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Serum Phospholipid Fatty Acids and Mammographic Density in Premenopausal Women

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ABSTRACT

Background: The role of fatty acids (FAs) on mammographic density (MD) is unclear, and available studies are based on self-reported dietary intake.

Objectives: This study assessed the association between specific serum phospholipid fatty acids (PLFAs) and MD in premenopausal women.

Methods: The cross-sectional study DDM-Madrid recruited 1392 Spanish premenopausal women, aged 39–50 y, who attended a screening in a breast radiodiagnosis unit of Madrid City Council. Women completed lifestyle questionnaires and FFQs. Percentage MD was estimated using a validated computer tool (DM-Scan), and serum PLFA percentages were measured by GC-MS. Multivariable linear regression models were used to quantify the association of FA tertiles with MD. Models were adjusted for age, education, BMI, waist circumference, parity, oral contraceptive use, previous breast biopsies, and energy intake, and they were corrected for multiple testing.

Results: Women in the third tertile of SFAs showed significantly higher MD compared with those in the first tertile (β_{T3vsT1} = 7.53; 95% CI: 5.44, 9.61). Elevated relative concentrations of palmitoleic (β_{T3vsT1} = 3.12; 95% CI: 0.99, 5.25) and gondoic (β_{T3vsT1} = 2.67; 95% CI: 0.57, 4.77) MUFAs, as well as high relative concentrations of palmitelaidic (β_{T3vsT1} = 5.22; 95% CI: 3.15, 7.29) and elaidic (β_{T3vsT1} = 2.69; 95% CI: 0.59, 4.79) *trans* FAs, were also associated with higher MD. On the contrary, women with elevated relative concentrations of n–6 (ω-6) linoleic (β_{T3vsT1} = −5.49; 95% CI; −7.62, −3.35) and arachidonic (β_{T3vsT1} = −4.68; 95% CI: −6.79, −2.58) PUFAs showed lower MD. Regarding desaturation indices, an elevated palmitoleic to palmitic ratio and a low ratio of oleic to steric and arachidonic to dihomo-γ-linolenic acids were associated with higher MD.

Conclusions: Spanish premenopausal women with high relative concentrations of most SFAs and some MUFAs and *trans* FAs showed an increased MD, whereas those with high relative concentrations of some n–6 PUFAs presented lower density. These results, which should be confirmed in further studies, underscore the importance of analyzing serum FAs individually. *J Nutr* 2020;00:1–10.

Keywords: breast density, fatty acids, desaturation index, premenopause, DDM-Madrid, biomarkers, fat, breast cancer, epidemiology

Introduction

The mammographic image reflects variations in the composition of the breast tissue: the darker areas correspond to the fatty tissue, and the lighter or denser areas represent the fibroglandular tissue. Mammographic density (MD) refers to the percentage of mammography composed of radiologically dense tissue, and it is an important risk factor for developing

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breast cancer (1, 2). Given that MD can be influenced by dietary factors, such as a Western dietary pattern, calorie intake, or olive oil consumption (3, 4), the identification of these specific nutrients may be of special interest for breast cancer prevention.

The role of fat intake in breast cancer risk has been widely investigated, but the evidence is too limited to draw any conclusion (5). Several studies have shown that some SFAs and n–6 PUFAs are associated with a higher breast cancer risk, whereas n–3 PUFAs and the n–3:n–6 PUFA ratio seem to be protective, mainly in Asian populations (6, 7). However, a meta-analysis of prospective cohort studies found no association with either fatty acid (FA) intake or serum FAs (8). More recently, elevated risk of breast cancer has been associated with high concentrations of circulating industrial *trans* FAs in the European Prospective Investigation in Cancer and Nutrition study (9) and in the American Nurses' Health Study II (10).

In relation to MD, most studies have focused on the study of large groups of FAs instead of analyzing their individual effect. The majority of these studies detected an association with SFAs (11–14), with the exception of 1 that detected an inverse association in premenopausal women (15). With respect to MUFAs, although Masala et al. (16) detected lower MD with elevated intake of these FAs, most authors did not report any association (12–15, 17, 18). Regarding PUFAs, the results are more inconsistent. Whereas some studies reported higher MD among premenopausal (15, 16) or lower MD among postmenopausal women with elevated PUFA concentrations (17), others found no association (13, 18). Finally, more recent studies showed that n–3 PUFAs might not be associated (19) or might be inversely associated with breast density among postmenopausal women (20, 21) and in animal models (22, 23), whereas n–6 PUFAs do not appear to be associated with MD (19, 20).

The biological connection between serum FAs and MD, although not well established, could arise through inflammatory processes. There is evidence that some SFAs and PUFAs have anti-inflammatory or pro-inflammatory properties (24, 25), and the expression of these inflammatory markers in normal breast tissue has also been associated with MD in pre- and postmenopausal women (26). FA concentrations could also exert a direct causal effect on breast density, helping increase/decrease the relative amount of nondense fatty tissue in the breast and, therefore, decrease/increase MD. On the other hand, this association could also reflect the indirect influence that steroid hormones have on MD because it has been observed that these hormones can modify the biosynthesis of unsaturated FAs, increasing the expression of stearoyl-CoA desaturase 1 (SCD-1) and decreasing the activity of the Δ5-desaturase and Δ6-desaturase enzymes (27). It is well known that small variations in the amount of endogenous sex hormones and insulin-like growth factors can also affect MD, even in premenopausal women (28, 29).

Epidemiological studies that have analyzed the association between FAs and MD have assessed FA intake using dietary questionnaires. Only Hudson et al.'s (19) study assessed PUFAs in erythrocyte membranes. Blood FA concentrations can be used as biomarkers of diet and metabolic processes. Many essential n–3 PUFAs, n–6 PUFAs, and *trans* FAs that cannot be endogenously synthesized by humans, and that must be obtained from diet, are good biomarkers of dietary intake. On the other hand, SFAs and MUFAs can be synthesized de novo in humans, and therefore circulating levels do not necessarily represent diet (30). However, it has been described that self-reported dietary habits are prone to systematic and random measurement errors (31, 32). Therefore, serum FA concentration may be a more accurate measure of the bioavailable amounts of these fats. The main objective of this study was to analyze the association between the relative concentrations of individual serum phospholipid fatty acids (PLFAs) and MD in Spanish premenopausal women.

Methods

Study population

DDM-Madrid is a cross-sectional study based on 1466 premenopausal women, aged 39–50 y, recruited between June 2013 and May 2015 at the Medical Diagnostic Centre of Madrid City Council (Madrid Salud), where these women went for their routine gynecological examination. The participation rate was 88%. Women were contacted by telephone and invited to participate. The same day that the women attended their medical examination, they signed a written informed consent and 3 interviewers interviewed them using a standardized epidemiological questionnaire that has been previously used in the DDM-Spain study (33, 34). This questionnaire contained sociodemographic information; data on childhood and youth; family and personal history; gynecological, obstetric, and occupational history; smoking; alcohol; and physical activity. Recreational physical activity during the previous year was assessed using a translation of a validated self-administered questionnaire that takes into account duration, frequency, and intensity of 26 activities (35). Total metabolic equivalents (MET-h/wk) were calculated according to the 2011 Compendium of Physical Activities (36). Finally, participants completed a 117-item semiquantitative FFQ, adapted and validated in several Spanish adult populations (37, 38), which included the eating habits of the previous 12 mo.

On the same day, the interviewers measured and weighed the participants using a certified scale. Following a standardized procedure, they also measured the women's waist and hip circumference. All these variables were measured twice, with a third measure if the first 2 were discrepant. The average anthropometric values were used in the analyses.

Interviewers also extracted a fasting blood sample from each woman, which was subsequently centrifuged, aliquoted, and stored at −80°C in the biobank of the Carlos III Institute of Health. The DDM-Madrid study was conducted in accordance with the Declaration of Helsinki guidelines and was formally approved by the Ethics and Animal Welfare Committee of the Carlos III Institute of Health.

