ORIGINAL ARTICLE

Consumption of animal products, their nutrient components and postmenopausal circulating steroid hormone concentrations

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Background/Objectives: Little is known about nutritional factors that influence circulating concentrations of steroid hormones, which are consistently associated with risk of breast cancer for postmenopausal women. We aimed to investigate the association between consumption of animal products and the plasma concentrations of steroid hormones and sex hormone-binding globulin (SHBG).
Subjects/Methods: Cross-sectional analysis was conducted on plasma from 766 naturally postmenopausal women. We measured plasma concentrations of steroid hormones and SHBG, and estimated dietary intakes using a 121-item food frequency questionnaire. Log-transformed values of hormone concentrations were regressed on quartiles of intake of meat and dairy products among food items, and fats, proteins and cholesterol among nutrient intake.
Results: Total red and fresh red meat consumption was negatively associated with SHBG levels (P for trend $= 0.04$ and < 0.01, respectively). Mean SHBG concentrations were $\sim 8\%$ and 13% lower for women in the highest quartile compared with the lowest quartile of total red and fresh red meat consumption, respectively. Positive associations were observed between dairy product consumption and total and free estradiol concentrations (P for trend $= 0.02$ and 0.03, respectively). Mean concentrations of total and free estradiol were 15 and 14% higher for women in the highest quartile of dairy product consumption than for those in the lowest quartile, respectively. No associations were observed with consumption of processed meat, chicken, fish, eggs, cholesterol, fats or protein.
Conclusions: Our study suggests that greater consumption of total red and fresh red meat and dairy products might influence circulating concentrations of SHBG and estradiol, respectively. Confirmation and further investigation is required.
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Introduction

High circulating concentrations of steroid hormones and low concentrations of sex hormone-binding globulin (SHBG) have been consistently associated with increased risk of

postmenopausal breast cancer (Rock *et al* 2008). It is less clear which factors influence circulating steroid hormone concentrations and subsequent risk of breast cancer (Carruba *et al.*, 2006), although dietary factors might affect metabolism and bioavailability of steroid hormones (Fung *et al.*, 2007).

Animal-derived foods possibly contain endogenous estrogens or estrogen metabolites, and as a result, their consumption might directly contribute to human circulating steroid hormone concentrations (Fritsche and Steinhart, 1998; Andersson and Skakkebaek, 1999). Alternatively, consumption of these foods might influence endogenous steroid hormone production indirectly through their nutrient components. Cholesterol is a major substrate for steroid

hormone synthesis (Lucenteforte et al., 2008), and animal products are rich sources of cholesterol as well as saturated fat and protein (Taylor et al., 2007). A dietary intervention study (Carruba et al., 2006) showed that changing from a diet high in animal fat and protein to one that was high in vegetable fat and protein reduced circulating estrogen levels by more than 40%.

Small epidemiological studies conducted in the 1980s reported higher circulating concentrations of 17-β-estradiol (Barbosa et al., 1990), androstenedione (Adlercreutz et al., 1989) and testosterone (Adlercreutz et al., 1989) for nonvegetarian compared with vegetarian postmenopausal women. Nonvegetarian postmenopausal women have also been found to have lower SHBG levels than vegetarians (Armstrong et al., 1981), but only when compared with nonobese vegetarians in one study (Adlercreutz et al., 1989). Moreover, compared with vegetarians, women who ate meat consumed higher total fat, saturated fat (Barbosa et al., 1990), monounsaturated fat, protein, cholesterol (Barbosa et al., 1990) and total calories (Adlercreutz et al., 1989).

In a larger more recent study (Thomas et al., 1999), the differences in circulating steroid hormone concentrations observed between meat eaters, vegetarian and vegan post-menopausal women disappeared after adjustment for body mass index (BMI). Adjusting for BMI also attenuated the associations between dietary factors and circulating steroid hormone concentrations observed by another study (Fung et al., 2007).

