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Maternal adiposity moderates associations between dietary, serum, and human milk n-3 and n-6 PUFA

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Overweight and obesity can alter the composition of human milk, including the fatty acid (FA) profile into more proinflammatory state. It is uncertain whether this is due to poor diet quality or the negative effects of obesity on FA metabolism. We examined the associations between dietary, serum, and human milk FA in mothers with normal and excessive body weight and investigated whether adiposity moderates the observed associations. A case-control study was conducted among 40 mothers (20 healthy weight (HW), 20 overweight/obese (OW/OB) 15.5 ± 1.2 weeks postpartum, matched by lactation duration and age. Dietary intake was analyzed based on 3-day food records, and adiposity was assessed by dual-energy X-ray absorptiometry (DXA), serum and human milk FA analyzed by gas chromatography (GC). Overweight/obese mothers had higher dietary and serum trans FA but lower serum arachidonic acid (AA) and human milk docosahexaenoic acid (DHA) compared to normal-weight mothers. Mediation analysis indicated that serum partially mediated the effect of dietary linoleic acid (LA), polyunsaturated fatty acid (PUFA) n-3, and alpha-linolenic acid (ALA) on human milk FA. Adiposity were found to negatively impact the dietary-to-human milk FA association but positively impact serum-to-human milk association. The obesity-related differences in human milk FA profile were not due to dietary differences. Our results suggest human milk PUFA levels may be influenced more by long-term diet than short-term intake, indicating a need for specific dietary guidelines for mothers with higher adiposity to minimze proinflammatory alterations in human milk composition.

Keywords BMI, DHA, Lactation, Mature milk, Mediation analysis, Overweight and obesity

Overweight/obesity rates have significantly risen in the last few decades, including in women of childbearing age[1]. This condition in mothers is linked to less favorable lactation outcomes such as lower initiation rates, exclusivity, and shorter duration of lactation[2]. Furthermore, maternal overweight/obesity and adiposity can influence the human milk composition, including macronutrients, fatty acids (FA), hormones, adipokines, cytokines, immunoglobulins, microRNAs, human milk oligosaccharides (HMOs), as well as human milk microbiota[3–5]. These alterations may give the human milk pro-inflammatory characteristics when compared to the human milk of mothers with a healthy weight[5,6].

Human milk's nutritional composition, especially its fatty acid profile, has been extensively researched concerning maternal overweight and obesity[7]. A recent systematic review found a link between a mother's body mass index (BMI) and specific components in human milk, such as leptin, insulin, and the omega-6 to omega-3 ratio[7]. Other authors showed that human milk produced by mothers with overweight/obesity tend to have higher levels of saturated fatty acids (SFAs)[8–11], n-6 polyunsaturated fatty acids (PUFA)[3,12–15], and less n-3 PUFA[3,6,8,12,16], but the results were often inconsistent[7].

Human milk FA could be derived from three sources: *de novo* lipogenesis in the mammary epithelia, maternal body stores (i.e., adipose tissue), and maternal diet[17,18]. Medium-chain FA (< 16 carbons; MCFA) are synthesized *de novo*, while PUFAs are taken up from circulation and are derived directly from the diet or maternal stores. Long-chain PUFAs, like arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), primarily come from the maternal diet and body stores[17,18]. Thus, changes in human milk FA profile observed in mothers with overweight/obesity (OW/OB) could be due to differences in FA *de novo* synthesis,

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release from endogenous stores, or dietary intake[3]. O'Sullivan et al.[19] found that human milk from mothers with excess gestational weight gain contains less monomethyl branched-chain fatty acids (C15:0 and C16:1), possibly due to reduced synthesis caused by obesity[19,20]. Obesity is also linked to the overproduction of pro-inflammatory adipocytokines (e.g., TNF-α, IL-6, and leptin), which can lead to chronic low-grade inflammation. Pro-inflammatory cytokines are involved in the pathogenesis of metabolic disorders and chronic diseases often accompanying obesity[21]. Inflammation may also inhibit lipoprotein lipase (LPL) activity, crucial to long-chain FA transfer through mammary epithelial cell basal membrane[22]. Recently, Walker et al.[22] confirmed that mothers with low milk production (< 300 mL/d) have obesity, elevated inflammatory biomarkers, and disturbed transfer of long-chain FAs from plasma to human milk.

The Western dietary pattern, with high energy, SFA, and n-6 PUFA intake and low n-3 PUFA, fiber, and antioxidants, is linked to obesity[23]. This results in poor n-3 PUFA status, increased n-6 PUFA, and oxidative stress and inflammation[24,25]. Moreover, a high-fat, western diet negatively affects the mammary synthesis of MCFA[17,26]. The recent systematic review found substantial evidence supporting the associations between dietary intake and DHA and EPA human milk levels, but low for ALA and AA[11]. Although the associations between dietary and human milk FA have been widely investigated, fewer studies investigated this in the context of maternal OW/OB. Mothers with pre-pregnancy OW/OB who participated in the PREOBE cohort study had a significantly higher AA intake and n-6/n-3 ratio compared to normal-weight mothers. However, it was not reflected in human milk FA composition[9]. Similarly, mothers with OW/OB from the USA had significantly lower human milk n-3 PUFAs and higher n-6/n-3 ratio with no difference in their intake[6]. Karbin et al.[10] did not find any correlations between dietary intake of saturated fatty acids, monounsaturated fatty acids (MUFA), n-3 and n-6 PUFA, and their human milk level in mothers with overweight and obesity. In our previous observational study, FA dietary intake, not maternal BMI or carotenoids, significantly predicted human milk PUFA, but only 15% of participants were overweight and obese[27]. More relevant findings regarding the modulating effect of OW/OB on the metabolic response to dietary PUFA were obtained in intervention studies. Monthé-Drèze et al.[28] found that mothers with pre-pregnancy overweight and obesity have an attenuated serum response to n-3 PUFA supplementation in pregnancy compared to lean mothers, resulting in 50% lower effect size, which supported results obtained within other subpopulations[29–31]. For example, Straka et al.[29] found that weight and BMI were inversely correlated with plasma, erythrocyte membrane EPA, breast adipose tissue EPA, and DHA changes after intervention [2015][29]. Authors hypothesized that OW/OB attenuates PUFA availability and metabolism due to higher n-3 PUFA utilization, lipid peroxidation, damage of DNA coding genes involved in PUFA metabolism, and hormonal changes[28,31]. Hence, it remains unclear whether the difference in human milk FA profile is due to their dietary intake or the adverse effects of obesity on PUFA metabolism.

We aimed to explore the associations between the dietary fatty acid intake and human milk fatty acid (FA) and whether the serum FA mediated these connections in mothers with healthy weight (HW) and those with overweight/obesity (OW/OB). Additionally, we investigated whether the maternal anthropometric and adiposity measures moderated these associations. Our specific hypotheses were: (1) OW/OB mothers would have lower n-3 PUFA and higher n-6 PUFA and n-6 to n-3 ratio in serum and human milk, with no differences in their dietary intake; (2) human milk n-3, n-6 PUFA, and its ratio would be associated with their dietary intake and mediated by maternal serum FA; (3) maternal OW/OB and adiposity would attenuate these associations.

Methods

Participants

Between October 2022 and April 2023, we recruited 40 predominantly or exclusively breastfeeding women for the case-control BLOOM (Breastmilk and the Link to Overweight/Obesity and Maternal diet) study from the local community of Warsaw (capital of Poland) through social media ads. Reporting of the study results followed the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidance. The participants were divided into two groups: 20 with HW (BMI 18.5–24.9 kg/m^2) and 20 with OW/OB (BMI ≥ 25.0 kg/m^2). We selected them from 160 respondents based on inclusion and exclusion criteria, matching them by age and breastfeeding duration (Fig. 1). The participants were 32.4 ± 3.9 years old and breastfeeding for 15.5 ± 1.2 weeks. Both groups had similar socio-demographic characteristics but significantly differed in body composition and anthropometric data (Supplementary Table 1).

The G*Power Software 3.1.9.7[32] was used to calculate the minimal sample size based on the results of Panagos et al.[6] for the DHA, n-3 PUFA, n-6/n-3 ratio. The calculations indicated that including 12 to 19 participants per group would be sufficient to detect significant differences between groups (two-tailed test, 80% power, and $\alpha = 0.05$), which aligned with our calculations for leptin[33].

The study was conducted following the Helsinki Declaration and approved by the Ethics Committee of the Faculty of Human Nutrition and Consumer Sciences at Warsaw University of Life Sciences (No. 53/2021, December 2021). Informed consent was signed by all participants involved in the study.

Outcome variables

Dietary assessment

The maternal diet was assessed using a 3-day dietary record, where mothers recorded all food, drinks, and dietary supplements consumed over three typical days before the study visit. Portion sizes of foods consumed were determined using home measurements and/or kitchen scales. Trained dietitian verified and analyzed records using Dieta 6.0 Software (National Institute of Public Health, National Research Institute, Warsaw, Poland) to determine energy value and nutrient intake. Intake from dietary supplements was calculated based on the manufacturer data, and total dietary fatty acid intake was expressed as a sum from both diet and supplements. More details about the dietary assessment we described elsewhere[33].

