of heart disease in this group was much, much lower.

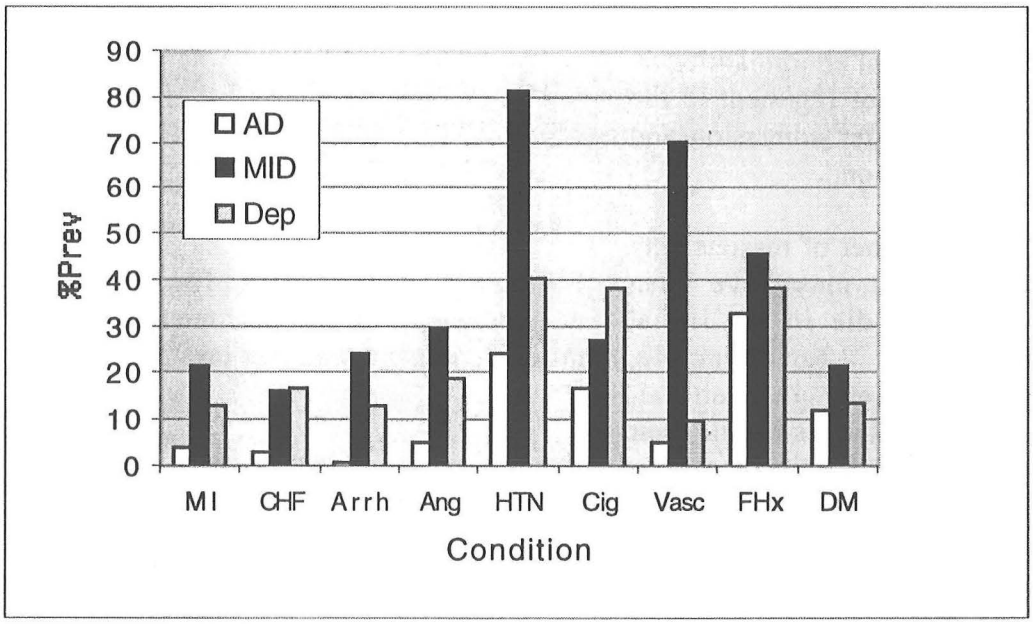

Figure 4. Percent prevalennce of cardiovascular conditions in patients with Alzheimer's disease (AD), multi-infarct dementia (MID) and Depressions (Dep). From (23).

PUTTING IT ALL TOGETHER

The Associations between Osteoporosis, Cancer and Cardiovascular Morbidity and Mortality. Almost a decade ago, Browner et al. evaluated non-traumatic mortality in elderly women with low bone mineral density in The Study of Osteoporotic Fractures

Research Group; in this study, 9704 ambulatory women (>99% Caucasian) aged 65 years and older were prospectively studied to determine whether low bone mineral density was associated with particular categories of non-trauma mortality (24). These women completed a questionnaire and bone mineral density was measured at distal radius, proximal radius and calcaneus by single photon absorptionometry. During 2.8 years of follow-up, 299 women died. One hundred and twenty deaths were due to cardiovascular disease (40%); 105 deaths were due to cancer (35%) and 10 deaths due to trauma (3%). Table VII shows the age-adjusted association between proximal radius bone mineral density and disease specific mortality. There was no statistically significant association between bone mineral density and disease specific mortality from cancers of the breast, endometrium or ovary or coronary heart disease. However, the association between low bone mineral density at the proximal radius and stroke mortality remained significant (RR= 1.91, 95%, CI 1.25-2.92, $p < 0.005$) after adjustment for age, previous stroke, hypertension, diabetes, cigarette smoking and use of hormone replacement therapy.

Table VII. Age-adjusted association between proximal radius bone mineral density and disease specific mortality.

Cause of Death (no.)	RR*	95% CI
All Cardiovascular Disease (120)	1.22	1.01-1.47
Coronary Heart Disease (70)	1.17	0.92-1.51
Stroke (25)	1.75	1.15-2.65
All Cancers (105)	1.04	0.85-1.27
Cancer of breast, uterus, ovary (26)	1.05	0.70-1.58

*Relative hazard per SD in bone mineral density. From (24).

Approximately one-third of these women were 65-69, one-third were 70-74 and the remaining one-third were 75 or greater years of age (with a mean age of 71.7 ± 5.3 years). It may be that the women who are the least estrogen susceptible (with the lowest bone mineral density and greatest risk of coronary heart disease) are already dead from coronary heart disease by the age of 65. This may also have been a particularly healthy group for a number of reasons: only 17% would characterize their overall health status as fair/poor/very poor, only 7% had diabetes and only 38% had hypertension.

Olson and Hagglund examined the morbidity and mortality in a prospective cohort of 677 women with distal forearm fracture diagnosed in 1974 and 1975 in Lund, Sweden (25). This group of women was younger than those described by Browner et al. (24). The median age of this group was 62 years with 19% of the women under the age of 50, 56% age 50-70 and 25% over the age of 75. They evaluated forearm fractures because they generally affect women earlier than hip fractures. From an earlier study conducted using absorptionometry in women in this health region with distal forearm fracture, they concluded that this group of women had reduced bone mineral content compared to healthy controls (26). The status of these women during the15 years of follow-up after this diagnosis was determined using census and death registries. Twenty two percent of the women had died. The incidence of various cancer diagnoses and causes of death in this group was compared to the expected incidence and standardized morbidity and

mortality ratios (SMR) were calculated. Table VIII lists morbidity and mortality in this prospective cohort of women who had distal forearm fractures.