Despite the reports that a diet high in animal products such as red and processed meat is associated with an increase in circulating steroid hormones and a reduction in SHBG concentrations, this relationship is yet to be fully elucidated (Fung et al., 2007). Similarly, reports from studies investigating the effect of dietary fat and protein on circulating steroid hormone concentrations have been unclear (Prentice et al., 1990; Holmes et al., 2000; Fontana et al., 2006). If any associations between these dietary factors and circulating steroid hormone concentrations exist, dietary intervention may be effective in modifying the hormonal profile and potential risk of breast cancer for postmenopausal women.

We aimed to investigate associations between the consumption of animal products and their major nutrient components on circulating steroid hormone and SHBG concentrations for postmenopausal women not taking any exogenous hormones.

Subjects and methods

The Cohort
The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41 514 people (24 469 women) aged between 27 and 81 years (99.3% aged between 40 and 69 years). Recruitment occurred between 1990 and 1994 in the Melbourne metropolitan area. All participants had their blood collected at baseline, of which 2 ml plasma was stored in liquid nitrogen using heparin as an anticoagulant. Details of the study have been published elsewhere (Giles and English, 2002). The Cancer Council Victoria's Human Research Ethics Committee approved the study protocol and all subjects provided a written consent to participate.

Selection of the study sample
Eligibility for this study was restricted to the 10 516 (43% of all women) women who were naturally postmenopausal at baseline and who had not had breast cancer or ovarian cancer before baseline (2%) and were not taking hormone replacement therapy (15%). A random sample of 916 women was selected for measurement of steroid hormones using baseline blood samples. Women with missing values for age at menopause ($n = 3$), energy from diet ($n = 1$) or potential confounders ($n = 18$) were excluded. We also excluded women who reported extreme values of total energy intake ($<$1st percentile or $>$99th percentile), or had a previous diagnosis of angina, myocardial infarction or diabetes, because their diets were not representative of the whole cohort, and we could not exclude the possibility that they had changed their diet after a recent diagnosis ($n = 109$). Hormone measurements were not possible for 19 women, because they had insufficient plasma. A total of 766 women were, therefore, included in the present study.

Measurement of potential determinants
Participants were asked questions about putative breast cancer risk factors such as reproductive history (age at menarche, age at menopause, parity, duration of lactation, oral contraceptive use, hormone replacement therapy use), demographic factors (age, country of birth, highest level of education) and lifestyle factors (physical activity, alcohol consumption, smoking habits). Height and weight were measured directly for each participant according to written protocols that were based on standard procedures. BMI was calculated by dividing weight in kilograms by height in meters square (Macinnis et al., 2004).

Measurement of dietary consumption
Subjects completed a 121-item food frequency questionnaire, specifically developed for the MCCS (Ireland et al., 1994). There were 22 items relating to intake of fresh red meat, processed meat, chicken and fish. Fresh red meat intake included: veal or beef schnitzel, roast beef or veal, beef steak, rissoles (meat balls) or meat loaf, mixed dishes with beef, roast lamb or lamb chops, mixed dishes with lamb, roast pork or pork chops, rabbit or game (English et al., 2004). Processed meat items included salami or continental sausages, sausages or frankfurters, bacon, ham (including prosciutto), corned beef and manufactured luncheon meats (including mortadella). Total red meat intake was calculated from the combination of all fresh red and processed red meat

items. Participants were asked whether they ate roast, fried, boiled or steamed chicken and mixed dishes with chicken. Fish intake included questions on steamed, grilled or baked, fried (including take-away), smoked and canned fish (tuna, salmon and sardines) and a generic seafood question (English *et al.*, 2004). Dairy products included cheese (cottage, ricotta, fetta, low fat, parmesan, cream and cheddar cheese), ice-cream, custard, cream or sour cream, yoghurt, milk drinks and milk consumed with cereal, tea and coffee (Ireland *et al.*, 1994). Butter was not included in the dairy products category because of its high fat content and lack of major nutrients associated with this group, for example, calcium and protein (Larsson *et al.*, 2006). Intake of energy was computed using Australian food composition tables (Lewis *et al.*, 1995), and included energy from the food frequency questionnaire and energy from alcohol consumption.

