Detailed fatty acid profile of serum lipid classes in lactating women and their relationship with milk fat

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Abbreviations: Ag⁺-HPLC: silver ion high performance liquid chromatography; BCFA: branched-chain fatty acid; CE: cholesterol ester; CLA: conjugated linoleic acid; DHA: docosahexaenoic acid; DMA: dimethyl acetal; FA: fatty acid; FAME: fatty acid methyl ester;

27 GC: gas chromatography; MMA: monomethylacetal; PC: phosphatidylcholine; PE:
28 phosphatidylethanolamine; PL: phospholipid; PUFA: polyunsaturated fatty acid; RA: rumenic
29 acid; SM: sphingomyelin; TG: triacylglycerol; TLC: thin layer chromatography; VA: vaccenic
30 acid.

ABSTRACT

Little is known about fatty acid (FA) distribution among triacylglyceride (TG), cholesterol ester (CE) and phospholipid (PL) plasma lipid fractions in lactating women, and the relationships between these fractions and the FA profile of the milk that mothers produce. Milk fat and serum lipid FA compositions in lactating women were compared to investigate which serum lipids are the source of FA in milk fat. A TLC methodology was used to fractionate serum lipids including PL subclasses, i.e. phosphatidylethanolamine (PE), phosphatidylcholine (PC) and sphingomyelin (SM). Plasma lipids consisted of CE (36.4%), TG (19.1%), PE (1.40%), PC (39.80%) and SM (3.29%). With regard to PL fractions, PE was characterized by the highest levels of 18:0, 20:4 n-6, 22:6 n-3 and alkenyl ethers. PC revealed 16:0 as the predominant FA, followed by 18:2 n-6, 18:0, *cis*-9 18:1 and 20:4 n-6, whereas saturated FA from 16:0 to 24:0 were characteristic of SM. Although n-6 and n-3 polyunsaturated FA were mostly found in PE and PC, strong correlations were found for 18:2 n-6 and 18:3 n-3 between plasma TG and milk lipids. This study also shows that *trans* 18:1 isomers may be selectively incorporated into milk fat from specific serum fractions.

Practical applications: An easy-to-use and inexpensive two-stage TLC methodology to fractionate plasma lipids requiring only 1 mL of blood serum has been developed. Moreover, this study provides valuable information on the composition of serum lipids in lactating women, particularly with regard to PL, and their influence on the milk fat FA profile.

1. INTRODUCTION

Triacylglycerols (TG) constitute more than 95% of the lipids in breast milk, and their fatty acids (FA) are the most variable component [1]. Milk medium-chain FA (10:0–14:0) are mainly produced within the mammary gland by *de novo* synthesis and they provide the breast-fed infant with a fuel that can be readily digested, absorbed and rapidly oxidized. In contrast, milk long-chain FA which are comprised of 16–22 C atoms are acquired from the diet or adipose stores. Long-chain polyunsaturated fatty acids (PUFA) such as omega-3 provide benefits for neurocognitive development in human beings [2], but high concentrations of partially hydrogenated *trans* fats could have a potentially harmful action in infant development [3]. Transport of all these long-chain FA in the bloodstream is complex and involves various plasma lipid fractions, mainly phospholipids (PL), cholesterol esters (CE) and TG.

Many surveys in the last decade have made a detailed investigation of the human milk FA profile, emphasizing the content of beneficial or detrimental compounds [4–7], but less is known about FA distribution among plasma lipid fractions in lactating women [8]. The FA profile of plasma lipids might influence breast milk FA composition, depending on which FA are preferentially incorporated from the bloodstream, which in turn could have health implications in breast-fed infants. Spear *et al.* [9] reported marked differences between milk and total serum lipid FA. Other authors [10–11] have found that the proportions of linoleic, α -linolenic, arachidonic (20:4 n-6) and docosahexaenoic (22:6 n-3, DHA) acids in serum PL are positively correlated with the percentages of these FA in milk lipids, which suggests that transfer of these critical FA from blood to milk is a relatively efficient process. However, those studies were limited to major FA and they did not report data about CE and TG serum lipid fractions. Furthermore, to our knowledge no information has been reported on the FA profile in lactating women of the three most important subclasses of PL, phosphatidylethanolamine (PE), phosphatidylcholine (PC) and sphingomyelin (SM).

The aim of this study was to examine in detail the FA composition of the plasma lipid fractions (TG, PL and CE), including PL subclasses, in lactating women and to investigate whether they were correlated with milk FA. Particular emphasis was placed on minor FA with a potential impact on human health, such as omega-3 n-3 PUFA, conjugated linoleic acid (CLA) and *trans* 18:1 isomers. The isolation of all classes of plasma lipids was carried out by a novel two-stage thin layer chromatography (TLC) followed by a gas chromatography (GC) analysis of each fraction.

