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Dietary intake of lactose as a strong predictor for secretor status of nipple aspirate fluid in healthy premenopausal non-lactating women[1]

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Abstract

Purpose—Nipple aspirate fluid (NAF) is considered a potential source for discovering breast cancer biomarkers. However, the success rate of obtaining NAF was reported to vary from 48 to 77%, and mechanisms for its secretion are not fully understood. The purpose of this study was to investigate dietary, demographic, reproductive, hormonal, and anthropometric factors that are associated with the ability to obtain NAF by aspiration (secretor status) from premenopausal women.

Study Design—NAF procedures were attempted for women who were 30–40 years old, not pregnant, not breastfeeding, and not taking contraceptive medications.

Results—Compared with non-secretors, secretors of NAF consumed significantly more dietary lactose (mainly from milk), were more likely to be parous, were older at first and last childbirth, breastfed their babies for a longer period of time, and had an earlier menarche and lower plasma concentrations of 17β-estradiol ($P<0.05$). Using multivariate logistic regression models, higher dietary intake of lactose [Odds Ratio (OR) =2.7; 95% Confidence Interval (CI): 1.5–4.8], earlier menarche (OR=0.8, CI: 0.7–1.0), being parous (OR=2.3, CI: 1.0–5.6), and older at first childbirth (OR=1.5, CI: 1.0–2.1) were found to be independent and positive predictors for being a secretor of NAF.

Conclusions—These findings suggest that dietary intake of lactose, a modifiable factor, may be used to change the NAF secretor status of women. This finding may facilitate the use of NAF as a diagnostic material for detecting breast diseases.

Keywords

nipple aspirate fluid; lactose; breast cancer

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Introduction

Breast cancer usually originates from epithelial cells that line the ducts of the breast. Fluid can be collected repeatedly by nipple aspiration (NAF) from the breast ducts of non-lactating women. The components of NAF include exfoliated epithelial cells, proteins, fats, and lactose (1,2). Prospective cohort studies showed that certain cytological findings in cells from NAF predicted breast cancer risk independently from traditional risk factors (3). In addition to cells, concentrations of various proteins, hormones, and lipids in NAF have also been associated with breast cancer risk (4–6). Potential tumor markers, including carcinoembryonic antigen, prostate specific antigen, c-erbB-2 (Her2/neu), basic fibroblastic growth factor, and vascular endothelial growth factor have been detected in NAF using ELISA and/or western blot (7–10). Recent advances in proteomic technologies, especially in mass spectrometry, have enabled further characterization of proteomes of NAF and identification of markers associated with breast cancer risk, such as α1-acid glycoprotein and vitamin D binding protein (4,11,12). Therefore, NAF provides a window to probe the biochemical, physiological, and pathological changes in the breast and has been considered a potential source for the early detection of breast cancer.

The reported success rate for obtaining NAF is suboptimal and ranges from 45% to 77% (13). Several large cross-sectional studies have investigated the association of NAF secretion with demographic and genetic features, reproductive history, and the hormonal status of women. Wrensch *et al* (14) associated easier secretion of NAF with earlier age of menarche, age in the range of 35 to 50 years, non-Asian ethnicity, and prior history of lactation. Miller *et al.* (13) reported that current oral contraceptive users were significantly less likely to yield NAF than those who had never taken those medications. Petrakis *et al.* (15) found no significant differences in serum concentrations of 17β-estradiol and estrone between secretors and non-secretors. Higgins *et al.* (16) reported that a reduced volume of NAF was associated with postmenopausal status, BRCA germline mutations and risk reduction therapies such as salpingo-oophorectomy and use of selective estrogen receptor modulators (SERMS). Lee *et al.* (17) reported a positive association between higher dietary fat and NAF secretion, but the influences of other nutritional variables were not studied. Petrakis *et al.* (18) found that consumption of soy increased the success rate for obtaining NAF and the volume of NAF obtained. These reports suggest that NAF production might be influenced by multiple factors. However, whether these factors were independent predictors has not been studied in multivariate-adjusted models.

