CLASSIFICATION OF HUMAN MILKS BASED ON THEIR *TRANS*
18:1 FATTY ACID PROFILE AND EFFECT OF MATERNAL DIET

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ABSTRACT

The diet of breastfeeding women influences the *trans* fatty acid (TFA) composition of the milk excreted. However, the effects associated to TFA are isomer-dependent and diverse TFA profiles may have different nutritional implications. The aim of this research was to evaluate whether certain TFA patterns in human milk fat can be used as indicators of TFA intake from different sources. To cover this proposal milk fat from 60 women were examined and classified based on their TFA profile. A Principal Component Analysis of the TFA data allowed the classification of the women into 3 groups depending on their content and profile. From the 60 subjects, 19 presented a TFA profile characteristic of ruminant products intake, 10 a typical TFA profile of industrial *trans* fats consumption and 31 a negligible *trans* content. Superimposed on this, 21 women presented high amounts of docosahexaenoic acid (22:6 n-3) which was related to fish intake.

INTRODUCTION

The World Health Organization recommends exclusive breastfeeding during the first 6 months of life due to its protective effect against infant mortality and its long-term health benefits.¹ Human milk provides 50-60% of its energy in the form of lipids, mainly fatty acids (FA). The diet of breastfeeding women can influence the FA profile of their milk and, consequently, it may have an impact on its protective effects.² In this regard, the levels of some FA in human milk, such as docosahexaenoic acid (DHA, 22:6 n-3) and *trans* FA (TFA), are clearly dependent on dietary intake.³ The European Food Safety Authority (EFSA) recommends a n-3 polyunsaturated fatty acids (PUFA) intake of 250 mg/day, with increases of (100-200) mg more per day if the woman is pregnant or lactating.⁴ DHA and other n-3 PUFA have been associated with the development of the retina, brain and nervous system during the first months of life, providing long-term benefits for neuro-cognitive development in human beings.⁵

In contrast to n-3 PUFA, high concentrations of *trans* fats could have a detrimental effect in infant development.⁶ The negative effects of TFA are mainly associated with isomers from partially hydrogenated vegetable oils (PHVO) or domestic frying, such as 6/7/8 *trans* 18:1, *trans*-9 18:1 and *trans*-10 18:1.⁷ However, the effects of TFA of natural origin could be different. Ruminant fats like meat, milk or dairy products are naturally rich in *trans*-11 18:1 (VA, Vaccenic acid) and higher levels of this VA in the mother milk has been correlated with lower rates of atopic manifestations and a reduced incidence of infant eczema in small children.^{8,9} Furthermore, this TFA can be converted endogenously in the human body¹⁰ to rumenic acid (RA, *cis*-9 *trans*-11 18:2), a conjugated linoleic acid (CLA) isomer with important biological properties.¹¹

A few surveys evaluating the effects on TFA profile in human milk that would reflect the type of fats consumed, whether derived from ruminants or PHVO, are currently available from Europe,^{12,13} Asia⁷ and America¹⁴ mothers. However, studies examining milk TFA profile of African women are very scarce and, unfortunately, the number of *trans* isomers reported is limited.¹⁵ In order to shed more light on this concern, milk fats from African mothers living in rural and urban areas of Northern Nigeria were characterized. FA composition was determined by high-resolution GC column paying particular attention to the TFA profile. The aim of this research was primarily to evaluate whether certain TFA patterns in human milk fat can be used as indicators of TFA intake from ruminant products or other sources and, secondly, to provide further information on the African human milk fat composition.

MATERIALS AND METHODS

Subjects

The study population was composed by 60 lactating women from rural and urban areas of Northern Nigeria. All subjects claimed to be in good health and gave their consent to participate in this study. The study was approved by the Human Ethics Committee of the Jos University Teaching Hospital. This research conformed to the principles outlined in the WMA Declaration of Helsinki.

The 30 rural subjects consisted of Fulani women who were recruited randomly from the rural villages of Bauchi State. They are situated around 40-50 km northeast of Jos, Nigeria. With regard to the urban subjects, 30 consecutive, lactating women visiting the Well-Baby Clinic at the Jos University Teaching Hospital were also recruited into the study. None of the urban subjects were of Fulani ethnicity.

Fulani women are semi-nomadic pastoralists whose diet is based on dairy products and consequently rich in natural *trans* fats. Their lifestyle is active, with no-smoking and alcohol consumptions, and they commonly nurse their infants for at least two years.¹⁶ Women from urban areas are generally more sedentary and with a higher caloric intake.¹⁵ Information regarding age, height, weight, BMI, parity and duration of lactation for each subject is presented in Table 1. Anthropometric data were taken before sample collection.

