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Sex hormone concentrations and the risk of breast cancer recurrence in postmenopausal women without hot flashes

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Abstract

Background—We examined if the reduced risk of breast cancer events seen among women without baseline hot flash symptoms in the Women’s Healthy Eating and Living (WHEL) dietary intervention trial was related to changes in sex hormone concentrations.

Methods—Baseline and year one concentrations of total and bioavailable estradiol and testosterone and sex hormone binding globulin (SHBG) were compared by intervention arm among 447 postmenopausal women without hot flashes. Cox proportional hazard models tested interaction terms between study arm and baseline hormone concentrations adjusted for study site, anti-estrogen use, positive nodes, tumor size, oophorectomy status, and hormone replacement therapy use.

Results—Sex hormone concentrations did not differ by study arm at baseline nor at year one. Twenty-two (9.8%) events occurred in the intervention arm vs. 42 (18.9%) in the comparison arm ($p=0.009$). Baseline bioavailable testosterone was significantly, positively associated with additional events (HR 1.69, 95% CI: 1.00-2.84; $p=0.049$). There were significant interactions between the intervention and total ($p=0.015$) and bioavailable ($p=0.050$) testosterone: the intervention was more protective among participants with higher baseline total (HR 0.3, 95% CI: 0.2-0.7) or bioavailable (HR 0.4, 95% CI: 0.2-0.7) testosterone than for participants with lower baseline total (HR 0.8, 95% CI: 0.4-1.5) or bioavailable (HR 0.8, 95% CI: 0.4-1.5) testosterone. No significant effects were seen for estradiol or SHBG.

Conclusions—The WHEL dietary intervention may have modified other risk factors of recurrence correlated with testosterone.

Impact—Sex hormones should be considered as part of a larger biological system related to the risk of breast cancer recurrence.

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CONFLICTS OF INTEREST: None to report.

Keywords

Postmenopausal; hot flashes; sex hormones; breast cancer

Introduction

In 2007, the Women's Healthy Eating and Living (WHEL) randomized trial reported that an intervention designed to increase fruit, vegetable, and fiber intake and decrease fat intake did not influence breast cancer prognosis (1). However, a subgroup analysis found that the WHEL dietary intervention did significantly reduce the risk of additional breast cancer events among women who did not report hot flash symptoms at baseline (2).

Hot flash symptoms, such as marked increases of body temperature and sweating, are a common disturbance for peri- and postmenopausal women(3). While the etiology of hot flashes is complex, evidence supports a relationship between hot flash symptoms and sex hormone concentrations (4-6). Specifically, among healthy postmenopausal women, low concentrations of estrogen and estrogen withdrawal can instigate symptoms (5). Additionally, there is evidence that androgens, particularly testosterone, may maintain the thermoregulatory set-point via endorphins and norepinephrine (5).

Although the data are conflicting, randomized trials suggest that a diet low in fat and high in fruits and vegetables can reduce circulating levels of estradiol and estrone among postmenopausal women (7-9). If true, this might translate to a decreased risk of breast cancer recurrence (10). Therefore, this analysis was conducted to determine if the reported protective effect of the WHEL dietary intervention for this subgroup was the result of an intervention-associated change in sex hormones. We focus our analysis on postmenopausal women to eliminate those who would not typically report hot flash symptoms (i.e., premenopausal women) and those where fluctuating diurnal hormonal concentrations would confound results (i.e., perimenopausal women). Additionally, we examined whether the impact of the dietary intervention varied by baseline concentrations of sex hormones in an effort to understand why differences were seen by reported hot flash status.

Methods

Subject Selection

Details regarding the WHEL study have been published (1). Briefly, women were randomized during 1995-2000. Inclusion criteria included age 18-70; successful treatment for invasive, operable, early stage breast cancer, and no more than four years past primary diagnosis. The study randomized 3,088 women who were followed for an average of 7.3 years; the trial concluded in mid-2006.