Table VIII. Morbidity and mortality in a prospective cohort of 677 women who had distal forearm fractures in 1974-1975.

Event	Obs.	Expt.	SMR	95% CI	p
All Cancer Dx	66	90.21	0.73	0.57-0.94	0.01
Breast Cancer Dx	11	20.31	0.54	0.27-0.97	0.03
Genital Organ Cancer Dx	5	11.84	0.42	0.14-0.99	0.04
Cerebrovascular Mortality	14	25.7	0.54	0.30-0.91	0.02
Coronary/Vascular Mortality	65	85.8	0.76	0.59-0.97	0.02
Total Mortality	146	191.7	0.76	0.64-0.90	0.001

Adapted from (26).

Thus, the risk of breast or uterine cancer was reduced in these women with lower bone mineral density as our hypothesis might predict. However, the risk of dying of cardiovascular or cerebrovascular disease was also reduced which is contrary to our prediction. The expected number of cases was determined by examining a population without fractures that was matched only by age in 5-year increments. The impact of factors that could confound the relationship between osteoporosis and coronary heart disease risk such as low body weight, smoking or treatment with estrogen replacement therapy were not taken into consideration in this study.

IS THIS CLINICALLY MEANINGFUL?

In summary then, these data suggest that some women may be more or less at risk for estrogen-associated diseases or conditions. Given the fact that many of these clinical diseases have important risk factors that are independent of estrogen such as cigarette smoking or diabetes, is the global effect of estrogen susceptibility powerful enough to be clinically meaningful? Would the identification of this kind of susceptibility have a significant impact on the way we practice medicine?

The ethnic paradox. In her Internal Medicine Grand Rounds in December 1999, Dr. Helen Hobbs discussed ethnic differences in the presentation, progression and prognosis of coronary artery disease (27). She described the ethnic paradox in which Blacks have a higher prevalence of cardiac risk factors such as hypertension, diabetes and obesity than Whites but not a proportionally higher rate of CAD. Table IX lists a comparison of prevalence of risk factor in Blacks and Whites form NHANES II and III (27, 28). The difference in prevalence between women is much more striking than in men.

Table IX. Comparison of Prevalence of Age-Adjusted Risk Factor Profiles in Men and Women Ages 20-74.

Risk Factor	% W M	%B M	%W W	%B W
Hypertension	43.1	48.5	31	45
Obesity	24.9	27.5	25	46.1
Diabetes	2.9	4.4	3.1	5.5

W=White, M= Men, B=Black, W=Women. From (27, 28).

Gillum et al. assessed associations of risk factors with coronary heart disease in Black and White participants who were enrolled in NHANES I between 1971 and 1975 and were followed for 19 years (29). Table X shows the features of Black and White women who developed incident cases of coronary heart disease during follow-up. A similar percentage of Black and White women developed coronary heart disease during the follow-up (17% in each group) despite the higher percentage of hypertension and current smoking among the Black women.

Table X. Characteristics of Black and White women who developed coronary heart disease during follow-up.

Risk Factor	BW	WW
Total No. Women	1041	5744
Serum Chol., mg/dL	218.3	220.8
Current smoking, %	38.2	31.3
History of hypertension, %	37.1	22.6

W=White, M= Men, B=Black, W=Women. Adapted from (29).

These findings may suggest that the Black women may be less vulnerable to standard risk factors because of some underlying protective mechanism. Could increased estrogen susceptibility in Black women account for this ethnic paradox? If in fact, Black women are more estrogen susceptible than White women, one might anticipate that Black women would be more likely than White women to develop breast cancer, endometrial cancer, fibroids and hot flashes but would be less likely than White women to develop osteoporosis and Alzheimer's.

Vasomotor symptoms during menopause in Black and White Women. One might infer that menopausal symptoms are lower in Black women than White women because the use of hormone replacement therapy is lower in this population (30, 31). However, the use rates may be lower because fewer physicians discuss hormone replacement therapy with Black women. In one study of primarily indigent inner city Black women, only 22% of the 276 women not receiving hormone replacement therapy recalled that their physician had discussed hormone replacement therapy with them (although they had good recall of other issues discussed during these visits) (14). Grisso et al. found that in a larger sample of 436 perimenopausal women, half of whom were Black and half of whom were White, 53% or the Black women had hot flashes compared with 29% of White women (4). These findings were not confirmed by Pham et al. who evaluated a much smaller group of 35 White women and 33 Black women and found that hot flashes

occurred with equal frequency (46% and 45% respectively) (32). Neither of these studies evaluated the interaction of obesity with vasomotor symptoms although it may be an important confounder because obesity is higher in Black women compared to White women and obesity may affect estrone levels.

Breast cancer risk in Black and White women. The age-adjusted incidence of breast cancer from 1993-1997 taken from the Surveillance, Epidemiology and End Result (SEER) Cancer Statistics from the National Cancer Institute was 102.8/100,000 in Black women and 115.3/100,000 in White women (33). These results are contrary to the hypothesis that Black women might be at higher risk of breast cancer due to greater estrogen susceptibility. Obviously, in order to be diagnosed with breast cancer, a woman needs to have access to and utilize screening opportunities such as mammograms. This incidence of breast cancer might be artificially lowered if Black women utilized mammography less than White women did. Even if this were the case, breast cancer might not necessarily be left undiagnosed, but simply present at a higher stage. Fifty-six and four tenths percent of White women in this SEER data set were diagnosed with in situ or Stage I breast cancer compared to 46.5% of Black women which may be a consequence of lower mammogram use by Black women or perhaps more aggressive tumor types (see below).