MD assessment

MD was assessed using 2-dimensional digital mammograms that workers undergo during their annual gynecological examination at the Madrid Salud center. The craniocaudal and mediolateral oblique views of the left and right breast mammograms of each woman were collected and anonymized. An experienced radiologist estimated the percentage of MD from the craniocaudal mammogram of the left breast assisted by DM-Scan, a free semi-automated computer tool that quantifies MD on digital mammograms, on a continuous scale and in DICOM format. This tool identifies the pixels that correspond to adipose tissue, dense tissue, and the background of the image. Based on these values,

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Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org/.
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Abbreviations used: FADS1, fatty acid desaturase 1; MD, mammographic density; PLFA, phospholipid fatty acid; SCD-1, stearoyl-CoA desaturase 1.

2 thresholds are created that allow estimating the proportion that corresponds to the dense area of the breast. DM-Scan has shown a high reproducibility and validity (39, 40). To assess the internal consistency of the radiologist, a sample of 100 mammograms were read again, and an intraclass correlation coefficient of 0.87 (95% CI: 0.82, 0.92) between the first and second reading was obtained.

Protocol for analysis of PLFAs

PLFAs were determined by using the protocol proposed by Criado-Navarro et al. (41), which is based on the isolation of PLs using 30-mg HybridSPE cartridges from Supelco, derivatization of the resulting extract to convert PLFAs into their more volatile PL-FAMEs, and GC-MS analysis. The NIST Mass Spectral Search Program version 11.0 was used for spectral search (Mainlib and Replib libraries). Tentative identification was reported when the correlation between experimental and database spectra was >0.75 in normal search mode. Confirmatory analysis was carried out by analysis of a FAMEs multistandard from Sigma–Aldrich.

Relative quantitation of PLFAs

The relative concentration of each PLFA, expressed as percentage of serum total PLFAs, was quantified by integrating the area under the peak and dividing the result by the total PLFA area. The variability of the determination, expressed as variation coefficient in percentage, ranged from 0.3% to 14.9%. A total of 21 individual PLFAs belonging to the following classes were determined: SFAs (14:0, 15:0, 16:0, 17:0, 18:0, and 20:0), *cis* MUFAs (16:1 n–7, 17:1, 18:1 n–9, and 20:1 n–9), ruminant *trans* FAs (16:1 n–7 and 18:1 n–7), industrial *trans* FAs (18:1 n–9), n–3 PUFAs (18:3, 20:5, and 22:6), and n–6 PUFAs (18:2, 18:3, 20:2, 20:3, and 20:4). Several desaturation indices were also calculated: the ratio between palmitoleic acid and palmitic acid (SCD-16 or DI_{16}) and the ratio between oleic acid and stearic acid (SCD-18 or DI_{18}), as biomarkers of SCD-1 (Δ9-desaturase) expression (42); the ratio of arachidonic to dihomo-γ-linolenic acid, an indicator of the activity of the fatty acid desaturase 1 (FADS1) (Δ5-desaturase); and the ratio of dihomo-γ-linolenic acid to linoleic acid, an indicator of the activity of Δ6-desaturase and elongase (43).

Statistical analysis

After excluding women whose mammogram could not be read, those with analogical images, those whose relative concentrations of serum PLFAs could not be measured, those who were not fasting when their blood was drawn, and those for whom information on the main confounding variables was lacking, the final sample size included 1392 participants (95%).

Descriptive characteristics of the participants were summarized as absolute values and percentages. Mean MD concentrations, as well as mean relative concentrations of the main FA classes (SFAs, *cis* MUFAs, *trans* MUFAs, n–3 PUFAs, and n–6 PUFAs) according to the women's characteristics, were also described and compared using the Wald test.

To analyze the association between MD and relative percentage of serum PLFAs, the latter were divided into tertiles. The second and third tertiles were compared with the first tertile (reference) using multivariable linear regression models. Two linear models were fitted. The first was adjusted for age (continuous variable) and BMI (in kg/m^2; continuous variable). The second model was further adjusted for educational level (primary school or less, secondary school, or university graduate) and variables that were associated with MD ($P < 0.05$): waist circumference (in tertiles), parity (nulliparous or 1, 2, or >2 children), oral contraceptive use (never, past use, and current use), previous breast biopsies (none, yes), and energy intake (continuous variable). The linear trend across tertiles was also tested with the Wald test. In addition to categorical analyses, each type of serum PLFA was modeled through a restricted quadratic spline with knots at the 5th, 50th, and 95th percentiles (44). These restricted quadratic splines allowed 2 different quadratic trends on either side of the median PLFA that were restricted to be linear below the 5th percentile and above the 95th percentile, so they could reproduce a large variety of smooth dose–response curves while avoiding implausible shapes at extreme relative concentrations of PLFAs. As a sensitivity analysis, we conducted a subset analysis by BMI and waist circumference [women with BMI <25 and waist circumference ≤80 cm (721 women) compared with women with BMI ≥25 and waist circumference >80 cm (396 women)], and tests of heterogeneity of associations were carried out. To account for the problem of multiple testing, *P* values were adjusted using the false discovery rate proposed by Benjamini and Hochberg (45). All analyses were performed using STATA/MP version 14.2 software.

Results

The mean MD of the women was 34.3% (IQR: 21.9–46.8). The general characteristics of the study population and the distribution of MD according to these characteristics are presented in **Table 1**. Briefly, women's mean age at recruitment was 44 y. Most of them attended university, and one-third were overweight or obese. Almost half of the participants had 2 children, and more than half had ever used oral contraceptives, were former or current smokers, and consumed <10 g of alcohol per day. Among the participants, 42% were sedentary, 7% had first-degree relatives with breast cancer, and 10% had previous breast biopsies. Hypercholesterolemia was treated by statins in 2% of the women, and the mean caloric intake was 1968 ± 593 kcal/d. Women with a higher BMI and larger waist circumference had a significantly lower MD. Higher MD was observed among nulliparous women, in those who never used oral contraceptives, in participants with previous breast biopsies, and in those with higher caloric intake.

Table 2 shows the distribution of serum PLFA according to women's characteristics. Relative concentrations of SFAs were higher in less educated women, with higher BMI, with larger waist circumference, and among physically inactive women. Relative concentrations of *cis* MUFAs were higher among university-educated women, in women with lower BMI and lower waist circumference, in those with higher alcohol consumption, and among physically active women. With regard to relative *trans* MUFA concentrations, these were also higher in women with higher education and lower BMI and waist circumference, in addition to women with previous breast biopsies and with lower caloric intake. Younger and slimmer women, current users of oral contraceptives, and women without hypercholesterolemia showed higher relative concentrations of n–6 PUFAs. Finally, older, university-educated, nulliparous, and physically active women, as well as those with higher alcohol and lower caloric intake, were the participants with higher relative concentrations of n–3 PUFAs.

The association between individual FAs and MD is shown in **Table 3**. Women in the third tertile of all SFAs showed a significantly higher MD compared with those in the first tertile, with the exception of palmitic acid, for which an inverse association was observed ($\beta_{T3vsT1} = -2.29$; 95% CI: −4.43, −0.15; *P*-trend = 0.058). Regarding *cis* MUFAs, we observed a significant association with palmitoleic, heptadecenoic, and gondoic acids. However, no association was detected with total *cis* MUFAs. The ruminant *trans* palmitelaidic acid ($\beta_{T3vsT1} = 5.22$; 95% CI: 3.15, 7.29; *P*-trend = 0.001) and the industrial *trans* elaidic acid ($\beta_{T3vsT1} = 2.69$; 95% CI: 0.59, 4.79; *P*-trend = 0.022) were also associated with higher MD. With regard to n–6 PUFAs, whereas high relative concentrations of γ-linolenic acid were associated with increased MD, women with elevated relative concentrations of linoleic acid and arachidonic acid showed lower density values. Therefore, the

TABLE 1 Descriptive characteristics of premenopausal women and mammographic density percentage according to women's characteristics[1]

