

Reversal of Relation Between Body Mass and Endogenous Estrogen Concentrations With Menopausal Status

Nancy Potischman, Christine A. Swanson, Pentti Siiteri, Robert N. Hoover*

For more than a decade, results from published studies have indicated that the risk of breast cancer may be lower among heavy premenopausal women than among their less obese counterparts (1-8). In heavy postmenopausal women, however, this risk is either increased or unchanged in comparison with non-obese postmenopausal women (9,10). The mechanism explaining this reversal in risk is unknown, although theoretical explanations have been proposed. Obese postmenopausal women may have elevated risk from higher levels of circulating estrogens secondary to increased conversion of androgen to estrogen in adipose tissue (11) and a higher proportion of bioavailable estrogen due to the low levels of sex hormone-binding globulin (SHBG) (12,13). Obese premenopausal women may exhibit a greater degree of anovulation, resulting in lower levels of both progesterone and estradiol, lower breast cell division rates, and, consequently, a lower risk of breast cancer (13-15).

We had the opportunity to evaluate the relation of body mass and hormonal profiles in a group of 195 community and 103 hospital control subjects from a study of endometrial cancer described elsewhere (16,17). Results of both control groups were similar; therefore, the two groups were combined. Body mass index (BMI) (kg/m^2) was calculated from reported current height and weight obtained during an interview. Fasting serum samples were generally drawn within 1 month of interview and prior to surgery for benign endometrial conditions for the hospital control subjects. Serum was extracted and separated by celite column chromatography. Estrone,

estradiol, androstenedione, estrone sulfate, progesterone, and SHBG were measured by radioimmunoassay (RIA). An enzymatic hydrolysis was performed before separation for estrone sulfate. Albumin-bound estradiol was assessed using ammonium sulfate precipitation, and percent-free estradiol was estimated with an equilibrium dialysis assay. The amount of free or albumin-bound estradiol was calculated by multiplying the total estradiol concentration by the percent in the other fractions. Serum was analyzed by Nichols Institute, Inc. (San Juan Capistrano, CA). We previously documented the reliability of this laboratory for steroid hormone assays (18). Blind quality control serum evaluated with each daily batch of samples demonstrated adequate reproducibility for all assays, particularly for estradiol in postmenopausal women (overall coefficient of variation = 16.6).

Written informed consent was obtained from all study participants, and procedures were approved by institutional review. Of the 477 and 253 eligible community and hospital control subjects, we were able to successfully interview 313 and 206 of these subjects, respectively, regarding demographic, medical, and reproductive factors. Following the interview, women were measured for a variety of anthropometric indices, and blood samples were obtained from 217 community and 139 hospital control subjects. As is common in population surveys, we obtained one blood sample from each subject. Clearly, multiple samples would better characterize a woman; however, one sample is generally adequate for epidemiologic purposes (19). Women were excluded from the analysis if they had no BMI data, if they had reported use of exogenous estrogens or oral contraceptives within 6 months, or if they had hormone values that indicated Premarin usage or perimenopausal status. Women were considered postmenopausal if they reported not having had a menstrual period for 6 or more months or if they reported having had a menstrual period within 6 months but exhibited a measured estradiol value of less than 20 pg/mL (laboratory cutoff for postmenopausal status). All premenopausal women reported having had a menstrual

period within 6 months and all but one had experienced menses within 1 month of the interview. Analysis of covariance was used to determine age-adjusted mean values within menopausal groups, and ordinary least-squares regression was used to test the trend of BMI with hormone concentration. An interaction term was entered into the regression to test the significance of noted differences of the association of BMI with hormones by menopausal status. All *P* values resulted from use of two-sided statistical tests.

Mean values across BMI tertiles revealed the expected decrease in SHBG with higher BMI in both menopausal groups (Table 1). As the BMI increased, however, total estradiol decreased in premenopausal women (*P* for trend = .11) and increased in postmenopausal women (*P* = .0001). Free estradiol and albumin-bound estradiol (i.e., the bioavailable fraction of this hormone) were found to increase with increasing BMI in postmenopausal women; a similar tendency for albumin-bound estradiol was observed in premenopausal women. Estrone and estrone sulfate were higher in heavy postmenopausal women but showed no relation with BMI among premenopausal women. Only the relation of BMI to estradiol differed significantly by menopausal status (*P* = .02).