A common feature between African-American and West African women who present with breast cancer is the early age and manifestation of the disease. Both African groups have their highest peak of incidence before menopause (34).

There is an interesting pattern of breast cancer risk by age in Black and White women shown in Figure 5. In young women, Black women are at greater risk for breast cancer whereas after the age of 40-44, White women are at great risk.

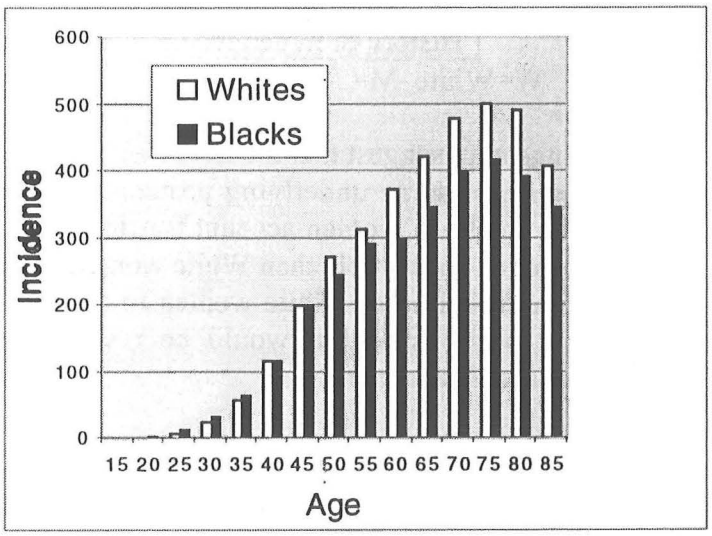

Figure 5. Diagnosis of breast cancer in Black and White women stratified by age. From (33).

Survival after breast cancer diagnoses is worse among Black women (35, 36). Survival data from the SEER program suggests that race is an independent predictor of survival from breast carcinoma, suggesting that there may be important racial differences in molecular biology of breast carcinoma (36). There may be specific pathologic breast tumor characteristics that could also account for Black/White differences and outcome (37). Estrogen receptors and progesterone receptors are important both as independent

indicators of prognosis (with estrogen positive tumors exhibiting less aggressive growth) and also as a basis for selecting treatment. A number of studies have found differences in estrogen receptor positive tumors between Black and White patients. Analysis of the NCIDWDSS found that Blacks were more likely to have tumors that lacked hormone receptors, had high-grade nuclear atypia and poorly differentiated tumors compared with Whites, all of which are tumor characteristics associated with a poor prognosis (38).

Endometrial cancer risk in Black and White women. From 1973 to 1988, the average age-adjusted incidence of endometrial cancer was 13.1/100,000 in Black women and 21.3/1000,000 in White women (39). From 1993 to 1997, the age-adjusted incidence of endometrial cancer was 14.5/100,000 in Black women and 22.3/1000,000 in White women (39). Clearly this data contradicts my working hypothesis.

Prevalence of uterine leiomyoma in Black and White women. Uterine leiomyomas appear during the reproductive years and regress during menopause suggesting an ovarian hormone growth potential. The prevalence of uterine leiomyomas increases with increasing age. A number of studies have suggested that Black women have an increased rate of leiomyoma with self-reported diagnosis (39) and they also have an increase rate of leiomyomas in those Black and White women having a hysterectomy (40). Kjerulss et al. evaluated 409 Black women and 836 White women who underwent hysterectomy under non-cancerous conditions (40). Eighty-nine percent of the Black women and 59% of the White women had leiomyomas. The Black women were diagnosed on average of 37.5 years compared to White women at 41.6 years. Black women were also more likely to have larger leiomyomas and greater numbers of leiomyomas per uterus (40).

Osteoporosis in Black and White Women. There are important racial differences in bone mineral density and osteoporosis. For the U. S. the overall rate of osteoporosis in non-Hispanic White women age 50 years and older is 21% compared to just 10% in African-American women of the same age (41). The incident rate for hip fracture and all fractures are also consistently lower in Black women than in White woman (42). A number of features may protect Black woman from osteoporosis including obesity, larger muscle mass and higher concentrations of endogenous sex hormones, particularly estrogen (42). Luckey et al. evaluated rates of bone loss prospectively in 122 White and 121 Black healthy non-obese pre and post-menopausal women and found that higher bone mass in Black women was largely due to the attainment of a greater bone mass by early adulthood (43). While some studies in Caucasian and Asian women suggest that polymorphisms in the vitamin D receptor may be associated with bone mineral density and rates of bone loss, this does not appear to have a major influence on osteoporotic risk in older African Americans women (44, 45).

THE IDEAL SCREENING TEST

If in fact this paradigm is correct, and there are differences between women in terms of their overall susceptibility to estrogen associated diseases, then again, one might expect to see these two clinical extremes as well as a population of women that has intermediate

risk. The ultimate value in identifying differences in the susceptibility to estrogen associated diseases is the ability to predict who will fall into which population and target preventive therapy well before clinical disease occurs. How then might one predict whether or not a woman will be less or more susceptible in her early premenopausal years? In an ideal world, this test would be simple such as blood draw, a x-ray or provocative maneuver such as a response to some physiologic stimulus like an exercise test. In order to identify what kind of test it might be reasonable to apply, a brief review of the basic mechanism of actions of estrogen is in order.