Secretor status relates to the volume of fluid produced in the breast ducts. Lactose, which is present in NAF (1), is an important nutrient in breast milk, and is also an osmotic agent. In the lactating mammary gland, its osmotic function causes water influx and increases the volume of milk (19). We hypothesized that the osmotic function of lactose in the non-lactating breast may be associated with NAF fluid volume and secretor status. In this study, we investigated the association of dietary intake of lactose with secretor status in a well-defined study population. In addition, we examined nutritional, demographic, reproductive, anthropometric, and hormonal factors that might be independent predictors of secretor status.

Materials and Methods

Study design

This cross-sectional study included healthy premenopausal women who were 30 to 40 years old and of all major races. The exclusion factors included use of contraceptive medications (pills, injections, or depots) during the past 6 months, pregnancy, lactation, irregular menstrual cycles, being a vegetarian and having first degree relatives with breast cancer. All women had normal mammograms at entry. All participating subjects (N=238) were recruited from

communities within a 50 mile radius of Galveston, Texas, by posted advertisements and postal mailings. Written informed consent was obtained from all subjects. A total of six study visits were scheduled for each subject during the luteal phases of two menstrual cycles, usually on the days between cycle day 20 and 24, with three visits during each menstrual cycle. The study protocol was approved by the Institutional Review Board of the University of Texas Medical Branch and the Human Subject Research Review Board of the US Army Medical Research and Materiel Command. The subjects for this study were volunteers for a dietary intervention study. Baseline information obtained prior to the onset of the dietary intervention were used for this analysis.

Methods

Procedures for obtaining NAF—A breast pump made in our laboratory was used to obtain NAF by gentle suction. Attempts to acquire NAF were made on three of the six separate study visits. On each of the three visits, the breast pump was applied to the breast, and suction was held for 15 seconds. A subject was classified as a "non-secretor" if no fluid was obtained from either breast on any of three visits. If fluid was successfully collected from at least one breast on at least one of three study visits, the patient was classified as a NAF secretor. For secretors, the volume of NAF obtained was recorded, and it was categorized as low (<10 μl), medium (10–30 μl), or high (>30 μl).

Measurements of nutrients, reproductive variables, anthropometrics, and hormones—Study subjects were instructed to record food intake (food item, brand name, and amount) for the 24 hours preceding three scheduled study visits. Three food records were obtained from each subject and later analyzed using the Nutrition Data System for Research software (developed by the Nutrition Coordinating Center, the University of Minnesota, MN). Using this software, daily intakes of 139 nutrients were estimated and reported for each food record. The average nutrient intake of three 24-hour food records was used for statistical analyses of nutritional influences on secretor status. A food frequency questionnaire (Harvard School of Public Health and the Brigham and Women's semi-quantitative food frequency questionnaire, 96/97 GP) was also administered at entry to the study, to assess dietary habits during the preceding year. Questionnaires were analyzed by the Nutrition Questionnaire Service Center at the Harvard School of Public Health (Boston, MA).

Reproductive history was obtained using a self-administered standard clinic questionnaire originally designed for gynecologic consultations. Age of menarche and parity (yes or no) were recorded for all subjects. For parous women, history of lactation (yes or no), cumulative length (months) of breastfeeding, age at first and last childbirth, and number of childbirths were also recorded.

During each study visit, body weight and height were measured. On one study visit, total body mass, body lean mass, and body fat mass were measured using dual energy X-ray absorptiometry (DEXA) (Model QDR4500A, Hologic, Waltham, MA). The subjects were examined with DEXA in a supine position in duplicate, with interval repositioning on the examination table, during one of the study visits. The average value for the duplicate measurements was used for statistical analyses.

Fasting blood samples were obtained at all six study visits. The first three samples from one luteal phase were used for measuring steroid hormones. All of the collected blood samples were immediately stored at −80°C until analysis.

