ORIGINAL PAPER

The Association of Reproductive and Lifestyle Factors with a Score of Multiple Endogenous Hormones

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Abstract

Introduction We recently reported that high levels of multiple sex and growth hormones were associated with increased postmenopausal breast cancer risk. Limited research has explored the relationship between reproductive, anthropometric, and lifestyle factors and levels of multiple hormones simultaneously.

Methods This cross-sectional analysis included 738 postmenopausal Nurses' Health Study participants who were controls in a breast cancer nested case-control study and had measured levels of estrone, estradiol, estrone sulfate, testosterone, androstenedione, dehydroepiandrosterone sulfate, prolactin, and sex hormone binding globulin (SHBG). A score was created by summing the number of hormones a woman had above (below for SHBG) each hormone's age-adjusted geometric mean. The association between lifestyle, anthropometric, and reproductive exposures and the score was assessed using generalized linear models.

Results The hormone score ranged from 0 to 8 with a mean of 4.0 (standard deviation=2.2). Body mass index (BMI) and alcohol consumption at blood draw were positively associated with the hormone score: a 5 unit increase in BMI was associated with a 0.79 (95%CI: 0.63, 0.95) unit increase in the score ($p<0.0001$) and each 15 g/day increase in alcohol consumption was associated with a 0.41 (95%CI: 0.18, 0.63) unit increase in the score ($p=0.0004$). Family history of breast cancer, age at menarche, and physical activity were not associated with the score.

Conclusions Reproductive breast cancer risk factors were not associated with elevated levels of multiple endogenous hormones, whereas anthropometric and lifestyle factors, particularly BMI and alcohol consumption, tended to be associated with higher levels of multiple hormones.

Introduction

Elevated levels of endogenous hormones, including estrogens, androgens, and prolactin, are associated with an increased risk of developing breast cancer among postmenopausal women [1–6]. A recent study by our group observed an increased risk of breast cancer associated with a score incorporating multiple plasma hormones simultaneously [7], suggesting that the interplay between hormones may be important in the development of breast cancer. Therefore, understanding the factors that lead to higher levels of multiple hormones using an integrative score has the potential to help reduce the risk of breast cancer.

Multiple studies have observed associations between individual endogenous hormones in postmenopausal women and a variety of anthropometric, lifestyle, and reproductive breast cancer risk factors. For example, higher body mass index (BMI), waist circumference and hip circumference were positively associated with levels of estrogens, but inversely associated with sex hormone binding globulin (SHBG) levels [8–12]. Increasing alcohol consumption has been associated with higher levels of estrogens, but not with androgens [11, 13]. Further, women who had a hysterectomy with bilateral oophorectomy tended to have lower levels of free testosterone

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and lower levels of androstenedione and testosterone [14, 15]. Additionally, parous women appeared to have lower levels of prolactin and estrone sulfate compared to nulliparous women [16, 17].

While many studies have examined breast cancer risk factors and endogenous hormones individually, no study has examined the association between breast cancer risk factors and levels of multiple risk-associated hormones. In this cross-sectional study, we examined the association between established breast cancer risk factors, including reproductive, anthropometric, and lifestyle factors, and a hormone score combining eight hormones, including estradiol, estrone, estrone sulfate, testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), prolactin and SHBG, among postmenopausal women not using hormones within the Nurses' Health Study (NHS).

Methods

Study Population

The NHS was established in 1976 when 121,700 US females, registered nurses aged 30–55 consented to participate by returning the baseline questionnaire. Since then, information on exposure status and disease diagnoses has been collected on biennial questionnaires. In 1989 and 1990, a subsample of the women ($n=32,826$) aged 43–70 provided a blood sample. The women were sent a blood collection kit including a short questionnaire. Blood samples were shipped with an ice pack overnight to our laboratory where the samples were processed. All samples were stored in liquid nitrogen freezers after collection.

In this cross-sectional analysis, we included postmenopausal controls who were not using hormone therapy (HT) at blood draw from a nested case-control study of breast cancer [1, 2]. The controls had been matched to breast cancer cases on age (±2 years), month of blood draw (±1 month), time of day of blood draw (±2 h), and fasting status (yes, no). Including follow-up from 1990 to 2004, 1,173 controls had their blood assayed for various hormones, of whom 744 had complete information for all eight hormones included in the score. This study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital, Boston, MA.

Exposures

Anthropometric

Questionnaire data was obtained as close to the 1989–1990 blood draw date as possible either from the main NHS questionnaires or from the blood collection questionnaire. BMI

was calculated in kilograms per square meter using weight at blood draw from the blood collection questionnaire and height reported in 1976. For women with missing weight at blood draw ($n=9$), weight was obtained from the 1990 main questionnaire. In 1980, women were asked to report their weight at age 18, which along with height reported in 1976, was used to calculate BMI at age 18. Weight change since age 18 was calculated in kilograms as weight at blood draw minus weight at age 18. Waist and hip circumference measurements were collected on the 1986 questionnaire. Women were asked to use a tape measure to measure around their waist while standing and the widest circumference of their hips. Waist circumference was divided by hip circumference to obtain waist-to-hip ratio (WHR). In 1988, women were asked to choose their body shape at ages 5 and 10 based on a provided diagram (somatotype pictogram) [18]. The reported body shapes at ages 5 and 10 were averaged together to obtain an overall youth body fatness estimate.