Approximately 15 mL of milk were collected in the morning (two hours after breakfast) 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.

Milk FA analysis

One mL of milk lipids was extracted with a chloroform/methanol/water mixture (2:2:1.8, v/v/v) according to Bligh & Dyer method.¹⁷ The extracted lipid residue was esterified using NaOCH₃/MeOH (Supelco 33080) as proposed by Kramer *et al.*¹⁸ Fatty acid methyl esters (FAME) were then analysed 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, Canada). All milk samples were run at two temperature programs as described Kramer *et al.*¹⁹ FAME quantification was carried out according to Li *et al.*⁷

Statistical analysis

Statistical analysis was conducted with JMP Version 9 (SAS Institute, Cary, NC). Paired comparisons, using Student's t test, were used to compare FA profiles. Pearson

correlation coefficients (r) were used to investigate the associations for some FA. Significant differences were declared at $P < 0.05$ and tendencies accepted if $P < 0.10$. Subsequently, the milk data were analyzed by principal component analysis (PCA) on all the 18:1 isomers and total CLA content of the 60 Nigerian subjects. A direct visual scree test performed on the eigenvalue distribution showed two factors were sufficient.

RESULTS

Mean values and standard deviations of the different FA quantified in Nigerian human milks are presented in Table 2. Saturated FA represented the primary group of FAME in all subjects, accounting for more than 50% of total FAME. The three major saturated FA, i.e. lauric (12:0), myristic (14:0) and palmitic (16:0) acids, showed no significant differences between rural and urban women ($P > 0.10$). Similarly, no differences were found in branch-chain saturated FA ($P > 0.10$).

Cis monounsaturated FA content was about 29% of total fat and oleic acid (*cis*-9 18:1) represented its most part (Table 2). Amounts of total *trans* monounsaturated FA detected in the present study, ranged between 0.46% (rural) and 0.75% (urban). Figure 1 shows the *trans* 18:1 FA profile determined by GC in three characteristic human milk samples. VA was the most prominent *trans* monounsaturated FA in lactating rural women (0.17% of total FAME) but its level were not significantly different from those found in urban women (0.20% of total FAME, $P > 0.10$).

18:2 TFA isomers were found in negligible amounts (Table 2) in both groups, urban and rural women. CLA content was very low for most of the breast milk studied. Its

individual values ranged from 0.04 to 0.61%, but only 4 out of the 60 Nigerian subjects presented a level greater than 0.3% of total FAME in milk fat.

Regarding essential FA, no differences were observed for linoleic or α -linolenic acids according to the women origin ($P>0.10$, Table 2). Their levels accounted for 13% and 0.6% for 18:2 n-6 and 18:3 n-3, respectively. Arachidonic acid (20:4 n-6) content was also unaffected (0.44% for rural and 0.39% of total FAME for urban subjects). In contrast, an interesting finding in the current survey would be the marked difference of long chain n-3 FAs between rural and urban lactating women. Compared to Fulani subjects, eicosapentaenoic (EPA, C20:5 n-3), docosapentaenoic acids (DPA, C22:5 n-3) and DHA contents were significantly higher in urban women from Jos city ($P<0.05$, Table 2). The greatest differences were found in DHA, which levels were twice as high in urban mothers (0.27%) than in Fulani women (0.13%) milk fats ($P<0.001$, Table 2). From the 60 subjects, 21 women (3 rural and 18 urban) presented a DHA content greater than 0.2% of total FAME.

DISCUSSION

Most milk FA presented large standard deviations and insignificant differences between rural and urban Nigerian areas which would indicate that they were not two homogeneous groups according to their diet but mixed groups in each region. Lauric, myristic and palmitic acids contents in milk fat were higher than that seen previously in human milk from different countries²⁰ but similar to those obtained previously by Glew *et al.* in Nigerian women.¹⁵ This finding would not be positive from a nutritional

perspective and suggests that the diet of Nigerian women was relatively high in carbohydrate and low in fat, similarly to Mexican or Philippine women.^{20,21}