Plasma was analyzed for progesterone (radioactive immunoassay, sensitivity 0.1 ng/ml), testosterone (ELISA, sensitivity 0.04 ng/ml), and 17β-estradiol (ELISA, sensitivity 7 pg/ml) using commercial immunoassay kits (all from Diagnostics Labs, Webster, TX) according to

the manufacturer's instructions. For all the assays, the average intra- and inter-assay coefficients of variations (CV) were less than 15%. Each sample was assayed at least twice, and results were averaged. Hormone concentrations from three different cycle days of the same luteal phase were averaged for each subject for statistical analyses. Triiodothyronine (T3), tetraiodothyronine (T4), and thyroid stimulating hormone (TSH) were measured in fasting blood samples from two visits by a certified hospital clinical lab, and the results were averaged for statistical analyses.

Statistical analyses—Means and standard deviations were computed for continuous data, and percentages were computed for categorical data. For univariate analyses, the two group *t*-test was performed on continuous variables for comparisons between secretors and non-secretors, and the χ^2 test was used for categorical variables to compare the distribution of secretors in each category. Pearson's correlation coefficients (r) were computed for associations among variables of interest. Factors that showed association with NAF secretor status in univariate analysis ($P<0.1$), as well as age and ethnicity, were further entered into multivariate logistic regression models to determine independent effects of each factor. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression for factors that independently predict secretor status. A trend test between lactose intake and the volume of NAF among secretors was conducted using contrast statement in the General Linear Model procedure. All statistical analyses were performed using SAS® (Version 9.1, SAS Institute Inc., Cary, NC).

Results

Prevalence and characteristics of secretors and nonsecretors in the study population

NAF was successfully obtained from 156 (66%) of 238 women participating in the study (Table 1). Our success rate for obtaining NAF was consistent with previous reports (13,16).

Nutritional, demographic, reproductive, hormonal, and anthropometric characteristics of the subjects are listed in Table 2. NAF secretors were slightly older (by 0.7 years, P=0.07), younger at menarche (by 0.5 years, P=0.03), older at first childbirth (by 2.1 years, P<0.001) and at last childbirth (by 1.6 years, P=0.005), and had lower concentrations of plasma 17β-estradiol (by 10.3 pg/ml, P=0.04) compared with nonsecretors. Compared with nulliparous women, parous women were more likely to secrete NAF (by 20%, P=0.04), and parous women who breastfed for more than 3 months were more likely to be secretors compared with those who had never breastfed or breastfed for 3 months or less (by about 20%, P=0.02). Data for nutrient intake was estimated from both the food records and food frequency questionnaires. Estimates, (shown in Table 2) from food records suggested that secretors consumed significantly more lactose (by 34%, P<0.001), and also had a significantly higher consumption of milk (by 37.8 g, P=0.01) and calcium (by 91mg, P=0.02), compared with nonsecretors. Data from the food frequency questionnaire further confirmed that secretors, on average, had a significantly higher consumption of lactose (11.1 ± 10.7 g) in the past year compared with non-secretors (8.5 ± 8.0 g, P=0.05, results not shown in Table 2).

There were no significant differences between the two groups in the distribution of ethnicity; anthropometric measurements; plasma concentrations of testosterone or progesterone; serum concentrations of T3, T4 or TSH; or dietary intake of calories, proteins, fats, or carbohydrates. Among parous secretors and parous non-secretors, there were no differences in the number of childbirths.

Many of the variables examined were significantly correlated with one another in our study. As expected, lactose intake strongly correlated with calcium intake (r=0.72, P<0.001) and milk consumption (r=0.89, P<0.001). In addition, lactose intake also moderately correlated with age

at first childbirth (r=0.22, P<0.01), length of breastfeeding (r=0.27, P<0.01), and age at last childbirth (r=0.16, P=0.02). Age at last childbirth also correlated significantly with age at first childbirth (r=0.52, P<0.001). Probably due to the age restriction of our study cohort, age at last childbirth also correlated significantly with age (r=0.22, P=0.01). This may not have been true if the population had had a wider age range.

