

The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States)

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(Received 28 June 1993; accepted in revised form 21 September 1993)

We undertook a cross-sectional study in 107 premenopausal women in Maryland (United States) of alcohol intake and hormonal status in order to evaluate whether plasma hormone levels might mediate the reported positive relation between alcohol ingestion and breast cancer risk. Alcohol ingestion was estimated using a drinking pattern questionnaire, a food frequency questionnaire, and seven-day food records. Fasting blood specimens were collected on days 5-7, 12-15, and 21-23 of each participant's menstrual cycle and pooled to create follicular, midcycle, and luteal phase samples, respectively, for analysis. Estrone, estrone sulfate, estradiol, androstenedione, and dehydroepiandrosterone sulfate (DHEAS) in plasma were measured by radioimmunoassay, and sex-hormone binding globulin (SHBG) was measured by an immunoradiometric assay. After adjusting for age, weight, and total energy intake, alcohol ingestion was not associated with plasma estrogens in the follicular, midcycle, or luteal phases of the menstrual cycle, nor with the level of SHBG or DHEAS in plasma averaged from the three phases of the cycle. Alcohol, however, was significantly positively associated with the average level of plasma androstenedione. Based on these cross-sectional findings among premenopausal women, the increased risk of breast cancer related to alcohol ingestion does not appear to be mediated by elevated plasma estrogen levels. Androstenedione, however, may mediate the alcohol/breast cancer-association. *Cancer Causes and Control* 1994, 5, 53 - 60.

Key words: Alcohol, androgens, estrogens, ethyl alcohol, United States.

Introduction

A majority of epidemiologic studies have shown that alcohol ingestion increases breast cancer risk in postmenopausal women.¹⁻⁶ Although results are less consistent,⁷⁻¹⁰ alcohol consumption also appears to increase

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risk in premenopausal women.^{1-3,5,11,12} Even for postmenopausal women, drinking patterns earlier in life may be important in determining risk. Harvey *et al*¹³ and Young *et al*¹⁴ both reported an increased risk of breast cancer in women over 50 years of age, who drank alcohol before they were 35, that was independent of current drinking patterns. The mechanism underlying the association of alcohol intake with breast cancer is unknown, but changes in blood levels of estrogens and androgens are a possible explanation. Elevated estradiol,¹⁵⁻¹⁸ testosterone,¹⁹⁻²⁴ androstenedione,²⁴ and dehydroepiandrosterone²⁵ in blood from breast cancer cases have been reported. The interrelations of these hormones are shown in Figure 1.²⁶ Depressed sex-hormone binding globulin (SHBG) levels^{15,27-30} and elevated percent non-protein bound estradiol^{15,16,27-34} and percent non-protein bound testosterone²⁹ also have been reported for cases.

Postmenopausal alcoholics have elevated blood levels of estradiol and estrone.^{35,36} Moderate alcohol consumption also has been related to elevated levels of estradiol and estrone in three of four cross-sectional studies of postmenopausal women.³⁷⁻⁴⁰ There was, however, no relation between alcohol ingestion and estrogen levels in a cross-sectional study of perimenopausal women.⁴¹ Premenopausal women who drink moderate amounts of alcohol have a higher frequency of menstrual abnormalities,⁴² and premenopausal alcoholics with amenorrhea have depressed blood levels of estradiol but elevated levels of estrone.⁴³ Metabolic studies indicate that acute alcohol administration to premenopausal women causes a transient rise in plasma estradiol levels.^{44,45}

The androgen, androstenedione, is elevated in postmenopausal alcoholics, whereas dehydroepiandrosterone is depressed.^{35,46} Moderate alcohol consumption, however, was not related to blood levels of androstenedione in a cross-sectional study of postmenopausal women.³⁷ We are not aware of any studies of moderate alcohol ingestion and blood androgen levels in premenopausal women.