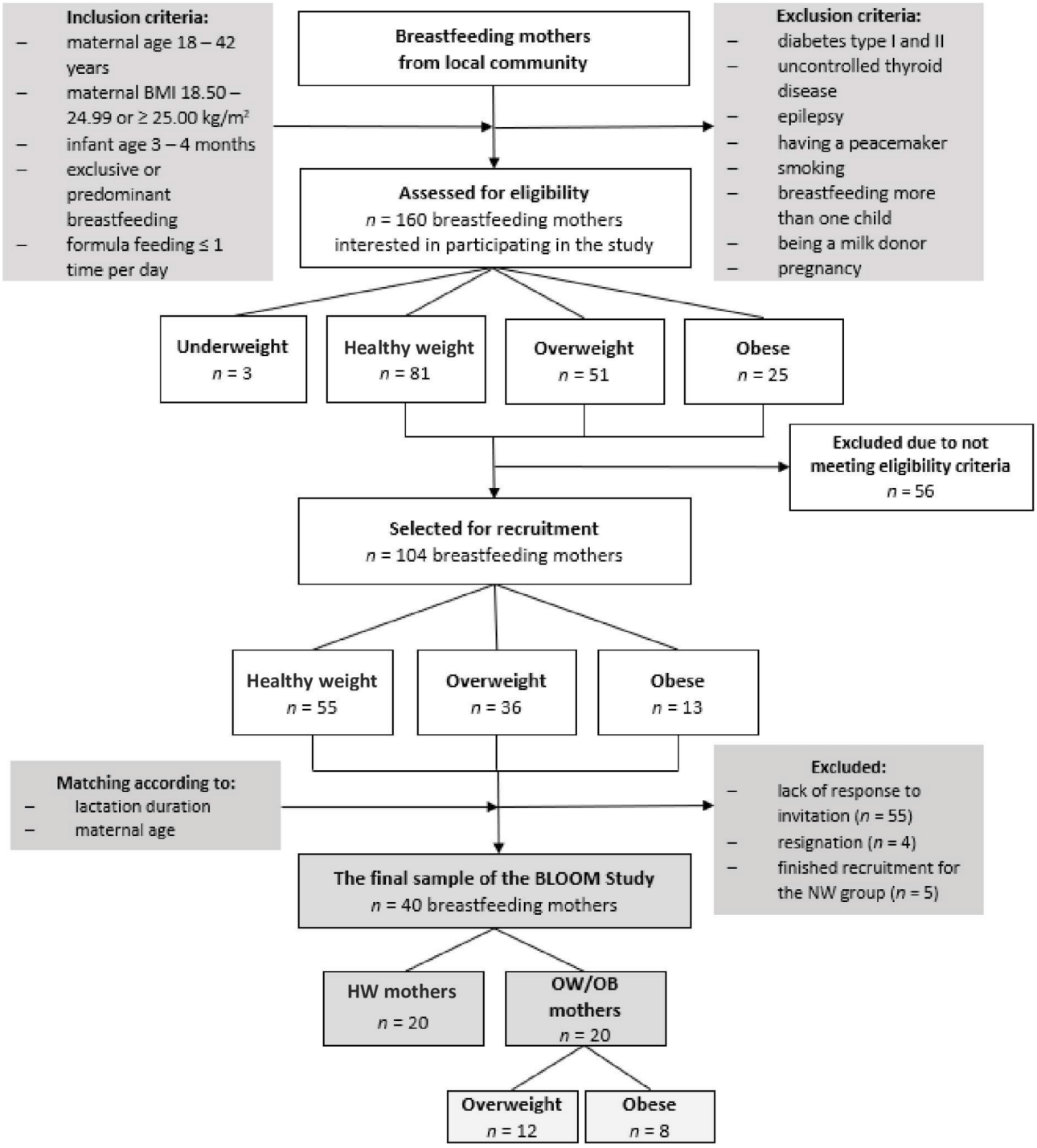

Fig. 1. Flowchart of the study recruitment process. BLOOM, Breastmilk and the Link to Overweight/Obesity and Maternal diet; BMI, body mass index; HW, healthy weight; OW/OB, overweight/obese.

Anthropometric assessment

Maternal body weight and height, waist circumference (WC) and hip circumference were measured twice as described previously[33]. We calculated BMI and waist-to-hip ratio (WHtR) based on measurements. Maternal body composition (fat mass (FM%), fat-free mass (FM), FM% in android and gynoid region) was measured using a dual-energy X-ray absorptiometry (Lunar Prodigy). Visceral (VAT) and subcutaneous (SAT) adipose tissue in the android region was calculated using Lunar Prodigy enCORE ver. 18 Software (GE HealthCare, Madison, WI, USA).

Human milk and blood collection

Human milk samples were collected within 24 h before the study visit. Mothers were asked to express equal volumes (5–10 mL) of foremilk and hindmilk from four time periods (6:00–12:00; 12:00–18:00; 18:00–24:00; 24:00–06:00) into one polypropylene bottle. The samples were stored and transferred to the laboratory under cooling and light protection. In the laboratory, pooled samples were vortexed and transferred into 2 mL Eppendorf tubes and frozen at − 80 °C for further FA analysis.

Blood was collected from the ulnar vein in the morning after overnight fasting. Blood samples were centrifuged, and serum was transferred into 0.2 mL Eppendorf tubes and stored at − 80 °C for further analysis.

Determination of FA profile

Total human milk lipids were extracted using modified Folch extraction[34]. Shortly, to 1 mL of human milk, we added 6 mL of chloroform/methanol (2:1, v/v) and vortexed the mixture for 5 min. Then, we added 1 mL of saturated NaCl solution, centrifuged the mixture (4,000 rpm, 15 min, 4 °C), and collected the lower fraction, which evaporated using CentriVap concentrator (Labconco, Kansas City, MO, USA). We added 1 mL of n-hexane to extracted lipids and transferred the mixture into a 4 mL vial. Then, we conducted saponification and esterification samples with potassium hydroxide in methanol to obtain fatty acid methyl esters (FAME). Total serum lipids were extracted by direct transesterification to FAME, according to Nikolic Turnic et al.[35].

The fatty acid profile was evaluated by gas chromatography (GC) technique using the YL6100 GC gas chromatograph equipped with a flame ionization detector (FID) and a BPX 70 capillary column (60 m length, 0.25 μm film thickness, and 0.25 mm internal diameter). The temperature of the oven was programmed as follows: 70 °C for 0.5 min, 15 °C/min to 160 °C, 1.1 °C/min to 200 °C, 200 °C for 12 min and 30 °C/min to 225 °C and then kept at 225 °C for 0.5 min. The injector and detector temperatures were set at 225 °C and 250 °C, respectively. The nitrogen was used as a carrier gas, and the flow was set at 1.2 mL/min. The abundance percentage of each fatty acid was determined, and each sample analysis was conducted in two repeated measurements. The results were expressed as relative percentages of each fatty acid, calculated using area normalization of the chromatographic peak area (% of the fatty acids peaks area to the total peak area was determined). Fatty acids were identified by comparing the relative retention times of peaks with external standard of fatty acid methyl ester mixture (Supelco 37 Component FAME Mix, Sigma-Aldrich, Germany). The analysis was conducted in duplicate for each sample.

Statistical analysis

All statistical analyses were conducted for all 40 participants, as we have no missing data. Quantitative variables were presented as a mean ± standard deviation (SD) or median (Me) and interquartile range depending on the normality of the distribution (checked by the Shapiro-Wilk test). Differences between the HW and OW/OB groups were assessed using the t-Student test (original or log-transformed variables) or the U-Mann Whitney test (original variables that did not follow a normal distribution after log-transformation). Correlations between log-transformed dietary, serum, and human milk FA and maternal anthropometrics were analyzed using Pearson correlations in the R corrplot package.

Then, we examined the relationships between dietary, serum, human milk fatty acids, and adiposity using mediation and moderation analyses, modern regression based techniques[36]. Prior to conducting the analyses, we checked for independence, linearity, homoscedasticity, normality of residuals, and multicollinearity. We used visual inspection of scatterplots, p-p plots, Durbin-Watson statistics, and variance inflation factors (VIF) as recommended by Clement and Bradley-Garcia[37]. Following this, we constructed mediation (model 4) and moderated mediation (model 59) models using the PROCESS Macro for SPSS version 4.2[38] based on the theoretical model illustrated in Fig. 2.

The independent variables (X) were dietary FAs, dependent variables (Y) were human milk FAs, mediators (M) were serum FAs, moderator (W) were adiposity measures. To check the robustness of analyses we conducted moderated mediation models with anthropometric measurements BMI, WC, WHtR, as well as body composition parameters (FM%, FFM%, android FM%, gynoid FM%, VAT [g], SAT [g]). We conducted analyses on log-transformed and centered variables to prevent multicollinearity. We created unadjusted and adjusted mediation models, as well as adjusted moderated models. We used a minimal set of confounders approach and directed acyclic graphs (DAGs) to identify covariates (Fig. 3). Adjusting for diet was sufficient to eliminate bias. Nuts and seeds intake was chosen as a covariate based on Pearson correlation analysis for the adjusted analyses.