Postmenopausal was defined as self-reported date of the last menstrual cycle at least one year prior to randomization. We further limited to women with baseline or year one total estradiol levels ≤ 100 pg/mL to exclude women who may have been biologically pre- or perimenopausal. Self-reported hot flash symptoms were categorized as none versus mild, moderate, or severe within the four weeks prior to baseline.

Laboratory Values

We report on serum samples that were analyzed for women without hot flash symptoms; data from a representative sample of women who reported hot flash symptoms was not available. Matched baseline and year one samples were analyzed in the same batch at the Reproductive Endocrine Research Laboratory at the University of Southern California by

analysts blinded to study group; using previously reported methods. (11). Briefly, estrogen and testosterone concentrations were determined by radioimmunoassay after organic solvent extraction and celite chromatography; intraassay and interassay coefficients of variation (CV) ranged from 6-9% and 12-14%, respectively. SHBG concentrations were determined “using the Immulite 2000 analyzer and a two-site chemiluminometric sandwich assay (Siemens Medical Solutions Diagnostics)” with intraassay and interassay CVs of 6.5% and 7.8%, respectively. Assay sensitivities were 3 ng/dL for estradiol, 20 pg/mL for testosterone, and 0.2 nmol/L for SHBG. Values below the assay sensitivities (n=1) were set to one unit below detection limit. Concentrations of bioavailable estradiol and testosterone were computed using law of mass action equations (12). Total estradiol and testosterone reflect hormone that is free or bound to albumin or SHBG; bioavailable fractions of estradiol and testosterone represent the fraction of total hormone that is free or bound to albumin. Nine women in our final subset did not have their blood samples analyzed at the lab sited above, yet did have their baseline and year one samples analyzed as part of an earlier study (13). We imputed their missing values with these observations, and found no meaningful differences in the results when these women were excluded.

Additional Events

Additional breast cancer event status was collected as previously described (1). Events were defined as local, regional, or distant recurrence, or new primary; the WHEL study did not include ductal carcinoma in situ or lobular carcinoma in situ as events. Time to outcome was date of randomization to date of additional event. Women who did not have an additional event were censored at the date of last contact or study end (June 1, 2006). Women who died due to conditions unrelated to breast cancer were censored at the date of death. We further excluded women who had a breast cancer recurrence within the first year of study enrollment.

Statistical Analysis

Univariate comparisons of baseline characteristics and time to additional event were completed with Cox proportional hazard modeling; the log-rank test was used to assess significance. Baseline measures significantly different by arm ($p < 0.10$) or significantly related to outcome ($p < 0.10$) were included as covariates in the adjusted models.

Baseline, year one, and year one change in sex hormone concentrations were compared by arm. Baseline and year one values were natural log (ln)-transformed for statistical comparisons; year one change data were not transformed. A series of Cox proportional hazard models were used to assess the impact of each sex hormone concentration on event status. Time to additional event was fit on intervention arm and baseline hormone concentration (ln-transformed), adjusted for anti-estrogen use, site, and any necessary covariates as described above. Likelihood ratio tests were used to test for the inclusion of an interaction term between baseline concentration and the intervention arm; p-values ≤ 0.05 were considered significant. Because BMI and oophorectomy status impacts sex hormone concentrations, we conducted sensitivity analyses by including baseline BMI in all models, and we repeated all analyses excluding women with any oophorectomies. All analyses were conducted with R, version 2.9.2.

Results

Of the 3,088 women randomized in the WHEL study, 577 postmenopausal women did not experience hot flash symptoms at baseline. Of these, we excluded 130 women: 22 experienced an additional event within one year, 93 did not have a year one blood sample, and 15 had a total estradiol level >100 pg/mL (5 in the intervention arm, 10 in the

comparison arm). The final sample size was 447 postmenopausal women, equally distributed by arm.