2. MATERIALS AND METHODS

2.1. Subjects and sampling

The study population consisted of 17 lactating women. They were recruited from the Well-Baby Clinic at the Jos University Teaching Hospital in Jos, Nigeria. All subjects claimed to be in good health and gave their written consent to participate in this study. The study was approved by the Human Ethics Committee of the Jos University Teaching Hospital and the University of New Mexico Human Research Review Committee. This research conformed to the principles outlined in the WMA Declaration of Helsinki.

Approximately 15 mL of milk was collected in the morning by manual pumping from each subject, aliquoted 5 min after initiation of pumping into 5 mL cryovials and stored at -43°C until they were transported frozen to Canada for analysis. Simultaneously, 10 mL of venous blood was obtained and allowed to clot for 45 min. The clotted specimens were centrifuged at $5,000 \times g$ in a clinical centrifuge, and the serum fraction (approximately 1.5 mL) was aliquoted into cryovials and stored at -43°C until they were transported together with the milk specimens to Canada.

2.2. Milk FA analysis

Milk samples were thawed to room temperature and vortexed vigorously to assure homogeneity. One mL of total milk was extracted with a chloroform/methanol/water mixture (2:2:1.8, v/v/v)

according to the Bligh and Dyer method [12]. The extracted lipid residue was esterified using NaOCH₃/MeOH (Supelco 33080) at room temperature for 20 min. Fatty acid methyl esters (FAME) were then quantified using a gas chromatograph (Hewlett-Packard model 5890 Series II, Palo Alto, CA 5890) equipped with an autoinjector, a 100 m CP-Sil 88 fused-silica capillary column (Varian Inc., Mississauga, ON) and a flame ionization detector. All milk samples were run using two temperature programs as described [13]. The first one (175 °C temperature program) consisted of two ramps: 45 °C (held for 4 min), 13 °C/min to 175 °C (held for 27 min) and 4 °C/min to 215 °C (held for 35 min). The second one (150 °C temperature program) was used to improve the resolution of the 18:1 and CLA regions: 45 °C (held for 4 min), 13 °C/min to 150 °C (held for 47 min) and finally 4 °C/min to 215 °C (held for 35 min). All peaks were identified by comparison of their retention times with analogous fat samples and standard mixtures purchased from Nu-Chek Prep. Inc. (Elysian, MN, USA). The identity of the alkenyl moieties was confirmed by converting the dimethyl acetals (DMA) to their stable 1,3-dioxolane derivatives and analysing by GC according to Santercole *et al.* [14].

2.3. Serum lipid analysis

Lipids were extracted from 1 mL of blood serum following the Bligh and Dyer procedure [12]. Then serum lipid classes were separated using two TLC stages (Figure 1). The first solvent system was hexane/ethyl ether/acetic acid (75:25:1). Afterwards, a second solvent system of chloroform/methanol/water (65:25:4) was used. After each development, the TLC plate was dried under a stream of nitrogen in a drying box. Then it was sprayed with 2',7'-dichlorofluorescein and observed under UV light at 254 nm. All serum lipid fractions were scratched off the TLC plate and eluted from the silica gel using 3 mL of chloroform. A known amount of internal standard (23:0) was added to each serum lipid fraction for quantification before its methylation. Following Cruz-Hernández *et al.* recommendations [15], principal serum fractions such as CE, TG and PC were methylated with acid (HCl/CH₃OH at 80 °C for 1 hour) to quantify total FAME, and also with base (NaOCH₃/CH₃OH at 50 °C for 30 min) to correct the CLA region. Minor PL fractions, PE and SM, were only catalysed with acid, because they were obtained in small quantities.

Afterwards, both neutral lipids and PL fractions were analysed by GC using the 175 °C temperature program described above.

2.4. Statistical analyses

Statistical analysis was conducted with SAS University Edition (SAS Institute, Cary, NC). Pearson correlation coefficients (r) were used to investigate the associations for some FA. Significant differences were declared at $P < 0.05$.

3. RESULTS AND DISCUSSION

A key strength of the present work was the application of a new TLC methodology which provides a complete fractionation of the plasma lipids and only needs a small sample of serum. Seven different lipid classes were separated using two TLC stages. The first solvent system yielded neutral serum lipid fractions (CE and TG), while the second solvent system allowed distinguishing between cholesterol, PE, PC, SM and lyso-PC fractions (Figure 1). In the 17 serum samples analysed, their average values (in % of total plasma lipids) were CE (36.42 ± 7.19), TG (19.09 ± 11.07), PE (1.40 ± 0.36), PC (39.80 ± 6.55) and SM (3.29 ± 1.25).

