Trans fatty acids and conjugated linoleic acids in the milk of urban women and nomadic Fulani of northern Nigeria

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Abstract

Background: Trans fatty acids (TFAs) and conjugated linoleic acids (CLAs) are present in dairy products and human milk and can have detrimental and beneficial effects in humans. The content of TFAs and CLAs in milk is determined largely by the diet of the mother.

Methods: We compared the proportions of TFAs and CLAs in the milk of rural Fulani in northern Nigeria who consume dairy products to that of women living in an urban center who consume little in the way of dairy products. Lactating Fulani women (n=41) and women residing in the city of Jos, Nigeria (n=41) were recruited into the study. We predicted that the milk of the Fulani pastoralists would contain higher amounts of TFAs and CLAs compared to their urban counterparts.

Results: The mean total TFA proportions for the Fulani and urban women were 0.22% and 0.34%, respectively, and were not significantly different. The percentages of CLAs in milk fat were not different between rural and urban women (0.16% vs 0.14%). These TFA and CLA values were 4- to 10-fold lower than for milk of women elsewhere in the world.

Conclusions: The percentages of TFAs and CLAs in milk were not different between rural and urban dwellers in northern Nigeria whose diets differ greatly in the amounts of dairy products they contain. However, the fact that the percentages of TFAs and CLAs in the milk of Nigerian women were much lower than the percentages reported from other parts of the world may have implications for the long-term growth and development of infants in the northern Nigeria and elsewhere in the Western Sahel.

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1. Introduction

Conjugated linoleic acids (CLAs) and trans fatty acids (TFAs) are found in a variety of foods of animal origin, including human milk and the milk of cows and other ruminant animals [1]. CLAs are a family of positional and geometric (trans) isomers of linoleic acid (cis-9,cis-12 octadecadienoic acid) (18:2n−6) in which a pair of carbon–carbon double bonds are conjugated. The major naturally occurring isomer of CLA is rumenic acid (c9,t11-18:2). CLA and TFA isomers with distinct chemical properties may have the same [2] or different [3] biological effects and mechanisms of action [3,4]. In humans, some detrimental effects of t10,c12-18:2 on blood lipids and insulin sensitivity have been reported [5]. In animal studies, c9,t11-18:2 has been shown to inhibit tumor cell growth [6–9], adipocyte proliferation and differentiation [4] and adipocyte lipoprotein lipase activity, and to reduce body fat [10–12]. There is evidence to suggest that certain CLAs inhibit carcinogenesis by inducing apoptosis through mechanisms involving inhibition of eicosanoid (e.g., prostaglandin) synthesis [13]. The t10,c12 isomer of CLA increases lipolysis in human adipocytes [14].
Trans fatty acids are unsaturated fatty acids containing one or more double bonds in the trans configuration. Most dietary TFAs are formed during hydrogenation of vegetable oils [15,16]. Trans fatty acids, such as elaidic acid (t9-18:1) have been shown to be associated with increased risk of cardiovascular disease. Studies in humans have shown that mixtures of dietary TFAs (e.g., t9-,t10-, and t11-18:1) are negatively correlated with the plasma HDL-cholesterol concentration, whereas the LDL-cholesterol concentration and TFA level are positively correlated [17–21]. Koletzko [20] determined the fatty acid composition of cholesterol esters in plasma from umbilical cord blood of premature infants and found that the proportions of arachidonic acid and docosahexaenoic acid (DHA) correlated inversely with the proportions of elaidic acid (t9-18:1). Furthermore, a low birth weight was associated with a high percentage of elaidic acid in the cholesterol ester fraction. Since arachidonic acid and DHA are critically important in the function and development of the central nervous system during the fetal and newborn periods [22] and because Elias and Innis [23] have shown an inverse relationship between the TFA and DHA levels of milk, there is concern that TFAs may interfere with growth and development during the neonatal period.

