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Influence of diet on nipple aspirate fluid production and estrogen levels

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Abstract

One possible mechanism how nutritional factors may affect breast cancer risk is through an influence on estrogen levels. Nipple aspirate fluid (NAF) is thought to provide a more direct insight into hormonal influences on breast tissue than serum. The ability to produce NAF may be an indicator of breast cancer risk. The current analysis was conducted as part of a soy trial in 92 premenopausal women and evaluated the relation of usual dietary intake with NAF volume and the most predominant steroidal estrogens in NAF and serum at baseline. Estradiol (E_2) and estrone sulfate (E_1S) were assessed in NAF and E_2, estrone (E_1), and E_1S, in serum using highly sensitive radioimmunoassays. The statistical analysis applied multivariate, log-linear regression models. Intake of saturated fat and cheese (p=0.06 for both) indicated a positive trend with NAF volume whereas isoflavonoid and soy—consumption suggested inverse associations (p=0.08 and p=0.01). For estrogens in NAF, total fat and monounsaturated fat intake was positively associated with E_2 (p=0.05 and p=0.02) and in serum, alcohol intake was associated with higher E_1S levels (p=0.02). These findings suggest a weak influence of dietary composition on NAF production and estrogen levels in serum and NAF.

INTRODUCTION

Nutritional factors, estrogen metabolism, and nipple aspirate fluid (NAF) production have been found to be associated with breast cancer risk. While NAF is thought to provide a more direct marker of hormonal influence on breast tissues than serum, limited research has investigated the influence of dietary intake on estrogen levels in NAF. Interestingly, the ability to produce NAF as well as the volume produced varies across individuals.[1–3] Longitudinal studies have observed a higher breast cancer risk in NAF producing, premenopausal women, especially if abnormal cytology was detected.[4–6] Dietary fat intake was associated with producing NAF especially epithelial cell containing NAF in two studies[7,8] and with circulating estrogen levels in a meta-analysis.[9] Similarly, intakes of lactose and soy were associated with NAF producer status in some studies[3,10] although our randomized crossover trial detected no effect of soy on NAF volume[2] and only a non-significant effect on NAF estrogens levels.[11] In contrast, dietary fiber, as well as fruits and vegetables, were inversely associated with yielding cellular NAF[8] while lowering circulating hormone levels.[12,13] Given that micronutrient levels measured in NAF, such as carotenoids and soy isoflavones, correlate with dietary intake,[14–16] dietary composition may influence estrogen levels in NAF. The current analysis was conducted as part of a randomized, crossover soy trial in premenopausal NAF-producing women. We evaluated the association

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between usual dietary intake of selected nutrients and foods with NAF volume and with estrogen levels in NAF and serum at baseline and paid particular attention to previously reported associations of NAF or breast cancer risk with fat, alcohol, dairy foods, and fruits and vegetables.

MATERIALS AND METHODS

Study design and data collection

As described in detail elsewhere,[2] we conducted a randomized, crossover soy intervention study consisting of two 6-month diet periods (high-soy and low-soy) separated by a 1-month washout period among premenopausal women aged 18–50 years. The study protocol was approved by the Committee on Human Subjects at the University of Hawaii and the participating clinics. All subjects signed an informed consent form at screening. Briefly, we excluded women who consumed more than 5 soy servings per week, had breast implants, used estrogen-containing oral contraceptives, were pregnant or breast-feeding, had been diagnosed with cancer, or did not have a uterus or regular menstrual periods. At the initial screening visit, the women completed a demographic questionnaire, weight and height measurements, and a NAF collection. Of the 310 women screened, 148 (48%) produced any NAF and 112 (36%) produced ≥10 μL.[17] After screening, 96 NAF participants proceeded to randomization and 94 of these completed a self-administered, 26-page food frequency questionnaire (FFQ)[18] that assessed dietary intake during the previous 12 months. Completed FFQs were analyzed for daily intake of nutrients (alcohol, lactose, α-carotene, total fat, saturated fat, isoflavonoid, and dietary fiber) and foods (alcoholic beverage, milk, cheese, yogurt, total dairy food, processed meat, meat, fish and poultry, soy, fruits and vegetables) using an ethnicity-specific food composition database with information from the U.S. Department of Agriculture (USDA) and from additional laboratory analyses in Hawaii and commercial publications.[12,19] Both food intake, expressed in USDA MyPyramid servings of cup, ounce or drink equivalents,[20] and nutrient intake were estimated as energy-density per 1,000 kilocalories (kcals). Two women with total energy intake of > 5000 kcals/day were excluded from all analyses (N=92), and another woman was excluded from estrogens analyses due to missing dietary data.