The data indicated that the FA were not equally distributed either in the milk or in the CE, TG, PE, PC and SM serum lipid fractions. Figure 2 shows the striking differences in major FA composition between the breast milk and the corresponding serum lipid fractions of the 17 mothers. Milk fat and serum TG presented a parallel FA composition with 3 predominant FA (16:0, *cis*-9 18:1 and 18:2 n-6), but these similarities were not found when compared with the other lipid fractions. This supports the idea that serum TG may be the major source of FA in human milk, while the composition of the remaining serum fractions would be characteristic of the lipid class.

The major differences between the milk lipids and the plasma TG profile were the percentages of 10:0, 12:0 and 14:0. The highest contents for these medium-chain saturated FA were found in breast milk (Table 1), which would be evidence of their *de novo* synthesis from acetyl-CoA in the mammary gland. On average, the milk lipid of the women in this study contained (21 ± 12)% of this group of FA, a figure that is within the limits previously reported in human milk from different countries [1, 7]. *De novo* FA in human milk appear to be important for infant survival by providing a high-energy source that is easily digested by the neonate. However, the high standard deviation observed indicates that *de novo* FA content can be very variable, depending on the individual. For instance, it is widely accepted that lactating women whose diets are high in carbohydrates tend to produce milk fat that is rich in 10:0–14:0 FA, whereas diets rich in fat generally contain low amounts [1].

The effect of the variations in the *de novo* FA in milk on the proportion of non-essential and essential FA in the milk lipids of the Nigerian women is shown in Table 2. The contents of omega-3 n-3 FA, α -linolenic acid, 20:5 n-3, 22:5 n-3, docosapentaenoic acid and DHA in the milk fraction were not negatively affected by the proportion of the sum of 10:0, 12:0 and 14:0. In contrast, two non-essential FA (18:0 and *cis* 18:1) showed significant ($P < 0.001$) negative correlations with the *de novo* FA ($r = -0.80$ and -0.94 , respectively). With regard to the n-6 FA, only a tendency to diminish ($P < 0.10$) was observed for 18:2 n-6 and 20:4 n-6 in breast milk when the *de novo* FA in milk increased. These observations indicate that omega-3 n-3 FA may be preferentially incorporated into milk at the expense of oleic acid and stearic acid. Thus the mammary gland may somehow regulate the synthesis of TG in milk in such a way as to ensure a greater incorporation of omega-3 n-3 FA that come exclusively from the diet to provide the breast-fed infant with the amounts of critical n-3 FA required for normal growth and development. Additional evidence that intermediate chain-length saturated FA may modulate the relative proportions of several FA in human milk was supplied by Schmeits *et al.* [16] and VanderJagt *et al.* [11]. Those studies also reported that when *de novo* FA synthesis is favoured in the mammary gland a decrease in the percentages of 18:0 and *cis*-9 18:1 is triggered.

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189 Plasma PL and CE contained several long-chain saturated FA (20:0, 22:0 and 24:0) as well as
190 alkenyl ethers not observed or scarcely present in breast milk, indicating that these FA may not
191 be preferentially incorporated by the mammary gland (Figure 2). Linoleic acid was the most
192 abundant FA in the CE fraction and accounted for more than half (52 ± 5)% of total FAME, in
193 agreement with previous studies in serum lipid classes in women [8, 17–18].

194
195 Although the FA composition of serum PL in lactating women has been studied previously [10–
196 11], to our knowledge this is the first report that separates and provides a detailed FA profile for
197 distinct PL subclasses. PC was the major PL fraction (Figure 1). It showed 16:0 as the
198 predominant FA, followed by linoleic, stearic and oleic acids, but 20:4 n-6 and 22:6 n-3 were also
199 found in significant amounts. SM, the second fraction in terms of percentage, consisted primarily
200 of long-chain saturated FA (from 16:0 to 24:0), their levels being greater than those of milk fat
201 and other serum lipids (Figure 2). PE, the smallest PL fraction analysed, was characterized by the
202 highest levels of 18:0, 20:4 n-6, 22:4 n-6, 22:5 n-3 and 22:6 n-3 when compared with breast milk
203 and other serum fractions (Table 1).