It is not only an oversimplification but moreover incorrect to view all CLAs as having beneficial effects on health and TFAs as having adverse effects. Illustrative of this point is the fact that dietary vaccenic acid (t11-18:1) is metabolized in humans, to c9,t11-conjugated linoleic acid by Δ9-desaturase [24–27]. Furthermore, in a rat model of mammary carcinogenesis, dietary vaccenic acid caused a dose-dependent increase in the tissue content of c9,t11-18:2, which was inversely correlated with a decreased risk of mammary tumors [25].

We have investigated various aspects of maternal/child health among the Fulani of northern Nigeria [28–33]. The Fulani are semi-nomadic pastoralists whose culture and economy are centered on cattle. As such, dairy products including milk, cheese and butter oil are widely consumed by all members of the community, including lactating women. Furthermore, it is common for Fulani mothers to nurse their infants for 2 years or more. In light of the extensive use of dairy products among the Fulani, we were interested in knowing the content of TFAs and CLAs in the milk of lactating Fulani women living in various hamlets dispersed throughout Bauchi State, Nigeria, with special interest in the proportions of TFAs and CLAs in the lipid fraction of the milk. We also compared the fatty acid composition of the milk lipids of Fulani women with that of their non-pastoral, sedentary counterparts living nearby in the city of Jos. In an earlier study [34], we compared the nutrient content of the diets of Fulani women in Bauchi State with those of their non-Fulani counterparts living in Jos. The urban dwellers in general consumed more calories and had a higher body mass than the rural men and women. Urban adults had carbohydrate intakes that were greater than those of the Fulani pastoralists, but considerably lower intakes of total fat and saturated fat than the Fulani adults. Our long-range goal is to identify factors that account for the high incidences of underweight and stunting we documented previously in Fulani children [35].

2. Methods

2.1. Subjects

The Nigerian subjects consisted of 41 nursing Fulani women living in the villages of Magama Gumau, Tilden Fulani and Toro located in Bauchi State 40–50km northeast of Jos, Nigeria, and 41 nursing mothers from the city of Jos. The Fulani women were recruited at random from the 3 villages. As for the urban subjects, 41 consecutive women visiting the Well-Baby Clinic at the Jos University Teaching Hospital were recruited into the study. The urban women were representative of 18 distinct ethnic groups, and none was Fulani. Jos is a cosmopolitan city of about 2 million people situated in the 2500-m high plateau of north central Nigeria. All subjects claimed to be in good health. Information regarding age, height, weight, ethnicity, parity and duration of lactation for each subject was obtained. Fifteen milliliters of milk were collected between 8:00 and 10:00 am by manual pumping 4 to 8 min after initiation of pumping. Following their collection, milk specimens were aliquoted into 5-ml cryovials and stored at −40°C until they were transported frozen to the U.S. for analysis. Specimens were gathered between the months of June and August, 2003. This study was approved by the Human Research Review Committee of the University of New Mexico Health Sciences Center and the Ethics Review Committee of the Jos University Teaching Hospital, Jos, Nigeria.

2.2. Anthropometric measurements

The weight of each subject was measured using a battery-operated scale that was accurate to 0.5 kg and height was measured to within 0.5 cm using a portable stadiometer. Body mass index (BMI) was calculated as weight (kg)/height (m)². Mid-upper arm circumference was measured with a tape measure (Creative Health Products, Plymouth, MI) and triceps skin-fold thickness using a body caliper (Caliper Company, Inc, Carson City, NV). Fat-free mass and body fat were estimated by bioelectrical impedance analysis conducted with a portable analyzer (RJL Systems, Inc, Detroit, MI) as described elsewhere [35]. Reactance and resistance values, weight, height, gender, and self-reported age were used to calculate fat-
free mass and body fat using the software provided by the manufacturer.