Logistic regression models to predict secretor status

Logistic regression models were constructed to predict NAF secretor status, as shown in Table 3. Since lactose intake exhibited a larger difference between secretors and non-secretors (Table 2), effect of lactose intake, rather than calcium or milk consumption, was the predictor variable entered into the logistic regression models. Model 1 included lactose intake only, which was a significant factor for being a secretor (OR=2.3, CI: 1.4–3.9). In model 2, the effect of lactose intake was adjusted for intake of total calories, proteins, fats and carbohydrates. High lactose intake remained an independent predictor for being a secretor of NAF (OR=2.6, CI: 1.4–4.7). Intake of total calories, proteins, fats, or carbohydrates was not a significant predictor. In model 3A, the effect of lactose was adjusted for other demographic and reproductive variables that had previously been reported to be associated with secretor status (14), including age, ethnicity, age of menarche, and parity. Higher lactose intake was still a strong predictor for being a secretor (OR=2.7, CI: 1.5–4.8) and was independent of age (OR=1.1, CI: 1.0–1.2), earlier age of menarche (OR=0.8, CI: 0.7–1.0), and being parous (OR=2.3, CI: 1.0–5.5). When milk or calcium intake was entered in model 3A to substitute for lactose intake as an independent variable (data not shown in Table 3), they were also strong predictors for secretor status (for milk, OR=1.4, CI: 1.0–1.8, increment of 100 g; for calcium, OR=1.3, CI: 1.0–1.6, increment of 200 mg), after adjusting for age (OR=1.1, CI: 1.0–1.2), earlier age of menarche (OR=0.8, CI: 0.7–1.0) and being parous (OR=2.3, CI: 0.9–5.4). The apparent effects of milk and calcium were expected, because intake of milk and calcium closely correlated with that of lactose, and therefore, served as surrogates for lactose intake in these models. To further ascertain whether the effect of lactose observed was independent of milk or calcium intake, we added the latter two variables to model 3A, and found that lactose intake remained a significant predictor (OR=1.6, CI: 1.1–2.4, increment of 10 g) for secretor status, after controlling for milk consumption (OR=0.91, CI: 0.75 – 1.11, increment of 100g), calcium intake (OR= 0.99, CI: 0.72 – 1.33, increment of 200 mg), age (OR=1.1, CI: 1.0–1.2), age of menarche (OR=0.8, CI: 0.7–1.0) and parity (OR=2.4, CI: 1.0–5.9).

Model 3B was restricted to parous women only and included all variables shown in model 3A plus the variable “age at first childbirth”. Age at first childbirth (OR=1.5, CI: 1.0–2.1) remained a strong predictor for secretor status in parous women, independent of lactose intake (OR=2.6, CI: 1.4–4.9), age (OR=1.1, CI: 1.0–1.2), and age at menarche (OR=0.8, CI: 0.6–1.0). Age at last childbirth was not entered in the model because of its strong correlation with “age at first childbirth”. When age at first childbirth was replaced by age at last childbirth in the model for parous women, the independent effect of age at last childbirth was not significant (OR=1.3, CI: 0.9–1.9, P=0.18, increment of 5 years), while the effects of lactose (OR=2.7, CI: 1.4–5.2, P=0.003) and age at menarche (OR=0.80, CI: 0.7–1.0, P=0.04) remained as strong predictors.

After mutual adjustment, an increment of 10 g per day in lactose intake and being parous both increased the odds of being a secretor by about 2 fold. An increment of 1 year in age of menarche decreased the odds of being a secretor by 0.8 fold. For parous women of 30 to 40 years of age, in addition to the above factors, an increment of 5 years in age at first childbirth increased the odds of being a secretor by 1.5 fold.