204
205 It is also important to highlight the abundance of monomethylacetals (MMA) and DMA in PE
206 (Figure 2), which confirms the presence of plasmalogens in this PL fraction. The largest contents
207 of these FA were observed for 16:0 (4.84 ± 2.75), 18:0 (6.77 ± 3.83) and 18:1 (1.65 ± 0.98) DMA
208 (Table 1). The high standard deviations may be evidence of the great individual variability of
209 these compounds. On the other hand, PC also presented small quantities of 16:0 and 18:0 DMA,
210 with values always lower than 1% of total FAME. Several studies suggest that plasmalogens
211 might act as antioxidant molecules in the metabolism [19–20]. The vinyl-ether bond of
212 plasmalogens could be one of the first targets of free radical attack and may represent a first shield
213 against oxidative damage by protecting other macromolecules.

The main *trans* FA in serum were *trans* 18:1, and their incorporation into serum lipids is presented in Figure 3. The distribution of total *trans* 18:1 in the different fractions was roughly 40% (TG), 30% (PC) and 20% (CE). The results presented here for total *trans* 18:1 among serum lipid fractions are in agreement with those of Vidgren *et al.* [18] in women consuming a diet supplemented with a source of *trans* FA, and of Shahin *et al.* [8], who also reported the highest proportion of total *trans* FA in the fraction of serum TG in lactating mothers.

It can be stated that *trans* FA content in human milk depends on dietary habits, as women do not synthesize these geometrical isomers, and there are sufficient data on *trans* FA to warrant caution against consumption of foods with partially hydrogenated fats by women who are pregnant or lactating [3]. Nevertheless, the incorporation of *trans* 18:1 FA from human serum into milk lipid seems to be somewhat selective since *trans*-10 and *trans*-11 isomers presented the opposite behaviour in the PC and CE fractions (Figure 3). Vaccenic acid (VA) tended to accumulate in PC (40% vs 18% for VA and *trans*-10 18:1, respectively), whereas *trans*-10 18:1 was preferentially incorporated into CE (34% vs 17% for *trans*-10 18:1 and VA, respectively). Moreover, the correlation between CE and milk for *trans*-10 18:1 was not significant ($r = -0.02$, $P = 0.93$), while the correlation between PC and milk for *trans*-11 18:1 was highly significant ($r = 0.78$, $P < 0.001$). These results suggest that the mammary gland prevents the incorporation of *trans*-10 18:1 into human milk fat and, in contrast, that VA is preferentially incorporated, which would be beneficial from the human health perspective. Although unfavourable effects of *trans*-10 18:1 or other *trans* isomers on LDL and other risk factors of atherosclerosis are well known, VA would not have these detrimental effects [21–22].

Rumenic acid (*cis*-9 *trans*-11 18:2, RA), the most important isomer of CLA in milk, only exceeded 0.1% in the milk of two of the 17 lactating women investigated and it accumulated predominantly in the TG fraction, confirming the results of previous studies [8, 23]. The presence of RA in the major PL groups (PC and SM) as well as in the CE fraction was very low (Table 1). Rumenic acid and its precursor VA were positively correlated in the serum TG fraction ($r = 0.60$,

$P < 0.05$) and even more strongly in breast milk ($r = 0.83$, $P < 0.001$). This result is evidence of their metabolic relationship. *Cis-9 trans-11* CLA isomer is endogenously synthesized from VA via Δ -9 desaturase in human tissues [24]. A significant correlation was also observed between milk RA and VA in the TG fraction ($r = 0.62$, $P < 0.01$), which could confirm a preferential incorporation of VA into milk fat and, afterwards, its conversion to RA in the mammary gland. No other significant correlations were found between RA and VA in the remaining serum fractions, either between them or with milk.

This study shows that n-6 and n-3 FA, with 18 or more C atoms, are selectively incorporated into the CE and PC fractions. Under normal conditions, PUFA in blood plasma of women appear to be incorporated into these fractions [8, 17–18]. However, important differences were observed, depending on the number of C atoms. As can be seen in Figure 4, linoleic and α -linolenic acids were preferentially incorporated into the CE fraction (62 and 45%, respectively). α -Linolenic acid was also found in the TG (37%) and PC (17%) fractions, and the PC fraction (27%) was the second source of linoleic acid in plasma. In contrast, half of 20:4 n-6 and 20:5 n-3 and more than three quarter of 22:4 n-6, 22:5 n-3 and 22:6 n-3 were detected in the PC fraction (Figure 3). Thus it is apparent that with an increasing number of C atoms PUFA are preferentially incorporated into plasma PL, with concomitant decreases in CE. Despite the very low presence of the PE fraction in plasma, it also contained a considerable proportion of those PUFA (3% for 20:4 n-6 and 20:5 n-3, 5% for 22:4 n-6 and 22:5 n-3 and 7% for 22:6 n-3, respectively).

