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Correlation between the content of intermediate chain-length fatty acids and copper in the milk of Fulani women

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Summary Intermediate chain-length fatty acids (C10-C14) in human milk triglycerides provide an easily absorbable fuel that provide a significant amount of the energy needed for growth during the first few months of life. The C10-C14 fatty acid and trace mineral content of human milk is variable. In this report we examined the relationship between the content of calcium, copper, magnesium, manganese, phosphorus, and zinc, and the weight percentage of C10-C14 fatty acids in milk from 33 Fulani women in northern Nigeria between 2 and 24 weeks post-gestation. The milk from these women contained proportions of C10-C14 fatty acids that were comparable to those reported for other populations around the world, as were the concentrations of Ca, Cu, Mg, Mn, Zn and P. Significant correlations were observed between the milk content of Cu and the wt% of C10 (P=0.005, r=0.475), C12 (P=0.001, r=0.539), C14 (P=0.44, r=0.352) and the total intermediate chain-length fatty acids (P=0.008, r=0.450). No correlations were observed between these fatty acids and any of the other five minerals. We speculate that the relationship between Cu and fatty acids could be related to a requirement for Cu by an enzyme required for C10-C14 fatty acid biosynthesis (e.g. decanoyl deacylase) in mammary tissue, or to some unique Cu binding properties of the intermediate chain length fatty acids. © 2000 Harcourt Publishers Ltd

INTRODUCTION

The salutary effects on the neonate of intermediate chain-length (C10-C14) fatty acids contained in the triglyceride fraction of human milk have been recognized for many years.[1,2] First, triglycerides that contain these fatty acids are hydrolyzed readily in the stomach by gastric lipase and in the intestine by pancreatic lipase,[1] and the C10-C14 fatty acid products of these lipase-catalysed reactions are transported across the gastric and intestinal mucosa directly into the blood circulation without having to undergo incorporation into chylomicrons.[1,2] Second, intermediate chain-length fatty acids can be taken up by mitochondria in a carnitine-independent manner.[1,3] Thus, the C10-C14 fatty acids synthesized in mammary gland and secreted into milk in the form of triglycerides provide easily absorbable fuel and one that can be readily oxidized to provide the energy needed to support growth and development during the first few months of life.

In studies of the nutrient quality of the milk of Fulani women that we conducted recently in northern Nigeria,[4,5] we uncovered evidence for another possible function or significance of the intermediate chain-length fatty acids in human milk. The Fulani are semi-normadic pastoralists who inhabit the western Sahel of Africa, and graze their herds of cattle and goats across Mali, Burkina Faso, Niger, Nigeria and Chad. We observed that the higher the proportion of C10-C14 fatty acids in the lipids in the milk of Fulani women, the higher was the proportion of critical polyunsaturated fatty acid docosahexanoic acid (DHA) in the serum phospholipids of the infants who were exclusively breast fed by these women. Docosahexanoic

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acid is the most abundant fatty acid in the membranes of the retina and central nervous system during the first 6 months of life.[6,7] This finding raises the possibility that intermediate chain-length fatty acids, also referred to as 'de novo' fatty acids because they are synthesized from acetyl-CoA in the mammary gland, could serve additional nutritionally relevant lactation-related functions.

In an effort to explore these other possibilities, we analyzed milk specimens obtained from 33 Fulani women for their content of six nutritionally essential minerals (calcium, copper, magnesium, manganese, phosphorus, and zinc) and correlated the concentrations of these minerals with proportions of C10-C14 fatty acids in the same milk specimens. Certainly, the finding of a correlation between two different analytes in a body fluid such as milk does not establish a metabolic or physiologic relationship; however, it would raise the possibility that such a relationship may exist. In the present report, we document a strong positive correlation between the copper content of the milk of Fulani women and the proportion of de novo fatty acids in their milk lipids.