When the data were analyzed using univariate analysis, secretors also had significantly lower concentrations of plasma estradiol. Data for estradiol concentrations were unavailable from 29 subjects because the kit used for measuring this hormone was discontinued by the manufacturer

before we had completed our analyses. Multi-variate analyses were thus performed only on the subset of subjects (N=209) with estradiol data. When demographic and reproductive variables (age, ethnicity, age of menarche, and parity) were included in the model, the independent effect of estradiol was not significant (OR=0.99, CI: 0.99–1.00, P=0.21), while high lactose intake (OR=3.4, CI: 1.7–7.1, P<0.001), earlier age of menarche (OR=0.8, CI: 0.7–1.0, P=0.03), and being parous (OR=2.5, CI: 1.0–6.5, P=0.05) remained strong and independent predictors for being a secretor of NAF.

Data for length of breastfeeding were missing from 35 parous women, but univariate analysis based on the data available (N=176) showed that women who breastfed for more than 3 months were more likely to be secretors. Logistic regression analyses were also performed on this subset of subjects. When demographic and reproductive variables (age, ethnicity, age of menarche, and age at first childbirth) were included in the model, the independent effect of breastfeeding was not significant (OR=1.2, CI: 0.5–3.3, P=0.13, breastfed for over 3 months vs. never breastfed). High lactose intake (OR=2.9, CI: 1.2–6.6, P=0.008), earlier age of menarche (OR=0.7, CI: 0.6–1.0, P=0.02), and older age at first childbirth (OR=3.0, CI: 1.2–7.3, P=0.02, increment of 5 years) remained independent predictors for being secretors.

The characteristics of the subjects with missing data (N=27 for estradiol concentrations and N=35 for breastfeeding data) did not differ from the remaining subjects included in the subsets of multivariate analysis models by anthropometric, reproductive, nutritional, or hormonal status (results not shown). Therefore, results of the multivariate analyses are not expected to be significantly affected by the missing data.

Association between volume of NAF and lactose intake

Since lactose has osmotic properties that may affect the volume of breast secretion, and therefore, secretor status, a trend analysis was performed between lactose intake and NAF volumes among secretors only. The fluid volumes among the secretors were categorized as low (<10 μl), medium (10 to 30 μl) and high (>30 μl), and the average intake of lactose was 7.6 ± 5.9, 8.3 ± 5.6, and 9.9 ± 9.8 g for these three secretor groups, respectively. Thus, the amount of lactose consumed appeared to be positively associated with NAF volume (P trend=0.08).

Discussion

In this study, we found that higher dietary intake of lactose and milk, earlier age of menarche, being parous, and older age at first childbirth are all independent and positive predictors for being a secretor of NAF. Moreover, among secretors, lactose intake was positively associated with the fluid volume of NAF that was obtained. These data suggest that the ability to secrete fluid and the fluid volume are under the regulation of multiple factors. We suggest that reproductive factors and osmotic effects of lactose intake both play important roles.

The reproductive factors we found to be associated with secretor status are consistent with those reported by others. Several studies have attempted to link NAF secretor status with breast cancer risk, but with mixed results (16,20). This is not surprising based on our observations that some of the reproductive factors associated with being secretors increase whereas others decrease the risk for breast cancer. For example, earlier age of menarche and older age at first childbirth have been shown to increase breast cancer risk, while also increasing the odds of being a secretor. However, being parous, having breastfed for a longer period of time, and having lower plasma estradiol concentrations were associated with lower risk of breast cancer, while increasing the likelihood of being a secretor. Therefore, we suggest that secretor status might not be related to breast cancer risk. Our suggestion deserves further study because, for

substances in NAF to be useful for early diagnosis of breast cancer, any effort to increase NAF yield should not also increase the risk of breast cancer.