Table 3 shows the correlations between the main n-6 and n-3 FA in the various plasma fractions and human milk. Milk 18:2 n-6 was strongly correlated ($P < 0.01$) with serum TG, although CE ($P < 0.01$), PC ($P < 0.05$) and PE ($P < 0.05$) were also positively correlated. In contrast, 18:3 n-3 was correlated only with the TG serum lipid fraction ($P < 0.05$). These relationships between the linoleic and α -linolenic acid contents of the mothers' serum fractions and their milk lipids underline the important role of plasma TG in the transfer of these two essential FA from blood to milk. No significant correlations were found between 20:4 n-6 in milk and plasma fractions (Table

3). On the contrary, as discussed above (Table 2), 22:6 n-3 may be preferentially incorporated into milk. In this regard, the DHA of all the serum lipid fractions, except for SM, which is typically rich in saturated FA, was positively correlated with milk DHA ($P < 0.05$, Table 3).

Although the incidence of the essential FA content in plasma PL in breast milk lipid composition in lactating women has already been documented [10–11], less has been reported about the predominant role of other plasma fractions in the milk n-3 and n-6 FA profile. Current research (Table 3) indicates that levels of linoleic and α -linolenic acids in breast milk are more closely linked to plasma TG than to CE or PL. Thus diets rich in linoleic and α -linolenic acids would favour their uptake and use in the mammary gland.

The balance between n-6 and n-3 PUFA in the diet is of great importance because of their competitive nature. The mammary gland tends to incorporate n-3 PUFA (Table 3), and as a result the lowest value for the n-6/n-3 ratio was observed for milk compared with blood plasma (Table 1). This effect indicates that the mammary gland favours excretion of n-3 PUFA, which is very beneficial for infant development.

There were also remarkable correlations between the content of total branched-chain fatty acids (BCFA) in milk fat and the various plasma lipid fractions. All serum lipids, except the smallest fraction, PE, showed a significant positive correlation with milk fat, especially PC ($r = 0.73$; $P < 0.01$) and CE ($r = 0.69$; $P < 0.01$). This suggests that the mammary gland promotes the mobilization of BCFA from the bloodstream to incorporate them into milk. BCFA are rare in internal mammalian tissues, but they are normal constituents of the human neonatal gut and have a potential role against inflammation in the premature intestine, alter the microbiota and increase expression of anti-inflammatory cytokines [25].

4. CONCLUSIONS

A diversity of FA profiles was found in the various plasma lipids in lactating women. This study supports the idea that serum TG is probably the major source of FA in human milk and that certain FA, such as BCFA, VA and DHA, may be selectively incorporated into milk fat from the diet. In contrast, other more controversial FA, such as *trans*-10 18:1, may be accumulated in the blood serum of the lactating mother and may not be easily transferred into milk. With regard to n-6 and n-3 PUFA, current research suggests that with an increasing number of C atoms PUFA are preferentially incorporated into the plasma PL, with concomitant decreases in the CE serum lipid fraction. Although the highest proportion of n-3 FA was found in the CE and PC fractions, this distribution would not impede a substantial transfer of α -linolenic acid from plasma TG to milk lipids.

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The authors declare that they have no conflicts of interest in the current research.

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396 **Table 1.** Fatty acid (FA) profile of breast milk and human serum lipid classes of Nigerian lactating women (n = 17). Data expressed as % of total FA methyl
 397 esters (mean $\pm$ SD).