2.3. Fatty acid analysis

Frozen milk was thawed and gently mixed to provide a uniform sample. Dry matter content was determined by drying 0.5g of milk in a forced-air oven at 60°C for 24h. An aliquot (2ml) of each sample was weighed in a 50-ml extraction tube prior to lipid extraction using chloroform/methanol (2:1, v/v). The extracted lipid residue was weighed after drying at 45°C under a stream of nitrogen. Fatty acids were transesterified to methyl esters using 0.5N NaOH in methanol and 14% (w/v) boron trifluoride in methanol [36]. Undecenoic acid (Nu-Check Prep, Elysian, MN) was added prior to methylation, and served as an internal standard.

Fatty acid methyl esters were quantified using a gas chromatograph (6890N, Agilent Technologies, Sunnyvale, CA) equipped with an autoinjector, a split/splitless capillary injection system, and a flame ionization detector as described elsewhere [26]. A customized fatty acid mixture described by Loor and Herbein [37], made with pure methyl ester standards (Nu-Check Prep, Elysian, MN; Sigma, St. Louis, MO), was used to identify peaks and determine response factors for integration with a Chem Station, version 8.0 (Agilent Technologies, Sunnyvale, CA). This procedure permits separation of trans 18:1 isomers. Co-eluted peaks of trans isomers of 18:1 were small; however, they do provide an indication that one or both of the co-eluting fatty acids are present in the milk fat.

2.4. Statistics

Descriptive statistics, group comparisons and correlations were made using the Number Cruncher Statistical Software (NCSS, ver 6, Kaysville, UT).

3. Results

3.1. Comments on the study populations

As shown in Table 1, the lactating Nigerian women who were recruited from the rural areas were, on average, 3 years older than their urban counterparts. Though the 2 groups of subjects were not distinguished by height, the Fulani (rural) women were about 10kg lighter than the women in Jos. Consequently, the mean BMI of the rural women was substantially less than that of the urban women. The mean body fat content of the urban women was greater than it was for the rural women, as was the case with respect to fat-free mass. These data indicate that the Fulani women were considerably leaner than the urban women. The difference between the mean phase angle of the rural (6.0°) and urban women (6.2°) was not statistically significant.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rural women (Fulani) (n=41)</th>
<th>Urban women (Jos) (n=41)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.4 (6.8)</td>
<td>25.4 (6.3)</td>
<td>0.05</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 (7)</td>
<td>161 (6)</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>49.6 (8.5)</td>
<td>60.1 (12.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.7 (2.6)</td>
<td>23.0 (3.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAC (cm)</td>
<td>23.4 (2.8)</td>
<td>26.6 (3.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SKF (mm)</td>
<td>14.0 (5.2)</td>
<td>18.6 (5.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parity</td>
<td>4.9 (2.7)</td>
<td>2.3 (1.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lactation (mo)</td>
<td>9.4 (4.7)</td>
<td>6.6 (4.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>25.5 (7.1)</td>
<td>31.1 (8.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>74.6 (7.1)</td>
<td>68.9 (8.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat (kg)</td>
<td>13.1 (5.5)</td>
<td>19.5 (8.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>37.1 (3.6)</td>
<td>40.6 (4.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phase angle (°)</td>
<td>6.0 (0.8)</td>
<td>6.2 (0.7)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Mean (standard deviation; BMI, body mass index; MAC, mid-arm circumference; TSK, tricep skin-fold thickness; FFM, fat-free mass; NS, not significant, p > 0.05.

The phase angle is widely regarded as being directly proportional to an individual’s overall wellbeing and nutritional status [38,39]. The parity of the Fulani women was significantly greater than that of their urban counterparts (parity, 4.9 vs 2.3, p < 0.001). On average, the rural subjects had been breastfeeding for a little more than 9 months, whereas the average period of breastfeeding for the urban subjects was 6.6 months (p < 0.01).

3.2. Comparison of the fatty acid composition of the milk fat of urban and rural populations

We first determined the fat content of the milk samples (Table 2). Though the mean fat content of the milk of the women in Jos was slightly greater than that of the Fulani women (3.63 vs 3.05 g/dl), the difference was not statistically significant. However, since milk samples were obtained without complete expression of milk, the values we report for the fat content of the milk of the 2 groups of women cannot be rigorously compared. These values for both rural and urban women compare favorably with the milk fat content that has been reported for other populations world-wide [40–43].