General Mechanisms of Action. As we discussed earlier, in premenopausal women, 17β-estradiol is the chief circulating estrogen produced by the ovaries. Serum estradiol levels increase with the onset of menarche. In premenopausal women they range from 100 pg/mL in the follicular phase to about 600 pg/mL at the time of ovulation. They may rise as high as 20,000 pg/mL during pregnancy. After menopause, serum estradiol concentrations fall to those values that are similar or lower to those of men of a similar age, 5 to 20 pg/mL.

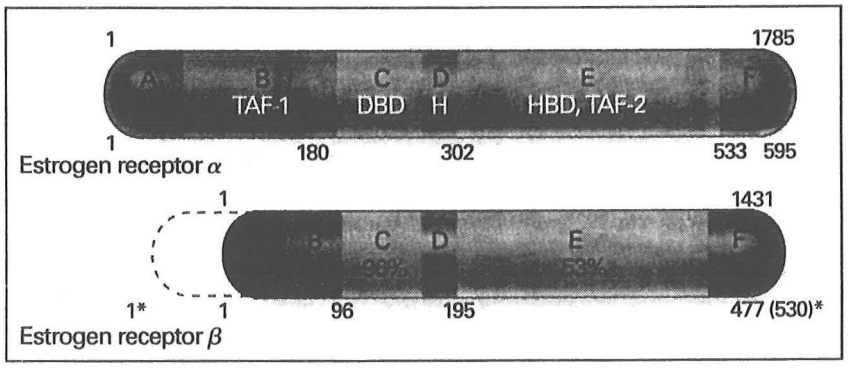

Figure 6. Structures of the human estrogen receptors α and β (see test). From (49).

There are two estrogen receptors, alpha and beta, both of which are members of the super family of steroid receptors (46, 47)]. The estrogen β-receptor is structurally and functionally distinct from the α-receptor. These receptors occur not only in the nucleus but also on the cell surface membrane (discussed below) (48). Figure 6 shows the structure of the human estrogen receptors α and β (49). These receptors are organized into domains. The A-B domains contain ligand-independent transcriptional activation function (TAF1); domain C is the DNA binding domain (DBD); domain D is the hinge region; domain E contains the hormone binding domain (HB) and the hormone-dependent transcriptional activation function TAF-2) and domain F is a variable region that is probably important for differences in the response of estrogen receptor to estradiol and selective estrogen modulators (49). The α-receptor is expressed in vascular and endothelial smooth muscle cells. The β-receptors found in prostate, uterus, ovaries, testes, bladder, lungs, brain, arteries, veins and myocardial cells. Estrogen receptors can be activated by estrogen binding but also by growth factors in the absence of estrogen.

Estrogen interacts with its receptors to generate what are termed "genomic" and "non-genomic" effects.

Genomic Effects of Estrogen. The genomic effects of estrogen occur via the classic estrogen receptor signal pathway: entry of estrogen into the cell with translocation to the nucleus and then interaction with the nuclear estrogen receptors to cause transcriptional activation or repression of estrogen responsive genes. This cell signaling may take hours or more to achieve its final downstream effects. Figure 7 depicts the mechanism of estrogen-receptor activation of gene expression (49). Estrogen enters the cytosol by passive diffusion and bind to high-affinity nuclear receptors. After estrogen binding, the estrogen receptors, which act as transcription factors, undergo conformational changes. These estrogen receptor complexes can then bind to specific sites in the control regions of their target genes (estrogen response elements). These complexes associate with proteins are capable of activating the general transcription apparatus (GTA) which contains the RNA polymerase that transcribes DNA into RNA. The estrogen-receptor-associated proteins include coactivator proteins (CoAct) and general integrators of transcription (Int). These estrogen receptors may also suppress the transcription of selected target genes by interaction with co-repressors. Table XI describes some estrogen related genes that may be important in cardiovascular disease (49).