Characteristic	*n* (%)	% MD	*P* value[2]
Total	1392 (100.0)	34.3 ± 17.5	
Age, y			0.303
<45	747 (53.7)	35.5 ± 17.4	
≥45	645 (46.3)	33.0 ± 17.5	
Educational level			0.435
Primary school or less	63 (4.5)	30.7 ± 17.4	
Secondary school	481 (34.6)	32.8 ± 17.0	
University graduate	848 (60.9)	35.5 ± 17.7	
BMI, kg/m^2			<0.001
<18.5	24 (1.7)	45.1 ± 18.5	
18.5–24.9	914 (65.7)	38.8 ± 16.7	
25–29.9	318 (22.8)	27.2 ± 14.7	
≥30	136 (9.8)	19.0 ± 13.4	
Waist circumference[3], cm			<0.001
<74.35	461 (33.1)	43.7 ± 16.5	
74.35–83.05	456 (32.8)	34.4 ± 15.9	
>83.05	456 (32.8)	24.8 ± 14.5	
Unknown	19 (1.4)		
No. of children			0.001
None	333 (23.9)	37.2 ± 18.4	
1	326 (23.4)	34.8 ± 18.5	
2	654 (47.0)	33.0 ± 16.5	
>2	79 (5.7)	31.8 ± 15.7	
Use of oral contraceptives			0.022
Never	529 (38.0)	36.1 ± 18.4	
Past use	807 (58.0)	33.5 ± 16.9	
Current use	45 (3.2)	30.9 ± 15.4	
Unknown	11 (0.8)	—	
Tobacco consumption			0.192
None	533 (38.3)	35.5 ± 18.0	
Former smoker	484 (34.8)	33.8 ± 16.9	
Current smoker	375 (26.9)	33.3 ± 17.3	
Alcohol consumption, g/d			0.782
None	251 (20.3)	34.1 ± 17.5	
<10	810 (65.6)	34.9 ± 17.4	
≥10	174 (14.1)	35.2 ± 17.0	
Physical activity, MET-h/wk			0.753
None	576 (41.6)	32.9 ± 17.0	
≤12	345 (24.9)	34.0 ± 17.2	
>12	465 (33.5)	36.4 ± 18.0	
Family history of breast cancer			0.759
None	1085 (77.9)	34.2 ± 17.2	
Second degree only	210 (15.1)	34.9 ± 18.5	
First degree	97 (7.0)	34.3 ± 17.9	
Previous breast biopsies			<0.001
None	1247 (89.6)	33.5 ± 17.3	
Yes	145 (10.4)	41.6 ± 17.5	
Hypercholesterolemia			0.808
No	1196 (86.9)	34.5 ± 17.5	
Yes, not treated	150 (10.9)	34.5 ± 18.0	
Treated with statins	31 (2.3)	28.5 ± 13.8	
Total energy intake[3], kcal/d			0.011
<1673	409 (29.4)	33.5 ± 17.5	
1673–2151	408 (29.3)	35.8 ± 17.2	
>2151	408 (29.3)	35.3 ± 17.2	
Unknown[4]	167 (12.0)		

[1] Values are number of women (%) and means ± SDs. MD, mammographic density; MET-h/wk, metabolic equivalent task hours per week.
[2] *P* values adjusted for age and BMI.
[3] In tertiles.
[4] Participants who did not answer the FFQ.

joint association of this subgroup turned out to be inverse and significant ($\beta_{T3vsT1} = -7.68$; 95% CI: −9.74, −5.62; *P*-trend = 0.001). Finally, although no significant association was found between MD and n–3 PUFAs, the n–6:n–3 PUFA ratio showed a negative trend ($\beta_{T3vsT1} = -2.52$; 95% CI: −4.64, −0.39; *P*-trend = 0.033). Regarding desaturation indices, a high ratio of palmitoleic acid to palmitic acid (SCD-16) was associated with higher MD, whereas the ratio of oleic acid to stearic acid (SCD-18) and the ratio between arachidonic and dihomo-γ-linolenic acids showed an inverse association.

Figure 1 shows the adjusted mean differences in MD for SFAs, *cis* MUFAs, *trans* MUFAs, n–3 PUFAs, n–6 PUFAs, and the log-transformed n–6:n–3 PUFA ratio. MD increased as the relative concentrations of serum SFAs increased, whereas MD decreased as the relative concentrations of n–6 PUFAs increased.

Analysis by BMI and waist circumference revealed no substantial difference in the association between individual FAs, FA groups, desaturation indices, and MD (**Supplemental Table 1**). It is worth noting that the association with total SFAs was slightly higher in obese women.

Discussion

This study aimed to analyze the association between the relative concentrations of individual PLFAs and percentage of DM in ∼1400 premenopausal women attending the breast radiodiagnosis unit of Madrid City Council. Our results show that high relative concentrations of several serum SFAs are associated with higher MD, whereas elevated relative n–6 PUFA concentrations, mainly linoleic acid and arachidonic acid, are associated with lower MD values.

In Spain, >70% of SFA intake comes from the almost equally distributed consumption of meat, dairy, oil, and fat products (46). However, long-term intake of SFAs does not appear to correlate well with blood concentrations because these can be synthesized endogenously (30). Note that the relative concentration of serum SFAs in our participants (55%) is considerably higher than that detected in a previous study (40%) (47). Our results show that women with high relative concentrations of SFAs presented higher MD. An association with breast cancer risk has already been suggested by several case–control and cohort studies (6). However, their association with MD has been less studied. The SFA most strongly associated with MD was arachidic acid. In a recent nested breast cancer case–control study of premenopausal women, a statistical interaction with BMI was also found for this FA, with lower breast cancer risk found among women with BMI <25 and higher risk among overweight/obese women (10). However, this interaction was not observed in our study. On the contrary, and in line with what was observed in our nonobese participants, these same authors observed an inverse association with palmitic acid (10), the most abundant SFA in serum.

MUFAs are biosynthesized from SFAs by the action of the enzyme SCD-1 in the liver, but they are also present in various foods. In Spain, oleic acid constitutes the most abundant MUFA, present in large quantities in olive oil (37% of the total MUFAs provided by the diet); however, MUFAs are also present in meat products, pastries, precooked foods, and other products in less quantity (46). Although we did not detect an association between this group of FAs and MD, we observed a higher MD associated with palmitoleic acid among nonobese women. This is an n–7 MUFA biosynthesized from palmitic acid by

TABLE 2 Mean percentage of serum phospholipid fatty acids according to premenopausal women's characteristics[1]