The main reason for performing the present study was to determine if the milk fat of Fulani women whose culture, diet and economy are centered on the dairy products derived from their cattle contain higher percentages of TFAs and CLAs than their counterparts who live in Jos and whose diet contains little in the way of dairy products. This prediction is not borne out by the data (Table 2): the percentages of both total TFAs and total CLAs were not different between the 2 groups of women. The percentage of total TFAs in the milk fat of the rural (Fulani) and urban women were 0.22% and 0.34%, respectively, while the percentages of total CLAs were 0.16% and 0.14%, respectively, in these same 2 groups. Vaccenic acid (t11-18:1) accounted for more than
The differences in the percentages of the 2 essential fatty acids, namely linoleic acid and α-linolenic acid, between the milk fat of the rural and urban women were not statistically significant (Table 2). Although the mean percentages of α-linolenic acid (0.77–0.80%) in the milk fat of the 2 populations of women were in the range of values generally regarded as nutritionally sound vis-à-vis the nursing infant or child, the percentages of linoleic acid (6.97–7.83%) in the milk fat of the 2 groups of women were higher in the milk fat of the rural women compared to the urban women (6.97% vs 6.81%, p<0.001). DHA accounted for only 0.15% of the fatty acid total for milk fat of the urban women but 0.42% of the milk fat of the urban women in Jos. In their study of the fatty acid composition of the milk fat of women in southern Nigeria, Koletzko et al. [48] reported much higher levels of DHA, namely 0.93%.

The percentage of arachidonic acid was significantly higher in the milk fat of the rural women compared to the urban women (0.62% vs 0.48%, p<0.001). These values are lower than the 0.82% value reported by Koletzko and associates for women in southern Nigeria [48–49]. DHA accounted for only 0.15% of the fatty acid total for milk fat of the Fulani but 0.42% of the milk fat of the urban women in Jos. In their study of the fatty acid composition of the milk fat of women in southern Nigeria, Koletzko et al. [48] reported much higher levels of DHA, namely 0.93%.

The percentage of the total C16–C14, intermediate chain-length fatty acids in the milk fat of the 2 groups of women was essentially the same, accounting for 25.2% and 26.6% of total fatty acids in the milk of the urban and rural women, respectively, which are within the range of values reported for women elsewhere in Africa [48,49] and in the world where carbohydrates constitute a high percentage of the diet [43]. The percentages of fatty acids in the lipid fraction of

### Table 2 (continued)

<table>
<thead>
<tr>
<th>Component</th>
<th>Rural women (Fulani) (n=41)</th>
<th>Urban women (Jos) (n=41)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polyunsaturated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c6,c9,c12 18:3n−6</td>
<td>0.15 (0.06)</td>
<td>0.13 (0.05)</td>
</tr>
<tr>
<td>c6,c9,c12 15:3n−3</td>
<td>0.30 (0.11)</td>
<td>0.45 (0.22)*</td>
</tr>
<tr>
<td>c9,c12,c15 18:3n−3</td>
<td>0.80 (0.57)</td>
<td>0.77 (0.47)</td>
</tr>
<tr>
<td>c11,10 20:1</td>
<td>0.33 (0.11)</td>
<td>0.53 (0.22)*</td>
</tr>
<tr>
<td>c11,10,c12 20:2n−6</td>
<td>0.50 (0.17)</td>
<td>0.51 (0.14)*</td>
</tr>
<tr>
<td>c8,c11 20:3n−6</td>
<td>0.13 (0.03)</td>
<td>0.11 (0.04)</td>
</tr>
<tr>
<td>c5,c8,c11,14 20:4n−6</td>
<td>0.62 (0.13)</td>
<td>0.48 (0.11)*</td>
</tr>
<tr>
<td>c5,c5,8,c11,14,17 20:5n−3</td>
<td>0.07 (0.04)</td>
<td>0.08 (0.05)</td>
</tr>
</tbody>
</table>