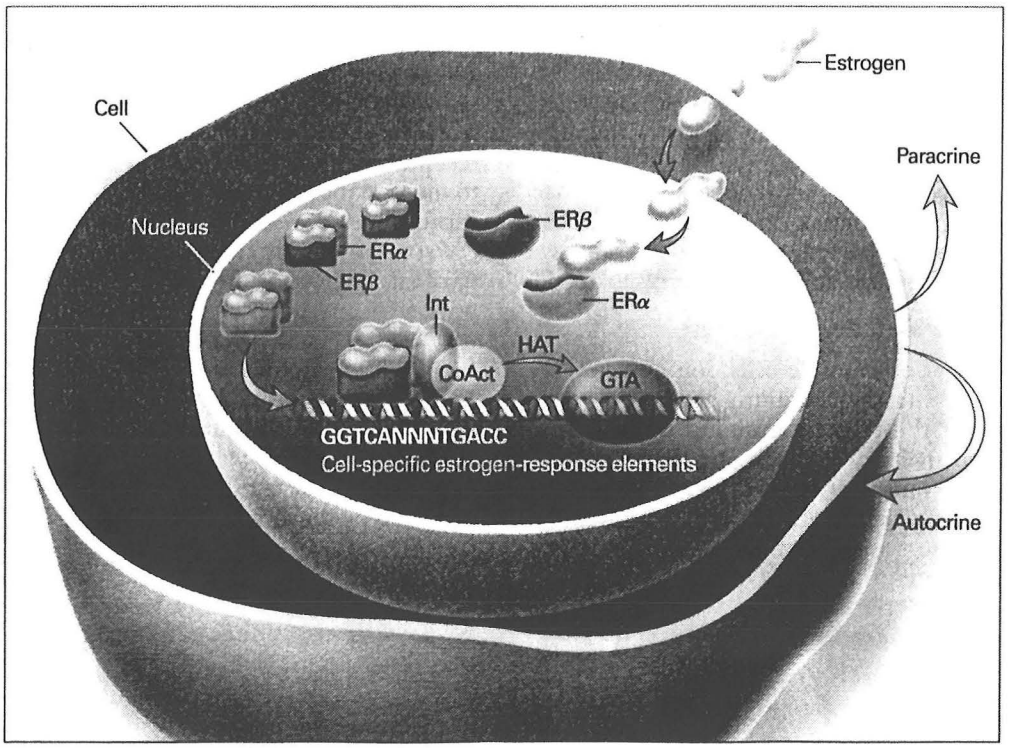

Figure 7. Mechanism of estrogen-receptor activiation of gene expression (see text). From (49).

Non-Genomic Effects of Estrogen. The documentation of rapid estrogen effects that occur in vasculature, breast, bone and neuronal tissue, further investigations into estrogen signaling mechanisms lead to the identification of cell surface forms of receptors that have been coupled with cytosolic signal transduction proteins (48). Figure 8 depicts the mechanism of rapid non-genomic receptor-dependent actions of estrogen in four cell types. Common to all of these cell types is activation of mitogen-activated protein kinase (MAPK).

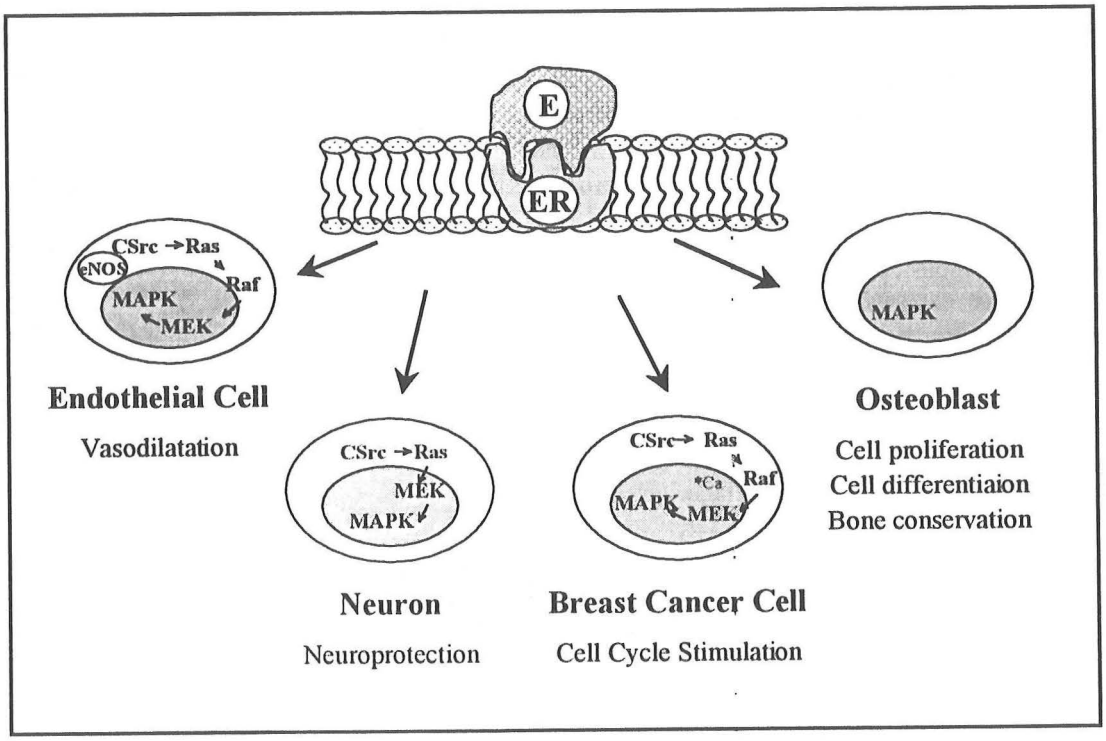

Figure 8. Mechanism of non-genomic actions of estrogen in four cell types (see text). From (48).

An example of a non-genomic effect is the rapid activation of nitric oxide synthase by estrogen in endothelial cells and vascular smooth muscle cells. This is illustrated in Figure 9, panels A and B (49). When estrogen interacts with plasma, membrane estrogen receptor and endothelial cells, there is sequential activation of Ras, Raf, MEK and MAPK. Endothelial nitrous oxide synthase may then be activated stimulating release of nitric oxide, which results in relaxation of the vascular smooth muscles. Estrogen can also rapidly activate calcium activated potassium channels that will then hyperpolarize and relax smooth muscle cells. Both of these effects account for the property of estrogen to cause short-term vasodilation. This is particularly pertinent because, as we will discuss shortly, the degree of vasodilation to a physiological challenge of estrogen might serve as a screening tool for estrogen susceptible women.

In summary, control of gene expression by complexes of estrogen and estrogen receptors involves a series of molecular interactions among estrogen, estrogen receptors, estrogen

receptor associated proteins and the control regions for different estrogen target genes present within the cell. Any of these sites could for example determine levels of estrogen susceptibility if you will.