METHODS

Human subjects

We obtained milk from 33 Fulani mother–infant pairs living in the town of Kaduna-Vom, which is located about 40 km south of the city of Jos. Ages, heights, weights, parity, and information regarding the general state of health of the subjects were recorded. All specimens and anthropometric data were gathered between June and August of 1998. Following collection, milk specimens were aliquoted into 1.5 ml cryovials and stored at -20°C until they were transported in the frozen state to the USA for analysis.

Analysis of minerals and trace elements

The milk specimens were thawed and vortexed vigorously and aliquots were quantitatively transferred to 50 ml Griffin beakers using 5% (v/v) nitric acid. The samples were transferred with successive rinses of first the caps, then the threads of the vials, and finally the sample vials. All of the samples, including the supplied blanks and a reagent blank, were digested with 4 ml concentrated HNO_3 and 1 ml of 70% $HClO_4$. The acids that were used throughout were Fisher Brand's Optima (Pittsburgh, PA, USA).

The beakers were covered with watchglasses and refluxed at 150°C for 4 h. The watch-glass covers were removed and the underside rinsed into the sample using a 5% HNO_3 solution. The samples were taken to near dryness at 150°C. One-fourth ml of 4:1 HNO_3–$HClO_4$ was used to redissolve each sample which was then quantitatively transferred to a graduated centrifuge tube and brought to a final volume of 5.0 ml with deionized water.

The resulting solutions were analyzed for their trace metal content by ICP-OES using a Spectre Analytical EOP (end on plasma-axial view) spectrometer which was equipped with a fixed cross-flow nebulizer and dual pass spray chamber.

Quality control samples, which were supplied by the National Institute of Occupational Safety and Health, consisted of the analyst's serum and breast milk from a co-worker. Three replicate aliquots (0.5 ml) of each were taken to establish baselines. Three additional aliquots were 'spiked' with multi-element ICP standards and analyzed concurrently with test samples.

Analysis of fatty acids

Milk samples were thawed, warmed to 37°C and vortexed vigorously before analysis. Lipids were extracted using the method described by Folch et al.[8] Boron trifluoride-methanol (14%, w/v) was used for transesterification of the total lipid fractions and the fatty acid methyl esters were extracted using hexane.[9]

Fatty acids were quantified using a Hewlett-Packard Gas Chromatograph (5890 series II; Mississauga, Ontario, Canada) equipped with a flame-ionization detector and a 50 m fused silica capillary column coated with 0.25 μm Supelcowax 10 (Supelco Inc., Bellefonte, PA, USA).[10] The injector and detector temperatures were 230°C. Commercial standards (Cu-Chek-Prep, Inc., Elysian, MN, and Supelco, Inc., Bellefonte, PA, USA) were used for identification and quantification of fatty acid methyl esters. Results are expressed as g/100g total fatty acid (i.e. weight %).

Statistical analysis

Group comparisons and correlations were done using NCSS 97 statistical system for windows (Kaysville, UT, USA). A P value < 0.05 was considered statistically significant.

RESULTS

Study population

Our study population was composed of 33 Fulani mother-infant pairs. Table 1 summarizes analysis of demographic data and includes maternal age (avg., 26.0 years), height (avg., 1.60 m), weight (avg., 49.3 kg), body mass index (BMI; avg., 19.4 kg), and parity (avg., 4), as well as infant age (avg., 11.0 weeks), length (avg., 56.9 cm), and weight (avg., 4.7 kg).

Mineral analysis

We analyzed the mineral concentrations of calcium, copper magnesium, manganese, phosphorus, and zinc in the breast milk of Fulani women and the results are summarized in Table 2. All values were similar to values reported for several other selected populations[11,12] (Table 2).