Because there is little information on the association of plasma hormones with moderate alcohol ingestion in premenopausal women, we undertook such a study to evaluate whether plasma estrogens or androgens might mediate the association of alcohol ingestion with breast cancer risk. Our study included a cross-sectional study and a controlled feeding study on a subset of the participants. In this paper, we present results of the cross-sectional study. Results of the controlled feeding study have been reported previously by Reichman *et al*.⁴⁷

Materials and methods

Participants for the cross-sectional study were recruited by posters and newspaper advertisements from communities around Beltsville, Maryland (United States) during 1988-90. Special emphasis was placed on recruiting women with a wide range of drinking patterns. Only women who met the following criteria were eligible: (i) 20 to 40 years of age; (ii) premenopausal with usual cycle length not more than 35 days; (iii) no history of cancer, diseases of the reproductive or endocrine systems, chronic liver or gastrointestinal disease, hypertension, diabetes, nephrolithiasis, gout, or hyperlipidemia; (iv) not pregnant or lactating during the past 12 months and not taking oral contraceptives during the past six months; (v) weight for height 85-130 percent of desirable based on 1983 Metropolitan Life Insurance tables; (vi) no history of alcohol abuse; (vii) not taking any medications other than an occasional analgesic; (viii) not following a vegetarian diet; (ix) not running competitively; and (x) not smoking.

All data and blood specimens were collected during a single menstrual cycle. Participants were asked to maintain their usual drinking patterns during this cycle and reported their alcohol ingestion on three questionnaires. The first was an extensive self-administered questionnaire on drinking patterns during the past year that included open-ended questions on usual intake

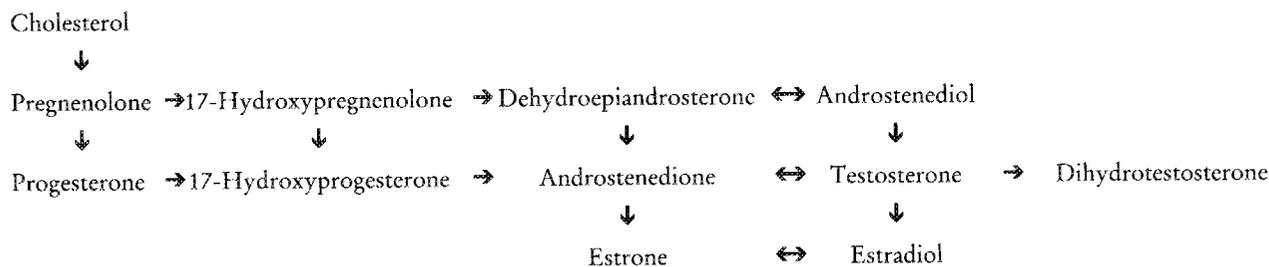


Figure 1. General scheme for synthesis of androgens and estrogens.²⁶

frequency and quantity for beer, wine, and liquor. The second was the food frequency component of the Health Habits and History Questionnaire version 2.1, which includes questions about usual intake frequency of standard quantities of beer, wine, and liquor during the past year.⁴⁸ Participants also completed seven-day food records on which they were asked to record any alcohol ingested. The seven-day records were analyzed by the University of Minnesota Nutrition Coordinating Center using version 19 of their nutrient database.⁴⁹ The drinking pattern questionnaire and the food frequency questionnaire were administered on day 14 of each participant's menstrual cycle and seven-day food records were completed on days 14 through 20. For all three questionnaires, percent alcohol by volume for beer, wine, and liquor was assumed to be 0.045, 0.115, and 0.40, respectively.⁵⁰

Blood was collected in the morning after an overnight fast. Equal volumes of plasma from days 5-7, 12-15, and 21-23 of the menstrual cycle were pooled to create, respectively, follicular, midcycle, and luteal phase specimens. All plasma specimens were stored at -70°C until hormone analyses were performed. Estrone, estradiol, and androstenedione in plasma were measured by radioimmunoassay (RIA) following solvent extraction and celite chromatography.⁵¹ Estrone sulfate also was measured by RIA after solvolysis, extraction of hydrolyzed estrone, and celite chromatography.⁵¹ Dehydroepiandrosterone sulfate (DHEAS) and progesterone were measured by RIA kits (ICN-Biomedical, Costa Mesa, California) and SHBG was measured by an immunoradiometric assay kit (Farnos Group Ltd, Oulunsalo, Finland). Percent unbound and albumin-bound estradiol were measured using centrifugal ultrafiltration,⁵² and SHBG-bound estradiol was calculated. Coefficients of variation for replicate quality-control samples averaged 9.5 percent for estrone, 11.1 percent for estradiol, 4.6 percent for estrone sulfate, 16.7 percent for androstenedione, 10.8 percent for DHEAS, 9.4 percent for progesterone, 10.6 percent for SHBG, and 10.6 percent for percent unbound, and 8.6 percent for percent albumin-bound estradiol.