85% of the trans 18:1 fatty acid in the milk fat of both groups of subjects, whereas rumenic acid (c9,11:18:2) accounted for about 40% of the CLA total for the 2 populations. The mean total percentages of TFAs in the milk of the 2 populations of Nigerian women (0.22–0.32%) were 7- to 10-fold lower compared to that of women in France [33] and other developed countries [34–36]; and the mean percentages of total CLAs in the milk fat of the Nigerian women were only about one-quarter the values that have been reported for women elsewhere in the world [44–47].
human milk fat can be affected by maternal body composition and other factors (e.g., parity, duration of lactation). However, the differences we found in the mean proportions of fatty acids between the 2 groups were still significant after controlling for maternal age and weight, parity and duration of lactation. When comparisons were made of the proportions of fatty acids other than arachidonic acid and DHA between the 2 groups of subjects, after correcting for the above-mentioned parameters (e.g., maternal age, parity, duration of lactation) no statistically significant differences were found.

3.3. Correlations between the amounts of particular fatty acids in milk fat vs anthropometric characteristics and duration of lactation

Because maternal age, weight, parity, length of lactation, and body composition can affect the fatty acid composition of human milk [50–55], we tested for possible relationships between the 52 fatty acids listed in Table 2 and those parameters of the Nigerian subjects. In order to increase sensitivity, the data for both populations of women (i.e., rural and urban) were pooled. With the exception of arachidonic acid and parity ($r=0.48$, $p<0.001$), no significant correlation was found between the percentage of any particular fatty acid, including the TFAs and the CLAs, and any of the above-mentioned parameters.

4. Discussion

In light of the fact that cow milk is known to contain substantial quantities of TFAs [1], the authors of this study expected that lactating Fulani women living in the rural areas of northern Nigeria and who consume relatively large quantities of dairy products would produce a milk that contained significant quantities of TFAs. We also expected to find lower proportions of TFAs in the milk of their lactating urban counterparts whose diets contain little in the way of milk or other dairy products. However, contrary to expectation, the milk of the Fulani women contained very low proportions of TFAs, and the percentage of total TFAs in the milk fat of the Fulani women was not significantly different from that of the urban women in Jos (0.22% vs 0.32%, respectively). Furthermore, the mean total percentages of TFAs in the milk of the 2 groups of Nigerian subjects in the present study were, on average, 5- to 20-fold lower than has been reported for the milk of women in economically advanced countries [44–47,56]. The percentages of total TFAs we report herein for the milk of 2 different populations of women in northern Nigeria are even much lower than the 1.20% of trans-isomeric fatty acids Koletzko et al. found in the milk of 10 women in Bendel State in southern Nigeria in 1991 [48].

The very low levels of TFAs we found in the milk of women living in the urban setting of Jos and whose diet is traditionally low in dairy products that are relatively costly indicates to us that those women were probably not consuming significant quantities of processed, hydrogenated oils. In western countries, the main source of TFAs is hydrogenated oils [15,16].

We speculated that the dairy products produced from the milk of the Fulani cattle at the same time our study was conducted may have contained little or no TFAs. It is well-known that the quality of the feed consumed by cows can have a marked influence on the fatty acid content of the milk they produce [1]. However, when we determined the fatty acid composition of the milk fat of 12 randomly selected cows among a herd of range-fed cattle belonging to the same Fulani community in which the rural women in the present study lived [57], we found that the percentages of total TFAs (4.61%) and the percentages of total CLAs (1.45%) were substantial and within the range of values reported for cattle elsewhere in the world [1].