		SFAs		*cis* MUFAs		*trans* FAs		n–6 PUFAs		n–3 PUFAs	
	n	% of total PLFAs	*P* value	% of total PLFAs	*P* value	% of total PLFAs	*P* value	% of total PLFAs	*P* value	% of total PLFAs	*P* value
Total	1392	54.80 ± 4.93		9.38 ± 2.25		1.43 ± 0.28		30.81 ± 4.22		3.57 ± 1.28	
Age, y			0.211		0.690		0.657		0.028		0.003
<45	747	54.64 ± 5.05		9.41 ± 2.48		1.43 ± 0.28		31.04 ± 4.27		3.48 ± 1.24	
≥45	645	54.98 ± 4.79		9.36 ± 1.95		1.43 ± 0.29		30.55 ± 4.14		3.69 ± 1.32	
Educational level			0.003		0.003		0.020		0.488		0.001
Primary school or less	63	56.24 ± 5.03		8.88 ± 2.20		1.39 ± 0.29		30.24 ± 4.33		3.26 ± 1.18	
Secondary school	481	55.07 ± 4.44		9.22 ± 1.89		1.41 ± 0.26		30.83 ± 3.78		3.46 ± 1.26	
University graduate	848	54.54 ± 5.16		9.51 ± 2.42		1.44 ± 0.29		30.85 ± 4.44		3.66 ± 1.29	
BMI, kg/m^2			<0.001		<0.001		<0.001		0.015		0.702
<18.5	24	53.13 ± 4.17		10.2 ± 1.61		1.54 ± 0.21		31.87 ± 4.27		3.26 ± 1.18	
18.5–24.9	914	54.45 ± 4.78		9.58 ± 2.08		1.45 ± 0.27		30.94 ± 4.07		3.58 ± 1.30	
25–29.9	318	55.18 ± 5.38		9.09 ± 2.77		1.41 ± 0.29		30.68 ± 4.81		3.63 ± 1.31	
≥30	136	56.52 ± 4.51		8.65 ± 1.81		1.30 ± 0.29		30.10 ± 3.54		3.43 ± 1.08	
Waist circumference[2], cm			0.025		<0.001		0.001		0.953		0.811
<74.3	461	54.49 ± 4.45		9.72 ± 2.10		1.45 ± 0.26		30.76 ± 4.00		3.58 ± 1.36	
74.3–83.0	456	54.64 ± 4.79		9.35 ± 1.83		1.45 ± 0.27		30.99 ± 3.92		3.57 ± 1.26	
>83.0	456	55.22 ± 5.49		9.09 ± 2.70		1.39 ± 0.30		30.74 ± 4.71		3.56 ± 1.24	
No. of children			0.073		0.175		0.068		0.788		<0.001
None	333	54.18 ± 4.53		9.54 ± 2.60		1.47 ± 0.28		30.92 ± 3.96		3.89 ± 1.44	
1	326	55.24 ± 5.28		9.33 ± 2.27		1.41 ± 0.30		30.47 ± 4.19		3.54 ± 1.29	
2	654	54.84 ± 4.99		9.35 ± 2.11		1.42 ± 0.27		30.93 ± 4.42		3.46 ± 1.18	
>2	79	55.18 ± 4.42		9.20 ± 1.55		1.45 ± 0.28		30.82 ± 3.61		3.35 ± 1.12	
Use of oral contraceptives			0.153		0.445		0.145		0.042		0.885
Never	529	55.01 ± 4.88		9.40 ± 2.01		1.41 ± 0.28		30.61 ± 4.08		3.57 ± 1.38	
Past use	807	54.64 ± 4.95		9.39 ± 2.43		1.44 ± 0.28		30.94 ± 4.27		3.59 ± 1.23	
Current use	45	54.40 ± 5.38		8.87 ± 1.47		1.43 ± 0.31		31.88 ± 4.64		3.42 ± 1.00	
Tobacco consumption			0.859		0.484		0.430		0.271		<0.001
None	533	54.92 ± 5.31		9.29 ± 2.44		1.43 ± 0.29		30.62 ± 4.55		3.74 ± 1.42	
Former smoker	484	54.58 ± 4.57		9.49 ± 2.17		1.43 ± 0.28		30.96 ± 3.96		3.54 ± 1.18	
Current smoker	375	54.90 ± 4.82		9.38 ± 2.06		1.42 ± 0.27		30.91 ± 4.04		3.40 ± 1.17	
Alcohol consumption, g/d			0.270		0.022		0.996		0.529		0.025
None	251	55.01 ± 4.95		9.24 ± 2.25		1.42 ± 0.28		30.84 ± 4.09		3.48 ± 1.26	
<10	810	54.83 ± 4.98		9.35 ± 2.00		1.44 ± 0.28		30.75 ± 4.24		3.62 ± 1.30	
≥10	174	54.46 ± 4.61		9.78 ± 3.17		1.41 ± 0.25		30.57 ± 4.10		3.77 ± 1.29	
Physical activity, MET-h/wk			0.003		0.038		0.005		0.203		0.002
None	576	55.33 ± 5.27		9.16 ± 2.20		1.40 ± 0.29		30.66 ± 4.51		3.45 ± 1.18	
≤12	345	54.38 ± 4.80		9.70 ± 2.76		1.46 ± 0.28		30.82 ± 3.99		3.63 ± 1.27	
>12	465	54.43 ± 4.54		9.43 ± 1.83		1.45 ± 0.27		31.00 ± 4.01		3.69 ± 1.40	

(*Continued*)

TABLE 2 (*Continued*)

		SFAs		*cis* MUFAs		*trans* FAs		n–6 PUFAs		n–3 PUFAs	
	n	% of total PLFAs	*P* value	% of total PLFAs	*P* value	% of total PLFAs	*P* value	% of total PLFAs	*P* value	% of total PLFAs	*P* value
Family history of breast cancer			0.336		0.870		0.461		0.177		0.239
None	1085	54.71 ± 4.90		9.41 ± 2.16		1.43 ± 0.28		30.89 ± 4.23		3.55 ± 1.28	
Second degree only	210	55.17 ± 5.04		9.13 ± 1.67		1.43 ± 0.29		30.61 ± 4.13		3.65 ± 1.28	
First degree	97	54.93 ± 5.06		9.61 ± 3.75		1.40 ± 0.28		30.39 ± 4.27		3.66 ± 1.30	
Previous breast biopsies			0.428		0.308		0.022		0.871		0.818
None	1247	54.83 ± 4.99		9.36 ± 2.26		1.42 ± 0.28		30.81 ± 4.24		3.57 ± 1.28	
Yes	145	54.49 ± 4.42		9.56 ± 2.11		1.48 ± 0.28		30.87 ± 3.99		3.60 ± 1.28	
Hypercholesterolemia			0.256		0.588		0.173		0.030		0.115
No	1196	54.76 ± 5.00		9.35 ± 2.06		1.42 ± 0.28		30.92 ± 4.30		3.55 ± 1.27	
Yes, not treated	150	54.91 ± 4.34		9.43 ± 2.07		1.48 ± 0.26		30.44 ± 3.47		3.74 ± 1.35	
Treated with statins	31	55.92 ± 4.65		9.48 ± 2.86		1.41 ± 0.28		29.51 ± 3.17		3.67 ± 1.34	
Total energy intake[2], kcal/d			0.077		0.153		0.026		0.885		0.001
<1673	409	54.50 ± 4.99		9.50 ± 1.94		1.45 ± 0.28		30.78 ± 4.14		3.77 ± 1.36	
1673–2151	408	54.86 ± 4.58		9.40 ± 2.20		1.44 ± 0.26		30.69 ± 3.70		3.61 ± 1.27	
>2151	408	55.11 ± 5.16		9.27 ± 2.57		1.41 ± 0.28		30.74 ± 4.65		3.47 ± 1.25	

[1]Values are mean percentages ± SDs. PLFAs, phospholipid fatty acids.
[2]In tertiles.

the action of the enzyme SCD-1 in the liver, and high plasma concentrations of this FA have been associated with an increased breast cancer risk (9).

We also found higher MD associated with *trans* palmitelaidic and *trans* elaidic acids in nonobese women. Elaidic acid is formed during the partial hydrogenation of vegetable oils, and it is found in a wide variety of industrial foods. Previous studies have described an association with total breast cancer (10, 48) and with estrogen receptor–negative tumors in particular (9). Elaidic acid has also been linked to lower risk of weight loss (49). Palmitelaidic acid is produced from biohydrogenation by bacteria in the rumen of ruminants, and consequently it is present in high-fat dairy products and meat of ruminants. Elevated blood concentrations of this *trans* FA have also been associated with higher breast cancer risk (10, 48). Whereas Hirko et al. (10) detected higher breast cancer risk only among obese women, we observed higher MD only among nonobese women. This discrepancy may be due to the fact that BMI behaves in the opposite way with these 2 endpoints: whereas it increases the risk of breast cancer in postmenopausal women, it is inversely associated with MD.

Another relevant finding of our study is the inverse association detected between MD and total n–6 PUFAs, mainly due to the lower MD associated with the 2 most common n–6 PUFAs: linoleic and arachidonic acids. These associations have not been detected in 2 previous studies (19, 20). One of them detected that a high n–6:n–3 PUFA ratio was associated with a higher MD (20), and the other found a trend toward increased percentage density with increased arachidonic acid (19). Regarding linoleic acid and breast cancer risk, whereas 1 meta-analysis concluded that high serum concentrations of this FA were associated with nonsignificant lower risk (36, 50), another suggested that the results from previous studies are too inconsistent to support this hypothesis (51). On the other hand, Sakai et al. (52), in a systematic review of arachidonic acid and cancer risk, concluded that this PUFA was not associated with breast tumors. The inverse association detected with MD in our study is difficult to explain. Both PUFAs are linked together through metabolism because arachidonic acid is obtained by desaturation and chain elongation of linoleic acid, an essential FA found in vegetable oils, nuts, and fatty seeds (53).

Regarding n–3 PUFAs, although these FAs seem to have an inhibiting role in the development and progression of breast cancer (7), their association with MD is less conclusive. Some studies have detected an inverse association between EPA and DHA intake and MD among postmenopausal women (20, 21), whereas others have not found such an association (54). Hudson et al. (19) also found no association between the concentration of these PUFAs in erythrocyte membranes and the percent density or dense breast area. The few studies that have analyzed premenopausal women have either detected a modest median decrease in absolute breast density (21) or, in line with our results, have not detected any association (20). In any case, it is important to highlight the low relative serum concentrations of n–3 PUFAs detected in the women under study. Their relative concentrations of EPA + DHA are below the phospholipid concentrations reported for other European countries (55). This leads to an n–6:n–3 PUFA ratio that is much higher than the dietary ratio estimated in other large Spanish studies (56).