The relation of plasma hormone levels to alcohol ingestion was evaluated using linear regression. To improve normality assumptions, all plasma hormone concentrations were converted to the \log_{10} scale before analysis. We also fitted models after categorizing participants into four drinking categories based on the drinking pattern questionnaire as follows: (i) *abstainers*, if they drank fewer than 12 alcoholic beverages during their life, never had 12 drinks in any one year, or, if they drank previously, did not drink any alcoholic beverages in the year prior to the study; (ii)

light drinkers, if they drank an average of 0.01 to 0.21 oz of alcohol/day; (iii) *moderate drinkers*, if they drank an average of 0.22 to 0.99 oz/day; and (iv) *heavy drinkers*, if they drank an average of 1.00 or more oz/day.⁵³ For the other two questionnaires, which did not ascertain lifetime drinking patterns, nondrinkers were included in the lowest category. For reference, an average drink of beer, wine, or liquor contains about 0.6 oz of alcohol. All models were adjusted for age, weight, and total energy intake. Total energy intake estimated by the food frequency questionnaire was used in models with alcohol from the drinking pattern and food frequency questionnaires, whereas total energy intake from seven-day food records was used in models with alcohol from these records. To evaluate whether the approach used to adjust for energy intake affected results,⁵⁴ energy intake from non-alcoholic sources only also was used in models with alcohol intake estimated from the drinking pattern questionnaire. Dietary fat consumption and physical activity did not modify associations between hormone levels and alcohol, and these variables were not included in models. All analyses were performed using SAS Statistical Software.⁵⁵

The drinking pattern questionnaire asked more precise information about alcohol ingestion than the food questionnaire, and the seven-day food records were sometimes completed over holidays when people might alter their drinking patterns. Therefore, findings based on the drinking pattern questionnaire are reported in detail and discrepancies generated by the other two questionnaires are noted.

Results

A total of 107 women with a mean age of 29.6 (± 5.1) years participated in the cross-sectional study. The women generally were well educated; all finished at least 12 years of school and 64 percent finished at least 16 years of school. Their mean body weight was 63.6 (± 11.4) kg and their mean body mass index (BMI) was 23.3 (± 4.1) kg/m^2 . Ninety-nine women (93 percent) reported regular menstrual cycles during the past year and their mean usual cycle length was 28.5 (± 2.3) days.

The median alcohol intake of participants was 1.8 oz/week (5-95 percentile: 0-9.9 oz) which is equal to about three drinks. Sixteen participants (15 percent) were abstainers, 34 (32 percent) were light drinkers, 46 (43 percent) were moderate, and 11 (10 percent) were heavy drinkers. Participants' median total energy-consumption from the food frequency questionnaire was 1,920 kcal/day (5-95 percentile: 1,000-3,378 kcal).

Geometric mean and plasma estrogen levels in the follicular, midcycle, and luteal phases of the menstrual

cycle are shown in Table 1. Plasma levels of SHBG during the different menstrual cycle phases were highly correlated. Pearson correlations were 0.94 and 0.87 for the follicular and midcycle, and follicular and luteal phases, respectively. The mean SHBG concentration for the combined follicular, midcycle, and luteal phases was used, therefore, for analysis of associations with alcohol intake. The geometric mean of the SHBG concentration, averaged across the three phases, was 39 nmol/l (95% confidence interval [CI] = 18-84 nmol/l). The androgens, androstenedione and DHEAS, also were highly correlated during the different menstrual cycle phases. For androstenedione, Pearson correlations were 0.81 for the follicular and midcycle phases and 0.72 for the follicular and luteal phases. For DHEAS, coefficients were 0.89 and 0.87 for the same phases. Therefore, mean androstenedione and DHEAS concentrations also were used in analyses. Geometric means of androstenedione and DHEAS, averaged across the three phases, were 9 nmol/l (CI = 5-17 nmol/l) and 8 μ mol/l (CI = 3-18 μ mol/l), respectively.