Sample collection and estrogen assays

For the current analysis, the NAF and serum samples obtained at the randomization visit by trained research staff were used; the NAF samples were carefully collected in capillary tubes and measured to the nearest μL. NAF sample collection was attempted during the mid-luteal phase (3–11 days before the next menstruation) based on previous menstruation dates. The actual date of the next menstruation after the visit was recorded in a follow-up phone call to calculate the number of days between NAF collection and the next menstruation. Due to scheduling problems with the women, many of whom were busy, e.g., working mothers, only 53% of the baseline NAF samples were collected during the mid-luteal phase. The intra-class correlation coefficient of NAF volume of 0.58 over 7 NAF collections throughout the study indicated stability of NAF volume within individuals across time. As reported previously,[11] blood collected at baseline was allowed to clot for 30 minutes and was centrifuged at 3000 rpm for 15 minutes before aliquoting into 1 ml cryovials and freezing at −80°C. Using highly sensitive radioimmunoassays (RIA), estradiol (E_2), estrone (E_1) and estrone sulfate (E_1S) were measured in the Reproductive Endocrine Research Laboratory at the University of Southern California. E_2, E_1 and E_1S were measured in serum, but, due to the small volume, NAF samples were analyzed for E_2 and E_1S only. Half the minimum detection limits of 1.0 pg/mL and 0.005 ng/mL were recorded for NAF E_2 and E_1S levels below the detection limits. Among the 82 women who completed the study, baseline NAF E_2 and E_1S measurements were available for 76 and 77 women, respectively; serum E_2 and

E_1 were available for 76 women; and E_1S was available for 75 women. NAF samples were missing for 2 women and one sample had abnormal E_2 levels due to assay issues. Blood samples were missing for 3 women, and another blood sample had missing E_1S due to high lipid content. Furthermore, one woman was excluded due to missing dietary data and 2 women because of total energy intakes of > 5000 kcals/day.

Statistical analysis

The statistical analysis was performed with the SAS statistical software package version 9.2. (SAS Institute, Inc., Cary, NC). Women were classified into three ethnic categories: Caucasian (42), Asian (13 Japanese, 5 Filipino, 4 Chinese, and 2 Korean), and Other (16 Native Hawaiian, 2 black, 1 American Indian, and 2 Others). Due to the non-normal distributions, dietary intake, NAF volume and estrogen concentrations were log-transformed prior to statistical analysis. The PROC GLM procedure was applied to evaluate the association of usual dietary intake and baseline NAF volume or estrogens in NAF and serum. The model for NAF volume included total energy intake, as well as characteristics previously associated with NAF production:[17] age at screening (continuous), parity (yes or no), ethnicity (Caucasian as reference, Asian, and Other), and body mass index (BMI; continuous). The model for estrogens included total energy intake and menstrual cycle phase (follicular, mid-cycle, mid-luteal, late-luteal, and >28 days), as well as age at screening, ethnicity, BMI, and age at menarche (continuous), which showed associations with at least one of the dietary or estrogen variables.

RESULTS

The mean age at screening (and standard deviation [std]) of the 92 women included in the current analysis was 39.4 (6.4) years with a mean BMI of 26.1 (5.6) kg/m^2 (Table 1). Mean baseline NAF volume was 31 (28) μL, and the respective mean E_2 and E_1S levels in NAF were 127 (141) pg/mL and 54 (111) ng/mL. In serum, mean E_2, E_1, and E_1S were 150 (91) pg/mL, 105 (53) pg/mL, and 2.21 (1.27) ng/mL. According to the FFQ, mean total energy intake (std) was 2,008 (871) kcals/day; mean intakes (std) of total fat, saturated fat, monounsaturated fat, and polyunsaturated fat were 36 (6), 12 (2), 13 (3), and 8 (8) g/1000 kcals/day, respectively. Mean dairy food and milk consumptions (std) were 0.7 (0.4) and 0.4 (0.3) cup/1000 kcals/day. Mean lactose intake (std) was 6 (4) g/1000 kcals/day. Consumption of alcoholic beverages was generally low, and only 12 women (13%) consumed ≥1 drink/day. Soy consumption was also low at a mean intake (and standard deviation) of 0.1 (0.2) oz/1000 kcals/day, which translated into a mean isoflavonoid intake (and standard deviation) of 3 (5) mg/1000kcals/day. Mean fruit and vegetable intakes (std) were 1.2 (0.7) and 2.4 (2.0) cups/1000 kcals/day, respectively, and the mean intake (std) of dietary fiber was 12 (4) g/day.