When the analysis was restricted to premenopausal women with progesterone values consistent with being in the follicular phase of their menstrual cycle (50 ng/dL progesterone or less), the results were similar (Table 2) with the exception that all three estradiol fractions decreased with increasing BMI (*P* for trend = .03 estradiol; *P* = .06 free estradiol). Interestingly, the proportion of subjects in the luteal phase actually increased as BMI increased; 40%, 44%,

*Affiliation of authors: Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

Correspondence to: Nancy Potischman, Ph.D., Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Institutes of Health, Executive Plaza North, Rm. 443, Bethesda, MD 20892.

See "Note" section following "References."

Table 1. Age-adjusted mean hormone values by body mass index tertile* among control subjects

Premenopausal age-adjusted mean (95% confidence interval)				
Hormone†	Tertile 1 (n = 30)	Tertile 2 (n = 27)	Tertile 3 (n = 31)	Test for trend, P value
SHBG, nmol/L	62 (53-72)	53 (45-63)	34 (29-40)	.0001
E2, pg/mL	123 (93-162)	109 (81-145)	96 (73-126)	.11
Free E2, pg/mL	1.67 (1.3-2.2)	1.48 (1.1-2.0)	1.49 (1.2-1.9)	.32
Albumin-E2, pg/mL	16.7 (13-22)	18.6 (14-25)	22.5 (17-29)	.41
E1, pg/mL	77.8 (65-93)	78.9 (65-95)	74.1 (62-88)	.81
E1 sulfate, pg/mL	1262 (953-1671)	1357 (1011-1821)	1368 (1038-1802)	.94
Androstenedione, ng/dL	101 (89-113)	103 (91-117)	87 (77-98)	.12
Postmenopausal age-adjusted mean (95% confidence interval)				
Hormone†	Tertile 1 (n = 69)	Tertile 2 (n = 75)	Tertile 3 (n = 66)	Test for trend, P value
SHBG, nmol/L	53 (46-60)	41 (36-47)	31 (27-35)	.0001
E2, pg/mL	5.1 (4.5-5.7)	6.1 (5.4-6.9)	10.5 (9.2-11.9)	.0001
Free E2, pg/mL	0.07 (0.06-0.08)	0.09 (0.08-0.10)	0.16 (0.14-0.19)	.0001
Albumin-E2, pg/mL	0.9 (0.7-1.0)	1.3 (1.1-1.6)	2.8 (2.3-3.4)	.0001
E1, pg/mL	27.1 (24-30)	28.4 (26-31)	40.1 (36-44)	.0001
E1 sulfate, pg/mL	271 (225-326)	286 (240-342)	458 (380-552)	.0001
Androstenedione, ng/dL	60 (53-68)	62 (55-69)	63 (56-71)	.56

*Body mass index tertiles based on all control subjects; tertile 1 <23.2 kg/m²; tertile 2 = 23.2-27.1 kg/m²; and tertile 3 >27.1 kg/m².

†SHBG = sex hormone-binding globulin; E2 = estradiol; E1 = estrone.

and 65% in tertiles 1, 2, and 3, respectively.

The finding of decreased levels of estradiol with increasing BMI among premenopausal women was surprising, but not unprecedented. Several small studies (20-24) (with seven to 25 subjects per group) showed that obese premenopausal women had lower estradiol values than those of women of normal weight. Most of these clinical studies involved subjects in the follicular phase of the menstrual cycle (21-24). In two epidemiologic studies, BMI was not associated with circulating estradiol in any phase of the menstrual cycle (25,26). However, these women were younger than most premenopausal

breast cancer patients (26), and there may have been few women with BMI values that have been found to be associated with the reduced risk of breast cancer (i.e., >27kg/m²) (25,26). In an earlier epidemiologic study (27), luteal phase urinary estrogens declined with increasing weight in premenopausal women. The results from these studies may have been inconsistent because of the ranges of BMI involved. Furthermore, inference from our study may be limited to groups with relatively high BMIs, such as Western populations.