Measurement of circulating concentrations of steroid hormones
Plasma samples were retrieved from liquid nitrogen, aliquoted into 450 µl amounts and shipped on dry ice in batches of 80 samples each to the laboratory of one of the authors (HAM) wherein SHBG, total estradiol, estrone sulfate, testosterone, dehydroepiandrosterone sulfate (DHEAS) and androstenedione were measured. Assignment to batches was done randomly. In total, 10% of the samples in each batch were aliquots from pooled plasma that had been stored with the samples from the participants. The laboratory was blind to status of the samples and one scientist carried out all the measurements. Samples were thawed in a warm water bath, vortexed rapidly for a few seconds and centrifuged at 2000 r.p.m. ($210 \times g$) for 10 min. Testosterone followed by total estradiol was measured by electrochemiluminescence immunoassay (Elecsys 2010 analyzer, Roche Diagnostics GmbH, Mannheim, Germany). Estrone sulfate was measured by radioimmunoassay (DSL-5400; Diagnostic Systems Laboratories, TX, USA). DHEAS was measured by competitive immunoassay (IMMULITE analyzer, DPC, Los Angeles, CA, USA). Andro-stenedione was analyzed by radioimmunoassay (DSL-4200, DSL). SHBG was measured by immunometric assay (IMMU-LITE analyzer, DPC). All hormones were measured between 6 and 13 years after blood collection (median, 9 years). Lower detection limits were 18 pmol/l for total estradiol, 0.03 nmol/l for estrone sulfate, 0.1 nmol/l for testosterone, 0.2 µmol/l for DHEAS, 0.02 nmol/l for androstenedione and 2 nmol/l for SHBG.

From the pooled plasma, the overall coefficients of variation were 10% (8% within batches and 6% between batches) for total estradiol at a concentration of 157 pmol/l; 15% (13% within batches and 8% between batches) for estrone sulfate at a concentration of 5.7 nmol/l; 7% (4% within batches and 5% between batches) for testosterone at a concentration of 4.3 nmol/l; 10% (9% within batches and 6% between batches) for DHEAS at a concentration of 4.0 µmol/l; 15% (11% within batches and 9% between

batches) for androstenedione at a concentration of 2.6 nmol/l; and 7% (6% within batches and 4% between batches) for SHBG at a concentration of 45 nmol/l.

Concentration of protein unbound estradiol (free estradiol) was calculated from the total concentration and from the concentration of SHBG using the law of mass action (Sodergard *et al.*, 1982) under the assumption of a fixed albumin concentration of 40 g/l (Endogenous Hormones Breast Cancer Collaborative Group 2003).

A reliability study was performed before study commencement. Plasma samples from 45 women who had given blood twice ∼1 year apart were each divided into two aliquots. The two aliquots were measured in separate batches a week apart. As a measure of reliability, we used the intraclass correlation, which is the proportion of total variance due to variation between persons, where the total variance included components due to between-persons, between-sampling occasions and residual variance. For total estradiol, the total variance did not include between-sampling occasions, because we had insufficient plasma samples. From the reliability study, the intraclass correlation (95% CI) for total estradiol was 0.93 (0.85, 1.00), 0.85 (0.78, 0.92) for estrone sulfate, 0.65 (0.52, 0.77) for testosterone, 0.87 (0.81, 0.93) for DHEAS, 0.61 (0.44, 0.78) for androstenedione and 0.90 (0.85, 0.95) for SHBG.

Statistical analysis
Nutrient intakes were defined as nutrient density (nutrient divided by energy) to minimize errors due to under- and overreporting of total energy intake and to rank women into quartiles that were independent of individual energy intake (Willett, 1998). Quartiles of animal products and nutrient intakes were created from the distributions across the entire cohort of participants.