Another reason for the finding of inordinately small amounts of TFAs in the milk of the Nigerian subjects in the present study could be their poor absorption of TFAs from the gastrointestinal tract. In a study conducted in the U.S. [58], it was shown that increasing the consumption of $c9,t11$-18:2 increased the concentration of this same CLA in human milk. It remains to be seen through future studies just how much of the various TFAs and CLAs the rural Fulani women and the women in Jos consume and what the levels of the TFAs are in the blood of these lactating women. It is also possible that Fulani women oxidize TFAs or metabolize them (e.g., Δ9-desaturase reaction) to other fatty acids before they have a chance of being incorporated into triglycerides in the mammary gland. Noteworthy is the fact that a variety of mammals can convert vaccenic to rumenic [7,24,26,27].

Another aim of the present study was to inquire regarding the content of CLAs in the milk of women in Nigeria. Once again, as was the case for the TFAs, the quantities of CLAs in the milk of the urban women and Fulani women were very low (e.g., 0.14–0.16%), both on a percentage basis and relative to the CLA levels in the milk of women elsewhere in the world [14,23,45,58–62]. Lacking information about the dietary and blood levels of CLAs of the Fulani women, we can say little at this point as to whether the low levels of CLAs in the milk of the Fulani women were the result of low dietary CLA intake, poor absorption of these particular fatty acids from the gastrointestinal tract, or because they were not extracted from the plasma by mammary tissue or incorporated into milk fat. In this regard, it would be useful to know how the substrate (i.e., triglyceride) specificity of the lipoprotein lipase associated with the capillary endothelium of the mammary gland of the women in the present study compares with that of the corresponding enzyme of women elsewhere in the world. Endothelial lipoprotein lipase in the mammary gland releases fatty acids from circulating lipoproteins (e.g., very low density lipoproteins and chylomicrons), thereby facilitating the uptake of the released fatty acids by the gland.
It is conceivable that the specificity of the lipoprotein lipase of the mammary gland of the Nigerian women in our study was such that it discriminated against triglycerides that contained TFAs and CLAs. Alternatively, since rumenic acid (c9,t11-18:1) is synthesized endogenously from vaccenic acid (t11-18:1)[7,24,26,27], the low levels of c9,t11-18:2 in the milk of the lactating women in the present study could be explained by a low intake or poor absorption of vaccenic acid, or low expression of Δ9-desaturase. These are questions we plan to address in a future study.

The physiological implications of these findings for the infants of the lactating women who participated in the present study are several. First, the results of our fatty acid analyses suggest that we would expect to find very low levels of TFAs and CLAs in the plasma and tissues of the infants who were being nursed by the urban and rural (Fulani) women who participated in this study. Second, if this prediction of low levels of TFAs in breastfed infants in northern Nigeria proves to be true, then they will have a low exposure to this particular risk factor for endothelial disease. On the other hand, with regard to the CLAs, if the plasma CLA levels in these same infants turn out to be low, then consuming human milk deficient in these particular fatty acid would have the effect of depriving them of the beneficial (e.g., growth-promoting, anticancer) effects of these CLAs. Although the results of this study were largely negative, the low levels of TFAs and CLAs we found in the milk of the urban and rural lactating women may have important public health implications, in particular with regard to the growth, body composition, neurodevelopment and cardiovascular health of their breastfed infants. Whereas rumenic acid (c9,t11-18:2) has no effect on lean body mass (63), t10,c12-18:2 depresses lipid accumulation [9,12]. Noteworthy is the fact that we did not find t10,c12-18:2 in the milk of the Nigerian women. In a future study we will investigate the relation between the quantities of the individual TFAs and CLAs consumed by breastfed infants in northern Nigeria and their growth (height, weight, head circumference) and body composition characteristics after the first year of life. Our longer-range interest is to correlate the amounts of TFAs and CLAs consumed by these infants through breastfeeding with their plasma lipid profiles in the adolescent period.

Finally, the results of the present study confirm our earlier findings of low levels of linoleic acid and DHA in the milk of Fulani women in northern Nigeria [48,49], and underscore the need for local public health officials to consider identifying ways of improving maternal nutrition with regard to these important fatty acids.

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References