High SCD-16 and SCD-18 desaturation indices were associated with higher and lower MD, respectively. Both indices reflect hepatic SCD-1 activity/expression, which converts SFAs to MUFAs, and they are biomarkers of endogenous lipogenesis

TABLE 3 Difference in mammographic density percentage in premenopausal women by tertiles of serum phospholipid fatty acids[1]

		Model 1[2]			Model 2[3]		
Fatty acids	% of total PLFAS	Tertile 2 β (95% CI)	Tertile 3 β (95% CI)	*P*-trend[4]	Tertile 2 β (95% CI)	Tertile 3 β (95% CI)	*P*-trend[4]
SFAs							
14:0 myristic acid	0.20 ± 0.16	7.19 (5.23, 9.14)	6.30 (4.32, 8.29)	0.001	6.79 (4.76, 8.81)	6.94 (4.84, 9.05)	0.001
15:0 pentadecanoic acid	0.09 ± 0.05	5.00 (3.03, 6.98)	4.68 (2.70, 6.65)	0.001	6.10 (4.05, 8.15)	6.20 (4.13, 8.26)	0.001
16:0 palmitic acid	32.91 ± 1.98	0.38 (−1.61, 2.38)	−2.03 (−4.04, −0.02)	0.091	0.67 (−1.41, 2.76)	−2.29 (−4.43, −0.15)	0.060
17:0 margaric acid	0.26 ± 0.46	3.72 (1.74, 5.70)	3.88 (1.90, 5.87)	0.001	3.99 (1.90, 6.08)	4.77 (2.69, 6.84)	0.001
18:0 stearic acid	21.21 ± 4.33	4.84 (2.88, 6.79)	8.13 (6.17, 10.10)	0.001	4.99 (2.94, 7.04)	7.71 (5.63, 9.78)	0.001
20:0 arachidic acid	0.12 ± 0.06	8.02 (6.11, 9.93)	10.53 (8.61, 12.44)	0.001	8.22 (6.23, 10.20)	10.73 (8.73, 12.73)	0.001
Total SFAs	54.80 ± 4.93	6.40 (4.45, 8.35)	7.77 (5.80, 9.73)	0.001	6.80 (4.76, 8.83)	7.53 (5.44, 9.61)	0.001
MUFAs							
16:1 n–7 palmitoleic acid	0.32 ± 0.18	1.91 (−0.08, 3.90)	1.92 (−0.09, 3.93)	0.109	2.39 (0.32, 4.46)	3.12 (0.99, 5.25)	0.009
17:1 heptadecenoic acid	0.02 ± 0.12	2.05 (0.06, 4.04)	3.62 (1.63, 5.60)	0.001	3.28 (1.21, 5.35)	4.95 (2.87, 7.03)	0.001
18:1 n–9 oleic acid	8.97 ± 2.19	−1.42 (−3.43, 0.59)	−0.73 (−2.77, 1.32)	0.582	−1.35 (−3.45, 0.76)	−0.81 (−2.95, 1.33)	0.553
20:1 n–9 gondoic acid	0.07 ± 0.03	3.22 (1.22, 5.22)	1.69 (−0.32, 3.70)	0.155	3.87 (1.78, 5.97)	2.67 (0.57, 4.77)	0.024
Total *cis* MUFAs	9.38 ± 2.25	−1.12 (−3.12, 0.89)	−0.74 (−2.78, 1.29)	0.582	−0.97 (−3.07, 1.12)	−0.71 (−2.84, 1.43)	0.574
trans fatty acids							
Ruminant *trans* fatty acids							
16:1 n–7 palmitelaidic acid	0.13 ± 0.05	2.53 (0.55, 4.51)	4.40 (2.41, 6.39)	0.001	3.57 (1.50, 5.65)	5.22 (3.15, 7.29)	0.001
18:1 n–7 vaccenic acid	1.16 ± 0.24	1.07 (−0.94, 3.08)	−1.56 (−3.58, 0.45)	0.175	1.30 (−0.81, 3.42)	−1.06 (−3.18, 1.06)	0.391
Total ruminant *trans* fatty acids	1.29 ± 0.26	1.96 (−0.05, 3.96)	−1.36 (−3.37, 0.66)	0.243	2.55 (0.45, −4.65)	−0.89 (−3.00, −1.22)	0.471
Industrial *trans* fatty acids							
18:1 n–9 elaidic acid	0.14 ± 0.06	2.17 (0.18, 4.16)	1.82 (−0.17, 3.82)	0.127	2.38 (0.30, 4.46)	2.69 (0.59, 4.79)	0.022
n–6 PUFAs							
18:2 linoleic acid	19.62 ± 3.69	−1.14 (−3.13, 0.84)	−4.67 (−6.70, −2.65)	0.001	−1.61 (−3.70, 0.48)	−5.49 (−7.62, −3.35)	0.001
18:3 γ-linolenic acid	0.05 ± 0.04	3.59 (1.61, 5.58)	3.91 (1.92, 5.90)	0.001	3.50 (1.42, 5.59)	3.77 (1.69, 5.85)	0.001
20:2 eicosadienoic acid	0.15 ± 0.05	−0.42 (−2.42, 1.57)	0.38 (−1.62, 2.37)	0.786	0.70 (−1.39, 2.78)	1.93 (−0.16, 4.03)	0.099
20:3 dihomo-γ-linolenic acid	2.15 ± 0.67	−1.33 (−3.32, 0.67)	−3.76 (−5.84, −1.69)	0.001	0.08 (−2.00, 2.16)	−1.33 (−3.57, 0.91)	0.340
20:4 arachidonic acid	8.84 ± 1.92	−3.73 (−5.71, −1.76)	−6.35 (−8.33, −4.38)	0.001	−2.84 (−4.93, −0.76)	−4.68 (−6.79, −2.58)	0.001
Total n–6 PUFAs	30.81 ± 4.22	−2.66 (−4.61, −0.71)	−8.07 (−10.03, −6.11)	0.001	−2.28 (−4.32, −0.24)	−7.68 (−9.74, −5.62)	0.001
n–3 PUFAs							
18:3 α-linolenic acid	0.04 ± 0.03	2.02 (0.02, 4.01)	0.88 (−1.14, 2.89)	0.504	2.09 (0.02, 4.16)	1.95 (−0.17, 4.06)	0.096
20:5 EPA	0.64 ± 0.56	0.74 (−1.26, 2.74)	0.33 (−1.68, 2.33)	0.802	0.76 (−1.34, 2.86)	0.61 (−1.50, 2.72)	0.611
22:6 DHA	2.90 ± 0.88	1.19 (−0.80, 3.19)	0.06 (−1.94, 2.06)	0.950	0.84 (−1.26, 2.94)	0.51 (−1.61, 2.63)	0.654
Total n–3 PUFAs	3.57 ± 1.28	1.85 (−0.14, 3.84)	0.23 (−1.77, 2.22)	0.845	1.65 (−0.45, 3.75)	0.47 (−1.65, 2.58)	0.669
n–6:n–3 PUFA ratio[5]	9.12 ± 1.51	0.48 (−1.51, 2.47)	−2.52 (−4.52, −0.53)	0.029	0.47 (−1.63, 2.56)	−2.52 (−4.64, −0.39)	0.033
Desaturation indices							
SCD-16: 16:1n–7c/16:0	0.01 ± 1.57	1.36 (−0.63, 3.35)	1.72 (−0.29, 3.72)	0.143	2.00 (−0.07, 4.07)	2.94 (0.83, 5.06)	0.012
SCD-18: 18:1n–9c/18:0[5]	0.42 ± 1.42	−1.70 (−3.69, 0.29)	−5.36 (−7.37, −3.35)	0.001	−0.82 (−2.91, 1.27)	−4.85 (−6.96, −2.74)	0.001
FADS1: 20:4n–6/20:3n–6[5]	4.21 ± 1.38	−1.90 (−3.91, 0.11)	−1.77 (−3.81, 0.28)	0.143	−1.89 (−4.02, 0.24)	−2.87 (−5.00, −0.73)	0.017
FADS2: 20:3n–6/18:2[5]	0.11 ± 1.48	0.04 (−1.97, 2.06)	−0.53 (−2.64, 1.58)	0.716	1.04 (−1.04, 3.13)	1.51 (−0.76, 3.78)	0.252

[1] Values are mean percentages ± SDs and mean differences in the percentage of mammographic density comparing tertile 2 and tertile 3 with tertile 1 (reference), $n = 1196$. FADS, fatty acid desaturase; PLFAs, phospholipid fatty acids; SCD, stearoyl-CoA desaturase.
[2] Adjusted for age and BMI.
[3] Adjusted for age, educational level, BMI, waist circumference, parity (with category of nulliparous), use of oral contraceptives, previous breast biopsies, and energy intake.
[4] *P* value for linear trend in tertiles following the Benjamini–Hochberg procedure (based on 31 independent models).
[5] Geometric means ± geometric SDs.