We used 10,000 bootstrap samples and determined the bias-corrected 95% confidence interval (CI) to evaluate the mediating and moderating effects. Results were expressed as coefficient, standard error, and p-value, the mediating effect was expressed as a bootstrapped effect, SE, and 95% CI. When the moderating effect of adiposity was significant, we analyzed conditional indirect effects of the focal predictors at Mean ± 1SD of moderators. We visualized the interactions of low (-1 SD), moderate (mean), and high (+ 1SD) values of moderator variables using the ggplot2. We also used the Johnson-Neyman (J-N) approach to determine the concrete values of the moderators at which the interaction with the predictor was significant. To evaluate the moderation of mediation, we analyzed the pairwise contrasts between conditional indirect effects. Mediation was considered moderate when bootstrapped the 95% CI did not cover zero.

The statistical analyses were considered significant if the *P* value was equal to or lower than 0.05 or if the bootstrapped 95% CI did not include 0. Descriptive statistics were calculated using STATISTICA 13.3 Software (TIBCO Software Inc., Paolo Alto, CA, USA), regression models were computed in IBM SPSS Statistics 29.0.2.0 (IBM Corp., Armonk, NY, USA), and data visualization was created in RStudio (version 4.3.3, 2024, RStudio, Inc., Vienna, Austria).

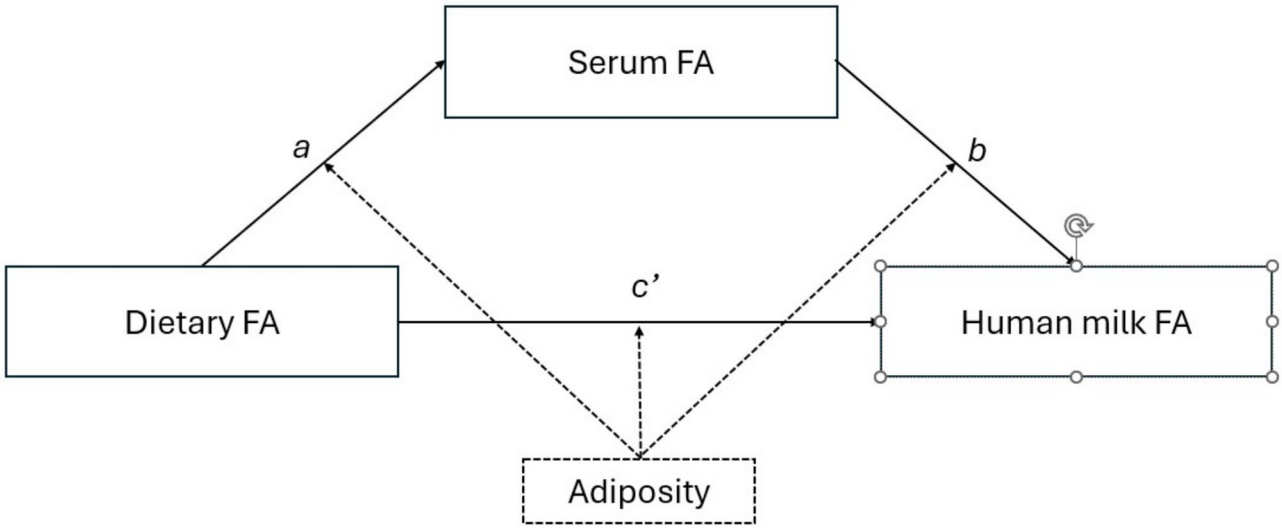

Fig. 2. Model of moderated mediation pathways of serum FA on the association between dietary and human milk FA. a, indirect pathway from dietary FA to serum FA; b, indirect pathway from serum to human milk FA; c', direct pathway from dietary FA to human milk FA; FA, fatty acid. The total effect of a model is given by c = [c' +(a x b)]

Results

Dietary, serum, and human milk FA

OW/OB mothers had higher trans-FA intake and serum dihomo-γ-linolenic acid (DGLA), MUFA, and trans-FA levels, as well as lower human milk DHA levels compared to HW mothers ($P \leq 0.05$). No other significant differences were found in the dietary, serum, and human milk FA composition between the two groups (Table 1).

Pearson correlations between dietary, serum, human milk FA, and adiposity markers

Dietary, serum, and human milk fatty acids for LA, n-3 PUFA, ALA, DHA, and n-6 to n-3 ratio showed positive correlations in unadjusted Pearson correlation analysis. Dietary LA/ALA ratio was significantly positively correlated with human milk but not with serum. Dietary n-6 PUFA was positively correlated with serum but not with human milk (Fig. 4A), but no other significant correlations were observed (Supplementary Fig. 1A).

Maternal BMI showed negative correlation with serum AA, n-3 PUFA, and DHA, as well as human milk n-3 PUFA and DHA (Fig. 4B). WC and FM% were negatively correlated with serum AA and human milk DHA, while FM% was also correlated with serum n-6 PUFA and LA (Fig. 4B). FFM% was positively correlated with serum n-6 PUFA, LA, AA, trans-FA, human milk n-3 PUFA, and DHA. FM% in the android region was correlated with serum LA, AA, LA/ALA ratio, trans-FA, and human milk DHA. FM% in the gynoid region was correlated with serum AA. VAT was significantly correlated with serum trans-FA and human milk DHA, while SAT was correlated with serum AA, trans-FA, DHA, and human milk DHA. WHtR was correlated with serum AA and human milk DHA. No other significant differences despite BMI, dihomo-γ-linolenic acid and saturated fatty acids (Supplementary Fig. 1B) were found.

Mediating effect of serum FA on association between dietary and human milk FA

We found that dietary FA had a significant association with serum FA (path a: diet → serum) for n-6 PUFA, LA, n-3 PUFA, ALA, DHA, and n-6/n-3 ratio, but after adjustment results for the n-6/n-3 ratio were no longer significant (Tables 2 and Supplementary Table 2). Serum FA significantly predicted human milk FA (path b: serum → human milk) such as n-6 PUFA, LA, n-3 PUFA, ALA, EPA, DHA, LA/ALA, and n-6/n-3 ratio, and those results were unchanged by adjustment. Dietary FA had a significant direct effect on human milk FA (path c': diet → human milk) only for LA and n-3 PUFA in unadjusted and adjusted models. Serum FA significantly mediated between dietary and human milk FA (path ab: diet → serum → human milk) for n-6 PUFA, LA, n-3 PUFA, ALA, DHA, and n-6/n-3 ratio, but after adjustment results for the n-6/n-3 ratio were no longer significant. The total effect of dietary FA on human milk FA was significant for LA, n-3 PUFA, ALA, LA/ALA ratio, and n-6/n-3 ratio. However, the total effect of dietary DHA on its human milk concentration after adjustment was marginally significant ($P = 0.064$).

Taking this together, the effects of dietary LA, n-3 PUFA, and ALA on their human milk concentrations were partially mediated by serum FA in the proportion of 20.4, 22.6, and 45.1%, respectively (in adjusted models). We also found that the effect of dietary DHA on human milk DHA was fully mediated by its serum concentration, but after adjustment, the total effect was marginally significant ($P = 0.064$).

Moderating effect of adiposity on association between dietary, serum, and human milk FA

Path a (diet → serum) was negatively moderated by WC only in the case of AA (β = -3.136, SE = 1.511, $P = 0.045$; Table 3 and Supplementary Tables 3–5). At the high WC levels, higher dietary AA was associated with a lower