Women in the intervention arm were more likely to have ever used hormone replacement therapy (HRT) (62.2% vs 52.0%; $p=0.038$) and to have had an oophorectomy (20.0% vs. 9.9%; $p=0.004$) versus the comparison arm. There was no difference in anti-estrogen therapy use by arm (63.4%). Tamoxifen accounted for the majority (98%) of all anti-estrogen therapies. Baseline measures significantly related to an additional event were number of positive nodes (3, 1-3, versus 0; $p<0.001$) and tumor size (>2 cm versus ≤ 2 cm; $p=0.029$). Therefore, all multivariate models were adjusted for site and anti-estrogen use (stated a priori); any previous HRT use and oophorectomy status (differed by arm); and number of positive nodes and tumor size (related to outcome). Overall, the risk of additional events remained lower in the intervention arm: there were $n=22$ (9.8%) events in the intervention arm and 42 (18.9%; $p=0.009$) in the comparison arm for an adjusted hazard ratio of 0.5 (95% CI: 0.3-0.9; $p=0.011$).

Concentrations of sex hormones did not differ by study arm at baseline nor did they change over one year (Table 1). Adjusting for anti-estrogen use, oophorectomy status, and hormone replacement therapy use did not alter these results. Additionally, we checked for any differential changes in sex hormones by anti-estrogen use by including an interaction term between the intervention arm and anti-estrogen use; no interactions were significant (all $p>0.630$, data not shown).

There was a significant, positive, main effect of baseline bioavailable testosterone on the risk of recurrence (HR for one unit increase in ln-transformed values 1.69, 95% CI: 1.00-2.84; $p=0.049$). Significant interactions between the intervention and baseline concentrations of total testosterone (likelihood ratio test, $p=0.015$) and bioavailable testosterone ($p=0.050$) were found (Table 2). Specifically, the intervention dietary pattern appeared more protective for those with higher baseline concentrations of total or bioavailable testosterone. For example, the event rate increased over increasing quartiles of baseline bioavailable testosterone for those in the comparison arm: Q1 n events=6 (10.3%), Q2 n =11 (19.0%), Q3 n =11 (24.4%), and Q4 n =14 (24.6%), respectively; while decreasing over increasing quartiles for those in the intervention arm: Q1, n events=7 (13%), Q2 n =6 (11.5%), Q3 n =5 (8.3%), and Q4 n =4 (7.7%), respectively. Figure 1 demonstrates this interaction, inserting values of testosterone that correspond to the 25th, 50th, and the 75th percentiles of baseline concentrations into models 4 and 5 from Table 2, and computing the hazard ratios for the intervention arm versus the comparison arm. The intervention was more protective for women at the 75th percentile of baseline total (HR 0.3, 95% CI: 0.2-0.7) and bioavailable (HR 0.4, 95% CI: 0.2-0.7) testosterone than for women at the 25th percentile of baseline total (HR 0.8, 95% CI: 0.4-1.5) and bioavailable (HR 0.8, 95% CI: 0.4-1.5) testosterone. No significant interactions were seen with baseline total estradiol, bioavailable estradiol, or SHBG. Sensitivity analyses showed that adjusting all models for baseline BMI did not impact the results. All results were similar when limiting to women without oophorectomies ($n=357$), with the interaction between study arm and baseline bioavailable testosterone (as in Table 2, model 5), becoming more significant: the likelihood ratio test for the interaction term was $p=0.015$, and the interaction term HR was 0.2, 95% CI: 0.1-0.7.

Discussion

These results report on a worse prognosis for women with increased bioavailable testosterone concentrations among postmenopausal women who do not report hot flash symptoms at baseline. However, these data also show that the WHEL dietary intervention was protective for this subset of women and was significantly more protective for women

with higher baseline testosterone concentrations. These results agree with previous studies that have reported significant, positive associations between testosterone and breast cancer recurrence among postmenopausal women (14,15), and add additional information related to a protective effect induced by a dietary intervention. As an example of this differential effect, the dietary intervention nearly halved the risk of an additional event for women with baseline concentrations near the 75th percentile of total (37.5 ng/dL) or bioavailable (15.5 ng/dL) testosterone. However, the dietary intervention did not change sex hormone concentrations over one year. One possible explanation for why we did not observe a change in concentrations might be that women in the WHEL trial did not lose weight (1), while other dietary studies that analyzed estrogen concentrations are confounded by weight loss (7,8). Regardless, these results imply the protective effect was not due to a change in circulating sex hormones, and suggest the intervention affected other pathways related to breast tumor formation, such as those related to glycemic control or inflammation (16-19).