Associations of alcohol ingestion with plasma estrogens during the follicular, midcycle, and luteal phases

of the menstrual cycle are shown in Table 2. After adjusting for age, weight, and energy intake, none of the plasma estrogens were related to alcohol ingestion. Similar results were obtained when alcohol was included in models as a continuous or categorical variable. SHBG and DHEAS also were not associated with daily alcohol intake. After adjusting for age, weight, and energy intake, SHBG increased by 4.6 percent (CI = -9.1 to 20.4 percent) and DHEAS decreased by 0.9 percent (CI = -15.3 to 15.8 percent) for each ounce of alcohol consumed daily.

The association of alcohol ingestion with plasma androstenedione was nonlinear, with androstenedione levels increasing with increasing alcohol at lower, but not at higher, levels of intake. Transformation of alcohol to the log₁₀ scale improved the fit of the model which indicated that, after adjusting for age, weight, and energy intake, a 50 percent increase in alcohol intake among drinkers was associated with a significant ($P = 0.001$) 2.3 percent (CI = 1.0-3.7 percent) increase in plasma androstenedione level. We also fit this model using categories of alcohol ingestion and the results are shown in Table 3. Moderate and heavy drinkers had increased plasma androstenedione levels relative to

Table 1. Geometric means and 95% confidence intervals (CI) for plasma estrogen levels by menstrual cycle phase

| | Follicular | | Midcycle | | Luteal | |
|--------------------------|------------|-------------|----------|-------------|--------|-------------|
| | Mean | (CI) | Mean | (CI) | Mean | (CI) |
| 17- β Estradiol | | | | | | |
| Total (pmol/l) | 141 | (57-346) | 256 | (92-712) | 254 | (93-693) |
| Free (pmol/l) | 2 | (1-6) | 4 | (1-12) | 4 | (1-12) |
| Albumin bound (pmol/l) | 26 | (10-69) | 48 | (16-141) | 48 | (15-150) |
| SHBG bound (pmol/l) | 112 | (45-276) | 202 | (72-568) | 200 | (75-539) |
| Estrone (pmol/l) | 210 | (95-465) | 324 | (143-732) | 314 | (135-730) |
| Estrone sulfate (pmol/l) | 1,364 | (517-3,597) | 2,449 | (752-7,976) | 2,240 | (707-7,097) |

Table 2. Percent differences in plasma estrogen levels by menstrual cycle phase related to increasing daily alcohol intake by one ounce^a

| | Follicular | | Midcycle | | Luteal | |
|--------------------------|--------------|-------------------|--------------|-------------------|--------------|-------------------|
| | % Difference | (CI) ^b | % Difference | (CI) ^b | % Difference | (CI) ^b |
| 17- β Estradiol | | | | | | |
| Total (pmol/l) | -2.6 | (-17.8-15.4) | 4.8 | (-13.7-27.2) | 11.0 | (-8.1-34.1) |
| Free (pmol/l) | -5.1 | (-20.4-13.3) | -0.6 | (-18.8-21.8) | 4.4 | (-14.9-28.1) |
| Albumin bound (pmol/l) | -5.0 | (-21.0-14.4) | 1.0 | (-17.6-23.8) | 7.8 | (-13.0-33.7) |
| SHBG bound (pmol/l) | -2.0 | (-17.3-16.3) | 5.6 | (-13.1-28.5) | 11.7 | (-7.4-34.7) |
| Estrone (pmol/l) | 9.6 | (-5.5-27.0) | 5.6 | (-9.7-23.5) | 14.3 | (-2.3-33.7) |
| Estrone sulfate (pmol/l) | -13.9 | (-28.4-3.6) | -6.1 | (-25.5-18.3) | 9.3 | (-12.2-36.1) |

^a Estimates from linear regression models including age, weight, and energy intake.

^b CI = 95% confidence interval.

Table 3. Percent difference in plasma androstenedione levels by category of alcohol intake^a

| Alcohol intake | % Difference | (CI) ^b |
|----------------------------|--------------|-------------------|
| Abstainers (n = 16) | ref | — |
| Light drinkers (n = 34) | 1.5 | (- 16.2-22.9) |
| Moderate drinkers (n = 46) | 27.4 | (5.6-53.5) |
| Heavy drinkers (n = 11) | 23.8 | (- 3.4-58.7) |

^a Estimates from linear regression models including age, weight, and energy intake.

^b CI = 95% confidence interval.

abstainers, although probably because of the smaller number of heavy drinkers, the association was significant only for moderate drinkers. A test of heterogeneity indicated that the relation of alcohol intake to plasma androstenedione level did not differ by whether the source was beer, wine, or liquor.