(42). Several epidemiological studies have described an association between a high blood SFA:MUFA ratio, indicating low SCD-1 activity, and lower breast cancer risk, suggesting that a reduction in the activity and expression of this enzyme in the liver could decrease the risk of developing this tumor (42). Our results show opposing associations of SCD-16 and SCD-18 indices with MD. Only the SCD-16 desaturation index has been associated with breast cancer risk in previous studies (9). Although FA desaturation indices accurately reflect the activity of the enzyme (endogenous synthesis), other environmental factors, such as the intake of other FAs, may influence these ratios. In this sense, given that the dietary content of palmitoleic acid is lower than the oleic acid content, several authors claim that the SCD-16 index could be a better marker of hepatic SCD-1 activity compared with the SCD-18 index (42, 57, 58). High-carbohydrate diets, insulin and estrogen concentrations, alcohol, or exercise training are other environmental factors that can modify the FA desaturation indices (42). Regarding MD, only 1 previous study found an association between decreasing concentrations of SCD-16 and SCD-18 and a progressive reduction in breast density, but only among obese women (59). When we analyzed these associations by BMI and waist circumference, we observed a stronger association with the SCD-16 index among nonobese women.

We also detected an inverse association between FADS1 and MD. This ratio is an indicator of the Δ5-desaturase activity, an enzyme encoded by the *FADS1* gene that converts dihomo-γ-linolenic acid to arachidonic acid. Although alterations in Δ5-desaturase activity have been associated with various diseases, these do not seem to influence the development of breast cancer (43). Only 2 previous studies have observed a statistically significant (60) or borderline (61) association with this tumor.

Hudson et al. (19) previously assessed the association between circulating erythrocyte n–6 and n–3 PUFA concentrations and MD in 248 postmenopausal women. Therefore, this is the first study to explore the association of relative serum concentrations of individual SFAs, MUFAs, and PUFAs with MD in premenopausal women. One of the main strengths of

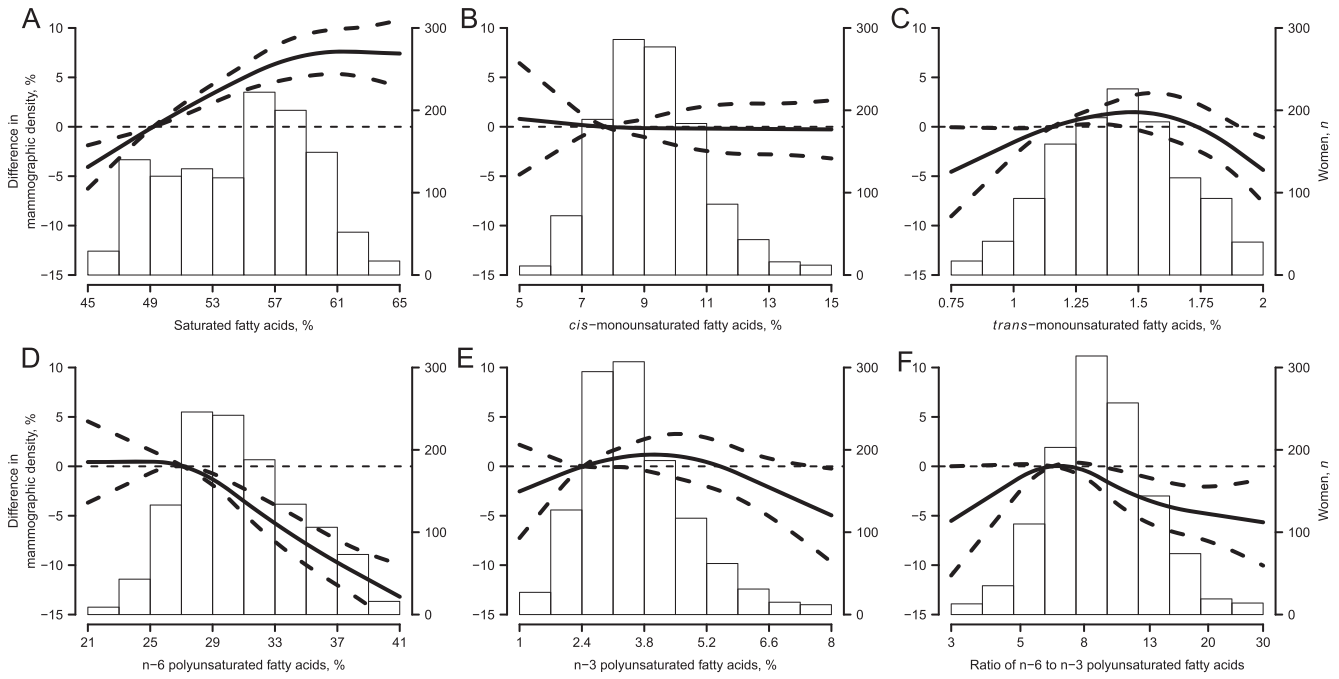

FIGURE 1 Difference in mammographic density percentage as a smooth function of the main fatty acid groups among premenopausal women in Madrid, Spain, 2013–2015, $n = 1196$. Curves represent adjusted mean differences (solid lines) and 95% CIs (dashed lines) based on restricted quadratic splines for saturated fatty acids, *cis* MUFAs, *trans* MUFAs, n–6 PUFAs, n–3 PUFAs, and the log-transformed ratio of n–6 to n–3 PUFAs with knots at their 5th, 50th, and 95th percentiles. The reference value for each type of fatty acid (mean difference = 0) was set at the median of the first tertile (49.2%, 7.75%, 1.16%, 27.1%, 2.44%, and 6.36%, respectively). Mean differences were obtained from linear regression models adjusted for age, educational level, BMI, waist circumference, parity, use of oral contraceptives, previous breast biopsies, and energy intake. Bars represent the histogram of each type of fatty acid.

the study is the high number of women included and the high participation rate. All mammograms of the participants were done in the same center and with the same equipment. MD was measured on a continuous scale using a validated computer-assisted method and by a single reader that showed high internal consistency. Another important strength is the wide range of FAs measured in serum phospholipids. Furthermore, compared to traditional self-reported assessment methods, which are more prone to measurement errors (31, 32), this biomarker can provide a more objective estimate of the intake of FAs that cannot be synthesized endogenously, such as some PUFAs and *trans* FAs.

This study also has several limitations. First, the cross-sectional design did not allow us to determine temporal associations. Second, serum PLFAs were evaluated only once, and although only fasting samples were used, their relative presence fluctuates with changes in dietary habits. However, although there are other biological specimens, such as adipose tissue, more suitable to reflect long-term dietary intake, the FA composition of serum phospholipids is considered a convenient alternative in epidemiological studies (62). Third, although we adjusted the models by all established predictors, unmeasured residual confounders, associated with relative PLFA concentrations (e.g., triglycerides, cholesterol, insulin, or other dietary factors) or with MD (e.g., time of the menstrual cycle) may have interfered with the detected associations. Fourth, although unlikely, women with previous breast biopsies could have modified their dietary habits, resulting in a reverse causation bias. For this reason, a sensitivity analysis excluding these women was carried out, and the results were very similar to those obtained using the entire sample (data not shown). Fifth, to assess whether the outliers may have influenced our results, a second sensitivity analysis was performed eliminating the most extreme density values. Although the estimators were slightly attenuated, no differences were observed with the associations detected in Table 3 (data not shown). Sixth, given the large number of tests performed, we cannot rule out the possibility of some of the results being detected by chance. However, we statistically accounted for multiple comparisons using the Benjamini correction (45). Finally, although we analyzed 21 individual FAs, the serum concentrations of most of them were very low. For this reason, we focused the discussion on those most abundant, which may have greater clinical relevance.