None of the foods and nutrients chosen for analysis showed statistically significant associations with NAF volume after adjustment for covariates except for isoflavonoids (p=0.01; Table 2). Similar to isoflavonoids, soy intake showed a non-significant inverse association (p=0.08), whereas saturated fat intake indicated a positive albeit non-significant trend (p=0.06). Intake of dairy foods, milk, or lactose was not related with NAF volume. Interestingly, cheese consumption was positively associated with NAF volume (p=0.06), probably reflecting its high fat content. A sensitivity analysis with only mid-luteal samples (N=49) showed similar trends of NAF volume with fat (β=1.5; p=0.06), saturated fat (β=1.4; p=0.04), and cheese (β=2.8; p<0.01).

In relation to estrogens in NAF, alcohol, total fat, monounsaturated fat, and processed meat intakes were positively associated with E_2 (p=0.10, p=0.05, p=0.02 and p=0.10, respectively) after adjustment for covariates; no other foods or nutrients including fruits and

vegetables and dietary fiber indicated any relation. In serum, alcohol consumption, both as a categorical and as a continuous variable, was associated with higher E_1S levels (p=0.02 for both), but not with E_2 or E_1. No other significant associations were detected for the measured serum estrogens. When analyses were limited to mid-luteal samples, alcohol intake was no longer associated with serum E_1S (p=0.80 for alcoholic beverage) but indicated weak associations with NAF E_2 and E_1S (β=1.1 and p=0.05 for both). Total fat (β=4.5; p=0.04), monounsaturated fat (β=5.6; p<0.01), polyunsaturated fat (β=3.9; p=0.03), and processed meat (β=9.5; p<0.01) intake also suggested positive associations with NAF E_2 whereas yogurt consumption indicated inverse associations with NAF E_2 (β=−9.4; p=0.03) and E_1S (β=−8.0; p=0.05). Intake of lactose, milk or cheese was not related to estrogen levels in serum from mid-luteal samples.

DISCUSSION

Unlike previous studies that linked dietary intake with NAF producer status,[3,7,8,10] we observed no strong associations between selected nutrients and foods and NAF volume except for the inverse trend of isoflavones, among women who were able to produce NAF. A similar, non-significant relation was observed for soy consumption; however, the overall consumption of soy, as well as the corresponding intake of isoflavones, was low. Moreover, no significant relations were apparent between the same nutrients and foods and estrogen levels in NAF and serum, except for fat, especially monounsaturated fat, and alcohol consumption. The positive trends for total fat and monounsaturated fat were limited to NAF E_2, and that of alcohol intake to serum E_1S in this population of generally low drinkers. Since dietary fat and alcohol intake have been linked with increased breast cancer risk,[21,22] the current results, despite the inconsistent findings, may suggest a possible dietary influence on endogenous estrogen metabolism.

To our knowledge, this was the first study to explore the association between usual dietary intake as assessed by a one-year FFQ and NAF volume. In the past, a few published studies had examined NAF producer vs. non-producer status by FFQs and food records.[3,7] Strengths of the present study include the diverse ethnic backgrounds of the women and the use of an FFQ that was previously validated within Hawaii's multiethnic population.[18] However, this study also had a number of limitations. Despite our constant efforts during the study, not all NAF and serum samples were collected in the mid-luteal phase. This could have confounded, despite adjustment, the observed findings for estrogens due to the wide variability in circulating estrogen levels among women throughout the menstrual cycle. Our sensitivity analysis based on mid-luteal samples suggested similar results on NAF estrogens, but no significant associations for serum E_1S as compared to the overall results. The observed differences may reflect the time lag in peak estrogen concentrations between NAF and serum;[23] however, they could also be chance findings due to small sample size or Type 1 errors due to multiple testing. The 30% missing E_2 levels are of concern; some samples were low because they were taken in the follicular phase and for others, problems in the lab may have been an issue. Nevertheless, the generally low levels are in agreement with other reports.[24–26] The high levels in one recent study[23] were described as unusually high and could not be explained (Dr. Robert Chatterton, personal communication). Measurement errors associated with the FFQ were also a concern given the limited sample size. In light of these confounding factors, the observed findings should be regarded as preliminary to encourage further investigations of dietary influences on NAF in larger populations. We also used NAF samples from a single collection although the ability to produce NAF varies over time.[2] However, a standardized NAF collection procedure was administered throughout the study, and NAF volume was fairly stable within individuals across time.