Age was included in all multivariate models as a co-variate, and older age was found to be marginally associated with being a secretor (OR=1.1, P=0.05 to 0.13). However, due to the narrow age range of our study population, this observation may have limited inference. Being parous and of an older age at first childbirth increased the odds of being a secretor. Number of childbirths was not associated with secretor status. Therefore, those who were older rather than younger at first childbirth were more likely to have had a child that was near the time of NAF samplings. This suggests that ever pregnancy and having a short time that has lapsed since last complete pregnancy are both important for remaining a secretor. Consistent with this hypothesis, we found, in univariate analysis, that secretors were also older at last childbirth (Table 2).

However, by multi-variate analysis, age at last childbirth was not a significant predictor for being a secretor, probably because this variable correlated well with age (r=0.22, P=0.01) and lactose intake (r=0.16, P=0.02). Consistent with the report by Petrakis *et al.* (14), a longer period of lactation was positively associated with being a secretor in our univariate analysis. However, it was not an independent predictor after adjustment for other variables, such as lactose intake, probably because women who were parous and had breastfed for a longer period of time also tended to consume more milk/lactose (P<0.01). Therefore, we suggest that some residual biochemical changes induced by a complete pregnancy persist even beyond lactation and may have an influence on secretor status, as discussed below.

In our study, the effect of lactose consumption on secretor status was independent of reproductive factors and intake of fats, proteins, carbohydrates, and total calories. This observation was found regardless of whether lactose intake was estimated from three 24- hour food records or from food frequency questionnaires. In addition, the effect of lactose remained strong after controlling for intake of milk and calcium. This suggests that lactose, not calcium or milk, might be the actual dietary source responsible for the secretion status of NAF in non-lactating women.

Lactose has been detected in NAF (1,2). However, lactose in NAF is not derived directly from dietary lactose in milk, but is synthesized *de novo* in the mammary gland. This is because lactose is a disaccharide, and cannot be absorbed intact by the intestine. Dietary lactose need to be first metabolized in the intestine to galactose and glucose, which can then be absorbed and enter into circulation. In the breast glands galactose and glucose are combined for the re-synthesis of lactose by lactose synthase. This enzyme is a complex of two proteins, protein A (also known as galactosyltransferase) and protein B (known as α-lactalbumin) (21). Lactose is an important energy source in milk for infants. Because it is osmotically active, its physiological effect in the lactating breast is to induce fluid influx, and increases milk volume. Lactose in the non-lactating breast can be expected to have a similar osmotic effect and increase fluid volume in the breast ducts, thus facilitating collection of NAF.

In the lactating mammary gland, 50 to 80% of galactose is synthesized *de novo* from glucose by a panel of enzymes that are highly expressed in the mammary gland during lactation (22). In the non-lactating breast, however, the enzymes for the *de novo* synthesis of galactose from glucose, such as UDP-galactose 4-epimerase, are of much lower activity than in the lactating mammary gland (23,24). Thus, circulating galactose from the breakdown product of dietary lactose might be a greater source for lactose synthesis, and therefore lactose content, in the non-lactating than in the lactating mammary glands. This may explain the positive association in our study between dietary intake of lactose and milk and being a secretor. Consistent with the ability of the non- lactating breast to synthesize lactose, is our prior observation that α-

lactalbumin (protein B of lactose synthase) was present abundantly in nipple aspirate fluids of 1/3 of our non- lactating secretors (25) and also that a strong correlation was found between lactose intake and volume of NAF among secretors in the present study. Therefore, it is tempting to speculate that a higher prevalence of α-lactalbumin might be more easily found in the mammary glands of non-lactating women with a more recent history of pregnancy. Consequently, higher intake of dietary lactose (a precursor for galactose) and a more recent history of pregnancy (with a greater residual amount of α-lactalbumin) might be important, but independent, predictors for having a higher volume of fluid in the breast ducts and being a secretor. Controlled studies of lactose feeding are needed to confirm a direct causal relationship between lactose intake and increased secretion of NAF.