Table 1 Characteristics of the mothers and infants

	Mean (SD)	Range
Mothers		
Age (years)	26.0 (7.5)	15–45
Height (m)	1.60 (0.07)	1.47–1.72
Weight (kg)	49.3 (7.0)	34.0–68.0
BMI (kg/m^2)	19.4 (2.4)	14.5–24.0
Parity	4 (3)	1–10
Infants		
Age (weeks)	11.0 (7.1)	2.0–24.0
Length (cm)	56.9 (5.5)	46.0–68.0
Weight (kg)	4.7 (1.6)	1.8–8.0

SD: standard deviation.

De novo fatty acid analysis

We also analyzed the proportions of C10:0 (decanoate), C12:0 (laurate), C14:0 (myristate), and total de novo (C10:0-C14:0) fatty acids in the milk of the Fulani women. These results are summarized in Table 2. The most notable difference between the milk of Fulani women and other populations selected for comparison is the lower weight % of C10:0 (avg., 0.28; SD, 0.10). The proportions of C12:0 and C14:0 in the Fulani milk were within the reported ranges of the comparison populations. The total de novo fatty acid proportion in the milk of the Fulani women was within the range of values reported by other investigators.[13-16]

Fatty acid–mineral correlations

The results of correlations performed using the proportion of intermediate chain-length (C10-14) fatty acids with the mineral concentrations of six different minerals and trace elements in milk are summarized in Table 3. Statistically significant correlations ($P<0.05$) were found

Table 2 Mineral content and proportions of de novo fatty acid concentrations in the milk of Fulani women and in selected populations

Mineral	**Fulani[a] (this study)**	**Nigeria[11,b] (Jos)**	**Guatemala[11,b]**	**Zaire[11,b]**	**Ethiopia[12,a]**
Ca (mg/L)	263 ± 56.6	226 ± 20	303 ± 7	274 ± 8	462 ± 133
Cu (μg/L)	399 ± 180	278 ± 25	246 ± 18	201 ± 16	370 ± 200
Mg (mg/L)	31.2 ± 7.8	29.0 ± 2.6	34.1 ± 0.9	37.8 ± 0.9	25.62 ± 2.87
Mn (μg/L)	16.0 ± 16.0	15.8 ± 4.1	3.79 ± 0.29	11.2 ± 2.5	NR
P (mg/L)	165 ± 34.2	146 ± 26	148 ± 6	155 ± 7	NR
Sn (mg/L)	2.93 ± 1.3	1.68 ± 0.22	2.61 ± 0.32	1.92 ± 0.12	6.59 ± 2.1
Fatty acid	**Fulani[a] (this study)**	**Nigeria[13,a] (Jos)**	**Nigeria[14,b] (Yoruba)**	**Gambia[15,a]**	**Rural S. Africa[16,a]**
C10:0 (wt%) (Decanoic)	0.28 ± 0.10	1.7 ± 0.64	2.1 ± NA	0.92 ± 0.10	1.30 ± 1.1
C12:0 (wt%) (Lauric)	9.10 ± 3.34	10.11 ± 4.12	31.4 ± 4.2	6.99 ± 0.59	7.4 ± 6.7
C14:0 (wt%) (Myristic)	12.5 ± 5.51	12.46 ± 5.45	9.0 ± 1.5	8.80 ± 0.85	15.9 ± 1.8
Total C10-C14 (wt%)	21.5 ± 8.24	24.27	42.5	16.71	24.6

[a](Mean ± 1 SD).
[b](Median ± 1 SD).
NR: not reported due to insufficient numbers of specimens containing measurable levels of mineral.