If average levels of estradiol are inversely related to BMI in premenopausal women, what is the likely mechanism? One possibility is that the

heavier women were more frequently anovular and therefore less likely to have blood samples taken when estradiol levels were higher. Evaluation of questionnaire data revealed no differences in cycle length or frequency of irregular menses among the heavy versus the thin women. On the basis of the assayed progesterone values, there was actually a higher proportion of heavy than thin women in the luteal phase. Further, previous data showing this inverse relationship have included both follicular and luteal phase specimens. Another possibility is that estradiol may be sequestered in adipose tissue, since injected radiolabeled estradiol has been recovered from the adipose tissue of

Table 2. Age-adjusted mean (95% confidence interval) hormone values by body mass index tertile* among 44 follicular phase subjects†

Hormone‡	Tertile 1 (n = 18)	Tertile 2 (n = 15)	Tertile 3 (n = 11)	Test for trend, P value
SHBG, nmol/L	60 (49-73)	48 (38-59)	34 (26-44)	.0005
E2, pg/mL	137 (94-200)	94 (62-142)	76 (47-124)	.03
Free E2, pg/mL	1.9 (1.3-2.7)	1.4 (0.9-2.0)	1.2 (0.8-1.9)	.06
Albumin-E2, pg/mL	19.5 (14-28)	18.8 (13-28)	16.7 (11-26)	.32
E1, pg/mL	76.6 (62-95)	63.6 (50-81)	68.1 (52-90)	.54
E1 sulfate, pg/mL	1212 (841-1746)	983 (659-1467)	1255 (787-2002)	.69
Androstenedione, ng/dL	102 (88-118)	100 (86-117)	83 (69-99)	.13

*Body mass index tertiles based on all control subjects; tertile 1 <23.2 kg/m²; tertile 2 = 23.2-27.1 kg/m²; and tertile 3 >27.1 kg/m².

†Follicular phase defined as progesterone values ≤50 ng/dL.

‡SHBG = sex hormone-binding globulin; E2 = estradiol; E1 = estrone.

obese women (28). Uptake of estradiol and other lipophilic steroids into adipocytes is likely to be concentration dependent, thus, the relatively high concentrations of non-SHBG-bound estradiol among heavy premenopausal women may result in substantial uptake of estradiol into fat. More important, the larger albumin-bound pool of estradiol in obese premenopausal women will be irreversibly cleared in the liver and other tissues, resulting in a higher metabolic clearance rate (29). Consequently, the total plasma estradiol concentration will be reduced in the absence of a compensatory increase in ovarian estradiol secretion.

At this point, it is provocative that the opposite roles obesity plays as a risk factor for breast cancer, depending on menopausal status, may in fact be explained by differences in plasma estrogen concentrations. The precise mechanistic relationships certainly deserve more attention. Larger studies specifically designed to evaluate the influence of BMI, and perhaps fat distribution, on estrogen concentrations by menopausal status should be undertaken.