While no association was found for lactose intake, the weak associations of saturated fat and isoflavonoid intakes with NAF volume support the possible influence of dietary intake on NAF production; the observed directions of association-positive for saturated fat and inverse for isoflavonoid and soy—with NAF volume parallel the previously-found trends of these dietary factors and breast cancer risk.[22,27] Additional investigations that further evaluate the link between varying NAF producer status and volume and dietary, as well as other known risk factors, may provide additional clues to understanding differences in breast cancer risk across women. Furthermore, evaluations of estrogen levels in NAF may offer important insight into endogenous estrogen metabolism within the breast. In the present analyses, total fat and monounsaturated fat intakes were positively associated with E_2 levels in NAF but not in serum. Alcohol intake, on the other hand, showed a positive relation with E_1S levels in serum but not so clearly in NAF. These apparent inconsistencies between NAF and serum estrogens may reflect the direct vs. indirect measures of estrogen levels in the breast and suggest the need for additional comparisons in larger populations given the ability of NAF to reflect breast tissue activity.

CONCLUSIONS

The current findings suggest a weak influence of dietary composition, mainly due to fat, alcohol, and soy, on NAF production and estrogen levels in NAF and serum.

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Table 1

Baseline characteristics of the participants[†]

N		92
Age at screening (years)		39.4, 6.4
Age at menarche (years)		12.5, 1.4
Body mass index (kg/m^2)		26.1, 5.6
Parity (N)	Yes	68
	No	24
Ethnicity (N)	Caucasian	47
	Asian	24
	Other	21
Randomization group (N)	A	46
	B	46
Total energy (kcals/d)		2008, 871
Nutrient intake[‡] (per 1000kcals/d)	Alcohol (g)	4, 7
	Lactose (g)	6, 4
	Total fat (g)	36, 6
	Saturated fat (g)	12, 2
	Monounsaturated fat (g)	13, 3
	Polyunsaturated fat (g)	8, 2
	Isoflavonoid (mg)	3, 5
	Dietary fiber (g)	12, 4
	α-Carotene (μg)	527, 515
Food intake[‡] (per 1000 kcals/d)	Alcoholic beverage (N)	
	<1 drink/mo	37
	1 drink/mo to <1 drink/d	43
	≥1 drink/d	12
	Milk (cup)	0.4, 0.3
	Cheese (cup)	0.3, 0.2
	Yogurt (cup)	0.1, 0.1
	Total dairy food (cup)	0.7, 0.4
	Processed meat (oz)	0.2, 0.1
	Meat, fish, and poultry (oz)	1.9, 0.9
	Soy (oz)	0.1, 0.2
	Fruits (cup)	1.2, 0.7
	Vegetables (cup)	2.4, 2.0

[†]Date are presented as N or mean and standard deviation (separated by a comma).

[‡]Usual intake of selected nutrients and foods in the United States Department of Agriculture's MyPyramid servings (as cup, ounce or drink equivalent) was estimated from a 1-year food frequency questionnaire (FFQ); 2 FFQs were excluded due to estimated total energy intake > 5000 kcals/day.

Table 2

Association of usual dietary intake of selected foods with NAF volume and estrogens in NAF and serum[†]