Lifestyle

Alcohol consumption was obtained from the 1990 food frequency questionnaire and calculated as grams of alcohol per day based on the reported consumption (ranging from never/ <1 month to 6+/day) of one serving of beer (13.2 g/12-oz can or bottle), red wine (10.8 g/glass), white wine (10.8 g/glass), and liquor (15.1 g/shot). For women with missing information on alcohol consumption in 1990, reported consumption in 1986 or 1994 was used ($n=32$). Physical activity was calculated as the average of the 1988 and 1992 reported metabolic equivalent hours (MET-hrs) per week based on the amount of leisure time physical activity including walking, jogging, and tennis reported on the main questionnaire [19]. Physical activity reported in 1990 was not used, as information was only obtained for walking and stair climbing. Smoking status was obtained from the 1990 questionnaire and included information on number of cigarettes smoked per day for current smokers, years since quitting for past smokers and pack-years of smoking. As a surrogate for healthy lifestyle, history of ever having a mammogram was obtained from the 1988 and 1990 questionnaires.

Reproductive/Hormonal

Past HT use and duration of HT use among past users (in years) was obtained from the 1990 questionnaire. Parity and age at first birth were reported from 1976 to 1984. In 1986, parous women reported the total number of months they had breastfed their children. Family history of breast cancer (mother or sister) was reported in 1976, 1982 and 1988. History of benign breast disease (BBD) was reported on each biennial questionnaire. Women who reported a diagnosis of BBD (1976–1980) or biopsy-confirmed BBD (1982–1990)

were considered to have a history of BBD. History of hysterectomy and oophorectomy were reported on the 1990 questionnaire. For women who were missing information on hysterectomy ($n=57$) and oophorectomy ($n=28$) status in 1990, the 1992 and 1994 questionnaires were used to fill in information for women who reported that they had not had a hysterectomy or oophorectomy after blood draw. Time since menopause was calculated as age at blood draw minus reported age at natural menopause or age at bilateral oophorectomy. Women who reported having a hysterectomy before menopause were set to missing for time since menopause. Age at menarche was obtained from the 1976 questionnaire. In 1984, women reported if they had used oral contraceptives in the past.

Outcome

Details of hormone assessment have been reported previously [1, 2]. Briefly, the first five batches of estradiol, DHEAS, and testosterone were assayed at Quest Diagnostics Nichols Institute (San Juan Capistrano, CA, USA) using extraction followed by radioimmunoassay. The remaining samples were assayed at the Mayo Clinic using liquid chromatography-tandem mass spectrometry (estradiol, testosterone; two batches) and solid-phase, competitive chemiluminescent enzyme immunoassay (DHEAS; one batch). The correlation between the two assays was greater than 0.9 [2]. The first estrone sulfate batch was assayed at the University of Massachusetts Medical Center's Longcope Steroid Radioimmunoassay Laboratory (Worcester, MA, USA) and the remaining six batches were assayed at the Nichols Institute. Estrone sulfate was measured by chromatography and radioimmunoassay at both laboratories. All seven estrone and androstenedione batches were assayed at the Nichols Institute using an extraction step followed by radioimmunoassay. Finally, the first two batches of SHBG and three batches of prolactin were assayed at the Longcope Laboratory with the remaining four SHBG and prolactin batches assayed at Massachusetts General Hospital's Reproductive Endocrinology Unit Laboratory (Boston, MA, USA) using a solid-phase two-site chemiluminescent enzyme immunometric assay. Based on blinded replicate quality control (QC) samples, the coefficient of variation (CV) was less than 10 % for 64 % of batches and 10–16 % otherwise. Hormone levels below the limit of detection were set to half of the limit ranging from none for SHBG and prolactin to 31 for DHEAS.

Estradiol did not exhibit batch variation. Testosterone was adjusted for batch by regression calibration based on replicate identical QC samples across batches [20]. No identical replicate QC samples were included for the other hormones, therefore average batch recalibration was used to adjust for batch and took account of participant factors that were important

predictors of each hormone and may have varied by batch, such as age and BMI at blood draw [21].

Statistical Analysis

Women with estradiol levels over 30 pg/ml ($n=6$) were excluded from analyses as estradiol levels above 30 pg/ml likely indicate current or very recent HT use. We natural-log transformed each of the eight hormones and created two versions of the hormone score. The primary score was calculated as the sum of the number of hormones with levels above (or below for SHBG) the age-adjusted geometric mean for each hormone. The score ranged from 0 to 8 with 0 signifying that a woman had no hormones above the age-adjusted geometric mean level (or below for SHBG) and 8 signifying that a woman had each hormone above the age-adjusted geometric mean level and below for SHBG. Additionally, separate estrogen (estradiol, estrone, and estrone sulfate) and androgen (testosterone, DHEAS, and androstenedione) hormone scores were created similar to the primary hormone score.

For the secondary hormone score, deciles were created for each of the eight hormones and the deciles were summed across the eight hormones (decile 1 was scored as 0 and decile 10 as 9). For SHBG, the rankings were reversed. The score ranged from 0 to 72, with a 72 signifying that the woman was in the top decile for each hormone and the bottom decile for SHBG. Separate estrogen and androgen decile hormone scores also were created.