For example, bioavailable testosterone has been positively correlated to markers of insulin resistance (20-23), which may then relate to tumor formation (16-18). In the DIANA randomized dietary intervention trial among post-menopausal women (24), significant reductions in, testosterone, insulin area, and fasting glucose and significant increases in SHBG were seen over 4.5 months among participants on the intervention diet that was “low in animal fat and refined carbohydrates and rich in low-glycemic-index foods.” However, once adjusted for weight loss, only fasting glucose levels remained significantly reduced in the intervention arm. Studies addressing dietary interventions and the multivariate role between sex hormones and additional biomarkers as related to breast cancer risk are needed.

Strengths of this study include the randomized design and the overall high compliance rates seen in the WHEL trial (1). Considering the significant, positive associations seen between testosterone and the risk of breast cancer recurrence reported in previous studies (14,15), it is possible that the differential protective effect of the WHEL diet as observed by hot flash symptoms (2) is related to differential baseline concentrations of sex hormones. Future studies that analyze all blood samples for participants in the WHEL study are needed to address this.

A limitation of this analysis is that these data are based on two, single measurements of hormones, and significant relationships between sex hormone concentrations over time and disease status may be attenuated. However, sex hormone measurements among post-menopausal women are considered reliable, especially when compared to other commonly used biomarkers (25). Additionally, we are not able to address if the absence of hot flash symptoms was due to inefficient metabolism of tamoxifen. As it is believed that <7% of women are poor metabolizers of tamoxifen based on genotype (26), we would expect only a minor proportion of women who were using tamoxifen at baseline to be inefficient drug metabolizers.

In summary, these results are encouraging in that a group of women at an increased risk of breast cancer recurrence may benefit from a low-fat, high fruit, vegetable, and fiber dietary intervention. Our results are limited due to the post-hoc nature of the analysis, the small number of events, and a lack of biomarkers to confirm other possible mechanisms. However, these data support further studies within this area.

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Figure 1.

Risk reduction for intervention arm versus comparison arm by baseline testosterone concentrations (ng/dL): postmenopausal women who did not report hot flash symptoms at baseline.

Hazard ratios with 95% confidence intervals for intervention arm versus comparison arm computed using models 4 and 5 from Table 2, inserting values of baseline hormone concentrations representing the 25th, 50th, and 75th percentiles as examples. Models adjusted for site, anti-estrogen use, number of positive nodes, tumor size, oophorectomy status, and previous hormone replacement therapy use. Sample size: n=22 (9.8%) events out of 225 participants in the intervention arm versus n=42 (18.9%) events out of 222 participants in the comparison arm; subset excludes women who recurred within the first year of the WHEL study.

Table 1

Summary of serum sex hormone concentrations (unadjusted) at baseline and year one by study arm: postmenopausal women who did not report hot flash symptoms at baseline