Alcohol was not a major source of energy for women in this study. The median percent of calories from alcohol was 2.4 (5-95 percentile: 0-8.7) from food frequency questionnaires and 1.9 (5-95 percentile: 0-9.8) from seven-day food records. Alcohol-hormone associations were unchanged when energy from non-alcoholic sources was used in place of total energy in regression models.

Seventeen women (16 percent) did not have a progesterone rise of at least 3 ng/ml during the luteal phase indicating that the cycle was anovulatory or that days 21 through 23 were not truly mid-luteal. Alcohol ingestion was not associated significantly with the probability of a progesterone rise ($P = 0.13$), and elimination of women without a rise did not affect associations between alcohol and plasma estrogens or androgens.

Alcohol intakes measured by the food frequency questionnaire and seven-day food records were correlated highly with intakes from the drinking pattern questionnaire; Pearson correlations were 0.77 and 0.66, respectively. Consequently, results generally were comparable regardless of the source of data on alcohol consumption. There were, however, a few exceptions. Alcohol intake only from the food frequency questionnaire showed a significant positive association with plasma estrone in the luteal phase; an increase of one ounce of alcohol per day was associated with a 39.8 percent (CI = 3.4-89.0 percent) increase in estrone. Additionally, alcohol intake only from seven-day food records showed a marginally significant negative association with plasma estrone sulfate level in the follicular phase. The positive association of alcohol intake with plasma androstenedione was highly significant using all three sources of data on intake. Data only

from seven-day records, however, indicated a linear relation. Percent differences in plasma androstenedione for light, moderate, and heavy drinkers relative to nondrinkers were, respectively, -2.9 percent (CI = -18.6-15.8 percent), 29.6 percent (CI = 8.0-55.6 percent), and 51.6 percent (CI = 16.3-97.7 percent).

Discussion

Our finding of a positive relation between alcohol ingestion and plasma androstenedione level is consistent with the elevated levels observed in female alcoholics.^{35,46} Alcohol could increase plasma androstenedione by stimulating secretion by the adrenals or ovaries or by altering metabolism by the liver and peripheral tissues. In premenopausal women, approximately half of the circulating androstenedione is produced by the adrenal and half by the ovary.⁵⁶ Alcohol's effect on secretion of androstenedione by the adrenals, *per se*, is not known, but at low levels, alcohol has been shown to stimulate the adrenals to produce cortisol, another steroid hormone which, like androstenedione, is synthesized from LDL-cholesterol.⁵⁷ Alcohol also enhances luteinizing hormone (LH) production which stimulates ovarian thecal cells to secrete androstenedione.⁴⁴ This androstenedione is metabolized to estrone and estradiol by ovarian granulosa cells in response to follicle stimulating hormone (FSH), which Mello *et al*⁵⁸ have hypothesized may be inhibited by alcohol. Our finding of an elevation in plasma androstenedione, but not estrone and estradiol, with alcohol ingestion, is consistent with inhibition of FSH secretion by alcohol.

Androstenedione is metabolized by the liver²⁶ and by peripheral tissues, particularly adipose tissue.⁵⁹ Alcohol also could affect plasma androstenedione levels by altering its metabolism and excretion. Little is known, however, about the effect of alcohol ingestion on metabolism of androstenedione in women.

The results from this cross-sectional analysis are not concordant with the analysis of the controlled feeding part of this study, in which Reichman *et al*⁴⁷ did not detect a change in plasma androstenedione with consumption of one ounce of alcohol/day. Ingestion of this level of alcohol was associated with increased plasma DHEAS in the follicular phase of the menstrual cycle and with increased estradiol and estrone mid-cycle. Alcohol consumption also was related to increased estradiol in pooled 24-hour urine specimens collected midcycle and with increased estradiol, estrone, and estriol in pooled 24-hour urine specimens collected during the luteal phase. In the current analysis, we did not evaluate associations of urinary estrogens with alcohol ingestion.