We used generalized linear models to assess the relationship between the reproductive and lifestyle factors and each of the hormone scores. For the primary hormone score, the results are presented as the unit difference in the hormone score for a given change in the exposure of interest. All analyses were adjusted for the matching factors from the original nested breast cancer case-control study, including age at blood draw (continuous), time of blood draw (midnight to 8 am, 8 am to noon, noon to midnight), month of blood draw (continuous) and fasting status (yes, no). Each breast cancer risk factor was assessed separately, controlling for the matching factors.

An anthropometric and lifestyle factor model was created by including BMI (per 5 kg/m^2), alcohol consumption (per 15 g/day), physical activity (per 3 MET-hours/week), smoking status (never, past, current), mammographic screening (never, ever) and the matching factors in one model. However, because the multivariate results were similar when mammographic screening was removed from the anthropometric and lifestyle model, results without this adjustment are presented. Waist circumference (per inch), hip circumference (per inch), WHR (per 0.1), BMI at age 18 (per 5 kg/m^2), weight change since age 18 (per 5 kg) and body shape (somatotypes: 1, 1.5–2, 2.5–3, 3.5–4, >4) were analyzed controlling for the lifestyle factors listed above except for

BMI at blood draw. We also considered categorical versions of each anthropometric measure as follows: BMI (<22, 22 to <25, 25 to <30, ≥30 kg/m^2), alcohol consumption (none, >0 to <5, 5 to <10, 10 to <15, 15 to <30, ≥30 g/day), physical activity (<6, 6 to <12, 12 to <24, ≥24 MET-hrs/week), waist circumference (<29, 29 to <32, 32 to <35, ≥35 in.), hip circumference (<38, 38 to <40, 40 to <43, ≥43 in.), WHR (<0.75, 0.75 to <0.80, 0.80 to <0.84, ≥0.84), BMI at age 18 (<20, 20 to <22, 22 to <25, ≥25 kg/m^2), and weight change since age 18 (<5, 5 to <10, 10 to <20, >20 kg).

A reproductive/hormonal model included past HT use (never, ever), parity (nulliparous, parous), history of BBD (yes, no), family history of breast cancer (yes, no), past oral contraceptive use (never, ever), age at menarche (per year), history of oophorectomy (no, unilateral, bilateral), history of hysterectomy (no, yes), time since menopause (per year), and the matching factors. Age at blood draw was assessed without adjusting for time since menopause. Age at first birth (per 5 years), breastfeeding (none, <1–3 months, 4–6 months, >6 months) and number of children (1, 2, 3, 4, ≥5 children) were assessed among parous women only. For all variables, we accounted for missing data in the following ways: for the continuous variables, the values for women with no information available were set to the median and a separate missing indicator was created and for categorical variables, a missing category was added to the variable.

In sensitivity analyses, smoking status was further categorized as never; current, <15 cigarettes/day; current, 15+ cigarettes/day; past, 1–19 years since quitting; and past, 20+ years since quitting. Additionally, we performed a sensitivity analysis restricted to women who reported natural menopause, which excluded women who experienced menopause due to radiation, bilateral oophorectomy or who had a hysterectomy before menopause. Analyses for the primary hormone score also were stratified by past HT use (never, ever) and by BMI (<25 kg/m^2, 25 to <30 kg/m^2, ≥30 kg/m^2).

All analyses were conducted using SAS, version 9.3 (SAS Institute Inc, Cary, North Carolina). All p values were two-sided and considered statistically significant if less than 0.05.

Results

The mean age of controls was 61.5 (SD=4.7) and did not differ by hormone score category (Table 1). Controls with a higher hormone score were more likely to have a higher alcohol intake, BMI, waist and hip circumference, and weight change since age 18 than women with a lower hormone score. Controls with a lower versus higher score were more likely to have a family history of breast cancer (17 vs. 12 %), have a history of hysterectomy (30 vs. 26 %) and have previously

used HT (37 vs. 32 %). The time since menopause did not appear to differ between the hormone score groups.

Anthropometric Risk Factors

The majority of the anthropometric factors was positively associated with the overall and estrogen hormone scores, but not with the androgen score (Table 2). BMI at blood draw was positively associated with the overall hormone score (p trend=<0.0001) and the estrogen score (p trend=<0.0001), with a 0.79 unit increase (95 % CI=0.63, 0.95) in the overall hormone score and a 0.55 unit increase (95 % CI=0.47, 0.64) in the estrogen score per 5 kg/m^2 increase in BMI. BMI was not significantly associated with the androgen score, with a 0.04 unit increase (95 % CI=−0.04, 0.13) in the score for a 5 kg/m^2 increase. Both BMI at age 18 and weight change since age 18 were associated with an increase in the overall (p trend=0.0004 and p trend=<0.0001, respectively) and estrogen (p trend=<0.0001 and p trend=<0.0001, respectively) hormone scores, but not with the androgen score (p trend=0.96 and p trend=0.32, respectively). Adjusting for BMI at age 18, a 5 kg increase in weight between age 18 and blood draw was associated with 0.33 unit increase (95 % CI=0.26, 0.40) in the overall hormone score and a 0.23 unit increase (95 % CI=0.19, 0.26) in the estrogen score. BMI at age 18 was associated with a 0.49 unit increase (95 % CI=0.22, 0.76) in the overall score and a 0.40 unit increase (95 % CI=0.27, 0.55) in the estrogen score per 5 kg/m^2 after adjusting for weight change since age 18. BMI at age 18 was associated with a 0.41 (95 % CI=−0.69, −0.12) decrease in the overall and a 0.21 (95 % CI=−0.36, −0.06) decrease in the estrogen hormone scores when adjusting for BMI at blood draw. After adjustment for BMI at blood draw, there was a suggestion of a decrease in the overall hormone score with increasing youth body fatness; however, this association was not significant (p trend=0.09) (data not shown).