	Milk	TG	CE	PE	PC	SM
<u>Saturated FA</u>						
10:0	1.08 $\pm$ 0.377	-	-	-	-	-
12:0	7.21 $\pm$ 2.825	-	-	-	-	0.04 $\pm$ 0.055
14:0	12.7 $\pm$ 9.112	1.14 $\pm$ 0.514	0.34 $\pm$ 0.149	0.37 $\pm$ 0.207	0.17 $\pm$ 0.057	0.39 $\pm$ 0.215
15:0 <i>iso</i>	0.02 $\pm$ 0.016	0.03 $\pm$ 0.036	0.02 $\pm$ 0.016	0.62 $\pm$ 0.229	0.03 $\pm$ 0.020	0.06 $\pm$ 0.045
15:0 <i>anteiso</i>	0.03 $\pm$ 0.019	0.03 $\pm$ 0.029	0.01 $\pm$ 0.013	0.05 $\pm$ 0.056	-	0.03 $\pm$ 0.058
15:0	0.17 $\pm$ 0.047	0.21 $\pm$ 0.080	0.11 $\pm$ 0.038	0.29 $\pm$ 0.161	0.11 $\pm$ 0.050	0.12 $\pm$ 0.143
16:0 <i>iso</i>	0.02 $\pm$ 0.015	0.02 $\pm$ 0.021	0.02 $\pm$ 0.014	0.03 $\pm$ 0.022	0.02 $\pm$ 0.013	0.01 $\pm$ 0.018
16:0	27.4 $\pm$ 3.438	28.8 $\pm$ 4.331	12.2 $\pm$ 1.272	13.1 $\pm$ 3.444	28.8 $\pm$ 4.067	25.4 $\pm$ 3.266
17:0 <i>iso</i>	0.06 $\pm$ 0.030	0.07 $\pm$ 0.057	0.06 $\pm$ 0.036	0.12 $\pm$ 0.040	0.08 $\pm$ 0.038	0.02 $\pm$ 0.014
17:0	0.25 $\pm$ 0.098	0.33 $\pm$ 0.091	0.10 $\pm$ 0.021	0.56 $\pm$ 0.201	0.35 $\pm$ 0.076	0.40 $\pm$ 0.087
18:0	5.17 $\pm$ 1.893	5.14 $\pm$ 0.790	0.90 $\pm$ 0.292	19.84 $\pm$ 7.197	14.9 $\pm$ 2.499	14.2 $\pm$ 1.984
19:0	0.02 $\pm$ 0.016	0.03 $\pm$ 0.023	0.01 $\pm$ 0.013	0.15 $\pm$ 0.131	0.08 $\pm$ 0.068	0.21 $\pm$ 0.248
20:0	0.22 $\pm$ 0.138	0.24 $\pm$ 0.144	0.01 $\pm$ 0.012	0.26 $\pm$ 0.084	0.08 $\pm$ 0.026	3.91 $\pm$ 1.109
21:0	0.01 $\pm$ 0.004	0.01 $\pm$ 0.013	-	-	0.01 $\pm$ 0.013	0.30 $\pm$ 0.072
22:0	0.08 $\pm$ 0.080	0.21 $\pm$ 0.237	0.03 $\pm$ 0.007	0.70 $\pm$ 0.244	0.06 $\pm$ 0.021	16.0 $\pm$ 2.807
24:0	0.05 $\pm$ 0.033	0.14 $\pm$ 0.114	0.04 $\pm$ 0.048	0.73 $\pm$ 0.252	0.07 $\pm$ 0.042	13.6 $\pm$ 1.701
25:0	-	-	-	0.30 $\pm$ 0.210	-	0.34 $\pm$ 0.137
Σ 10:0, 12:0, 14:0	21.0 $\pm$ 11.709	1.14 $\pm$ 0.514	0.34 $\pm$ 0.149	0.37 $\pm$ 0.207	0.17 $\pm$ 0.057	0.42 $\pm$ 0.262
<u>Monounsaturated FA</u>						
<i>cis</i> -9 14:1	0.05 $\pm$ 0.024	-	-	-	-	-
<i>trans</i> -9 16:1	0.02 $\pm$ 0.016	-	-	-	-	-
<i>cis</i> -7 16:1 + 17:0 <i>anteiso</i>	0.31 $\pm$ 0.101	0.60 $\pm$ 0.126	0.41 $\pm$ 0.120	-	0.16 $\pm$ 0.044	0.25 $\pm$ 0.487
<i>cis</i> -9 16:1	1.11 $\pm$ 0.527	1.85 $\pm$ 0.856	1.88 $\pm$ 1.013	0.13 $\pm$ 0.083	0.27 $\pm$ 0.119	0.10 $\pm$ 0.100