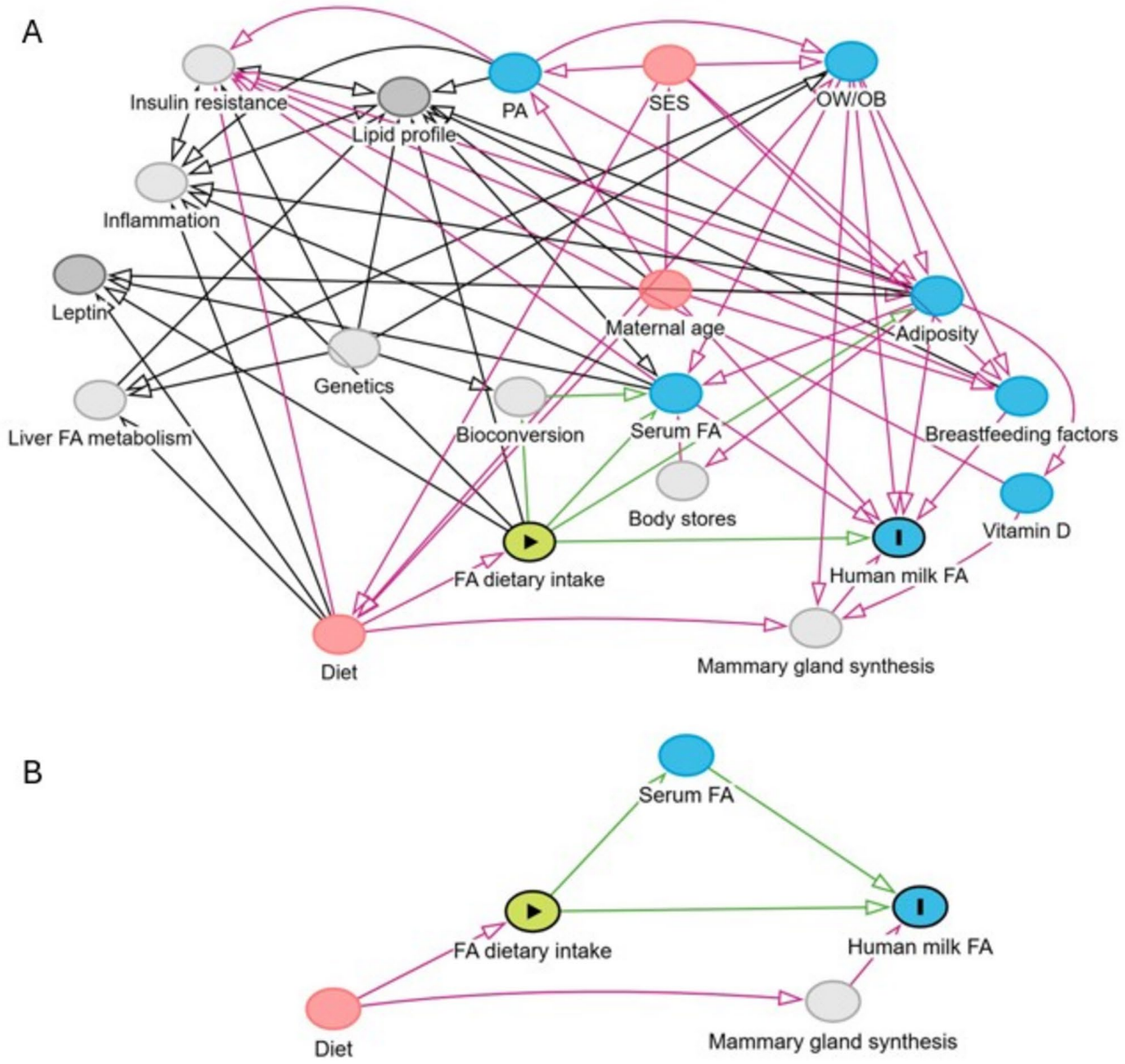

Fig. 3. Directed acyclic graph (DAG) of variables potentially associated with dietary, serum, and human milk FA: (**A**) full; (**B**) simplified. FA, fatty acid; PA, physical activity; OW/OB, overweight or obesity; SES, socioeconomic status.

level of serum AA (conditional effect (CE) = -0.663, $P = 0.016$; Fig. 5A). The effect of dietary AA was significant when WC was above 89 cm (Supplementary Table 6).

Path b (serum → human milk) was significantly and positively moderated by BMI (for EPA (Supplementary Fig. 2A), ALA (Fig. 5B), DHA (Fig. 5C), n-6 PUFA (Fig. 5D)), WC (EPA (Supplementary Fig. 2C), n-6/n-3 ratio (Fig. 6A)), WHtR (EPA (Supplementary Fig. 2E), n-6 PUFA (Supplementary Fig. 2I), n-6/n-3 ratio (Fig. 6D)), FM% (n-6 PUFA (Fig. 5E), n-6/n-3 ratio (Fig. 6E)), VAT (EPA (Supplementary Fig. 2F), n-6 PUFA (Fig. 5F), n-6/n-3 ratio (Fig. 6G)), SAT (EPA (Supplementary Fig. 2H), n-6 PUFA (Fig. 5G), n-6/n-3 ratio (Fig. 6I)), and FM% in android (n-6 PUFA (Fig. 5G), n-6/n-3 ratio (Fig. 6L)) and gynoid (n-6 PUFA (Fig. 5I), n-6/n-3 ratio (Fig. 6M)) region (Tables 3 and 4). Despite mediation models for EPA being insignificant (Table 2), they significantly predict human milk EPA after adding interactions with moderators (Table 3 and Supplementary Table 2). FFM% negatively moderated the association between serum and human milk n-6 PUFA. Among all tested moderators, FM% in the android region, SAT, and VAT were the weakest predictors of human milk EPA, n-6 PUFA, and n-6/n-3 ratio, while anthropometry indices, FM%, and FFM% seemed to have a stronger effect. The positive impact of serum n-6 PUFA on its human milk concentration increased as BMI increased from low to high levels (respectively, from CE = 0.137 to CE = 1.096; Fig. 5D). In other analyzed moderators, CE was insignificant at low levels of moderator but increased from moderate to high values of moderators (Fig. 5B,

	Diet [g/d]			Serum [% of FA]			Human milk [% of FA]		
Fatty acid	HW	OW/OB	*P* value	HW	OW/OB	*P* value	HW	OW/OB	*P* value
MCFA (< 16 C)	7.3 ± 3.7	7.7 ± 3.2	0.778[a]	1.2 ± 0.4	1.0 ± 0.4	0.283[b]	11.2 ± 2.8	11.0 ± 3.9	0.732[b]
SFA	29.6 ± 8.7	32.2 ± 8.7	0.336[a]	40.9 ± 2.7	39.7 ± 2.3	0.150[a]	41.1 ± 4.7	42.7 ± 5.0	0.302[a]
MUFA	33.3 ± 11.1	33.3 ± 9.5	0.992[a]	19.2 (18.1–19.9)	21.6 (19.6–23.5)	0.017[c]	42.9 (40.3–45.6)	42.8 (40.7–45.5)	0.799[c]
n-6 PUFA	10.8 ± 4.1	9.7 ± 3.4	0.371[b]	31.3 ± 2.8	31.6 ± 3.2	0.831[a]	12.3 ± 2.0	11.4 ± 2.2	0.200[a]
18:2 (LA)	10.7 ± 4.1	9.6 ± 3.4	0.394[b]	20.5 ± 2.4	19.7 ± 2.9	0.170[a]	11.4 ± 2.0	10.6 ± 2.2	0.213[a]
18:3 (GLA)	NA	NA	NA	0.21 ± 0.08	0.27 ± 0.11	0.111[b]	1.4 ± 0.4	1.3 ± 0.5	0.233[b]
20:3 (DGLA)	NA	NA	NA	8.7 ± 1.5	9.9 ± 1.9	0.037[a]	0.49 (0.45–0.58)	0.48 (0.43–0.58)	0.799[c]
20:4 (AA)	0.11 ± 0.06	0.09 ± 0.06	0.315[b]	2.52 (1.50–3.25)	1.25 (0.80–2.56)	0.046[c]	0.22 ± 0.06	0.20 ± 0.04	0.414[b]
22:2 (EDA)	NA	NA	NA	0.058 (0.020–0.140)	0.020 (0.0–0.193)	0.301[c]	2.38 (1.70–2.70)	1.94 (1.43–2.44)	0.301[c]
n-3 PUFA[$]	3.5 ± 1.3	3.8 ± 2.2	0.957[b]	6.2 ± 1.8	5.7 ± 1.2	0.305[a]	2.3 ± 0.6	2.0 ± 0.6	0.127[a]
18:3 (ALA)	2.0 (1.7–3.0)	1.8 (1.2–4.0)	0.968[c]	0.43 ± 0.09	0.47 ± 0.15	0.542[b]	1.4 ± 0.4	1.3 ± 0.5	0.430[a]
20:5 (EPA)[$]	0.064 (0.034–0.107)	0.086 (0.036–0.098)	0.989[c]	1.3 ± 0.41	1.2 ± 0.47	0.341[b]	0.13 ± 0.06	0.13 ± 0.07	0.668[b]
22:6 (DHA)[$]	0.494 (0.003– 0.604)	0.397 (0.003– 0.603)	0.583[c]	4.5 ± 1.5	4.1 ± 0.82	0.474[b]	0.76 ± 0.27	0.59 ± 0.19	0.030[a]
Trans FA	0.36 ± 0.22	0.56 ± 0.21	0.003[b]	0.17 ± 0.06	0.26 ± 0.10	0.002[a]	0.37 ± 0.15	0.34 ± 0.17	0.604[a]
LA/ALA ratio	4.2 (3.7–6.0)	4.3 (3.2–6.5)	0.620[c]	49.7 ± 10.2	46.2 ± 13.5	0.355[a]	8.8 ± 2.2	9.7 ± 3.4	0.519[b]
n-6/n-3 ratio	3.3 ± 1.2	3.0 ± 1.1	0.385[a]	5.4 ± 1.6	5.7 ± 1.3	0.537[a]	5.6 ± 1.2	6.2 ± 1.5	0.235[a]

Table 1. Fatty acid profile of diet (g/d), serum, and human milk (% of fatty acids) regarding maternal weight status ($n = 40$). [$] - total intake from diet and dietary supplements; [a] – t-Student test; [b] – t-Student test on log-transformed variables; [c] – U-Mann Whitney test. AA, arachidonic acid; ALA, α-linoleic acid; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GLA, γ-linolenic acid; LA, linoleic acid; MCFA, mid-chain fatty acids; MUFA, monounsaturated fatty acid; NA, not applicable; HW, healthy weight; OW/OB, overweight or obese; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

C, E-I, and 6B, D, G, L, M). This indicates that the relationship between serum and human milk FA only holds for mothers with moderate and high levels of adiposity (the specific values of moderators are shown in Supplementary Table 3).