Dietary intake		Original unit per 1000kcals/d	NAF[‡]						Serum[‡]					
			Volume (μL)	*P value*	E_2 (pg/mL)	*P value*	E_1S (ng/mL)	*P value*	E_2 (pg/mL)	*P value*	E_1 (pg/mL)	*P value*	E_1S (ng/mL)	*P value*
N			92		76		77		76		76		75	
Below detection limit (N)			--		27		19		0		0		0	
Mean, SD			31, 28		127, 141		54, 111		150, 91		105, 53		2.21, 1.27	
Minimum[‡]			0		1		0.005		14		21		0.87	
Maximum			149		95		850		470		315		9.50	
Regression estimate[†]	**Nutrient**													
	Alcohol	g	−0.1	*0.93*	0.4	***0.10***	0.3	*0.16*	0.1	*0.79*	0.1	*0.22*	0.1	***0.02***
	Lactose	g	0.1	*0.98*	−0.3	*0.46*	−0.1	*0.73*	−0.1	*0.74*	−0.1	*0.50*	−0.1	*0.50*
	Total fat	g	1.0	*0.12*	3.2	***0.05***	1.0	*0.50*	0.2	*0.66*	0.1	*0.84*	−0.1	*0.72*
	Saturated fat	g	1.2	***0.06***	1.9	*0.20*	0.7	*0.61*	0.1	*0.99*	−0.3	*0.42*	−0.3	*0.39*
	Monounsaturated fat	g	0.9	*0.19*	3.8	***0.02***	1.5	*0.31*	0.3	*0.57*	0.2	*0.68*	−0.1	*0.75*
	Polyunsaturated fat	g	0.4	*0.47*	1.6	*0.19*	0.5	*0.63*	0.1	*0.79*	0.3	*0.31*	0.1	*0.97*
	Isoflavonoid	mg	−0.5	***0.01***	−0.1	*0.72*	−0.1	*0.78*	−0.1	*0.27*	0.1	*0.38*	0.1	*0.32*
	Dietary fiber	g	−0.6	*0.17*	−0.5	*0.58*	0.2	*0.84*	−0.3	*0.34*	0.1	*0.87*	−0.1	*0.52*
	α-carotene	μg	−0.2	*0.16*	−0.1	*0.98*	0.2	*0.49*	0.1	*0.87*	0.1	*0.24*	0.1	*0.63*
	Food[§]													
	Alcoholic beverage	drink	−0.1	*0.98*	0.3	*0.45*	0.3	*0.38*	0.1	*0.85*	0.1	*0.22*	0.2	***0.02***
	Milk	cup	−0.1	*0.86*	−1.0	*0.38*	−0.7	*0.52*	−0.1	*0.79*	−0.1	*0.81*	−0.1	*0.59*
	Cheese	cup	1.8	***0.06***	0.9	*0.68*	0.8	*0.68*	−0.5	*0.46*	−0.8	*0.11*	−0.5	*0.30*
	Yogurt	cup	−0.7	*0.62*	−4.5	*0.15*	−0.5	*0.85*	−0.8	*0.36*	−0.9	*0.20*	−0.6	*0.41*
	Total dairy food	cup	0.2	*0.62*	−1.0	*0.39*	−0.4	*0.68*	−0.2	*0.50*	−0.3	*0.28*	−0.2	*0.34*
	Processed meat	oz	0.3	*0.78*	4.0	***0.10***	1.5	*0.49*	0.5	*0.50*	0.3	*0.64*	0.1	*0.89*
	Meat, fish and poultry	oz	−0.2	*0.58*	−0.1	*0.94*	0.3	*0.73*	0.1	*0.93*	0.1	*0.61*	0.2	*0.28*
	Soy	oz	−1.4	***0.08***	−1.0	*0.56*	−0.2	*0.90*	−0.8	*0.12*	0.2	*0.58*	0.1	*0.79*
	Fruits	cup	0.1	*0.93*	−0.5	*0.54*	−0.1	*0.96*	−0.1	*0.49*	−0.1	*0.56*	−0.1	*0.42*

Dietary intake	Original unit per 1000kcals/d	NAF‡						Serum‡					
		Volume (μL)	*P value*	E_2 (pg/mL)	*P value*	E_1S (ng/mL)	*P value*	E_2 (pg/mL)	*P value*	E_1 (pg/mL)	*P value*	E_1S (ng/mL)	*P value*
Vegetables	cup	−0.4	*0.15*	−0.4	*0.61*	0.2	*0.72*	−0.1	*0.83*	0.2	*0.16*	0.1	*0.40*

†Regression estimates were calculated on a log-log model between log-transformed NAF volume or estrogens and log-transformed dietary intake (density per 1000 kcals/day) of nutrients and foods from a 1-year FFQ; covariates included total energy intake, age at screening, body mass index, ethnicity, age at menarche, and menstrual cycle phase.

‡E_2=estradiol, E_1=estrone, E_1S=estrogen sulfate; for NAF E_2 and E_1S, half the minimum detection limits of 1 pg/mL and 0.005 ng/mL, respectively, were recorded for observations below the detection limits.

§Usual food intake was estimated in the United States Department of Agriculture's MyPyramid servings (as cup, ounce or drink equivalent) from a 1-year food frequency questionnaire.