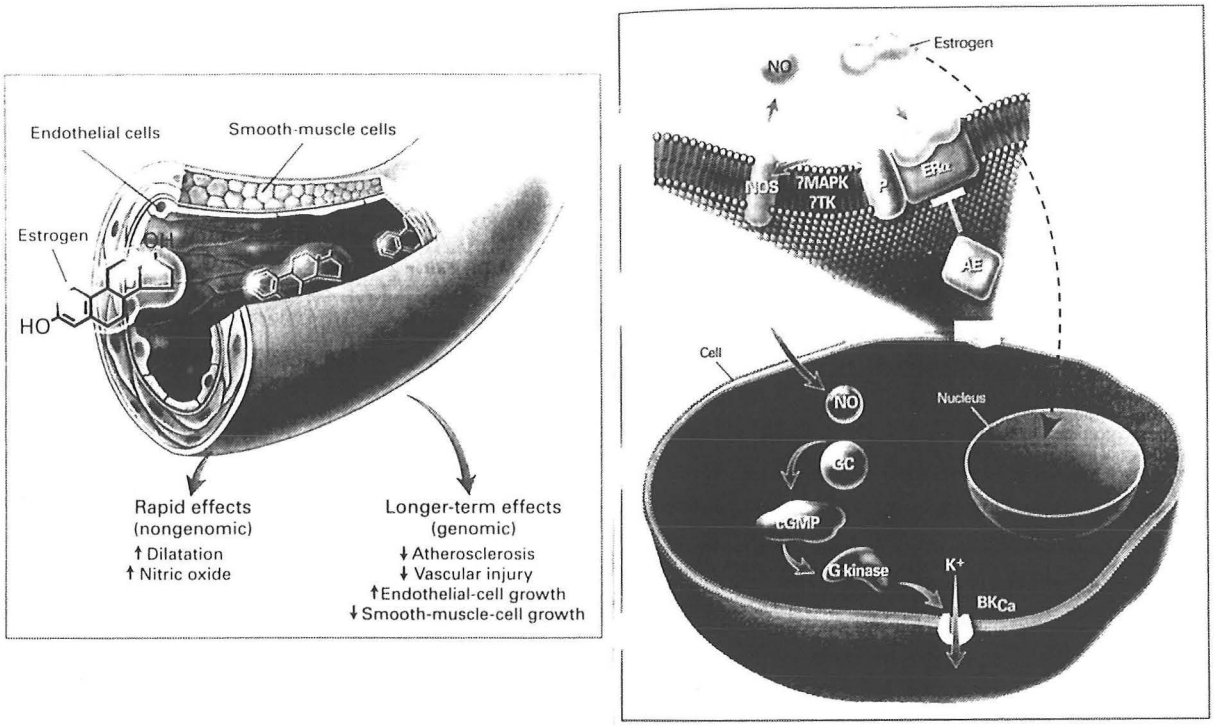

Figure 9. A. Direct Effect of estrogen on blood vessels (see text). B. Mechanism of non-genomic activation of nitric oxide synthase by estrogen in endothelial cells and vascular smooth muscle cells (see text). From (49).

Circulating Premenopausal Estradiol Levels. The simplest mechanism of stratifying women according to susceptibility would potentially be something simple like premenopausal estradiol levels. Again, given the changes in estradiol levels during the menstrual period, use would obviously have to be standardized to the cycling. To support estradiol levels being an important determinant of estrogen susceptibility, we might expect that premenopausal women and estradiol levels would be higher in those women with breast cancer for example, and lower in those women osteoporosis or coronary heart disease. It is important to differentiate studies that evaluate the effect of premenopausal versus postmenopausal hormone levels on the incidence of disease. It very well may be that the action of estrogen on tissue or cells in the healthy state may be different from that on diseased tissue or atypical cells (normal versus atherosclerotic arteries for example).

Sex Hormones and the Risk of Breast Cancer. As mentioned previously, the risk of breast cancer increases with an increased lifetime exposure of women to significant levels of reproductive hormones, including age at first pregnancy, number of children, age at menarche (50). Wysowski and colleagues evaluated the relationship between sex

hormone levels and the development of breast cancer in approximately 13,000 residents of Washington county, Maryland who donated blood in 1974 and were evaluated for the presence of a breast cancer diagnoses over the following 7 years. Mean serum levels of estrone, estradiol, estriol, and androstenedione, progesterone and testosterone were not significantly different in the 39 postmenopausal cases compared to controls or in the 17 premenopausal cases vs. controls. Obviously, the number of premenopausal women in this study was quite low (51).

Country of birth can influence the risk of breast cancer with women in North America and Northern European countries having the highest the risk for breast cancer. Women in Southern European and Latin American countries are at intermediate risk and women in African and Asian countries have the lowest risk (52). A number of studies have been conducted to try to determine if low rates of breast cancers in different populations are associated with low levels of estrogens. Key et al., (53) found that premenopausal high-risk British women had estradiol concentrations that are 36% higher on average than those of Chinese women who have extremely low rate of breast cancer. Key and Pike (54) reviewed five case control studies of estrogen profiles in premenopausal women relating estrogen profiles to breast cancer risk. The number of cases in these studies have been extremely low averaging between five and 36 cases per study. The difficulty with these kinds of studies is that usually only one or a few samples are drawn to represent the overall hormonal milieu in the face of cyclic hormonal variations.