Using a linear regression model, natural log-transformed values of steroid hormone concentrations were regressed on quartiles of each food and nutrient intake, and least-squared means were calculated for each quartile. Regressions were adjusted for age, country of birth, laboratory batch, BMI, total energy from diet, alcohol consumption, level of education, age at menarche, age at menopause, age at first birth (live birth or gestation >24 weeks) and parity, duration of lactation, oral contraceptive use, hormone replacement therapy use, physical activity and smoking. We performed tests for trend (using likelihood ratio test) to investigate a possible linear dose–response relation between food and nutrient intakes and steroid hormone concentrations by regressing the outcome variables onto the median of the quartiles for each dietary variable (pseudo-continuous variable). The relative contribution of each food and nutrient intake to the variation in steroid hormone concentrations was assessed using the coefficient of determination, R^2.

Statistical analyses were performed using Stata/SE 10.0 (Stata Corporation, College Station, TX, USA). All *P*-values were two-sided and $P \leqslant 0.05$ was considered to be statistically significant.

Results

The study involved 766 naturally postmenopausal women not currently taking hormone replacement therapy (11% were past users). The mean age was 61 years (range, 46–70 years) and the mean age at menopause was 50 years (range, 34–62 years). In total, 73% were born in Australia, New Zealand or the United Kingdom and 27% in either Italy or Greece. Table 1 summarizes the reproductive history, demographic and lifestyle characteristics of the women, and the plasma concentrations of steroid hormones and SHBG.

Table 2 shows the least square means for each hormone and SHBG concentrations by quartile of intake of total red and fresh red meat and dairy products for the model adjusted for all potential confounders. Total red and fresh red meat consumption were inversely associated with the circulating concentration of SHBG. Mean circulating concentrations of SHBG were $\sim$8 and 13% lower for women in the highest quartile compared with women in the lowest quartile of total red meat and fresh red meat consumption, respectively (P for trend $= 0.04$ and < 0.01, respectively). Weak positive associations were observed between consumption of total red meat and estrone sulfate (P for trend $= 0.07$), and between fresh red meat and androstenedione (P for trend $= 0.08$). Higher consumption of dairy products was associated with higher concentrations of total and free estradiol (P for trend $= 0.02$ and 0.03, respectively). Compared with women in the lowest quartile, women in the highest quartile for consumption of dairy products had mean circulating concentrations of total and free estradiol that were 15 and 14% higher, respectively (P for trend $= 0.02$ and 0.03, respectively).

No associations were found between circulating concentrations of steroid hormones or SHBG and consumption of processed meat, chicken, fish, eggs or butter under the fully adjusted model, as seen in Table 2.

We investigated the major nutrient components of animal products, which included cholesterol, protein, total fat and categories of fat, saturated fat, monounsaturated fat, polyunsaturated fat, transunsaturated fat, stearic acid, palmitoleic acid, linoleic acid, arachidonic acid and translinoleic acid. None of these components were associated with steroid hormones or SHBG concentrations (Table 3).

None of the food items or nutrients explained more than 1% of the total variation in concentration of any steroid hormone or SHBG (results not shown).

Adjusting for total consumption of fruit and vegetables did not materially change the observed associations between circulating steroid hormones and SHBG and food items or nutrients (not shown).

Discussion

Analysis of our sample of 766 naturally postmenopausal women showed a negative association between total red and fresh red meat consumption and circulating concentrations of SHBG and a positive association between consumption of dairy products and total and free estradiol concentrations. We did not detect any associations between circulating steroid hormones or SHBG concentrations and consumption of processed meat, chicken, fish, eggs, butter, fat or fatty acids, cholesterol or protein.

Previous studies (Fritsche and Steinhart, 1998; Andersson and Skakkebaek, 1999) report that meat possibly contains estrogens and increases circulating concentrations of androgens (Adlercreutz et al., 1989). In the Nurses' Health Study (Fung et al., 2007), total estrogen and free estradiol concentrations were significantly higher among women in the highest category for consumption of the Western dietary pattern that comprised of high intakes of red and processed meats. However, this association disappeared after adjustment for BMI. We only found evidence of a weak association between consumption of total red meat and estrone sulfate and between fresh red meat and androstenedione, neither of which were statistically significant.