<i>cis</i> -11 16:1	0.03 ± 0.011	0.02 ± 0.014	0.01 ± 0.013	0.01 ± 0.022	0.01 ± 0.008	-
<i>cis</i> -7 17:1	0.03 ± 0.011	0.03 ± 0.023	0.01 ± 0.010	-	0.05 ± 0.022	-
<i>cis</i> -9 17:1	0.08 ± 0.033	0.12 ± 0.057	0.11 ± 0.048	-	0.03 ± 0.014	-
<i>trans</i> (6+7+8) 18:1	0.07 ± 0.070	0.09 ± 0.075	0.02 ± 0.015	0.08 ± 0.239	0.03 ± 0.012	0.01 ± 0.012
<i>trans</i> -9 18:1	0.10 ± 0.106	0.24 ± 0.131	0.05 ± 0.015	0.06 ± 0.042	0.07 ± 0.025	0.03 ± 0.021
<i>trans</i> -10 18:1	0.08 ± 0.088	0.13 ± 0.103	0.05 ± 0.017	0.05 ± 0.030	0.02 ± 0.008	0.03 ± 0.026
<i>trans</i> -11 18:1	0.14 ± 0.140	0.14 ± 0.121	0.03 ± 0.015	0.09 ± 0.048	0.07 ± 0.044	0.03 ± 0.028
<i>trans</i> -12 18:1	0.03 ± 0.026	0.01 ± 0.021	0.01 ± 0.018	0.05 ± 0.029	0.02 ± 0.011	0.01 ± 0.014
<i>cis</i> -9 18:1	25.0 ± 7.293	36.4 ± 3.352	19.4 ± 2.663	5.30 ± 2.063	9.21 ± 1.733	2.25 ± 1.584
<i>cis</i> -11 18:1	1.12 ± 0.374	1.18 ± 0.383	0.82 ± 0.176	0.29 ± 0.122	0.91 ± 0.251	0.16 ± 0.073
<i>cis</i> -12 18:1	0.02 ± 0.023	0.04 ± 0.033	0.01 ± 0.011	0.03 ± 0.052	0.02 ± 0.018	0.02 ± 0.050
<i>cis</i> -13 18:1	0.04 ± 0.015	0.19 ± 0.270	0.11 ± 0.093	0.16 ± 0.178	0.09 ± 0.063	0.04 ± 0.045
<i>cis</i> -11 20:1	0.34 ± 0.175	0.31 ± 0.102	0.02 ± 0.068	0.12 ± 0.039	0.16 ± 0.062	0.05 ± 0.020
Σ <i>trans</i> 18:1	0.52 ± 0.472	0.61 ± 0.389	0.16 ± 0.052	0.33 ± 0.273	0.21 ± 0.076	0.12 ± 0.083
<u>18:2 FA</u>						
<i>cis</i> -9 <i>trans</i> -12	0.05 ± 0.017	0.12 ± 0.030	0.08 ± 0.019	0.02 ± 0.023	0.02 ± 0.017	0.01 ± 0.011
<i>trans</i> -9 <i>cis</i> -12	0.02 ± 0.015	0.02 ± 0.018	0.02 ± 0.017	-	0.02 ± 0.018	-
<i>trans</i> -11 <i>cis</i> -15	0.03 ± 0.018	0.02 ± 0.023	-	-	-	-
<i>cis</i> -9 <i>cis</i> -12	13.4 ± 4.488	17.1 ± 4.443	52.0 ± 4.683	7.94 ± 1.726	21.2 ± 4.243	2.30 ± 1.082
<i>cis</i> -9 <i>trans</i> -11	0.06 ± 0.045	0.08 ± 0.049	0.03 ± 0.024	0.10 ± 0.058	0.03 ± 0.024	-
<i>trans</i> -11 <i>trans</i> -13	0.03 ± 0.014	0.04 ± 0.028	-	0.02 ± 0.023	0.01 ± 0.019	-
Other <i>trans-trans</i>	0.01 ± 0.008	-	-	-	0.01 ± 0.009	-
<u>Polyunsaturated FA</u>						
18:3 n-6	0.09 ± 0.047	0.43 ± 0.180	0.98 ± 0.422	0.06 ± 0.074	0.08 ± 0.047	0.02 ± 0.025
18:3 n-3	0.52 ± 0.246	0.40 ± 0.129	0.28 ± 0.092	0.07 ± 0.036	0.10 ± 0.030	0.01 ± 0.014
20:2 n-6	0.35 ± 0.266	0.17 ± 0.053	0.02 ± 0.010	-	0.34 ± 0.102	-
20:3 n-6	0.38 ± 0.156	0.29 ± 0.112	0.72 ± 0.219	0.98 ± 0.291	3.32 ± 1.144	0.51 ± 0.279
20:4 n-6	0.38 ± 0.137	1.35 ± 0.480	7.16 ± 1.679	13.0 ± 3.632	8.95 ± 2.199	3.11 ± 1.810
20:5 n-3	0.05 ± 0.053	0.13 ± 0.160	0.65 ± 0.666	0.94 ± 1.113	0.60 ± 0.595	-
22:4 n-6	0.09 ± 0.085	0.18 ± 0.066	0.01 ± 0.013	0.88 ± 0.352	0.42 ± 0.153	-