Among the 139 dietary nutrients analyzed and reported using the Nutrition Data System for Research Software, lactose showed the strongest association with secretor status, independent of milk and calcium intake. Since milk is not an independent predictor from, and not a stronger predictor than, lactose, this suggests that the effect of lactose may be independent of milk intake and any other milk components not included in the nutrient database. In addition, lactose has a physiologic role in influencing fluid volume in the NAF. Therefore, we speculate that intake of lactose may be the major factor in diet that can influence the secretion of NAF. However, other chemicals/constituents in milk, which can not be estimated from our nutrient analysis software, may also contribute to the secretor status and deserve future studies.

Other dietary factors that have been associated with being a secretor of NAF include higher consumption of dietary fats, as reported by Lee *et al.* (17). However, we could not confirm this association in our study. In addition, Petrakis *et al.* (15) found no differences in serum concentrations of 17β-estradiol between secretors and non-secretors. In our study cohort, a lower plasma concentration of estradiol was observed in secretors; however, an independent effect of estradiol was not evident after adjusting for other reproductive and demographic factors.

The strength of our study includes a well-defined population and a variety of variables, which made it possible to evaluate effects of dietary, demographic, reproductive, and hormonal factors in multivariate-adjusted analyses. However, our exclusion criteria limited the inferences that could be made from our study since we did not include women who had abnormal mammograms, were outside the age range of 30 to 40 years old (including postmenopausal women), or were on contraceptive medications.

In summary, we found that consumption of dietary lactose, in the presence of residual factors from pregnancy, has a strong influence on secretor status. Thus, we showed that diet influences the secretory activity of the breast. NAF might be a suitable source of material for probing pathophysiologic changes in the breast, and modulation of secretor status by diet may have important implications for the detection of biomarkers for breast cancer.

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Table 1

The frequency distribution of NAF secretors

Secretor status	N	Percentage
Secretors	156	66%
Non-secretors	82	34%
Total	238	100%

Table 2

Characteristics of the study population, by NAF secretor status

A. Continuous variables	**All subjects (N=238)**	**Non-secretors (N=82)**	**Secretors (N=156)**	**P**[1]
Nutrients and food groups[2]				
Lactose (g)	7.6 ± 6.7	5.7 ± 4.6	8.6 ± 7.4	<0.001
Total caloric intake (kcal)	1751.4 ± 520.7	1736.1 ± 616.2	1759.4 ± 464.7	0.74
Total protein intake (g)	67.7 ± 21.5	67.8 ± 23.3	67.6 ± 20.6	0.94
Total fat intake (g)	75.3 ± 26.7	75.4 ± 29.2	75.3 ± 25.4	0.99
Total carbohydrate intake (g)	200.9 ± 72.5	196.9 ± 85.9	203.0 ± 64.6	0.54
Calcium (mg)	617.2 ± 295.5	554.3 ± 270.9	651.0 ± 303.4	0.02
Milk (g)	71.0 ± 122.3	48.9 ± 82.3	86.7 ± 142.9	0.01
Age and reproductive history				
Age (yr)	36.1 ± 2.7	35.7 ± 2.7	36.4 ± 2.6	0.07
Age of menarche (yr)	12.6 ± 1.6	13.0 ± 1.6	12.5 ± 1.5	0.03
Number of childbirths[3]	2.6 ± 1.1	2.5 ± 1.1	2.6 ± 1.2	0.92
Age at first childbirth[3]	23.0 ± 5.0	21.6 ± 4.0	23.7 ± 5.3	<0.001
Age at last childbirth[3]	29.4 ± 4.5	28.3 ± 4.8	29.9 ± 4.3	0.02
Hormones				
Testosterone (ng/ml)	0.8 ± 0.9	0.8 ± 0.6	0.8 ± 1.0	0.9
17β-Estradiol (pg/ml)[4]	78.2 ± 34.2	85.0 ± 39.7	74.7 ± 30.7	0.04
Progesterone (ng/ml)	10.8 ± 5.5	10.5 ± 5.6	10.9 ± 5.5	0.56
T3 (ng/dl)	124.8 ± 36.3	126.8 ± 35.3	123.7 ± 37.0	0.55
T4 (μg/dl)	8.3 ± 1.4	8.2 ± 1.5	8.3 ± 1.4	0.68
TSH (μIU/ml)	2.4 ± 5.4	2.3 ± 1.8	2.4 ± 6.6	0.82
Anthropometrics				
Height (cm)	161.8 ± 6.8	162.2 ± 6.3	161.6 ± 7.0	0.52
Weight (kg)	74.7 ± 14.8	76.8 ± 16.3	73.6 ± 13.9	0.12
BMI (kg/m^2)	28.6 ± 5.5	29.2 ± 6.1	28.3 ± 5.3	0.21
Body fat mass (kg)	27.9 ± 9.7	29.2 ± 10.5	27.2 ± 9.3	0.16
Body lean mass (kg)	46.5 ± 6.2	46.8 ± 6.2	46.2 ± 6.2	0.5