Table 3 Statistical analysis of the correlations between the metal concentration and proportion de nova fatty acids in the milk of Fulani women

Metals	**De novo fatty acid (wt %)**			
	C10:0	**C12:0**	**C14:0**	**Total de novo (C10-14)**
	P-value (r)			
Copper	0.005 (0.475)	0.001 (0.539)	0.044 (0.352)	0.008 (0.450)
Calcium	0.48 (0.015)	0.69 (0.005)	0.73 (0.004)	0.95 (0.000)
Magnesium	0.06 (0.109)	0.09 (0.087)	0.68 (0.005)	0.33 (0.030)
Manganese	0.27 (0.039)	0.48 (0.016)	0.87 (0.001)	0.68 (0.005)
Phosphorus	0.13 (0.137)	0.21 (0.049)	0.896 (0.001)	0.55 (0.012)
Zinc	0.45 (0.180)	0.99 (0.000)	0.32 (0.303)	0.53 (0.125)

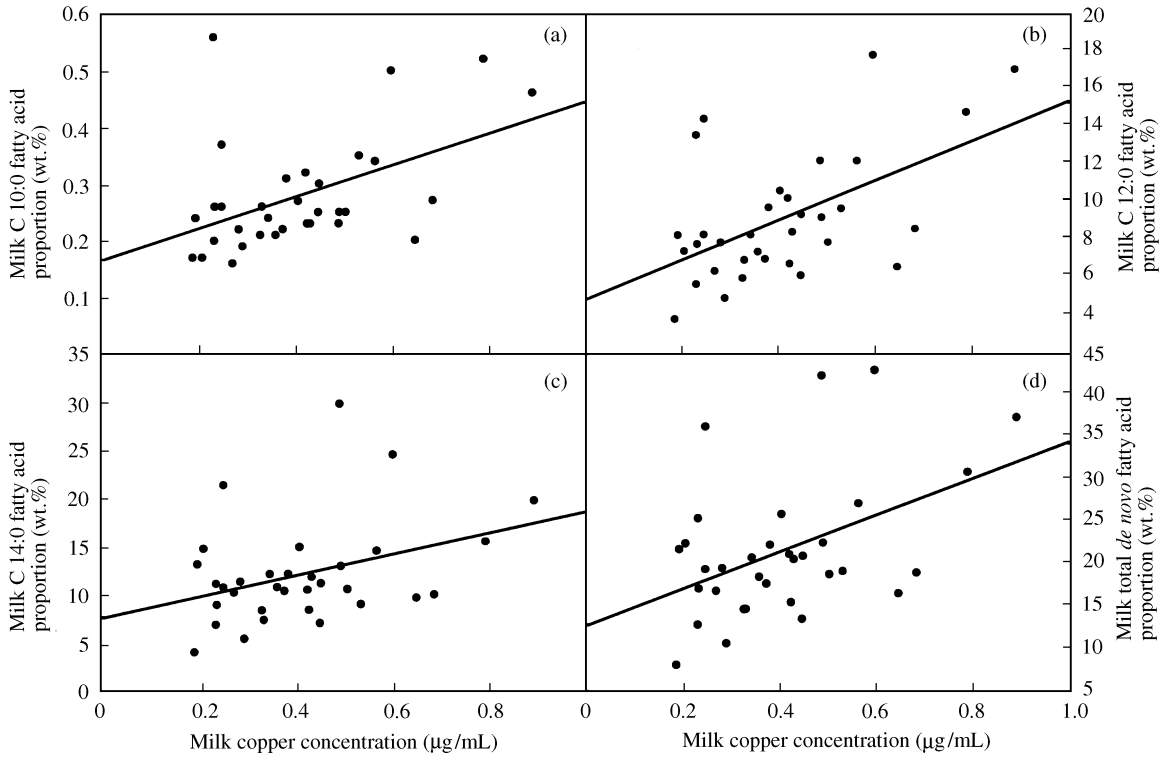

Fig. 1 Panel A: milk C10:0 fatty acid proportion (wt%) vs milk copper concentration (μg/mL); line eq.: Y=0.28x+0.17; P=0.005, r=0.475.
Panel B: milk C12:0 fatty acid proportion (wt%) vs milk copper concentration (μg/mL); line eq.: Y=10.41x+4.77; P=0.001, r=0.539.
Panel C: milk C14:0 fatty acid proportion (wt%) vs milk copper concentration (μg/mL); line eq.: Y=10.66x+7.83; P=0.044, r=0.352.
Panel D: milk total de novo fatty acid proportion (wt%) vs milk copper concentration (μg/mL); line eq.: Y=21.24x+12.76; P=0.008, r=0.450.