The total TFA levels may not appear to be a major concern compared to the much higher levels found in western countries.^{13,14,22}. Leaving aside VA, all *trans* 18:1 percentages were significantly greater for urban women ($P < 0.05$) which would suggest a greater TFA consumption from industrial sources. Non-conjugated 18:2 TFA are mainly produced by frying oil rich in linoleic acid (e.g. soybean, sunflower or rapeseed oils) at high temperature while cooking. In the current research, 18:2 TFA percentages in human milk were low which should be considered positive as these FA may result in a greater risk factor for coronary heart disease than monounsaturated TFA. Furthermore, 18:2 TFA have been shown to be metabolized to other *trans* containing PUFA that interfere with brain and retinal function.²³

RA was quantitatively the main CLA isomer found and its percentages were not significantly different between groups. RA is the most biologically active CLA isomer and its main dietary source is ruminant fat.¹¹ In addition, a significant relationship was observed between VA and RA ($r = 0.83$; $P < 0.001$). Turpeinen *et al.*¹⁰ indicated that approximately 20% of VA is converted to RA by Δ -9 desaturase in human tissues. In the present study, the high correlation observed between both FA would point out in the same direction. Concerning other CLA isomers such as *trans*-9 *cis*-11, *trans*-11 *cis*-13, *cis*-9 *cis*-11 and *trans*-11 *trans*-13, quantities were also detected but in very low amounts (Table 2). These minor CLA isomers are currently found in different dairy products^{24,25} originated in the rumen by enzymatic conversion of dietary FAs.

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176 In order to obtain a more complete overview of the 60 Nigerian subjects and be able to
177 group them according to their origin (rural vs urban) and their *trans* fat content (no
178 *trans*, natural *trans* vs industrial *trans*), a PCA was performed on all 18:1 isomers and
179 total CLA milk contents (Figure 2). In the PCA map, the first two principal components
180 accounted for 43.0% and 26.9% of the variance in the first and second dimension,
181 respectively. No differences were observed among Nigerian women according to their
182 origin confirming previous statements (Figure 2A). In contrast, subjects were
183 differentiated in three groups according to their *trans* isomers profile (Figure 2B). The
184 first group was characterized by samples close to the origin of the PCA system that
185 indicate a low amount of total *trans* 18:1 isomers and CLA according to a very limited
186 *trans* fat intake. On the other hand, industrial and natural *trans* fat intakes tended to
187 separate by particular FA mostly in the second dimension of the PCA. Based on the
188 loadings plot (Figure 2C), natural *trans* consumption would promote milk fat with
189 higher concentrations of VA, total CLA, *trans* (13 + 14) 18:1 and *trans*-16 + *cis*-14
190 18:1. In contrast, industrial *trans* consumption would increase milk fat with higher
191 contents of *trans* (6 + 7 + 8), *trans*-9, *trans*-10 and *cis*-12 18:1 isomers. The three
192 groups differentiated in the present study could be extrapolated to any other nutritional
193 study, but good chromatographic separations of the 18:1 area is needed (i.e. *trans* (6 + 7
194 + 8), *trans*-9, *trans*-10 and *trans*-11 18:1 isomers have to be well resolved) (Figure 1).

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196 To summarize, a negligible *trans* 18:1 amount was noticed in 31 women (18 rural and
197 13 urban) out of the 60 Nigerian mothers studied. The fact that only 10 women had a
198 typical partially hydrogenated *trans* fat consumption profile (2 rural and 8 urban) would

indicate a low westernized diet in northern Nigeria. On the other hand, it was unexpected that only 10 from the 30 Fulani milks analyzed had a natural *trans* intake pattern. Fulani are semi-nomadic pastoralists whose culture and economy are centered on cattle and their diet is rich in milk, cheese and other dairy products with high content in natural TFA.²⁶ Glew *et al.* speculated with a poor absorption of TFA from the gastrointestinal tract or a specificity of the lipoprotein lipase of the mammary gland of the Fulani women such that it discriminated against triglycerides that contained TFA.¹⁵ However more research would be needed to clarify this concern.

Conclusions

Although the results of this work confirm the presence of low levels of TFA in Nigerian breast milk, we could sort milk samples into 3 groups based on their dissimilar TFA profile. The first group would be related to a natural *trans* fat consumption and high levels of VA, total CLA, *trans* (13 + 14) 18:1 and *trans*-16 + *cis*-14 18:1 while industrial *trans* fat intake would be related with higher contents of *trans* (6 + 7 + 8), *trans*-9, *trans*-10 and *cis*-12 18:1 isomers. Finally, the third group would be linked to low TFA isomers in breast milk. This classification could be convenient to other clinical studies dealing with TFA analysis and samples of different nature (e.g. biological samples).

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