22:5 n-6	0.05 ± 0.032	0.17 ± 0.087	0.06 ± 0.025	0.84 ± 0.490	0.43 ± 0.245	1.25 ± 0.568
22:5 n-3	0.09 ± 0.035	0.15 ± 0.086	0.02 ± 0.014	1.14 ± 0.505	0.66 ± 0.263	0.09 ± 0.066
22:6 n-3	0.24 ± 0.149	0.31 ± 0.371	0.47 ± 0.280	6.03 ± 3.457	2.64 ± 1.628	0.21 ± 0.118
Σ Long chain n-6	1.69 ± 0.773	2.15 ± 0.725	7.98 ± 1.861	15.7 ± 4.303	13.8 ± 3.629	4.87 ± 2.118
Σ Long chain n-3	0.47 ± 0.251	0.59 ± 0.597	1.13 ± 0.924	8.1 ± 4.778	3.90 ± 2.358	0.31 ± 0.170
n-6/n-3	4.47 ± 2.307	5.83 ± 3.717	11.1 ± 7.110	3.59 ± 5.15	5.03 ± 3.328	23.2 ± 24.47
<u>Acetals</u>						
MMA 16:0	-	-	-	0.65 ± 0.383	-	-
DMA 16:0	-	-	-	4.84 ± 2.746	0.50 ± 0.218	-
MMA 16:1	-	-	-	0.24 ± 0.134	-	-
DMA 16:1	-	-	-	0.58 ± 0.264	-	-
MMA 18:0	-	-	-	1.44 ± 0.687	-	-
DMA 18:0	-	-	-	6.77 ± 3.835	0.09 ± 0.033	-
MMA 18:1	-	-	-	0.71 ± 0.404	-	-
DMA 18:1	-	-	-	1.65 ± 0.984	-	-
DMA 18:2	-	-	-	0.17 ± 0.117	-	-
DMA 20:0	-	-	-	0.87 ± 0.533	-	-

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399 CE: cholesterol esters; TG: triacylglycerides; PE: phosphatidylethanolamine; PC: phosphatidylcholine; SM: sphingomyelin; MMA: monomethylacetal; DMA:

400 dimethyl acetal.

Table 2. Analysis of the correlation in breast milk between the proportion of total *de novo* fatty acids (10:0 + 12:0 + 14:0) and other selected fatty acids.

Fatty Acid	<i>r</i>	<i>P</i> value
16:0	-0.04	0.878
18:0	-0.80	0.000
<i>cis</i> -9 18:1	-0.94	<.0001
18:2 n-6	-0.47	0.056
20:4 n-6	-0.44	0.078
22:4 n-6	-0.32	0.213
22:5 n-6	-0.10	0.712
18:3 n-3	-0.14	0.588
20:5 n-3	0.43	0.083
22:5 n-3	-0.10	0.692
22:6 n-3	0.34	0.188

Table 3. Analysis of the correlation between the proportion of main n-3 and n-6 FA in milk and different blood plasma fractions (CE, cholesterol esters; TG, triacylglycerides; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SM, sphingomyelin).

Fatty Acid	Plasma Fraction	<i>r</i>	<i>P</i> value
18:2 n-6	CE	0.68	0.003
	PC	0.49	0.044
	PE	0.51	0.042
	SM	0.18	0.503
	TG	0.73	0.001
18:3 n-3	CE	0.03	0.910
	PC	0.08	0.768
	PE	-0.41	0.115
	SM	-0.25	0.344
	TG	0.53	0.029
20:4 n-6	CE	0.30	0.242
	PC	0.08	0.774
	PE	-0.01	0.983
	SM	0.21	0.443
	TG	-0.10	0.697
22:6 n-3	CE	0.82	<.0001
	PC	0.80	0.000
	PE	0.71	0.002
	SM	-0.03	0.902
	TG	0.54	0.026

FIGURE LEGENDS

Figure 1. Separation of plasma lipid classes by two TLC stages in human samples.

Figure 2. Main fatty acid (FA) profile of breast milk and human serum lipid classes of Nigerian subjects. Data expressed as % of total FA methyl esters ($\bar{X} \pm SD$).

CE: cholesterol esters; TG: triacylglycerides; PE: phosphatidylethanolamine; PC: phosphatidylcholine; SM: sphingomyelin.

Figure 3. Plasma distribution of total *trans* 18:1, *trans*-10 18:1 and *trans*-11 18:1. Data expressed as % of individual FA methyl ester concentration in the various plasma lipid subclasses.

CE: cholesterol esters; TG: triacylglycerides; PE: phosphatidylethanolamine; PC: phosphatidylcholine; SM: sphingomyelin.

Figure 4. Plasma distribution of linoleic acid (18:2 n-6), α -linolenic acid (18:3 n-3), arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3), adrenic acid (22:4 n-6), docosapentaenoic acid (22:5 n-3), and docosahexaenoic acid (22:6 n-3). Data expressed as % of individual FA methyl ester concentration in the various plasma lipid subclasses.

CE: cholesterol esters; TG: triacylglycerides; PE: phosphatidylethanolamine; PC: phosphatidylcholine; SM: sphingomyelin.

434 **Figure 1.**

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Figure 2.

Figure 3.

445 **Figure 4.**

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