Both waist and hip circumference were positively associated with the overall and estrogen hormone scores; waist circumference ≥35 compared to <29 in. was associated with 2.00 unit increase (95 % CI=1.48, 2.52) in the overall hormone score and hip circumference ≥43 versus <38 in. increase was associated with 1.44 unit increase (95 % CI=0.91, 1.98) in the overall hormone score (Table 2). However, the association with hip circumference was attenuated when hip and waist circumference were mutually adjusted in the model and when adjusted for BMI at blood draw (data not shown).

Lifestyle Risk Factors

Increasing alcohol consumption was associated with an increase in the overall (0.41 unit increase per 15 g/day; 95 % CI=0.18, 0.63), estrogen (0.18 unit increase; 95 % CI=0.06, 0.30) and androgen (0.16 unit increase; 95 % CI=0.04, 0.29)

Table 1 Characteristics of postmenopausal women not on HT at the time of blood draw in 1989–1990 by hormone score level (*n*=738)

	Hormone score group		
	Score 0–2 (*n*=203)	Score 3–5 (*n*=325)	Score 6–8 (*n*=210)
Means (SD)[a]			
Age at blood draw, years[b]	61.4 (4.5)	61.5 (4.5)	61.6 (5.2)
Time since menopause, years	11.6 (6.1)	11.7 (5.7)	11.7 (6.2)
Age at menarche, years	12.7 (1.5)	12.7 (1.5)	12.7 (1.4)
Duration of HT use, years[c]	4.4 (6.0)	3.6 (4.1)	3.5 (4.0)
Age at first birth, years	25.4 (3.1)	25.7 (3.7)	25.8 (3.3)
Body mass index, kg/m^2	24.4 (3.7)	26.0 (4.7)	28.4 (4.9)
Body mass index at age 18, kg/m^2	21.6 (2.8)	21.5 (2.8)	21.8 (3.1)
Weight change since age 18, kg	8.0 (9.5)	12.4 (12.3)	17.9 (11.9)
Waist circumference, inches	31.0 (3.9)	31.9 (4.4)	34.5 (4.3)
Hip circumference, inches	39.2 (4.3)	40.2 (3.8)	42.1 (5.1)
Waist-to-hip ratio	0.8 (0.2)	0.8 (0.1)	0.8 (0.2)
Alcohol consumption, g/day	4.3 (8.1)	5.0 (9.0)	6.6 (12.9)
Physical activity, MET-hrs/week	20.1 (17.8)	18.3 (18.8)	17.2 (17.4)
Pack-years of smoking	26.3 (18.9)	26.4 (19.2)	25.9 (22.2)
Number of children	3.8 (1.9)	3.5 (1.6)	3.5 (1.9)
Percentages			
Ever parity	92	94	94
Ever breastfeed	63	73	63
Past HT use	37	36	32
Past oral contraceptive use	26	33	27
History of benign breast disease	27	32	25
Family history of breast cancer	17	15	12
History of bilateral oophorectomy	10	15	12
History of hysterectomy	30	26	26
Ever smoker	51	56	50

Hormone score created as the number of hormones above (or below for SHBG) the age-adjusted geometric mean level for each hormone and includes estradiol, estrone, estrone sulfate, testosterone, androsteinedione, DHEAS, prolactin, and SHBG

[a] Values are means (SD) or percentages and are standardized to the age distribution of the study population

[b] Value is not age adjusted

[c] Among past hormone therapy (HT) users

scores (Table 3), with the highest mean hormone score observed among women who consumed ≥30 g/day. There was a borderline significant association (*p*=0.07) between current smoking and the androgen score: current smokers had a 0.25 unit higher (95 % CI=−0.02, 0.52) androgen score compared to never smokers. However, there was no difference in the association of current smoking and the androgen score by the number of cigarettes smoked per day (data not shown). Physical activity was not associated with the hormone scores.

Reproductive Risk Factors

No significant differences in the overall, estrogen, or androgen hormone scores were observed for nulliparous versus parous women (Table 4). However, among parous women, there was a modest, but significant, decreasing trend in the overall (*p* trend=0.03) and androgen (*p* trend=0.02) hormone scores across categories of number of children. Women with five or more children had a 0.33 unit lower (95 % CI=−1.10, 0.45) overall hormone score and a 0.23 unit lower (95 % CI=−0.66, 0.20) androgen score compared to women with one child. Hysterectomy was borderline associated with a lower androgen score (0.49 unit decrease; 95 % CI=−1.00, 0.01), but was not associated with the overall (*p*=0.23) or estrogen (*p*=0.62) scores. Age at blood draw, not adjusting for time since menopause, was not associated with the hormone score, with a 0.01 unit increase (95 % CI=−0.02, 0.05) per year in the overall score. Among past HT users, each year of HT use was associated with a borderline significant 0.07 unit decrease

Table 2 Association between anthropometric factors and the overall, estrogen and androgen hormone scores represented as unit change in the hormone score among 738 postmenopausal women