Consistent with findings from other studies, we observed lower mean concentrations of SHBG for women in the highest quartile of total red and fresh red meat consumption. A previous study consisting of 93 postmenopausal women (Armstrong et al., 1981) reported lower concentrations of SHBG for nonvegetarians (16%) compared with vegetarians. Another study (Thomas et al., 1999) also reported concentrations of SHBG that were 6 and 12% higher for vegetarian and vegans, respectively, than for nonvegetarians.

A recent study of Asian American women (Wu et al., 2009) also observed similar results to ours. No associations were observed with circulating estrogen concentrations, but SHBG concentrations were 23% lower for women in the highest category for meat intake and this inverse association remained after adjustment for BMI. Our models included adjustment for BMI, which being inversely associated with SHBG and directly associated with meat consumption by the total energy intake could be a confounder of the association between SHBG and meat consumption (Kaaks et al., 2003). Furthermore, our findings persisted after adjusting for total consumption of fruit and vegetables, thus ruling out the possibility that they were due to lower consumption of fruit and vegetables by women in the highest quartile of meat intake. However, further investigation is required as to why there was the slightly stronger effect observed for fresh red meat compared with total red meat, for example, differences in nutrient composition, preparation/cooking processes and preservatives.

The higher mean concentrations of total and free estradiol associated with the high dairy consumption that we observed, lends support to the hypothesis that milk and dairy products are major sources of estrogens in the human diet (Ganmaa and Sato, 2005). According to one study (Ganmaa and Sato, 2005), this may be due to the fact that current milk supplies are obtained from cows in late pregnancy when circulating estrogen levels are higher than

Table 1 Characteristics of the study population

Characteristic	Category	Values
Age in years, mean (s.d.)		61 (6)
Age at menopause in years, mean (s.d.)		50 (4)
Country of birth, n (%)	Australia/New Zealand	516 (67.4)
	UK	42 (5.5)
	Italy	115 (15.0)
	Greece	93 (12.1)
Level of education, n (%)	Primary school	207 (27.0)
	Some high school	338 (44.1)
	Completed high school	124 (16.2)
	Degree/diploma	97 (12.7)
Age at menarche, n (%)	<12 years	99 (12.9)
	12 years	145 (18.9)
	13 years	195 (25.5)
	14+ years	327 (42.7)
Parity (age at first pregnancy and number of full-term pregnancies), n (%)	Nulliparous	91 (11.9)
	<25 and 1	11 (1.4)
	<25 and >1	316 (41.3)
	⩾25 and 1	48 (6.3)
	⩾25 and >1	300 (39.2)
Duration of lactation, n (%)	Never	197 (25.7)
	Up to 6 months	165 (21.5)
	7–12 months	132 (17.2)
	13–24 months	155 (20.2)
	More than 24 months	117 (15.3)
OC use, n (%)	Never	433 (56.5)
	Ex	333 (43.5)
HRT use, n (%)	Never	679 (88.6)
	Ex	87 (11.4)
Physical activity category, n (%)	None	155 (20.2)
	Low	160 (20.9)
	Medium	305 (39.8)
	High	146 (19.1)
Smoking, n (%)	Never	545 (71.1)
	Current	68 (8.9)
	Former	153 (20.0)
Alcohol consumption, n (%)	Abstainers	356 (46.5)
	Ex drinkers	26 (3.4)
	Low	310 (40.5)
	Medium	61 (8.0)
	High	13 (1.7)
BMI (kg/m^2), mean (s.d.)		27.2 (4.6)
Energy from diet (MJ/day), mean (s.d.)		8.7 (2.9)
Hormones and SHBG, geometric mean (95% CI)[a]		
Total estradiol (pmol/l)		58.07 (56.04–60.17)
Free estradiol (pmol/l)		0.80 (0.77–0.83)
Estrone sulfate (nmol/l)		2.91 (2.81–3.01)
Testosterone (nmol/l)		0.67 (0.64–0.71)
DHEAS (μmol/l)		1.43 (1.35–1.51)
Androstenedione (nmol/l)		2.11 (2.02–2.21)
SHBG (nmol/l)		50.24 (48.74–51.77)