In conclusion, this study shows that relative concentrations of most SFAs, some MUFAs, as well as γ-linolenic acid were associated with higher MD, whereas high relative concentrations of palmitic, linoleic, and arachidonic acids were associated with lower breast density. Low endogenous synthesis of palmitoleic acid and high endogenous production of oleic and arachidonic acids were also associated with lower MD. This study emphasizes the importance of analyzing the association with serum PLFAs individually. Given that this is an exploratory analysis, and that there are hardly any previous studies that have analyzed these associations, our results should be taken with caution and confirmed in future studies.

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for important intellectual content, and read and approved the final manuscript.

References

1. Huo CW, Chew GL, Britt KL, Ingman WV, Henderson MA, Hopper JL, Thompson EW. Mammographic density: a review on the current understanding of its association with breast cancer. Breast Cancer Res Treat 2014;144:479–502.
2. Pettersson A, Graff RE, Ursin G, Santos Silva ID, McCormack V, Baglietto L, Vachon C, Bakker MF, Giles GG, Chia KS, et al. Mammographic density phenotypes and risk of breast cancer: a meta-analysis. J Natl Cancer Inst 2014;106:dju078.
3. Castelló A, Ascunce N, Salas-Trejo D, Vidal C, Sanchez-Contador C, Santamariña C, Pedraz-Pingarrón C, Moreno MP, Pérez-Gómez B, Lope V, et al. Association between Western and Mediterranean dietary patterns and mammographic density. Obstet Gynecol 2016;128:574–81.
4. García-Arenzana N, Navarrete-Muñoz EM, Lope V, Moreo P, Vidal C, Laso-Pablos S, Ascunce N, Casanova-Gómez F, Sánchez-Contador C, Santamariña C, et al. Calorie intake, olive oil consumption and mammographic density among Spanish women. Int J Cancer 2014;134:1916–25.
5. World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project expert report 2018. Diet, nutrition, physical activity and breast cancer. [Internet]. Available from: https://www.aicr.org/wp-content/uploads/2020/01/breast-cancer-report-2017.pdf.
6. Shapira N. The potential contribution of dietary factors to breast cancer prevention. Eur J Cancer Prev 2017;26:385–95.
7. Zanoaga O, Jurj A, Raduly L, Cojocneanu-Petric R, Fuentes-Mattei E, Wu O, Braicu C, Gherman CD, Berindan-Neagoe I. Implications of dietary ω-3 and ω-6 polyunsaturated fatty acids in breast cancer. Exp Ther Med 2018;15:1167–76.
8. Cao Y, Hou L, Wang W. Dietary total fat and fatty acids intake, serum fatty acids and risk of breast cancer: a meta-analysis of prospective cohort studies. Int J Cancer 2016;138:1894–904.
9. Chajès V, Assi N, Biessy C, Ferrari P, Rinaldi S, Slimani N, Lenoir GM, Baglietto L, His M, Boutron-Ruault MC, et al. A prospective evaluation of plasma phospholipid fatty acids and breast cancer risk in the EPIC study. Ann Oncol 2017;28:2836–42.
10. Hirko KA, Chai B, Spiegelman D, Campos H, Farvid MS, Hankinson SE, Willett WC, Eliassen AH. Erythrocyte membrane fatty acids and breast cancer risk: a prospective analysis in the Nurses' Health Study II. Int J Cancer 2018;142:1116–29.
11. Brisson J, Verreault R, Morrison AS, Tennina S, Meyer F. Diet, mammographic features of breast tissue, and breast cancer risk. Am J Epidemiol 1989;130:14–24.
12. Knight JA, Martin LJ, Greenberg CV, Lockwood GA, Byng JW, Yaffe MJ, Tritchler DL, Boyd NF. Macronutrient intake and change in mammographic density at menopause: results from a randomized trial. Cancer Epidemiol Biomarkers Prev 1999;8:123–8.
13. Nagata C, Matsubara T, Fujita H, Nagao Y, Shibuya C, Kashiki Y, Shimizu H. Associations of mammographic density with dietary factors in Japanese women. Cancer Epidemiol Biomarkers Prev 2005;14:2877–80.
14. Qureshi SA, Couto E, Hilsen M, Hofvind S, Wu AH, Ursin G. Mammographic density and intake of selected nutrients and vitamins in Norwegian women. Nutr Cancer 2011;63:1011–20.
15. Vachon CM, Kushi LH, Cerhan JR, Kuni CC, Sellers TA. Association of diet and mammographic breast density in the Minnesota breast cancer family cohort. Cancer Epidemiol Biomarkers Prev 2000;9:151–60.
16. Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, Palli D. Dietary and lifestyle determinants of mammographic breast density: a longitudinal study in a Mediterranean population. Int J Cancer 2006;118:1782–9.
17. Thomson CA, Arendell LA, Bruhn RL, Maskarinec G, Lopez AM, Wright NC, Moll CE, Aickin M, Chen Z. Pilot study of dietary influences on mammographic density in pre- and postmenopausal Hispanic and non-Hispanic white women. Menopause 2007;14:243–50.
18. Ursin G, Sun C-L, Koh W-P, Khoo K-S, Gao F, Wu AH, Yu MC. Associations between soy, diet, reproductive factors, and mammographic density in Singapore Chinese women. Nutr Cancer 2006;56:128–35.
19. Hudson AG, Reeves KW, Modugno F, Wilson JW, Evans RW, Vogel VG, Gierach GL, Simpson J, Weissfeld JL. Erythrocyte ω-6 andω-3 fatty acids and mammographic breast density. Nutr Cancer 2013;65:410–6.
20. Diorio C, Dumas I. Relations of ω-3 and ω-6 intake with mammographic breast density. Cancer Causes Control 2014;25:339–51.
21. Fabian CJ, Kimler BF, Phillips TA, Nydegger JL, Kreutzjans AL, Carlson SE, Hidaka BH, Metheny T, Zalles CM, Mills GB, et al. Modulation of breast cancer risk biomarkers by high-dose ω-3 fatty acids: phase II pilot study in postmenopausal women. Cancer Prev Res 2015;8:922–31.
22. Khadge S, Thiele GM, Sharp JG, McGuire TR, Klassen LW, Black PN, DiRusso CC, Talmadge JE. Long-chain ω-3 polyunsaturated fatty acids modulate mammary gland composition and inflammation. J Mammary Gland Biol Neoplasia 2018;23:43–58.
23. Zhu Z, Jiang W, McGinley JN, Prokopczyk B, Richie JP, El Bayoumy K, Manni A, Thompson HJ. Mammary gland density predicts the cancer inhibitory activity of the N-3 to N-6 ratio of dietary fat. Cancer Prev Res 2011;4:1675–85.
24. Santos S, Oliveira A, Lopes C. Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines. Nutr Res 2013;33:687–95.
25. Calder PC. Polyunsaturated fatty acids and inflammation. Prostaglandins Leukotrienes Essent Fatty Acids 2006;75:197–202.
26. Hanna M, Dumas I, Orain M, Jacob S, Têtu B, Sanschagrin F, Bureau A, Poirier B, Diorio C. Association between expression of inflammatory markers in normal breast tissue and mammographic density among premenopausal and postmenopausal women. Menopause 2017;24:524–35.
27. de Alaniz MJT, Marra CA. Steroid hormones and fatty acid desaturases. Prostaglandins Leukotrienes Essent Fatty Acids 2003;68:163–70.
28. Walker K, Fletcher O, Johnson N, Coupland B, McCormack VA, Folkerd E, Gibson L, Hillier SG, Holly JM, Moss S, et al. Premenopausal mammographic density in relation to cyclic variations in endogenous sex hormone levels, prolactin, and insulin-like growth factors. Cancer Res 2009;69:6490–9.
29. Bertrand KA, Eliassen AH, Hankinson SE, Rosner BA, Tamimi RM. Circulating hormones and mammographic density in premenopausal women. Horm Cancer 2018;9:117–27.
30. Chavarro JE, Kenfield SA, Stampfer MJ, Loda M, Campos H, Sesso HD, Ma J. Blood levels of saturated and monounsaturated fatty acids as markers of de novo lipogenesis and risk of prostate cancer. Am J Epidemiol 2013;178:1246–55.
31. Heitmann BL, Frederiksen P. Imprecise methods may both obscure and aggravate a relation between fat and breast cancer. Eur J Clin Nutr 2007;61:925–7.
32. Prentice RL. Dietary assessment and the reliability of nutritional epidemiology reports. Lancet 2003;362:182–3.
33. Lope V, Pérez-Gómez B, Sánchez-Contador C, Santamariña MC, Moreo P, Vidal C, Laso MS, Ederra M, Pedraz-Pingarrón C, González-Román I, et al. Obstetric history and mammographic density: a population-based cross-sectional study in Spain (DDM-Spain). Breast Cancer Res Treat 2012;132:1137–46.
34. Pollán M, Lope V, Miranda-García J, García M, Casanova F, Sánchez-Contador C, Santamariña C, Moreo P, Vidal C, Peris M, et al. Adult weight gain, fat distribution and mammographic density in Spanish pre- and post-menopausal women (DDM-Spain). Breast Cancer Res Treat 2012;134:823–38.
35. Chasan-Taber L, Erickson JB, Nasca PC, Chasan-Taber S, Freedson PS. Validity and reproducibility of a physical activity questionnaire in women. Med Sci Sports Exerc 2002;34:987–92.
36. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR, Tudor-Locke C, Greer JL, Vezina J, Whitt-Glover MC, Leon AS. 2011 Compendium of physical activities: a second update of codes and MET values. Med Sci Sports Exerc 2011;43:1575–81.
37. Vioque J, Weinbrenner T, Asensio L, Castelló A, Young IS, Fletcher A. Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. Br J Nutr 2007;97:977–86.
38. Vioque J, Navarrete-Muñoz E-M, Gimenez-Monzó D, García-de-la-Hera M, Granado F, Young IS, Ramón R, Ballester F, Murcia M, Rebagliato M, et al. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. Nutr J 2013;12:26.