Anthropometric factors		N	Change in overall hormone score[a] (95 % CI)	Change in estrogen hormone score[b] (95 % CI)	Change in androgen hormone score[c] (95 % CI)
Body mass index (kg/m^2)	<22	133	Ref.	Ref.	Ref.
	22 to <25	210	0.66**	0.45**	0.12
	25 to <30	253	1.25**	0.89**	0.03
	≥30	142	2.30**	1.57**	0.21
	Per 5 kg/m^2	738	0.79	0.55	0.04
			(0.63, 0.95)**	(0.47, 0.64)**	(−0.04, 0.13)
Body Mass Index at age 18 (kg/m^2) [d,e]	<20	188	Ref.	Ref.	Ref.
	20 to <22	267	0.42*	0.25*	0.03
	22 to <25	174	0.22	0.23*	−0.09
	≥25	76	0.84**	0.65**	0.02
	Per 5 kg/m^2	705	0.49	0.40	0.00
			(0.22, 0.76)**	(0.26, 0.55)**	(−0.15, 0.15)
Weight change since age 18 (kg) [e,f]	<5	185	Ref.	Ref.	Ref.
	5 to <10	114	0.49*	0.25	0.02
	10 to <20	233	1.22**	0.72**	0.16
	≥20	173	2.17**	1.40**	0.19
	Per 5 kg	705	0.33	0.23	0.02
			(0.26, 0.40)**	(0.19, 0.26)**	(−0.02, 0.06)
Waist-to-hip ratio[e]	<0.75	129	Ref.	Ref.	Ref.
	0.75 to <0.80	163	0.57*	0.21	0.11
	0.80 to <0.84	101	0.82**	0.29	0.18
	≥0.84	132	1.13**	0.56**	0.19
	Per 0.1 units	525	0.10	0.08	0.00
			(−0.04, 0.24)	(0.00, 0.16)*	(−0.07, 0.07)
Hip circumference (inches)[e]	<38	128	Ref.	Ref.	Ref.
	38 to <40	109	0.43	0.29	−0.01
	40 to <43	165	0.98**	0.77**	0.03
	≥43	124	1.44**	1.02**	0.12
	Per 1 in.	526	0.12	0.08	0.02
			(0.08, 0.17)**	(0.06, 0.11)**	(0.00, 0.04)
Waist circumference (inches)[e]	<29	120	Ref.	Ref.	Ref.
	29 to <32	135	0.70**	0.27	0.20
	32 to <35	131	0.75**	0.45**	−0.02
	≥35	143	2.00**	1.18**	0.38**
	Per 1 in.	529	0.15	0.10	0.02
			(0.11, 0.19)**	(0.07, 0.12)**	(0.00, 0.05)*

Categorical variables adjusted for age at blood draw (cont.), time of blood draw (midnight to 8 am, 8 am to noon, noon to midnight), fasting status (yes, no), alcohol consumption (none, >0 to <5, 5 to <10, 10-<15, 15 to <30, ≥30 g/day), physical activity (>6, 6 to <12, 12 to <24, ≥24) and smoking status (never, current, past) unless otherwise noted

Continuous variables adjusted for age at blood draw (per year), time of blood draw (midnight to 8 am, 8 am to noon, noon to midnight), fasting status (yes, no), alcohol consumption (g/day), physical activity (MET-hrs/week) and smoking status (never, current, past) unless otherwise noted

*$p<0.05$; **$p<0.01$

[a] Includes estradiol, estrone, estrone sulfate, androstenedione, testosterone, DHEAS, prolactin and SHBG

[b] Includes estradiol, estrone and estrone sulfate

[c] Includes androstenedione, testosterone and DHEAS

[d] Additionally adjusted for weight change since age 18

[e] Categories do not add up to total (N=738) due to missing data

[f] Additionally adjusted for BMI at age 18

Table 3 Association between lifestyle factors and the overall, estrogen and androgen hormone scores represented as unit change in the hormone score among 738 postmenopausal women

Lifestyle factors		N	Change in overall hormone score[a] (95 % CI)	Change in estrogen hormone score[b] (95 % CI)	Change in androgen hormone score[c] (95 % CI)
Alcohol consumption (grams/day)	0 (no alcohol)	303	Ref.	Ref.	Ref.
	<5	241	−0.09	−0.07	−0.05
	5 to <10	64	0.14	−0.06	0.02
	10 to <15	53	0.11	−0.03	−0.10
	15 to <30	42	0.38	0.15	0.13
	≥30	35	1.16**	0.53**	0.51*
	Per 15 g/day	738	0.41 (0.18, 0.63)**	0.18 (0.06, 0.30)**	0.16 (0.04, 0.29)*
Physical activity (MET-hrs/week)	<6	201	Ref.	Ref.	Ref.
	6 to <12	147	0.16	0.12	0.08
	12 to <24	191	−0.01	0.00	0.06
	≥24	199	−0.03	−0.01	0.03
	Per 3 MET-hrs/wk	738	−0.01 (−0.03, 0.02)	0.00 (−0.01, 0.01)	0.00 (−0.02, 0.01)
Smoking status	Never	346	Ref.	Ref.	Ref.
	Current smoker	89	−0.04 (−0.53, 0.45)	−0.05 (−0.32, 0.21)	0.25 (−0.02, 0.52)
	Past smoker	303	−0.22 (−0.54, 0.10)	−0.11 (−0.28, 0.07)	−0.11 (−0.29, 0.07)