Path c' (diet → human milk) was negatively moderated by WC (EPA (Fig. 5A), n-6/n-3 ratio (Fig. 6A)), WHtR (EPA, n-6/n-3 ratio(Fig. 6C)), FM% (n-6/n-3 ratio (Fig. 6E)), VAT (n-6/n-3 ratio (Fig. 6F)), SAT (EPA, n-6/n-3 ratio (Fig. 6H)), and FM% in android (n-6/n-3 ratio (Fig. 6K)) and gynoid (n-6/n-3 ratio (Fig. 6M)) region and positively by FFM% (n-6/n-3 ratio; Table 4). Interactions between dietary FA and FM% in the android region, SAT, and VAT had the weakest effect on human milk FA. Additionally, interactions and CE were lower when compared to interactions with serum. The analysis of the CE showed that the direct effect of dietary FA on human milk concentrations gradually decreased as adiposity increased (Figs. 5A and 6A, C, E, F, H, K and M), and associations mainly were significant at low or moderate levels of adiposity. Additionally, the CE for interactions between dietary, milk EPA and WC, WHtR, and SAT were insignificant and low, moderate, and high levels of adiposity (Supplementary Fig. 2B, D, G), but the J-N approach identified the values of significance of CE for these interactions (Supplementary Table 5). Specifically, mothers with moderate and/or lower adiposity and higher dietary n-6/n-3 ratio had a higher human milk n-6/n-3 ratio, while the direct effect was insignificant in mothers with high adiposity.

The last step in moderated mediation analysis was the evaluation of whether indirect effects of dietary FA on human milk through serum FA were moderated by adiposity, what was confirmed only for DHA (by BMI) and EPA (by SAT). The indirect effect of dietary DHA was substantial only in mothers with moderate and high BMI (indirect effects and bootstrapped 95% CI were 0.038 and 0.008–0.089; 0.094 and 0.033–0.191, respectively). The indirect effect of EPA was significant only in mothers with high SAT (0.382 and 0.039–0.993).

Discussion

In this study, we found that mothers with overweight/obesity had lower human milk DHA despite no differences in dietary intake or serum concentration. Differences in dietary and serum FA were not reflected in human milk FA profile. We also found that the effect of dietary LA, n-3 PUFA, and ALA on its human milk concentrations was partially mediated by serum, and the direct effect of DHA was fully mediated by serum (with a marginally significant total effect). Full mediation indicates that all milk DHA is transported through circulation from diet to milk, while partial mediation suggests additional mobilization of FA from maternal stores. Moreover, maternal adiposity moderated the associations between dietary, serum, and human milk AA, ALA, EPA, n-6 PUFA, and n-6/n-3 ratio. Specifically, adiposity weakened the associations between diet and human milk but surprisingly strengthened the associations between serum and human milk. This means that the effect of serum FA on human milk FA increased, while the effect of dietary FA on human milk decreased with the increase in the adiposity measures. In addition, we observed that body composition and anthropometric indices were stronger moderators than adipose tissue distribution. Taking this together, our results only partially align with our initial hypotheses that (1) OW/OB is associated with lower n-3 PUFA and higher n-6 PUFA and n-6 to n-3

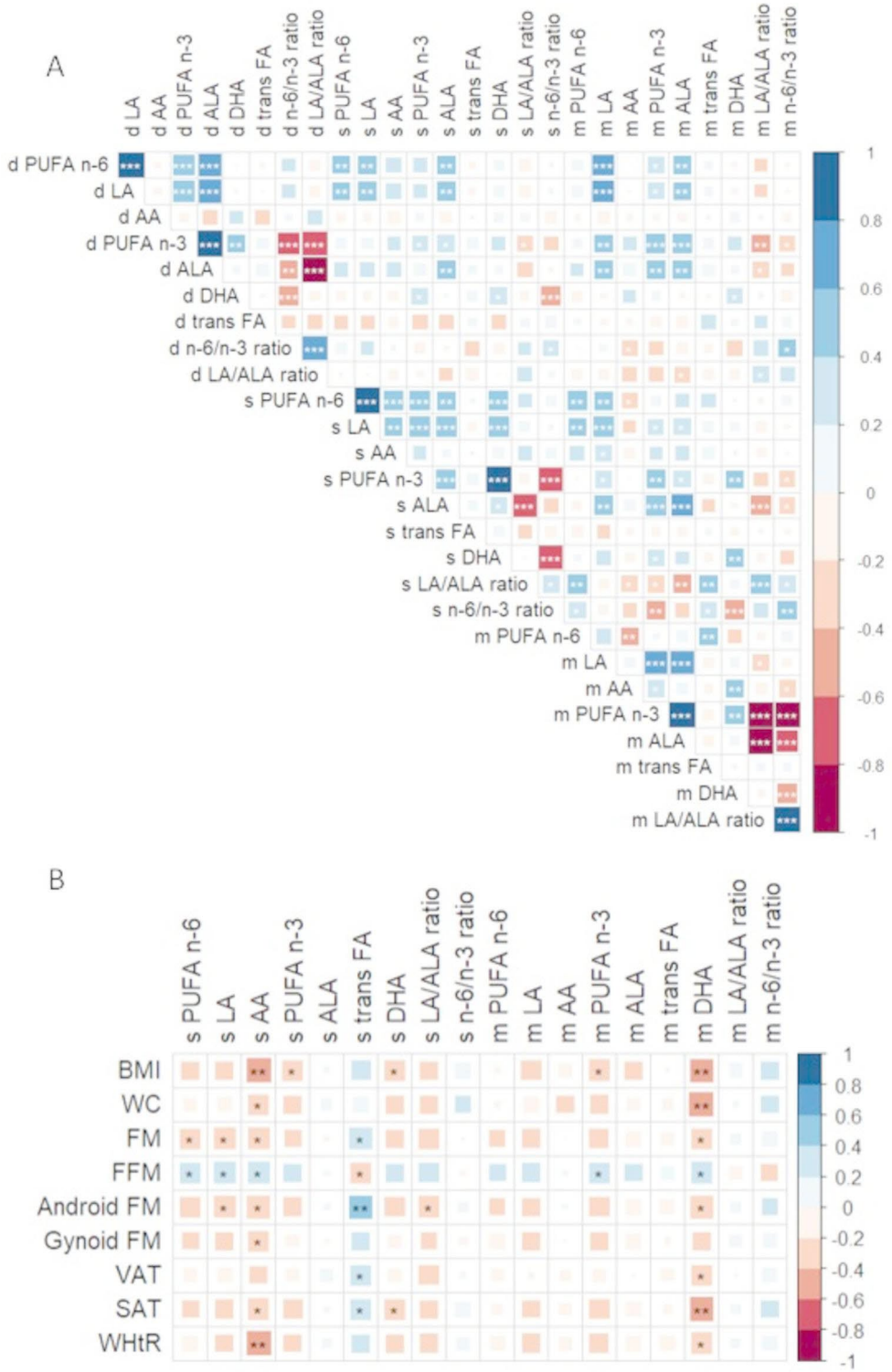

Fig. 4. Results of the Pearson correlations between (A) dietary, serum, and human milk FA and (B) adiposity, serum, and human milk FA. Dietary FA intake was calculated based on a 3-day food record and summed with dietary supplements. AA, arachidonic acid; ALA, α-linoleic acid; BMI, body mass index; d, dietary; DHA, docosahexaenoic acid; FFM, fat-free mass; FM, fat mass; LA, linoleic acid; m, human milk; PUFA, polyunsaturated fatty acids; s, serum; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WC, waist circumference. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

FA	Effect of dietary FA on serum FA		Effect of serum FA on human milk FA		The mediating effect of serum FA[&]		Direct effect of dietary FA on human milk FA		Total effect of dietary FA on human milk FA	
	β_A (SE)	*P* value	β_B (SE)	*P* value	$Effect_{AB}$ (BootSE)	Boot95% CI	$\beta_{C'}$ (SE)	*P* value	β_C (95% CI)	*P* value
n-6 PUFA	0.186 (0.091)	0.048	0.234 (0.080)	0.006	0.043 (0.029)	-0.009–0.108	-0.025 (0.047)	0.601	0.019 (0.048)	0.698
18:2 (LA)	0.250 (0.101)	0.016	0.233 (0.075)	0.004	**0.060 (0.030)**	**0.008–0.127**	0.241 (0.115)	0.043	0.293 (0.072)	< 0.001
20:4 (AA)	-0.230 (0.171)	0.187	-0.043 (0.062)	0.496	0.010 (0.017)	-0.024–0.046	-0.027 (0.066)	0.689	-0.017 (0.064)	0.794
n-3 PUFA	0.226 (0.108)	0.042	0.344 (0.137)	0.017	**0.078 (0.037)**	**0.015–0.160**	0.267 (0.095)	0.008	0.345 (0.096)	0.001
18:3 (ALA)	0.238 (0.082)	0.006	0.615 (0.170)	0.001	**0.146 (0.056)**	**0.048–0.268**	0.178 (0.094)	0.065	0.324 (0.097)	0.002
20:5 (EPA)	0.124 (0.086)	0.158	0.663 (0.187)	0.001	0.082 (0.069)	-0.031–0.242	0.073 (0.100)	0.471	0.155 (0.111)	0.174
22:6 (DHA)	0.052 (0.020)	0.012	0.418 (0.178)	0.024	**0.022 (0.013)**	**0.003–0.053**	0.021 (0.023)	0.366	0.043 (0.023)	0.064
LA/ALA	0.168 (0.101)	0.105	0.509 (0.159)	0.003	0.085 (0.056)	-0.011–0.208	0.144 (0.101)	0.163	0.229 (0.109)	0.042
n-6/n-3	0.203 (0.115)	0.087	0.343 (0.129)	0.012	0.069 (0.058)	-0.005–0.219	0.147 (0.094)	0.126	0.217 (0.097)	0.032

Table 2. Adjusted mediation analysis models of the associations between dietary, serum, and human milk FA ($n = 40$). Models were adjusted for the minimal set of covariates identified by DAG.[$]- total intake from diet and dietary supplements; [&]- BootSE and Boot95% CI were obtained based on 10000 Bootstrap samples; A – path A (dietary FA → serum FA); AB – path AB through the mediator (dietary FA → serum FA → human milk FA); B – path B (serum FA → human milk FA); C – total effect of model given by C=[C'+(AxB)]; C' – path C' (dietary FA → human milk FA). AA, arachidonic acid; ALA, α-linoleic acid; DHA, docosahexaenoic acid; DGLA, dihomo-γ-linolenic acid; EDA, eicosadienoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GLA, γ-linolenic acid; LA, linoleic acid; MCFA, mid-chain fatty acids; MUFA, monounsaturated fatty acid; HW, healthy weight; OW/OB, overweight or obese; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. Bolded values in $Effect_{AB}$ and Boot95% CI columns were significant at the P-value ≤ 0.05.

ratio in serum and breastmilk, with no differences in their dietary intake (we observed this only for DHA); (2) breastmilk PUFA n-3, n-6, and its ratio would be associated with their dietary intake and mediated by maternal serum FA (we confirmed this for LA, n-3 PUFA, and ALA); (3) maternal OW/OB and adiposity would attenuate these associations (this was observed for association between diet and serum, while the association between serum and human milk was strengthened).