only between copper and all four fatty acid fractions tested: C10:0 (P=0.005, r=0.475), C12:0 (P=0.001, r=0.539), C14:0 (P=0.44, r=0.352), and total de novo fatty acids (P=0.008, r=0.450). Calcium, magnesium, manganese, phosphorus, and zinc all failed to show significant correlations with the proportion of C10:0, C12:0, C14:0, or total de novo fatty acids. Plots of copper concentration vs. the proportions of C10-14 and total de novo fatty acids (Fig. 1) illustrate the strong positive correlations we observed between these parameters.

DISCUSSION

The main finding of this study was the correlation between the copper concentration and the proportions of the three major intermediate chain-length fatty acids in the milk of Fulani women in northern Nigeria. Furthermore, this correlation was specific for copper; the concentration of none of the other five minerals or trace elements we analyzed (Ca, Mg, Mn, P, and Zn) exhibited such a correlation. It is axiomatic in statistics that a statistically significant correlation, even one that enjoys the apparent characteristic of specificity of the C10-C14/copper correlation reported herein, only points to a possible biological relationship between two parameters; it does not prove that such a relationship exists. Moreover, even if a physiological relevance between the two variables does exist, the correlation informs nothing of the directionality of the relationship; that is, in this particular case, whether copper regulates the content of medium chain-length fatty acids in human milk or vice versa.

Since we could find no reports in the literature linking copper or any other essential metal micronutrient to the content of C10-C14 fatty acids in the human mammary gland, we can only speculate as to how copper and intermediate chain-length fatty acids might be related metabolically. First, assuming copper is the dominant partner in the relationship, one possibility is that this metal is required for one or more of the steps in the pathway of de novo fatty acid biosynthesis in mammary tissue, for example, the one catalyzed by decanoyl deacylase (thioesterase II). Decanoyl deacylase catalyzes the terminal step in the biosynthesis of intermediate chain-length fatty acids in mammary tissue,[17,18] namely the premature termination of the fatty acid synthase reaction when the growing fatty acyl chain is 10–14 carbons long. Copper might be required for the expression of this gene or for the activity of the enzyme. However, although an extensive search of the literature

turned up numerous citations related to thioesterase II, none reported any involvement of copper in the expression or function of this enzyme. Alternatively, copper could be required for the transport of C10-C14 fatty acids between subcellular or cellular compartments in the mammary gland, or in the secretion of triglycerides rich in these intermediate chain-length fatty acids into the lumen of the lobules that secrete milk. Could it be that mammary tissue contains a fatty acid binding protein (FABP) that is specific for C10-C14 fatty acids and that the expression or activity of this FABP is dependent on copper?

On the other hand, what if intermediate chain-length fatty acids are the dominant player in the copper/de novo fatty acid relationship? That is, could it be that intermediate chain-length fatty acids play some role in copper transport or in maintaining or regulating the concentration of copper in the mammary gland? Since fatty acids and their coenzyme A derivatives are capable of binding copper,[19,20] perhaps copper, when complexed to intermediate chain-length fatty acids, is recognized by a transport system in the mammary gland.

The data in this report raise questions about the apparently specific and special relationship between the levels of copper and intermediate chain-length fatty acids in human milk. First, is the relationship manifest in the milk of women in other parts of the world? If so, then what is the biological basis of this phenomenon and what are its implications vis-à-vis the nutrition of lactating women and the infants they breastfeed? Those questions are being addressed in our ongoing studies of the nutrient quality of the milk of women in Nigeria[4,5] and other countries.[21] The question of whether the copper concentration of the milk of Fulani women is correlated statistically with the content of essential n-3 and n-6 polyunsaturated fatty acids is currently under investigation by us.

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