Categorical variables adjusted for BMI (<22, 22 to <25, 25 to <30, ≥30 kg/m^2), age at blood draw (cont.), time of blood draw (midnight to 8 am, 8 am to noon, noon to midnight), fasting status (yes, no) and all categorical variables in the table

Continuous variables adjusted for BMI (per 5 kg/m^2), age at blood draw (per year), time of blood draw (midnight to 8 am, 8 am to noon, noon to midnight), fasting status (yes, no) and all continuous variables in the table

*$p<0.05$; **$p<0.01$

[a] Includes estradiol, estrone, estrone sulfate, androstenedione, testosterone, DHEAS, prolactin and SHBG

[b] Includes estradiol, estrone and estrone sulfate

[c] Includes androstenedione, testosterone and DHEAS

(95 % CI=−0.13, 0.00) in the overall hormone score. The remaining reproductive factors were not significantly associated with the hormone score.

Stratification by past HT use did not substantially change the results for most of the anthropometric, lifestyle, and reproductive factors (data not shown) with the exception of age at menarche and oophorectomy. Among past HT users, each 1-year increase in age at menarche was associated with a 0.20 unit increase (95 % CI=0.03, 0.38) in the overall hormone score. However, age at menarche was associated with a non-significant decrease in the overall score among never HT users (*p* interaction=0.01). Among past HT users, women who had had a bilateral oophorectomy compared to women with both ovaries intact had a higher overall (0.91; 95 % CI=−0.44, 2.27) hormone score. Conversely, among never HT users, bilateral oophorectomy was associated with a decrease in the overall (−0.29; 95 % CI=−1.59, 1.00) hormone score (*p* interaction=0.04).

Stratification by BMI did not substantially change the results for any of the lifestyle or reproductive factors (data not shown). For the anthropometric variables, the effect of increasing BMI on the overall hormone score appeared to be strongest for lean women (BMI <25 kg/m^2) compared to overweight and obese women. Among lean women, each 5 kg/m^2 increase in BMI was associated with a 1.24 unit increase (95 % CI=0.61, 1.87) in the overall score. However, among obese women, each 5 kg/m^2 increase in BMI was associated with a non-significant 0.17 unit increase (95 % CI=−0.28, 0.62). Additionally, when stratified by BMI, the associations with waist and hip circumference for the overall hormone score were only observed among women with a BMI <30 kg/m^2 (*p* interaction=0.06 for waist circumference and *p* interaction=0.19 for hip circumference). Among non-obese women (BMI <30 kg/m^2), each 1-in. increase in waist circumference was associated with a 0.13 unit increase (95 % CI 0.07, 0.18) in the overall hormone score, while

Table 4 Association between reproductive factors and the overall, estrogen and androgen hormone scores represented as unit change in the hormone score among 738 postmenopausal women

Reproductive factors		N exp/N unexp[a]	Change in overall hormone score[b] (95 % CI)	Change in estrogen hormone score[c] (95 % CI)	Change in androgen hormone score[d] (95 % CI)
Parous vs. nulliparous		683/55	−0.09 (−0.67, 0.49)	0.03 (−0.28, 0.34)	0.00 (−0.33, 0.32)
Number of children[e]	1	38	Ref	Ref	Ref
	2	158	0.32	0.27	0.09
			(−0.42, 1.06)	(−0.13, 0.67)	(−0.32, 0.50)
	3	181	−0.20	0.00	−0.23
			(−0.95, 0.54)	(−0.40, 0.40)	(−0.64, 0.19)
	4	154	−0.19	0.12	−0.27
			(−0.95, 0.58)	(−0.29, 0.53)	(−0.69, 0.16)
	≥5	152	−0.33	−0.09	−0.23
			(−1.10, 0.45)*	(−0.50, 0.33)	(−0.66, 0.20)*
Age at first birth (per 5 years)[e]		683	0.09 (−0.15, 0.33)	−0.01 (−0.14, 0.12)	0.05 (−0.08, 0.19)
Breastfeeding (months)[e]	None	231	Ref	Ref	Ref
	<1 to 3	196	0.04	0.01	0.08
			(−0.36, 0.43)	(−0.20, 0.22)	(−0.13, 0.30)
	4 to 6	75	−0.11	−0.09	0.03
			(−0.65, 0.42)	(−0.38, 0.20)	(−0.27, 0.33)
	>6	181	0.01	0.11	0.01
			(−0.39, 0.41)	(−0.10, 0.33)	(−0.21, 0.23)
Family history of breast cancer vs. no family history		111/627	−0.19	−0.18	−0.02
			(−0.61, 0.22)	(−0.41, 0.04)	(−0.25, 0.21)
History of BBD vs. no history		213/525	0.01	−0.02	0.08
			(−0.32, 0.34)	(−0.20, 0.16)	(−0.11, 0.26)
Past HT use vs. never use		255/483	0.01	−0.02	0.09
			(−0.32, 0.35)	(−0.20, 0.16)	(−0.10, 0.27)
Duration of past HT use (per year)[f]		255	−0.07	−0.02	−0.03
			(−0.13, 0.00)	(−0.06, 0.01)	(−0.07, 0.01)
Past use of oral contraceptives vs. never use		220/518	0.01	−0.08	0.06
			(−0.33, 0.35)	(−0.26, 0.10)	(−0.12, 0.25)
Age at menarche (per year)		738	0.02	−0.01	0.01
			(−0.08, 0.12)	(−0.06, 0.05)	(−0.05, 0.07)
History of oophorectomy	Both intact	607	Ref.	Ref.	Ref.
	One removed	37	−0.03 (−0.75, 0.69)	−0.07 (−0.46, 0.31)	−0.01 (−0.41, 0.39)
	Both removed	94	0.55 (−0.36, 1.46)	0.12 (−0.37, 0.60)	0.27 (−0.23, 0.78)
History of hysterectomy vs. no history		201/537	−0.55 (−1.46, 0.35)	−0.12 (−0.61, 0.36)	−0.49 (−1.00, 0.01)
Time since menopause (per year)		738	0.00	−0.01	0.01
			(−0.04, 0.04)	(−0.03, 0.01)	(−0.01, 0.03)
Age at blood draw (per year)[g]		738	0.01	0.01	0.01
			(−0.02, 0.05)	(−0.01, 0.03)	(−0.01, 0.02)