Abbreviations: BMI, body mass index; CI, class interval; DHEAS, dehydroepiandrosterone sulfate; HRT, hormone replacement therapy; OC, oral contraceptive; SHBG, sex hormone-binding globulin.
[a]The number of missing measures were 15 for total estradiol, 16 for free estradiol, 15 for estrone sulfate, 4 for testosterone, 3 for DHEAS, 3 for androstenedione and 1 for SHBG.

usual. However, a German market basket survey (Hartmann and Steinhart, 1998) that examined the hormone content of the local food supply found that concentrations of steroid hormones from dietary sources were insignificant compared with daily endogenous production. As no other studies, to our knowledge, have specifically investigated the association between dairy products and steroid hormone concentrations, our results should be interpreted with caution. Given that the consumption of dairy products has been positively correlated with the risk of breast cancer ($r = 0.82$) (Ganmaa and Sato, 2005), more studies are clearly needed on this food group.

Table 2 Least-squared means of circulating hormones and SHBG concentration by quartiles of red meat and dairy products[a]

Food item	Quartile[b]	E2	E2_free	E1S	T	DHEAS	AD	SHBG
Total red meat	1	60.74	0.81	1.1	0.74	1.63	2.08	53.32
	2	61.92	0.86	1.16	0.75	1.61	2.29	49.64
	3	57.16	0.81	1.19	0.69	1.67	2.19	48.23
	4	63.75	0.89	1.22	0.77	1.67	2.34	48.88
	P for trend	0.53	0.16	0.07	0.69	0.74	0.13	0.04
Fresh red meat	1	62.13	0.83	1.13	0.71	1.56	2.02	54.43
	2	60.03	0.83	1.12	0.74	1.61	2.23	49.52
	3	59.42	0.83	1.21	0.75	1.71	2.3	49.71
	4	60.95	0.86	1.17	0.74	1.7	2.3	47.35
	P for trend	0.79	0.39	0.31	0.7	0.27	0.08	<0.01
Dairy products[c]	1	55.42	0.77	1.15	0.68	1.55	2.15	49.18
	2	62.54	0.86	1.17	0.73	1.64	2.18	51.02
	3	61.75	0.85	1.2	0.81	1.84	2.37	50.64
	4	63.93	0.88	1.08	0.71	1.51	2.09	50.18
	P for trend	0.02	0.03	0.21	0.47	0.88	0.91	0.79

Abbreviations: AD, androstenedione; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E2, total estradiol; E2_free, free estradiol; EIS, estrone sulfate; HRT, hormone replacement therapy; SHBG, sex hormone-binding globulin; T, testosterone.
[a]Estimates are from the linear regression model of the logarithm of hormone concentration including each food item separately. Estimates were adjusted for laboratory batch, age, country of birth, level of education, age at menarche, parity, duration of lactation, oral contraceptive use, HRT, age at menopause, physical activity, smoking, alcohol consumption, BMI and energy from diet.
[b]For total red meat intake, quartile 1 is $\geqslant 0$ times/week, quartile 2 is $\geqslant 5$ times/week, quartile 3 is $\geqslant 7$ times/week and quartile 4 is $\geqslant 10$ times/week. For fresh red meat intake, quartile 1 is $\geqslant 0$ times/week, quartile 2 is $\geqslant 3$ times/week, quartile 3 is $\geqslant 4.5$ times/week, and quartile 4 is $\geqslant 6.5$ times/week. For dairy products intake, quartile 1 is $\geqslant 0$ times/week, quartile 2 is $\geqslant 18$ times/week, quartile 3 is $\geqslant 32.5$ times/week and quartile 4 is $\geqslant 48$ times/week.
[c]Dairy products include cheese, ice cream, custards, cream, yoghurt and milk drinks.