B. Categorical variables	**All subjects**	**Secretors**	**% of secretors**	**P**[5]
Race/ethnicity				
Caucasian	122	88	72.1	0.19
Hispanic	72	46	63.9	
Black	35	18	51.4	
Asian	3	2	66.7	
Other	6	3	50	
Parity				
No	27	13	48.2	0.04
Yes	211	143	68.2	
Length of breastfeeding[6]				

A. Continuous variables	All subjects (N=238)	Non-secretors (N=82)	Secretors (N=156)	P[1]
Never	46	31	67.4	0.02
3 months or less	35	21	60	
More than 3 months	95	78	82.1	

[1] P value for two group t-test;

[2] Data obtained from food records;

[3] For parous subjects only, N=211;

[4] N=209, with 139 secretors (67%), and 70 non-secretors (33%);

[5] P value for Chi-square test;

[6] A total of 176 parous women had data for length of breastfeeding; 130 secretors (73.9%), and 46 non-secretors (26.1%).

Table 3

Logistic regression models to predict NAF secretor status (N=238)

	Models[2]			
Variables[1]	**Model 1**	**Model 2**	**Model 3A**	**Model 3B**
Lactose intake	2.3 (1.4, 3.9)	2.6 (1.4, 4.7)	2.7 (1.5, 4.8)	2.6 (1.4, 4.9)
Total caloric intake	NE[3]	1.2 (0.6, 2.2)	NE	NE
Protein intake	NE	0.9 (0.6, 1.2)	NE	NE
Fat intake	NE	0.9 (0.5, 1.6)	NE	NE
Carbohydrate intake	NE	0.9 (0.7, 1.2)	NE	NE
Age	NE	NE	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)
Ethnicity	NE	NE	0.6 (0.3, 1.3)	0.9 (0.4, 1.8)
Menarche	NE	NE	0.8 (0.7, 1.0)	0.8 (0.6, 1.0)
Parity	NE	NE	2.3 (1.0, 5.6)	NE
Age at first childbirth	NE	NE	NE	1.5 (1.0, 2.1)

[1]Lactose, increment of 10 g; total caloric intake, increment of 100 kcal; protein intake, increment of 10 g; fat intake, increment of 10 g; carbohydrate intake, increment of 10 g; age at first childbirth, increment of 5 years; parity, yes vs. no.

[2]Model 1: the effect of lactose was estimated. Model 2: the effect of lactose was adjusted by other nutrients including intake of total calories, proteins, fats and carbohydrates. Model 3A: the effect of lactose was adjusted for demographic and reproductive variables, including age, ethnicity, age of menarche, and parity.

Model 3B: for parous women only (N=211), the effect of lactose was adjusted by demographical and reproductive variables, including age, ethnicity, age of menarche, and age at first childbirth.

[3]NE, variable not allowed to enter the model