Sex Hormones and the Risk of Osteoporosis. Because of postmenopausal bone loss is clearly related to estrogen deficiency and can be prevented by estrogen replacement, premenopausal estrogen levels may play an important role in achieving peak bone mass and thus may be predictors of the risk of osteoporosis. Arnamentho-Villareal et al. evaluated the effect of serum estrogen levels on vertebral bone density in 63 premenopausal women (55). Compared with women with normal bone density, subjects with low estrogen bone density had significantly lower serum estradiol and estrone levels. Low bone mineral density was associated with a lower estrogen score, which is the sum of age at menarche, average length of menstrual cycle and the use of birth control pills. They also had a later of age of menarche or more were more likely to have menstrual irregularities and had lower serum estradiol and estrone levels (55). Zatterman et al., (56) evaluated the effect of physiological variations in sex hormone levels during menstrual cycle in ten young, healthy Caucasian women on biomarkers of bone turnover (56). They found that normal menstrual cycling in young women was associated with monthly fluctuations in bone turnover. For example, they found that the excretion of bone reabsorption DP was inversely associated with serum estradiol level

Sex Hormones and the Risk of Coronary Heart Disease. Do studies support a link between menopausal estradiol levels and the risk of coronary artery disease in women? Hanke et al. evaluated estradiol concentrations in 14 premenopausal women with coronary heart disease (57). Compared with the healthy control group, matched for age and other cardiac risk factors, premenopausal women with coronary heart disease had significantly a lower plasma estradiol. However progesterone conditions were not significantly different. These findings were confounded by the fact that 8 in the patient

group had had tubal ligations, which can impact on ovarian function (57). The association between estradiol and coronary heart disease can be evaluated somewhat indirectly looking at the clinical syndrome of women with polycystic ovaries. Given the fact that women with polycystic disease are more likely to have insulin resistance, lower HDL and high serum triglycerides, these women may be at increased risk for coronary heart disease (58). However, some of these women may have low estradiol because of increased anovulation while others tend to be obese and therefore can have normal circulating levels of estrogen but as estrone (48). Surprisingly, an exhaustive Medline search was unable to produce information regarding estradiol levels in premenopausal women and the risk of coronary heart disease mortality from either the Nurses Health Study or the Framingham Database.

Sex Hormones in Black and White Women. A few studies have evaluated the effect of race on sex hormone levels. Pham et al., (32) measured hormones in 35 White and 33 Black women 47 years of age and found no difference in mean values of follicular phase, plasma levels of estradiol, FSH, PHEA sulfate, intracycle variations of these hormones or the rate of anovulatory cycles. There are studies that suggest that there may be differences in hormones during the menstrual cycle in Black and White women. Harlow et al., (59) found ethnic differences in the length of the menstrual cycle in 125 Black and 123 White girls ages 12-14. Mills and Berry (60) examined cardiovascular recovery from two standard laboratory stressors in healthy Black and White normotensive women mean age 33 years, once during the follicular phase and once during the luteal phase of the menstrual cycle. In the White women, there were no differences in blood pressure recovery during the cycle during Black women showed a greater diastolic blood pressure recovery on the luteal as compared to the follicular phase (60). Freedman and Girgis (61) looked at the effects of the menstrual cycle and race on peripheral vascular responses to intra-arterial infusion of phenylephrine and clonidine. During the clonidine effusion, White women showed significantly more vasoconstriction in the follicular phase then the luteal phase whereas Black women had similar responses to both phases. While these various small studies are suggestive that there may be racial differences in cycling of hormones in premenopausal women, there are certainly no large studies addressing this important issue.

Differences in Estrogen Receptors. Susceptibility to circulating estrogen could be a function of differences in numbers of receptors in a given tissue or of differences in receptor structure and function. There has been some preliminary reports looking at the distribution of estrogen receptors in different tissues obtained at autopsy or during surgical interventions such a coronary artery bypass grafting. These studies are limited in that the distribution of receptors may be altered by the development of disease and the need for surgical specimens significantly reduces the attractiveness of this approach as a screening tool in the general population. A number of studies have evaluated the effect of estrogen polymorphisms on the risk of disease.

Several studies have been published that have shown a lack of association of estrogen polymorphisms with coronary heart disease (62), Alzheimer's disease (63) or breast cancer (64). There have been contrary findings in association with bone mineral density

(141,144,147,400)(65-68). A positive association of estrogen receptor polymorphisms with the onset of both natural and surgical menopause (due to fibroids) has been reported in a group of 900 postmenopausal Scandinavian women (69).

Allelic Differences in Estrogen-Associated Genes. There have been a number of studies in which the presence of apolipoprotein E (APOE) episilon4 allelle has been associated with increased risk of Alzheimer's disease (70-72), reduced bone mineral density (73), increased risk of fracture (74) and increased risk of atherosclerosis (75).

Differences in the Non-Genomic Effects of Estrogen. Estrogen administration has been shown to vasodilate coronary arteries in a number of animal models as well as in humans (76-80). For obvious reasons, the instrumentation of coronary arteries is not the optimal primary prevention screening tool. A much more accessible arterial vessel for measuring the vasodilatory effects of acute administration is the brachial artery. A number of studies have shown that acute estrogen administration improves flow-mediated endothelium-dependent vasodilation in postmenopausal women without cardiovascular risk factors (81-84) with cardiovascular risk factors (82) and stable coronary artery disease (85).