The proportion of SFA, n-3 PUFA, and EPA in our study was similar to the global human milk FA profile reported in meta-analyses by Zhang et al.[39]. However, we found higher proportions of MUFA, GLA, DGLA, EDA, ALA, and DHA and lower proportions of n-6 PUFA, LA, and AA. Additionally, n-3 PUFA was higher, and the ratios of n-6/n-3 and LA/ALA were lower compared to our previous longitudinal analyses of human milk samples from healthy mothers with similar sociodemographic background[27]. A meta-regression analysis found a positive correlation between sampling year and concentration of LA, ALA, and PUFA, probably due to increased dietary intake, while the effect on DHA was marginally significant[39]. Our study participants had higher DHA intake, compared to our previous study[27] and other authors[6,9], which may explain the observed differences in human milk FA profile. We assume that higher DHA intake was related to the increased popularity of DHA supplementation and its concentration in supplements intended for use in the third trimester of pregnancy and lactation. Mothers from the OW/OB group had significantly higher trans-FA intake, but no other FA differences were observed, and this was reflected in serum but not human milk. On the contrary, the only difference in the human milk FA profile was a lower DHA level in the OW/OB group compared to the HW group, consistent with previous studies[6,9,24] but other studies did not confirm this[10,16,40], and/or reported other alterations in FA profile into more pro-inflammatory direction[6,9,24,41] not observed in this study. Similarly, Ćwiek et al.[40] found that mothers with higher body fat had lower DHA levels in their human milk. This difference was not observed when stratifying by BMI category. Several possible explanations exist for differences in the DHA level between the HW and OW/OB groups. Recently, Walker et al.[22] reported that mothers with low milk production (< 300 mL/d) and obesity along with inflammation had disturbed transfer of FAs ≥ 16 C from circulation to the mammary gland. They suggested that this was caused by TNF-α-related disruption of LPL activity, crucial for proper mammary gland fatty acid uptake[22,42]. Although we did not measure TNF-α, the OW/OB group had a significantly elevated serum and human milk leptin, also a biomarker of systemic inflammation[33]. Moreover, a small amount of human milk DHA comes from low-efficient endogenous synthesis from ALA (while LA can be converted to AA)[43]. However, obesity and its comorbidities can reduce desaturases (FADS1 and 2) activity involved in this conversion along with elongases (ELOVL 2 and 5)[3,24]. Certain genetic variations in FADS1, FADS2, and ELOVL are related to OW/OB risk and less favorable PUFA metabolism and status[44,45]. In addition, a recent untargeted metabolome-wide association study from Guatemala[46] indicated that maternal BMI was linked to changes in fatty acid metabolism and biosynthesis (and galactose and xenobiotic metabolism and amino acids biosynthesis). However, in our study, we did not observe differences in other LC PUFA, and the association between serum and human milk fatty acids is strengthened by adiposity, which does not support this explanation. This could be partially explained by the fact that our study group comprised mothers who had not faced severe lactation difficulties, as one of our inclusion criteria was exclusive or predominant breastfeeding with no more than 1 formula-fed per day. In addition, the observed difference in breastmilk DHA, despite its similar dietary intake, could result from increased uptake by adipose tissue or utilization for metabolic processes related to inflammation[28,47], which we discussed in detail in the following paragraphs.

FA	M	Predictors	Serum FA[#]		Human milk FA[#]		Moderated mediation[£]
			Adjusted β (SE) [&]	*P* value	Adjusted β (SE) [&]	*P* value	
AA	WC	Diet FA[$] x M	-3.136 (1.511)	0.045	-0.461 (0.669)	0.495	NS
		Serum FA x M			-0.351 (0.586)	0.553	
EPA	BMI	Diet FA[$] x M	0.522 (0.470)	0.274	-1.048 (0.518)	0.051	NS
		Serum FA x M			2.544 (1.008)	0.017	
	WC	Diet FA[$] x M	0.844 (0.709)	0.216	-2.073 (0.764)	0.011	NS
		Serum FA x M			4.000 (1.606)	0.018	
	WHtR	Diet FA[$] x M	0.983 (0.890)	0.174	-2.030 (0.937)	0.038	NS
		Serum FA x M			3.565 (1.696)	0.043	
	VAT	Diet FA[$] x M	0.114 (0.109)	0.303	-0.071 (0.122)	0.567	NS
		Serum FA x M			0.393 (0.182)	0.022	
	SAT	Diet FA[$] x M	0.245 (0.183)	0.189	-0.483 (0.202)	0.022	≤0.05
		Serum FA x M			1.111 (0.418)	0.012	
ALA	BMI	Diet FA[$] x M	-0.423 (0.444)	0.347	0.794 (0.393)	0.052	NS
		Serum FA x M			1.709 (0.820)	0.045	
DHA	BMI	Diet FA[$] x M	0.101 (0.105)	0.724	-0.182 (0.140)	0.201	≤0.05
		Serum FA x M			3.240 (1.492)	0.037	
n-6 PUFA	BMI	Diet FA[$] x M	-0.415 (0.396)	0.302	-0.016 (0.144)	0.911	NS
		Serum FA x M			2.553 (0.387)	<0.001	
	WHtR	Diet FA[$] x M	-0.305 (0.722)	0.676	0.107 (0.324)	0.744	NS
		Serum FA x M			2.953 (0.959)	0.004	
	FM%	Diet FA[$] x M	-0.460 (0.437)	0.300	-0.237 (0.131)	0.080	NS
		Serum FA x M			3.338 (0.359)	<0.001	
	FFM%	Diet FA[$] x M	1.061 (0.754)	0.168	0.446 (0.250)	0.084	NS
		Serum FA x M			-5.579 (0.649)	<0.001	
	VAT	Diet FA[$] x M	-0.117 (0.078)	0.141	-0.013 (0.040)	0.742	NS
		Serum FA x M			0.322 (0.079)	<0.001	
	SAT	Diet FA[$] x M	-0.092 (0.147)	0.536	-0.033 (0.058)	0.574	NS
		Serum FA x M			1.028 (0.188)	<0.001	
	A FM%	Diet FA[$] x M	-0.303 (0.269)	0.267	-0.129 (0.089)	0.157	NS
		Serum FA x M			1.901 (0.239)	<0.001	
	G FM%	Diet FA[$] x M	-0.245 (0.626)	0.698	-0.161 (0.254)	0.532	NS
		Serum FA x M			3.784 (0.782)	<0.001	

Table 3. Results of adjusted moderated mediation regression models for AA, EPA, ALA, DHA, and n-6 PUFA ($n=40$). Full models were presented in Supplementary Tables 3 and Supplementary Table 4. [#] - models were adjusted for the minimal set of covariates identified by DAG. [£] - mediation was significantly moderated when the bootstrapped 95% CI did not include 0 for the pairwise contrasts between conditional indirect effects. $ - total intake from diet and dietary supplements. AA, arachidonic acid; A FM%, fat mass in the android region; ALA, α-linoleic acid; BMI, body mass index; EPA, eicosapentaenoic acid; FA, fatty acids; DHA, docosahexaenoic acid; FFM%, fat-free mass; FM%, fat mass; G FM%, fat mass in the gynoid region; M, moderator; PUFA, polyunsaturated fatty acid; SAT, subcutaneous adipose tissue; SE, standardized effect; VAT, visceral adipose tissue; WC, waist circumference; WHtR, waist to hip ratio. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Mediation analysis, the modern regression technique, allows for exploring mechanisms of exposure-outcome effects also in cross-sectional and case-control studies[36]. Previously, Christensen et al.[48], using this statistical approach, demonstrated that circulating leptin mediated the association between maternal adiposity and human milk leptin. Here, we used this analysis to examine associations between dietary and human milk FA, which showed that circulating FA mediates this relationship in cases of LA, n-3 PUFA, ALA (partially), and probably DHA (results on full mediation were marginally significant). Our results align with previous studies showing that LC PUFAs are taken from maternal circulation (derived directly from diet or maternal stores, i.e., adipose tissue)[49,50]. Studies using isotopically labeled fatty acids found that approximately 20% of LA and DHA[50,51], as well as 65% of ALA[43] are directly transferred from the diet. Our study supports that more ALA than LA is directly transferred from the diet into human milk throughout circulation, as mediation rates were at 20% for LA and 45% for ALA. The remaining ingested FA could be oxidized, converted to other PUFA, and incorporated into body stores, mainly adipose tissue, with slow turnover[52,53]. Previous studies have shown that the human milk PUFA profile responds rapidly to dietary FA[54] and reflects the long-term dietary intake through mobilization from adipose tissue[49,55]. Our results seemed to confirm these observations, but they must