39. Llobet R, Pollán M, Antón J, Miranda-García J, Casals M, Martínez I, Ruiz-Perales F, Pérez-Gómez B, Salas-Trejo D, Pérez-Cortés J-C. Semi-automated and fully automated mammographic density measurement and breast cancer risk prediction. Comput Methods Programs Biomed 2014;116:105–15.
40. Pollán M, Llobet R, Miranda-García J, Antón J, Casals M, Martínez I, Palop C, Ruiz-Perales F, Sánchez-Contador C, Vidal C, et al. Validation of DM-Scan, a computer-assisted tool to assess mammographic density in full-field digital mammograms. Springerplus 2013;2:242.
41. Criado-Navarro I, Mena-Bravo A, Calderón-Santiago M, Priego-Capote F. Profiling analysis of phospholipid fatty acids in serum as a complement to the comprehensive fatty acids method. J Chromatogr A 2020;1619:460965.
42. Chajès V, Joulin V, Clavel-Chapelon F. The fatty acid desaturation index of blood lipids, as a biomarker of hepatic stearoyl-CoA desaturase expression, is a predictive factor of breast cancer risk. Curr Opin Lipidol 2011;22:6–10.
43. Tosi F, Sartori F, Guarini P, Olivieri O, Martinelli N. δ-5 and δ-6 desaturases: crucial enzymes in polyunsaturated fatty acid-related pathways with pleiotropic influences in health and disease. Adv Exp Med Biol 2014;824:61–81.
44. Greenland S. Dose–response and trend analysis in epidemiology: alternatives to categorical analysis. Epidemiology 1995;6:356–65.
45. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc Ser B (Methodological) 1995;57:289–300.
46. Ruiz E, Ávila JM, Valero T, Del Pozo S, Rodriguez P, Aranceta-Bartrina J, Gil Á, González-Gross M, Ortega RM, Serra-Majem L, et al. Macronutrient distribution and dietary sources in the Spanish population: findings from the ANIBES study. Nutrients 2016;8:177.
47. Aglago EK, Biessy C, Torres-Mejía G, Angeles-Llerenas A, Gunter MJ, Romieu I, Chajès V. Association between serum phospholipid fatty acid levels and adiposity in Mexican women. J Lipid Res 2017;58:1462–70.
48. Chajès V, Thiébaut ACM, Rotival M, Gauthier E, Maillard V, Boutron-Ruault M-C, Joulin V, Lenoir GM, Clavel-Chapelon F. Association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC study. Am J Epidemiol 2008;167:1312–20.
49. Chajès V, Biessy C, Ferrari P, Romieu I, Freisling H, Huybrechts I, Scalbert A, Bueno de Mesquita B, Romaguera D, Gunter MJ, et al. Plasma elaidic acid level as biomarker of industrial trans fatty acids and risk of weight change: report from the EPIC study. PLoS One 2015;10:e0118206.
50. Zhou Y, Wang T, Zhai S, Li W, Meng Q. Linoleic acid and breast cancer risk: a meta-analysis. Public Health Nutr 2016;19:1457–63.
51. Arab A, Akbarian SA, Ghiyasvand R, Miraghajani M. The effects of conjugated linoleic acids on breast cancer: a systematic review. Adv Biomed Res 2016;5:115.
52. Sakai M, Kakutani S, Horikawa C, Tokuda H, Kawashima H, Shibata H, Okubo H, Sasaki S. Arachidonic acid and cancer risk: a systematic review of observational studies. BMC Cancer 2012;12:606.
53. Tallima H, El Ridi R. Arachidonic acid: physiological roles and potential health benefits—a review. J Adv Res 2018;11:33–41.
54. Sandhu N, Schetter SE, Liao J, Hartman TJ, Richie JP, McGinley J, Thompson HJ, Prokopczyk B, DuBrock C, Signori C, et al. Influence of obesity on breast density reduction by ω-3 fatty acids: evidence from a randomized clinical trial. Cancer Prev Res 2016;9:275–82.
55. Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N. Global survey of the ω-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. Prog Lipid Res 2016;63:132–52.
56. Ros E, López-Miranda J, Picó C, Rubio MÁ, Babio N, Sala-Vila A, Pérez-Jiménez F, Escrich E, Bulló M, Solanas M, et al. Consensus on fats and oils in the diet of Spanish adults: position paper of the Spanish Federation of Food, Nutrition and Dietetics Societies. Nutr Hosp 2015;32:435–77.
57. Peter A, Cegan A, Wagner S, Lehmann R, Stefan N, Königsrainer A, Königsrainer I, Häring H-U, Schleicher E. Hepatic lipid composition and stearoyl-coenzyme A desaturase 1 mRNA expression can be estimated from plasma VLDL fatty acid ratios. Clin Chem 2009;55:2113–20.
58. Vessby B, Gustafsson I-B, Tengblad S, Berglund L. Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat. Br J Nutr 2013;110:871–9.
59. Manni A, Richie JP, Schetter SE, Calcagnotto A, Trushin N, Aliaga C, El-Bayoumy K. Stearoyl-CoA desaturase-1, a novel target of ω-3 fatty acids for reducing breast cancer risk in obese postmenopausal women. Eur J Clin Nutr 2017;71:762–5.
60. Pouchieu C, Chajès V, Laporte F, Kesse-Guyot E, Galan P, Hercberg S, Latino-Martel P, Touvier M. Prospective associations between plasma saturated, monounsaturated and polyunsaturated fatty acids and overall and breast cancer risk—modulation by antioxidants: a nested case–control study. PLoS One 2014;9:e90442.
61. Wirfält E, Vessby B, Mattisson I, Gullberg B, Olsson H, Berglund G. No relations between breast cancer risk and fatty acids of erythrocyte membranes in postmenopausal women of the Malmö Diet Cancer cohort (Sweden). Eur J Clin Nutr 2004;58:761–70.
62. Zeleniuch-Jacquotte A, Chajès V, Van Kappel AL, Riboli E, Toniolo P. Reliability of fatty acid composition in human serum phospholipids. Eur J Clin Nutr 2000;54:367–72.