Few studies have looked at these effects in premenopausal women (86, 87). Hashimoto et al. examined brachial artery diameters using ultrasound in 34 young, healthy female and male volunteers at rest, during reactive hyperemia and during sublingual nitroglycerine administration (86). Table XII shows the percent change in flow mediated diameter (FMD) in men at baseline and in women during three points in the menstrual cycle: during menses (M), the follicular phase (F) and luteal phase (L) and the associated sex hormone levels.

Table XII. %FMD and associated sex hormone levels in men and women during three points in the menstrual cycle.

Characteristic	Men	Women-M	Women-F	Women-L
Estradiol, pmol/L	14.5 ± 9.9	121.9 ± 12.5	632.1 ± 74.5	533.8 ± 33.4
Progesterone, nmol/L	1.6 ± 1.16	1.6 ± 0.19	3.2 ± 0.60	48.7 ± 4.8
%FMD	10.60 ± 0.75	11.22 ± 0.58	18.20 ± 0.81	17.53 ± 0.74

FMD=flow mediated diameter. M=menses, F=follicular phase, L= luteal phase.
From (86).

English et al. evaluated the effect of the menstrual cycle on endothelium-dependent vasodilation of the brachial artery in 20 normal young women (87). Blood sampling and measurement of flow-mediated vasodilation as well as nitroglycerine-induced dilation were performed 3 times during the menstrual cycle: follicular phase, luteal phase and at ovulation (midcycle). Table XIII shows data organized in a similar fashion to that of Hashimoto et al seen in Table XII.

Table XIII. %FMD and associated sex hormone levels in women during three points in the menstrual cycle.

Characteristic	Follicular	Midcycle	Luteal	P value
Estradiol, pmol/L	49 ± 5	174 ± 27	157 ± 14	< 0.001
Progesterone, nmol/L	0.7 ± 0.07	0.9 ± 0.16	9.4 ± 1.0	<0.001
%FMD	8.0 ± 1.1	10.9 ± 1.4	7.6 ± 1.1	0.041

FMD=flow mediated diameter. From (87).

Figure 10 shows the flow-mediated vasodilation in each of the 28 women. What is fascinating about this figure is the heterogeneity in the responses. One might be able to separate these individuals into two populations: those who are more estrogen susceptible and those who are less. This could in fact be an ideal screening tool in healthy premenopausal women. There has been some criticism of this technique in that the % changes in FMD are of the same magnitude of the standard error of the technique. With the use of an intravaginal probe, measurements of blood flow in the uterine artery are possible (88, 89). If the uterine artery were more sensitive to the effects of endothelial-dependent vasodilation, the relative changes might be greater and thus this might be a reasonable screening tool (90).

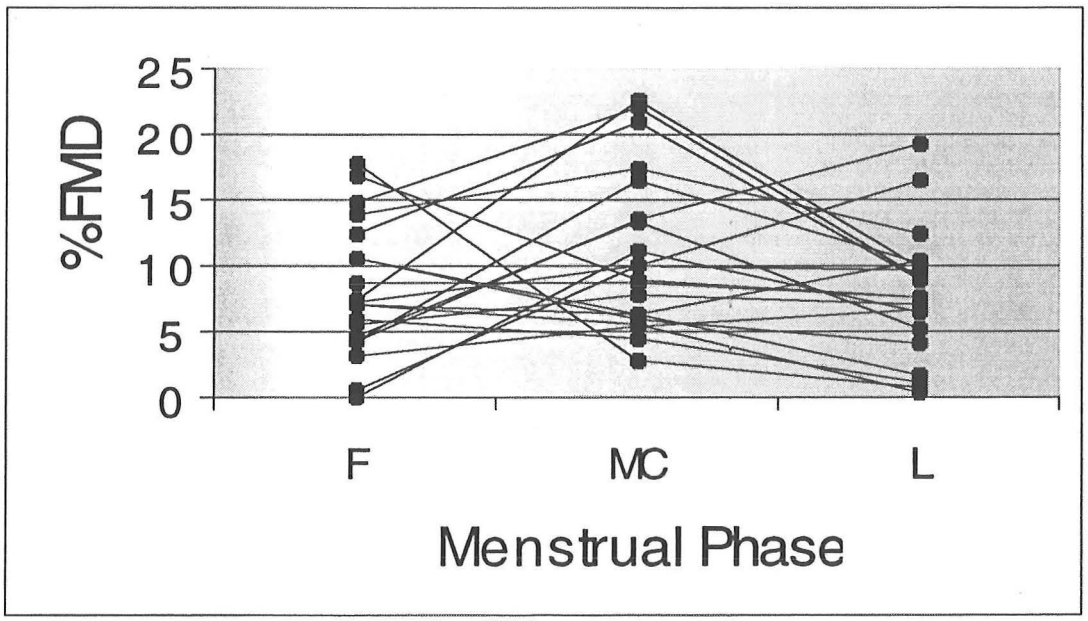

Figure 10. %Flow-mediated vasoconstriction in women during the menstrual cycle (see text). From (87).

CONCLUSION

In summary, there is some evidence to suggest that some women may be more susceptible to the overall effects of estrogen than others. This susceptibility affects the risk to important health risks to women including breast cancer, coronary heart disease and osteoporosis. Clearly additional research needs to be performed on large databases already in existence to better define these associations in large populations of ethnically diverse women. The next major hurdle will be the identification of a screening tool which can be applied to premenopausal women well in advance of the clinical presentations of these diseases so that preventive therapy can be aggressively targeted to those women at greatest risk.

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