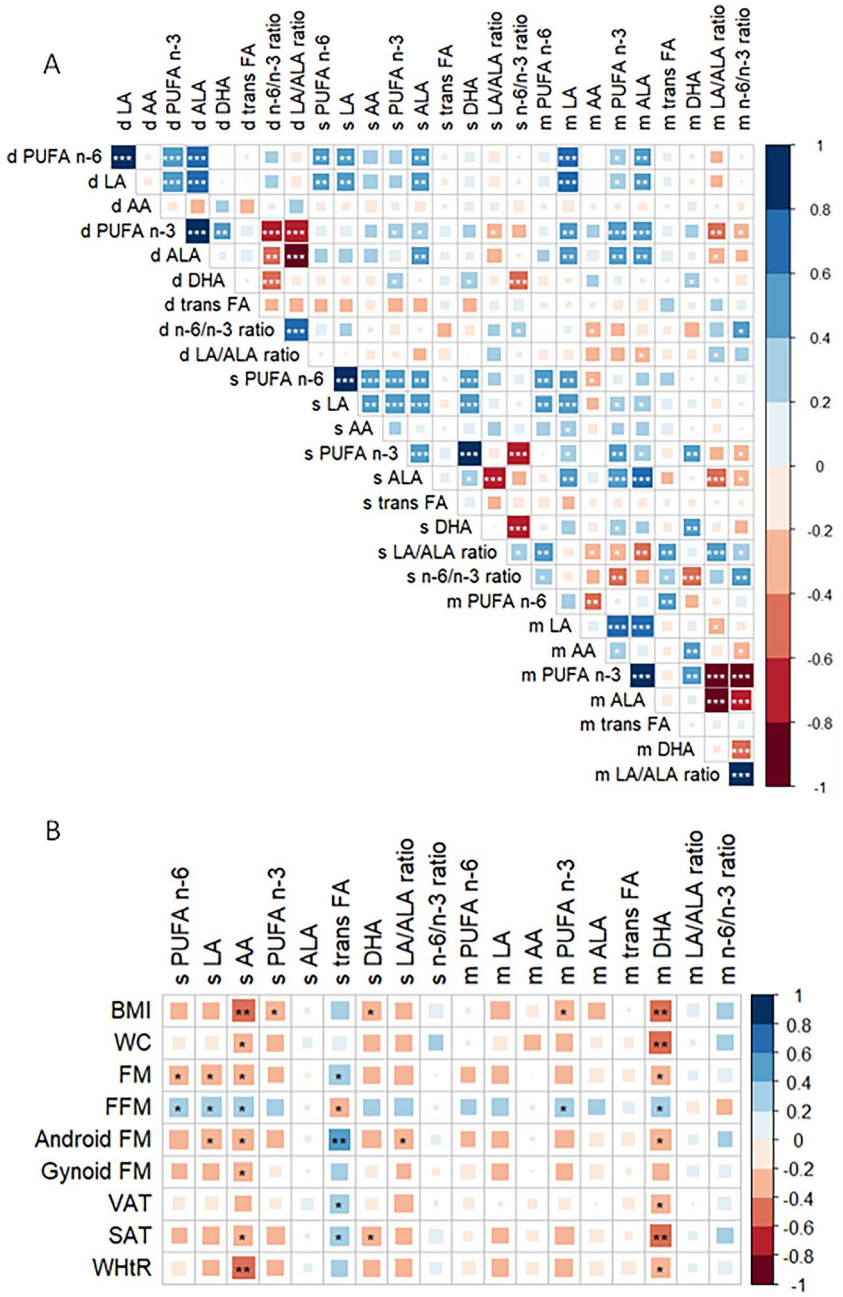

Fig. 5. Visual representation of moderating effect of low, moderate, and high adiposity on associations (conditional effects) between dietary/serum and human milk AA, ALA, DHA, and n-6 PUFA. AA, arachidonic acid; ALA, α-linoleic acid; BMI – body mass index; d – dietary; FM - fat mass; m – human milk; PUFA, polyunsaturated fatty acid; s – serum; SAT – subcutaneous adipose tissue; WC – waist circumference. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

be interpreted cautiously as we assessed only short-term dietary intake (based on 3-day food records) and status (based on plasma FA reflecting PUFA intake over 2–3 weeks[52].

Another important finding from this study was that adiposity measures moderated associations between dietary/serum FA and its human milk concentration. Our initial hypotheses assumed that adiposity would weaken the association between dietary or serum FA and human milk, presumably due to obesity-related inflammation and metabolic alteration[3,56]. Nonetheless, increasing adiposity strengthened associations between serum and human milk ALA, EPA, n-6 PUFA, and n-6/n-3 ratio. Moreover, the results for EPA became significant only when a moderator was added to the model. Visualizations of moderating effects (Figs. 5 and 6) showed that the effect of serum FA on its human milk concentration was significant only among mothers with moderate and/or high adiposity but not in those with lower adiposity measures. We assume that positive moderation of associations between plasma and human milk FA supports FA release from maternal stores in adipose tissue. Previous in vivo

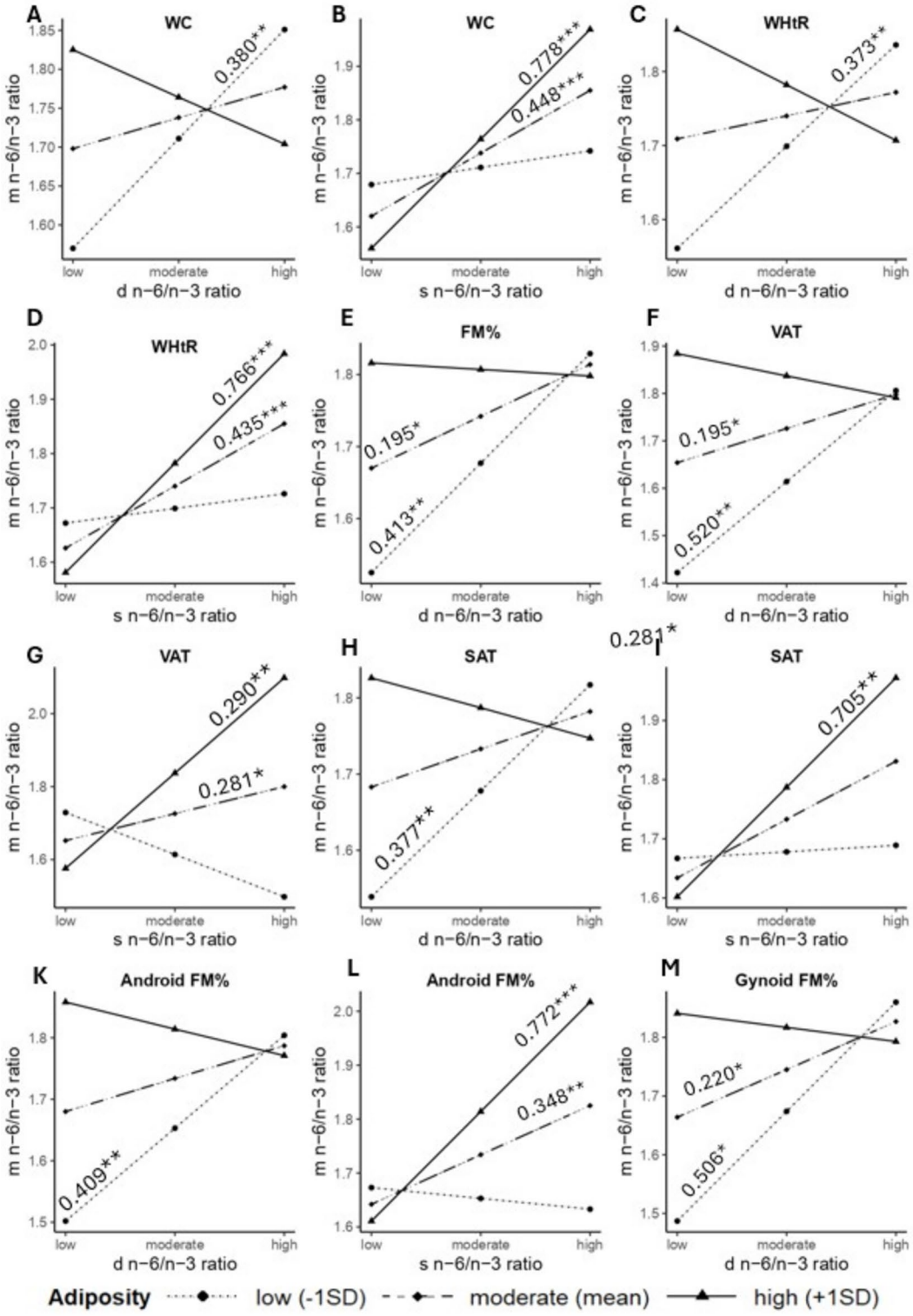

Fig. 6. Visual representation of moderating effect of low, moderate, and high adiposity on associations between dietary/serum and human milk n-6/n3 ratio. d – dietary; FM%, fat mass; m – human milk; s – serum; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; WC, waist circumference; WHtR, waist to hip ratio. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

studies showed that a significant amount of ingested FA is incorporated into adipose tissue[43,50,52,57]. Interestingly, adipose tissue in different regions incorporates FA with varying efficiency[52] and has distinct metabolic activity. SAT is more responsive to insulin's lipolytic effect than VAT[58], which may account for observed differences in the moderating effect of different adiposity measures. Furthermore, Villapando et al.[57] showed that mothers with