Although we found no evidence of an association between fish intake and any of the steroid hormones or SHBG, one intervention study (Jacques *et al.*, 1992) reported elevated concentrations of SHBG for women who replaced their regular intake of beef, pork, eggs and milk with lean white fish. Fish contains omega 3 (*n*-3) polyunsaturated fatty acids and high-density lipoprotein-cholesterol (Kabir *et al.*, 2007), which has been positively correlated with SHBG (Thomas *et al.*, 1999). A low intake of fish by our study subjects (median, 1.5 times per week) may make it difficult to detect an association for this food group. Similarly, no associations were observed for other low intake foods in our study such as processed meat, chicken, eggs and butter (median, 0–2 times per week).

We did not detect any associations between concentrations of steroid hormones or SHBG and dietary fat or fatty acids. This agrees with the findings from another Australian study of 33 women (Ingram *et al.*, 1987), but is inconsistent with a Japanese study of 324 postmenopausal women (Nagata *et al.*, 2005) that found that a high intake of fat was associated with higher serum concentrations of estrone and DHEAS. Discrepancies between these studies could be due to population differences in consumption patterns and thresholds (Boyd *et al.*, 1996; Li *et al.*, 2003). It has been reported that large differences in fat intake are required to cause a significant change to circulating hormone concentrations (Adlercreutz *et al.*, 1989). One study suggested that fat intake had to be <20% of total energy before it can influence circulating hormone concentrations (Holmes *et al.*, 2000). This may be more relevant to Japanese women who traditionally have a lower dietary fat intake compared with women from the Western populations such as Australia (Holmes *et al.*, 2000; Li *et al.*, 2003). Interestingly, the two- to fourfold increase in breast cancer mortality in Japan since World War II has been attributed to changes to a more Western diet that is higher in animal products such as meat, eggs and dairy products (Li *et al.*, 2003).

A diet high in fat is usually associated with high protein consumption (Adlercreutz *et al.*, 1989). As with fat, we found no association between consumption of protein and concentrations of circulating steroid hormones or SHBG. Although similar to fat, it may be more important to differentiate between measures from animal and vegetable sources than to focus solely on total values (Adlercreutz *et al.*, 1989).

Major strengths of our study included the large sample of women (766), the restriction to women who had reached menopause naturally and the availability of extensive information on potential confounding factors. Whereas other studies have focused predominantly on estrogens (Ganmaa and Sato, 2005), we also measured androgens and SHBG concentrations. Androgens are important for postmenopausal women, as they can be converted to estrogens through aromatization in adipose tissue (Adlercreutz *et al.*, 1989). In addition, we were able to collect enough data from our 121-item food frequency questionnaire to provide sufficient detail on consumption of animal products and corresponding nutrient components. Unlike previous studies that have compared hormonal profiles between vegetarian and nonvegetarian postmenopausal women, we were able to use more sensitive assessment of dietary exposures and associations (Willett, 1998). Assigning our participants to categories on the basis of dietary intake provided information on variation according to the level of intake and on possible linear trends (Willett, 1998).

A limitation of our study was the lack of repeated measures of both steroid hormones and dietary intakes. However, the high intraclass correlations for SHBG and steroid hormones indicate that our measures of these were generally good. Reports of good within-subject reproducibility for serum androgens and SHBG over a relatively long period of time suggest that a single blood sample measure is adequate to

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Table 3 Least-squared means of circulating hormones and SHBG concentration by quartiles of nutrients[a]