	Comparison Arm (n=222) Median [IQR]	Intervention Arm (n=225) Median [IQR]	p-value*	p-value [†]
Total Estradiol (pg/mL)				
Baseline	8.0 [5.0,12.0]	8.0 [5.0,12.0]	0.991	0.582
Year one	9.0 [6.0, 12.0]	8.0 [6.0,12.0]	0.532	0.836
Year one change	0.0 [-1.0, 2.0]	0.0 [-2.0,2.0]	0.512	0.426
Bioavailable Estradiol (pg/mL)				
Baseline	4.4 [2.8, 7.1]	4.5 [2.8, 7.1]	0.991	0.483
Year one	4.8 [2.9, 7.6]	4.6 [2.7, 7.4]	0.500	0.856
Year one change	0.1 [-0.8, 1.4]	0.2 [-1.1, 1.2]	0.353	0.261
Total Testosterone (ng/dL)				
Baseline	26 [19.3, 37.8]	27.5 [20.8, 37.3]	0.787	0.341
Year one	28.4 [20.2, 43.5]	27.0 [21.2, 38.9]	0.855	0.561
Year one change	0.7 [-3.9, 7.4]	0.3 [-4.4, 5.4]	0.257	0.476
Bioavailable Testosterone (ng/dL)				
Baseline	10.8 [7.4, 16.2]	11.3 [7.8, 15.2]	0.729	0.163
Year one	11.5 [8.3, 17.1]	12.2 [8.5, 15.1]	0.990	0.319
Year one change	0.2 [-1.4, 3.0]	0.4 [-1.4, 2.2]	0.440	0.509
SHBG (nmol/L)				
Baseline	63.5 [44.0-91.5]	59.0 [43.3, 90.0]	0.891	0.474
Year one	62.0 [39.0, 87.0]	60.0 [39.0, 83.0]	0.988	0.528
Year one change	-2.3 [-10.0, 7.2]	-2.0 [-9.0, 5.0]	0.899	0.958

IQR: Interquartile range.

Baseline and year one values were ln-transformed for inclusion in models; year one change values were not transformed. Subset excludes women who recurred within the first year of the WHEL study.

* Unadjusted 2-sample T-test using ln-transformed values or non-transformed change values.

[†] p-value based on between group difference for intervention arm term in linear regression model, adjusted for anti-estrogen use, oophorectomy status, and hormone replacement therapy use. Models for year one change also adjusted for ln-transformed baseline value.

Table 2

Risk of additional event by study arm, baseline serum sex hormone concentration, and the study arm and baseline concentration interaction: postmenopausal women who did not report hot flash symptoms at baseline

	HR	p-value	Likelihood Ratio Test*
Model 1: Sex Hormones Not Included			
Intervention Arm	0.51 (0.30-0.85)	0.011	--
Model 2: Total Estradiol (pg/mL)			
Intervention Arm	0.50 (0.29-0.85)	0.010	0.294
Baseline concentration	1.01 (0.61-1.67)	0.960	
Interaction: Intervention Arm × Baseline concentration	0.63 (0.27-1.50)	0.300	
Model 3: Bioavailable Estradiol (pg/mL)			
Intervention Arm	0.51 (0.30-0.86)	0.012	0.322
Baseline concentration	1.07 (0.72-1.61)	0.729	
Interaction: Intervention Arm × Baseline concentration	0.72 (0.37-1.39)	0.324	
Model 4: Total Testosterone (ng/dL)			
Intervention Arm	0.51 (0.30, 0.88)	0.014	0.015
Baseline concentration	1.45 (0.81, 2.59)	0.212	
Interaction: Intervention Arm × Baseline concentration	0.24 (0.08, 0.77)	0.016	
Model 5: Bioavailable Testosterone (ng/dL)			
Intervention Arm	0.54 (0.31, 0.91)	0.022	0.050
Baseline concentration	1.69 (1.00, 2.84)	0.049	
Interaction: Intervention Arm × Baseline concentration	0.37 (0.14, 0.99)	0.049	
Model 6: SHBG (nmol/L)			
Intervention Arm	0.52 (0.31-0.89)	0.016	0.851
Baseline concentration	0.66 (0.38-1.14)	0.137	
Interaction: Intervention Arm × Baseline concentration	0.92 (0.36-2.31)	0.851	

HR: Hazard ratio. Represents one unit increase in ln-transformed baseline concentrations for each serum sex hormone. Models adjusted for site, anti-estrogen use, number of positive nodes, tumor size, oophorectomy status, and previous hormone replacement therapy use.

Sample size: n=22 (9.8%) events out of 225 participants in the intervention arm versus n=42 (18.9%) events out of 222 participants in the comparison arm; subset excludes women who recurred within the first year of the WHEL study.

* Likelihood ratio test used to test the inclusion of the interaction term of baseline concentration and intervention arm in the model.