M	Predictors	Serum FA[#]		Human milk FA[#]		Moderated mediation[£]
		Adjusted β (SE) [&]	*P* value	Adjusted β (SE) [&]	*P* value	
WC	Diet FA[$] x M	0.907 (0.838)	0.286	-2.088 (0.738)	0.008	NS
	Serum FA x M			2.527 (1.087)	0.026	
WHtR	Diet FA[$] x M	1.267 (0.966)	0.198	-2.385 (0.915)	0.014	NS
	Serum FA x M			2.740 (1.186)	0.027	
FM%	Diet FA[$] x M	0.040 (0.766)	0.959	-1.344 (0.596)	0.032	NS
	Serum FA x M			1.736 (1.072)	0.115	
FFM%	Diet FA[$] x M	-0.039 (1.247)	0.975	2.287 (0.988)	0.027	NS
	Serum FA x M			-3.076 (1.832)	0.103	
VAT	Diet FA[$] x M	0.011 (0.106)	0.916	-0.174 (0.077)	0.031	NS
	Serum FA x M			0.385 (0.154)	0.017	
SAT	Diet FA[$] x M	0.172 (0.224)	0.449	-0.483 (0.197)	0.020	NS
	Serum FA x M			0.660 (0.311)	0.041	
A FM%	Diet FA[$] x M	0.213 (0.478)	0.658	-1.015 (0.377)	0.011	NS
	Serum FA x M			1.633 (0.612)	0.012	
G FM%	Diet FA[$] x M	0.259 (1.165)	0.825	-2.151 (1.039)	0.042	NS
	Serum FA x M			1.046 (1.554)	0.505	

Table 4. Results of interactions between focal predictors and moderators for n-6/n-3 ratio ($n = 40$). Full models were presented in Supplementary Table 5. [#] - models were adjusted for the minimal set of covariates identified by DAG. [£] - mediation was considered as significantly moderated when the bootstrapped 95% CI did not include 0 for the pairwise contrasts between conditional indirect effects. A FM%, fat mass in the android region; FA, fatty acids; FFM%, fat free mass; FM%, fat mass; G FM%, fat mass in the gynoid region; M, moderator; SAT, subcutaneous adipose tissue; SE, standard error; VAT, visceral adipose tissue; WC, waist circumference; WHtR, waist to hip ratio.

higher BMI oxidized and secreted more dietary LA into their milk than women with lower BMI within 12 h after [^{13}C]LA ingestion, with no differences in fat and LA intake or human milk concentrations. This suggest that adipose tissue may play a role in controlling human milk FA concentrations. Additionally, a recent multi-center European study[49] showed that adipose tissue reflecting long-term dietary intake (~ 1 year) was a better source of FAs ≥ 16 C than erythrocytes (reflecting 2–3 monthly intake). Hence, we speculate that mothers with higher adiposity had more long-term lipid stores released into circulation and transferred into milk instead of direct transfer from the diet.

On the contrary, adiposity weakens the direct effect of short-term dietary intake on human milk levels only in the case of EPA and n-6/-n-3 and at very low levels of moderators (below − 1 SD, Figs. 3 and 4 and Supplementary Table 5). This is consistent with previous intervention studies showing that overweight/obesity attenuates the response to n-3 PUFA supplementation[29–31,59] leading to changes in n-6/n-3 ratio[28], presumably due to obesity-related inflammation. OW/OB is linked to decrease n-3-derived and an increase n-6-derived oxylipins, which are involved in inflammatory reactions[60] and potentially contribute to lactation regulation[22]. A recent study found that short-term supplementation of DHA-rich oil in women with obesity increased n-3 PUFA and DHA-derived oxylipins, but did not decrease inflammatory biomarkers[47]. However, to the best of our knowledge, no study investigated oxylipins during lactation in mothers with different BMI. Nevertheless, this could be an important research direction to clarify the metabolic pathways of adverse lactation outcomes in mothers with OW/OB[61].

Understanding how maternal factors affect human milk PUFA profile is crucial as they play a pivotal role in healthy infant growth and development. Previously, we showed that human milk n-3 PUFA supported infant motor development[62] and Hahn-Holbrook et al.[63] linked them with infant temperament, while de la Garza Puentes[9] found that effect on cognitive score was dependent on maternal pre-pregnancy BMI. However, evidence regarding child growth and body composition is inconsistent due to methodological issues, except for the association with head circumference (except DHA)[64]. Furthermore, LC PUFA may support the development of the immune system[65], and intestine[66] and protect against the development of type 1 diabetes-related autoimmunity[67]. Although breastfeeding is associated with a variety of health benefits for infants, obesity-related alterations in human milk composition could mediate in the OW/OB transmission from mother to infant[68]. Previous studies found that obesity-related changes in human milk FA profile may affect infant growth trajectories[41,69]. Hence, it is important to analyze the composition of human milk in both the context of maternal and infant health, as recently called the "mother – human milk – infant triad"[68]. Furthermore, human milk nutrients and bioactive are often intercorrelated (e.g., PUFA, oxylipins, and cytokines), and a more "systemic" view of the human milk composition would provide better insight into obesity-related alterations in human milk composition and their consequences for developing infants[64].

The major strength of this study was that we included data about dietary FA intake from 3-day food records and collected both serum and human milk FA profiles. This comprehensive approach allowed us to analyze the difference in FA status in mothers with normal and excessive body mass. Unlike many other studies that only

looked at differences in human milk FA profile, we could determine if dietary differences or other factors caused the observed discrepancies. We took our analysis a step further by using mediation and moderated mediation analyses, allowing us to explore these associations more deeply than regular regression techniques. Furthermore, we used different measures of adiposity, including anthropometry indices and body composition analyzed using DXA, a gold standard in body composition measurements[70], as moderators. We also collected 24-hour human milk samples of both fore- and hindmilk to minimize diurnal and intra-feeding variations in human milk composition[71,72].

However, our study had some significant limitations. We lacked follow-up, which would have improved our understanding of FA metabolism across lactation and its association with maternal nutritional status. Additionally, the limited number of participants restricted our ability to observe significant results and led to low power for some associations. Hence, our study provided important exploratory insight and identified potential relationships to be investigated in further larger studies. However, because of the lack of correction for multiple testing due to the small number of observations our findings should be inerpreted with caution, as the potential for inflated type I error (false positives) remains. Nevertheless, the participants were matched by lactation duration and maternal age. It's worth noting that most participants had a high socio-economic status, which limits the generalizability of the results but also reduces the impact of other maternal factors (e.g. lactation duration) on human milk composition. Also, our study group may be limited to the OW/OB group of mothers without significant lactation difficulties and metabolic alterations related to overweight and obesity, which may decrease the generalizability of our results. Furthermore, we did not analyze low-grade inflammation biomarkers that could mediate the effect of increased adiposity on PUFA metabolism[22]. Future studies should include an erythrocyte membrane FA profile and, ideally, an adipose tissue FA profile, which may provide a more accurate biomarker than a serum FA profile, reflecting only short-term FA status.

Conclusions

In our study, we conducted exploratory analysis of the relationship between dietary fatty acid intake, serum and human milk fatty acid profiles, and maternal adiposity. Firstly, we hypothesized that OW/OB mothers would have lower PUFA n-3 and higher PUFA n-6 and n-6 to n-3 ratio in serum and breastmilk, with no differences in their dietary intake. We confirmed this hypothesis only for DHA, but no other PUFAs n-3 and n-6. Secondly, we hypothesized that breastmilk PUFA n-3, n-6, and its ratio would be associated with their dietary intake and mediated by maternal serum FA. However, we found that only the associations between dietary intake and the composition of human milk LA, n-3 PUFA, and ALA were partially mediated by serum. Hence, this hypothesis was partially confirmed. Our final hypothesis assumed that maternal OW/OB and adiposity would attenuate these associations. This hypothesis also was partially confirmed as, we noticed that adiposity has a dual effect, positively moderating the relationship between serum and human milk FAs, while negatively moderating the connection between diet and human milk FAs. This implies that in mothers with elevated adiposity, the composition of human milk PUFA may be more influenced by long-term dietary habits rather than short-term intake, but it requires further studies. Furthermore, elevated adiposity could attenuate the direct association between dietary and human milk FA.

Our study emphasizes the importance of including mothers with overweight or obesity in further research, as obesity seems to affect FA metabolism. This is consistent with existing literature highlighting the diverse nutritional requirements of individuals with overweight or obesity[73], signaling the need for tailored dietary recommendations. Moreover, developing tailored strategies will also contribute to shaping infant health through interactions within the mother-human milk-infant triad.

Data availability

The datasets generated and/or analyzed during the current study are available in the RepOD repository, https://doi.org/10.18150/SY25JG.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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