The reason for discrepancies in findings from the cross-sectional and controlled feeding studies is unclear but could be due to differences in study design, the exposure measured, or participants' responses to alcohol. In the controlled feeding study, each woman's hormones were measured during periods of alcohol ingestion and no alcohol ingestion and individual differences in plasma hormone levels between periods were evaluated. In the cross-sectional study, no attempt was made to control alcohol ingestion, but plasma hormone levels of women who consumed different amounts of alcohol were compared after adjusting for characteristics, such as age and body weight, that could affect observed associations. Energy and fat ingestion were kept constant in the controlled feeding study because of reported associations between dietary fat and plasma estrogen levels.⁶⁰ In the cross-sectional study, we adjusted for energy, but not fat, intake because dietary fat did not affect alcohol-hormone associations in our analysis. Women in the controlled feeding study consumed a constant amount of alcohol each day, whereas women in the cross-sectional study reported their average consumption, which probably varied by day and may have included binge drinking. Exposures, therefore, were not exactly the same in both studies. The 34 women who participated in the controlled feeding part of the study also may have had a different response to alcohol than the entire sample of participants in the cross-sectional study. Variability in alcohol metabolism has been reported by others.⁶¹

The hormonal effects of chronic alcohol ingestion in non-alcoholic premenopausal women have been investigated in only one other study. Mendelson *et al*⁶² reported no menstrual cycle or hormonal abnormalities in women living in a research ward who consumed an average of less than three drinks (approximately 1.8 oz alcohol) per day for 35 days. Women who consumed more than three drinks/day, however, exhibited an increase in anovulatory cycles characterized by smaller increments in estradiol just prior to the expected time of ovulation. Our midcycle plasma specimens, collected on days 12 through 15 of the menstrual cycle, were not pre-ovulatory for most women, which may explain discrepancies in findings from the study by Mendelson *et al*⁶² and both our cross-sectional and controlled feeding studies.

Acute administration of alcohol to premenopausal women results in an increase in plasma estradiol in the follicular⁴⁵ and luteal⁴⁴ phases of the menstrual cycle. In one study of acute administration, subjects consumed approximately 1.7 oz of alcohol in 19 minutes⁴⁵ and in a second study, approximately three ounces were consumed in three hours.⁴⁴ In the studies of chronic intake discussed above, women ingested substantially lower

alcohol dosages for longer periods of time, which may explain differences in findings.

The lack of an association between alcohol ingestion and plasma estrogen levels in this cross-sectional study could be due, at least in part, to limitations in study design. Although we attempted to recruit heavy drinkers, only 11 participants reported consuming the equivalent of two or more drinks per day. Blood specimens were collected on the same day of the menstrual cycle measured from the start of last menses to account for the wide fluctuations in plasma estrogens over the menstrual cycle. Because of the variability in timing of ovulation and menstrual cycle length, however, a more powerful design which times blood collections in relation to the LH peak may be necessary to detect an association between alcohol and plasma estradiol levels. Furthermore, evaluation of the relation of alcohol ingestion to plasma hormone levels over multiple menstrual cycles would distinguish sporadic from sustained differences and enhance interpretability of results. Better measures of alcohol ingestion, as well as more sensitive and specific hormone assays, also may be necessary, particularly if the magnitude of the hypothesized association is small.

Although estrogens are believed to play a role in the etiology of breast cancer,⁶³ the relation of plasma hormones to risk is unclear. Breast-fluid estradiol levels are not correlated with plasma levels,⁶⁴ and results of epidemiologic studies on the association of plasma hormone levels to breast cancer risk have been inconsistent.⁶⁵ The findings of this cross-sectional study do not support the hypothesis that the increased risk of breast cancer related to alcohol ingestion is mediated by elevated plasma-estrogen levels premenopausally. Androstenedione can be aromatized to estrone and subsequently reduced to estradiol in the breast⁶⁶ and breast cancer patients have been reported to have elevated blood levels of androstenedione.²⁴ Alcohol ingestion, therefore, possibly could increase breast cancer risk by increasing the blood level of androstenedione. Alcohol has a wide range of metabolic effects and other non-endocrine modes of action are possible.⁶⁷ Further studies are needed to determine the mechanism by which alcohol ingestion increases breast cancer risk.

Footnote

Estradiol (pmol/l) = Estradiol (pg/ml)/272.37 × 1000;
Estrone (pmol/l) = Estrone (pg/ml)/270.36 × 1000;
Estrone Sulfate (pmol/l) = Estrone Sulfate (pg/ml)/350.42 × 1000; Androstenedione (nmol/l) = Androstenedione (ng/ml)/286.40 × 1000; DHEAS (μmol/l) = DHEAS (μg/ml)/368.47 × 1000.

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