Adjusted for age at blood draw (per year), time of blood draw (midnight to 8 am, 8 am to noon, noon to midnight), fasting status (yes, no) and all other variables in the table unless otherwise noted

*p trend<0.05

[a] N unexp number in reference group; N exp number in comparison group

[b] Includes estradiol, estrone, estrone sulfate, androstenedione, testosterone, DHEAS, prolactin and SHBG

[c] Includes estradiol, estrone, and estrone sulfate

[d] Includes androstenedione, testosterone, and DHEAS

[e] Among parous women (n=683)

[f] Among past HT users (n=255)

[g] Not adjusted for time since menopause

among obese women, the comparable change was 0.02 units (95 % CI=−0.08, 0.13). Similar results were observed for hip circumference (data not shown).

Results were similar when analyses for the reproductive factors were restricted to women with a natural menopause (data not shown). Additionally, similar results were obtained for the secondary hormone score, with the exception that age at blood draw was associated with a significant decrease in the overall and androgen hormone scores when not adjusting for time since menopause (data not shown).

Discussion

In the first study of its kind, we examined the association between a hormone score comprising circulating levels of prolactin, SHBG, estrogens, and androgens with breast cancer risk factors. The majority of the anthropometric measures were significantly positively associated with the overall hormone score; this appeared to be driven primarily by associations with the estrogens. Further, increased alcohol consumption was associated with a higher overall hormone score as well as with both the estrogen and androgen scores. While the majority of the reproductive factors were not associated with the three hormone scores, there was a significant trend for decreasing overall and androgen hormone scores with increasing number of children.

Increasing BMI and weight gain consistently has been associated with increasing levels of circulating estrogens and decreasing levels of SHBG among postmenopausal women [8–12, 22–27]. The findings for BMI and androgens are less consistent. Some studies have reported a positive association for testosterone and/or androstenedione levels [8, 10, 24–26], while other studies have shown no association [9, 11, 22, 24, 26]. DHEAS and prolactin have not been associated with BMI in previous studies [8–11, 22, 24–26, 28]. A higher estrogen score among women with higher BMI is unsurprising given that, among postmenopausal women, the main source of estrogen is conversion of androstenedione to estrone by aromatase activity in peripheral tissues [29]. Increases in adiposity lead to increased aromatase activity, thereby increasing estrogen levels [30]. Only one other study has examined the association between BMI at age 18 and postmenopausal hormone levels. Su and colleagues [31] observed that increasing body fatness at ages 5 and 10 was associated with decreasing prolactin; however, no association between BMI at age 18 and prolactin levels was observed. In our population, BMI at age 18 was inversely associated with the overall and estrogen hormone scores when adjusting for current BMI, possibly reflecting the potential decreased risk of postmenopausal breast cancer with increasing early life body size [32–36].

Few studies have assessed the relationship between waist and hip circumference and specific hormones. The patterns for waist and hip circumference generally are consistent with positive associations for estrogen levels and inverse associations with SHBG [9–12], while all but one study have observed no association between androgens and waist and hip circumference [9–11]. The null findings for hip circumference after adjustment for waist circumference in our study suggest that central adiposity is more strongly associated with circulating hormone levels than gluteal adiposity. Body fat distributions and aromatase activity have been observed to change after menopause, with increased central adiposity and visceral aromatase activity [37–39]. Interestingly, in our study, waist circumference appeared to only be associated with the overall and estrogen hormone scores among women with a BMI of <30 kg/m^2. Among 1,180 postmenopausal women, Liedtke and colleagues [40] observed similar findings for free testosterone and free estradiol levels. These findings suggest that, among non-obese women, the increase in aromatase activity in visceral adipocytes may lead to an increase in hormone levels, whereas among obese women, overall body fat aromatase activity may overwhelm any independent impact of abdominal aromatase activity.

In our analyses, alcohol consumption, particularly above 30 g/day, was associated with an increase in all three hormone scores. Studies of the associations between individual hormones and alcohol consumption are inconsistent [11, 13, 14, 23, 25, 27, 41–44]. Increased estrone and estrone sulfate levels tended to be associated with higher alcohol consumption in most studies [11, 13, 25, 27, 28, 41, 44]. The lack of association in some studies may be due to small sample sizes—several of these studies were randomized feeding studies with fewer than 50 participants [23, 27, 41, 42]. The majority of studies did not find an association between alcohol consumption and androgens, with the exception of DHEAS, for which five of the eight studies observed an association [11, 13, 14, 25, 28, 41, 44]. Thus, the increase observed in the androgen score may have been driven by DHEAS. The largest study, with over 6,000 post-menopausal women, observed an association between alcohol consumption and each of the hormones they assessed, including estradiol, estrone, estrone sulfate, androstenedione, testosterone, DHEAS, and SHBG [25].