References

- (1) Helmrich SP, Shapiro S, Rosenberg L, Kaufman DW, Slone D, Bain C, et al. Risk factors for breast cancer. *Am J Epidemiol* 1983;117:35-45.
- (2) Willett WC, Browne ML, Bain C, Lipnick RJ, Stampfer MJ, Rosner B, et al. Relative weight and risk of breast cancer among premenopausal women. *Am J Epidemiol* 1985;122:731-40.
- (3) Le Marchand L, Kolonel LN, Earle ME, Mi MP. Body size at different periods of life and breast cancer risk. *Am J Epidemiol* 1988;128:137-52.
- (4) Kampert JB, Whittemore AS, Paffenbarger RS Jr. Combined effect of childbearing, menstrual events, and body size on age-specific breast cancer risk. *Am J Epidemiol* 1988;128:962-79.
- (5) Swanson CA, Brinton LA, Taylor PR, Licitra LM, Ziegler RG, Schairer C. Body size and breast cancer risk assessed in women participating in the Breast Cancer Detection Demonstration Project. *Am J Epidemiol* 1989;130:1133-41.
- (6) London SJ, Colditz GA, Stampfer MJ, Willett WC, Rosner B, Speizer FE. Prospective study of relative weight, height, and risk of breast cancer [see comment citations in Medline]. *JAMA* 1989;262:2853-8.
- (7) Pathak DR, Whittemore AS. Combined effects of body size, parity, and menstrual events on breast cancer incidence in seven countries. *Am J Epidemiol* 1992;135:153-68.
- (8) Swanson CA, Coates RJ, Schoenberg JB, Malone KE, Gammon MD, Stanford JL, et al. Body size and breast cancer risk among women under age 45. *Am J Epidemiol*. In press.
- (9) Harris JR, Lippman ME, Veronesi U, Willett W. Breast cancer [see comment citation in Medline]. *N Engl J Med* 1992;327:319-28.
- (10) Ballard-Barbash R. Anthropometry and breast cancer. Body size—a moving target. *Cancer* 1994;74:1090-100.
- (11) MacDonald PC, Edman CD, Hemsell DL, Porter JC, Siiteri PK. Effect of obesity on conversion of plasma androstenedione to estrone in postmenopausal women with and without endometrial cancer. *Am J Obstet Gynecol* 1978;130:448-55.
- (12) Siiteri PK, Nisker JA, Hammond GL. Hormonal basis of risk factors for breast and endometrial cancer. In: Iacobelli S, King RJ, Lindner HR, Lippman ME, editors. *Hormones and cancer*. New York: Raven Press, 1980.
- (13) Key TJ, Pike MC. The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer. *Eur J Cancer Clin Oncol* 1988;24:29-43.
- (14) Pike MC, Spicer DV, Dahmouh L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. *Epidemiol Review* 1993;15:17-35.
- (15) Henderson BE, Ross RK, Pike MC. Hormonal chemoprevention of cancer in women. *Science* 1993;259:633-8.
- (16) Brinton LA, Berman ML, Mortel R, Twiggs LB, Barrett RJ, Wilbanks GD, et al. Reproductive, menstrual, and medical risk factors for endometrial cancer: results from a case-control study. *Am J Obstet Gynecol* 1992;167:1317-25.
- (17) Swanson CA, Potischman N, Wilbanks GD, Twiggs LB, Mortel R, Berman ML, et al. Relation of endometrial cancer risk to past and contemporary body size and body fat distribution. *Cancer Epidemiol Biomarkers Prev* 1993;2:321-7.
- (18) Potischman N, Falk RT, Laiming VA, Siiteri PK, Hoover RN. Reproducibility of laboratory assays for steroid hormones and sex hormone-binding globulin. *Cancer Res* 1994;54:5363-7.
- (19) Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. *Cancer Epidemiol Biomarkers Prev* 1995;4:649-54.
- (20) Kopelman PG, Pilkington TR, White N, Jeffcoate SL. Abnormal sex steroid secretion and binding in massively obese women. *Clin Endocrinol (Oxf)* 1980;12:363-9.
- (21) Zumoff B. Relationship of obesity to blood estrogens. *Cancer Res* 1982;42(8 Suppl):3289s-94s.
- (22) Dunaif A, Mandeli J, Fluhr H, Dobrjansky A. The impact of obesity and chronic hyperinsulinemia on gonadotropin release and gonadal steroid secretion in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1988;66:131-9.
- (23) De Pergola G, Giorgino F, Cospite MR, Giagulli VA, Cignarelli M, Ferri G, et al. Relation between sex hormones and serum lipoprotein and lipoprotein(a) concentrations in premenopausal obese women. *Arterioscler Thromb* 1993;13:675-9.
- (24) Grenman S, Ronnema T, Irjala K, Kaihola HL, Gronroos M. Sex steroid, gonadotropin, cortisol, and prolactin levels in healthy, massively obese women: correlation with abdominal fat cell size and effect of weight reduction. *J Clin Endocrinol Metab* 1986;63:1257-61.
- (25) Erster VL, Wrensch MR, Petrakis NL, King EB, Miike R, Murai J, et al. Benign and malignant breast disease: initial study results of serum and breast fluid analyses of endogenous estrogens. *J Natl Cancer Inst* 1987;79:949-60.
- (26) Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, et al. The relation of body size to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control* 1995;6:3-8.
- (27) Trichopoulos D, Polychronopoulou A, Brown J, MacMahon B. Obesity, serum cholesterol, and estrogens in premenopausal women. *Oncology* 1983;40:227-31.
- (28) Twombly GH, Scheiner S, Levitz M. Endometrial cancer, obesity, and estrogenic excretion in women. *Am J Obstet Gynecol* 1961;82:424-7.
- (29) Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW. The serum transport of steroid hormones. *Recent Prog Horm Res* 1982;38:457-510.

Note

Manuscript received November 1, 1995; revised March 1, 1996; accepted March 7, 1996.