Fatty acid	Quartile	E2	E2_free	E1S	T	DHEAS	AD	SHBG
Total fat	1	61.05	0.85	1.14	0.76	1.64	2.27	49.72
	2	59.9	0.82	1.15	0.71	1.66	2.13	50.68
	3	60.93	0.86	1.18	0.69	1.55	2.04	47.63
	4	60.65	0.83	1.17	0.74	1.68	2.24	51.7
	P for trend	0.97	0.8	0.53	0.54	0.95	0.71	0.58
Saturated fat	1	64	0.88	1.15	0.78	1.62	2.29	50.24
	2	58.75	0.81	1.17	0.7	1.62	2.16	49.52
	3	58.47	0.8	1.14	0.73	1.7	2.2	50.49
	4	62.74	0.85	1.18	0.76	1.67	2.15	51.93
	P for trend	0.83	0.55	0.7	0.86	0.62	0.38	0.32
Mono-unsaturated fat	1	62.2	0.85	1.13	0.74	1.63	2.22	51.42
	2	58.64	0.82	1.15	0.71	1.62	2.07	48.36
	3	61.58	0.86	1.25	0.76	1.74	2.49	49.08
	4	59.7	0.82	1.17	0.73	1.65	2.17	50.46
	P for trend	0.55	0.67	0.4	0.99	0.68	0.83	0.73
Polyunsaturated fat	1	61.96	0.85	1.15	0.73	1.69	2.23	51.25
	2	58.36	0.82	1.19	0.76	1.66	2.23	48.36
	3	60.43	0.82	1.15	0.75	1.62	2.18	51.3
	4	61.64	0.85	1.14	0.7	1.55	2.15	50.23
	P for trend	0.82	0.89	0.58	0.41	0.2	0.48	0.93
Transun-saturated fat	1	61.32	0.84	1.14	0.76	1.66	2.16	51.39
	2	61.78	0.85	1.14	0.74	1.56	2.19	50.67
	3	58.5	0.8	1.12	0.69	1.55	2.19	51.32
	4	60.42	0.84	1.22	0.73	1.74	2.28	48.37
	P for trend	0.57	0.87	0.24	0.55	0.42	0.42	0.2
Cholesterol	1	61.65	0.85	1.12	0.72	1.58	2.26	51.19
	2	58.53	0.8	1.19	0.74	1.67	2.16	51.38
	3	59.98	0.83	1.13	0.67	1.51	2.07	49.21
	4	61.59	0.85	1.19	0.8	1.79	2.28	49.64
	P for trend	0.75	0.54	0.33	0.2	0.11	0.78	0.32
Protein	1	59.85	0.82	1.2	0.74	1.72	2.29	50.93
	2	59.1	0.81	1.11	0.7	1.62	2.13	51.22
	3	62.07	0.86	1.11	0.75	1.63	2.2	50.05
	4	61.41	0.85	1.22	0.76	1.63	2.21	49.06
	P for trend	0.47	0.3	0.55	0.54	0.54	0.75	0.28

Abbreviations: AD, androstenedione; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E2, total estradiol; E2_free, free estradiol; EIS, estrone sulfate; HRT, hormone replacement therapy; SHBG, sex hormone binding globulin; T, testosterone.
[a]Estimates are from the linear regression model of the logarithm of hormone concentration including each nutrient separately. Estimates were adjusted for laboratory batch, age, country of birth, level of education, age at menarche, parity, duration of lactation, oral contraceptive use, HRT, age at menopause, physical activity, smoking, alcohol consumption, BMI and energy from diet.

assess long-term levels for postmenopausal women (Lukanova et al., 2003). Although there can be a temporal issue with the cross-sectional nature of the study, the food frequency questionnaire used to collect dietary data has been found to be an effective tool for measuring usual dietary intake over a 12-month period and ranking individuals based on consumption levels (Hodge et al., 2000). However, our findings need to be replicated to exclude the possibility that they occurred by chance or because of unknown residual confounders.

In conclusion, greater consumption of red meat and dairy products might influence circulating concentrations of SHBG and estradiol, respectively. Given the well-established role of steroid hormones in breast cancer etiology for postmenopausal women, these findings may have important health implications and therefore require confirmation and further investigation.

Conflict of interest

The authors declare no conflict of interest.

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