The mechanism through which alcohol consumption impacts hormone levels is not fully understood. Alcohol can lead to decreased oxidation of estradiol to estrone in the liver and possibly increased extraction of testosterone in the liver, although this would lead to lower circulating testosterone levels [45, 46]. Based on the results of a feeding study among 11 young men, Gordon and colleagues [46] hypothesized that the observed decrease in testosterone may be due to changes in aromatase activity induced by alcohol consumption, with an increase in conversion of androgens to estrogens. These two hypotheses would explain the elevated estrogen hormone score observed in our study, but not the elevated androgen

hormone score. Alcohol has been shown to directly and indirectly stimulate the adrenal gland, which is the main source of DHEAS production, and may explain the increase in the androgen score [41, 47].

Most prior studies among postmenopausal women not on HT did not report an association between reproductive factors and the eight hormones included in our hormone score [16, 17, 24, 43, 48]. For parity, there was a suggestion that DHEAS varied by number of pregnancies in one study, but with no clear trend [25]. In the NHS, Eliassen and colleagues [16] observed an inverse association between parity and prolactin among postmenopausal women; however, approximately 35 % of these women were current hormone therapy users. Additionally, Hankinson and colleagues [17] noted a weak inverse association between estrogens and prolactin and parity among a smaller sample of postmenopausal women within the NHS. Other studies have reported no association between parity and levels of estrogens or androgens [24, 43, 48–50]. One possible explanation is that the effect of parity on hormone levels is not strong for a single hormone but, in combination, an association can be observed.

Unexpectedly, we observed that associations for age at menarche and bilateral oophorectomy differed by history of HT use. For bilateral oophorectomy, the expected inverse association was observed among never HT users for the overall hormone score; however, the opposite association was observed among past HT users although neither of these associations was statistically significant. While it is unclear whether this is a chance finding or reflects underlying biology, certain breast cancer risk factors, such as BMI, have weaker associations with breast cancer among ever HT users [51]. Additional research should consider the long-term impact of HT use on hormone levels and the potential interaction with other risk factors, although past HT use was not associated with current hormone levels in our analysis.

The recent study by Tworoger and colleagues [7] observed that a score of multiple plasma hormones was associated with a nearly three-fold increased risk of breast cancer for having seven or eight as compared to zero hormones above the geometric mean level. Taken together with the results from our study, it appears that certain breast cancer risk factors, such as BMI, waist and hip circumference, and alcohol consumption, may influence the development and/or progression of breast cancer through alterations of multiple hormonal pathways. Further, both estrogen and androgen scores were associated with risk of breast cancer, although the strength of association was weaker than the score combining all the hormones. With respect to risk factors, we hypothesized that certain factors would be associated with only certain hormones, such as BMI and estrogens. The association observed in our study between the overall hormone score and BMI was most likely driven by the

strong association between BMI and estrogens. However, for other risk factors, such as alcohol consumption, we observed an association with the overall and component hormone scores suggesting that the interplay between both estrogens and androgens may be important in the pathway from alcohol consumption to breast cancer development and may help us to better understand the underlying biological mechanism.

One of the main strengths of our study was our relatively large sample size with information on the circulating levels of eight hormones. Precise assays were used to assess each of the hormones, with the majority of CV's below 10 %. However, the hormones were only measured at one time point, which may not reflect long-term hormone levels. In a reproducibility study within this cohort, we observed interclass correlations over a 3-year period of 0.68 for estradiol, 0.74 for estrone, 0.75 for estrone sulfate, 0.92 for SHBG, 0.66 for androstenedione, 0.88 for testosterone, 0.88 for DHEAS and 0.53 for prolactin, suggesting that current hormone levels fairly consistently reflect long-term hormone levels [52].

Our study also had several limitations. As this study is cross-sectional, the temporality of the relationship between the hormone levels and the risk factors cannot be determined. Additionally, the Nurses' Health Study is predominantly white, reflecting those who entered nursing in the 1960s. Setiawan and colleagues [53] reported that hormone levels differed between African Americans, Native Hawaiians, Japanese Americans, Latinas, and Whites and that many of these differences were consistent with the known associations between ethnicity and breast cancer risk. Future research should investigate the associations between anthropometric, lifestyle and reproductive factors, and the hormone score in ethnically diverse populations. Finally, waist and hip circumference were self-measured in this study and a substantial number of women were missing information on both waist and hip circumference; however, the results for both measures were consistent with the literature for single hormones.

Overall, the strongest association between the hormone scores and breast cancer risk factors was for BMI, possibly reflecting increased aromatase activity and, thus, conversion of androstenedione to estrone in adipose tissue among postmenopausal women. Alcohol consumption was significantly associated with the overall, estrogen, and androgen hormone scores, particularly among women consuming two or more drinks per day. The majority of the reproductive factors were not associated with the hormone score. These results suggest that differences in postmenopausal hormone levels may mediate the association between postmenopausal BMI and alcohol consumption and breast cancer risk. Future research should investigate the association between earlier life hormone levels, in particular premenopausal levels, and reproductive, anthropometric, and lifestyle breast cancer risk factors.

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