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Fatty acid composition in the mature milk of Bolivian forager-horticulturalists: controlled comparisons with a US sample

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Abstract

Breast milk fatty acid (FA) composition varies greatly among individual women, including in percentages of the long-chain polyunsaturated FAs (LCPUFA) 20:4n-6 (arachidonic acid, AA) and 22:6n-3 (docosahexaenoic acid, DHA), which are important for infant neurological development. It has been suggested that owing to wide variation in milk LCPUFA and low DHA in Western diets, standards of milk FA composition should be derived from populations consuming traditional diets. We collected breast milk samples from Tsimane women at varying lactational stages (6–82 weeks). The Tsimane are an indigenous, natural fertility, subsistence-level population living in Amazonia Bolivia. Tsimane samples were matched by lactational stage to samples from a US milk bank, and analysed concurrently for FA composition by gas-liquid chromatography. We compared milk FA composition between Tsimane (n = 35) and US (n = 35) mothers, focusing on differences in LCPUFA percentages that may be due to population-typical dietary patterns. Per total FAs, the percentages of AA, DHA, total n-3 and total n-6 LCPUFA were significantly higher among Tsimane mothers. Mean percentages of 18:2n-6 (linoleic acid) and *trans* FAs were significantly higher among US mothers. Tsimane mothers' higher milk n-3 and n-6 LCPUFA percentages may be due to their regular consumption of wild game and freshwater fish, as well as comparatively lower intakes of processed foods and oils that may interfere with LCPUFA synthesis.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

MAM, WDL, SJG, MG, HK, JGW and ALM made substantial contributions to conception and design of study. MAM, WDL, RWE, MG, HK, JGW, ALM, BSD and SRG made substantial contributions to the acquisition and/or analysis and interpretation of data. The paper was drafted by MAM and critically revised by SJG, WDL, RWE, MG, HK, JGW and ALM. All authors gave final approval of the version submitted for publication.

Keywords

lactation; diet; docosahexaenoic acid; arachidonic acid; breast milk; infant and child nutrition

Introduction

Infant growth and development require adequate sources of fatty acids (FAs), all of which are available in maternal breast milk. For breastfed infants, breast milk is the only source of the long-chain polyunsaturated FAs (or LCPUFA) 20:4n6 (arachidonic acid, AA) and 22:6n3 (docosahexaenoic acid, DHA), which are elevated in the infant brain both pre- and postnatally (Smit *et al.* 2002; Innis 2007b; Milligan *et al.* 2008). DHA in particular is important in the development of the central nervous and visual systems, and early deficiencies may have long-term effects on behavioural and cognitive function (Bazan & Scott 1990; Innis 2007a). However, breast milk FA composition is highly variable among women, with maternal diet the main factor affecting the percentages of many specific FAs, including DHA, in milk (Jensen 1999; Innis 2007b).

FAs secreted in milk are mobilized from adipose tissue stores, absorbed directly from maternal dietary lipids and/or endogenously synthesized (Del Prado *et al.* 2001).The essential PUFA 18:2n-6 (linoleic acid, LA) and 18:3n-3 (α-linolenic acid, ALA) can only be obtained through dietary sources. The major portion of PUFA in milk, LA and AA, originate primarily from maternal fat stores, influenced by long-term dietary intake (Demmelmair *et al.* 1998; Del Prado *et al.* 2001). Dietary sources of preformed DHA may be especially important for DHA content in milk. DHA milk percentages may be increased with short-term intake and supplementation (Makrides *et al.* 1996; Fidler *et al.* 2000, Brenna & Lapillonne 2009), although long-term intakes affect composition of bodily stores (Sauerwald *et al.* 2001). N-6 and n-3 LCPUFA (more than 20 carbon chains in length) are also synthesized from their respective precursors, LA and ALA, although conversion of AA, eicosapentaenoic acid (EPA) and DHA is low (Del Prado *et al.* 2001; Brenna *et al.* 2009). Supplementing nursing mothers with ALA, for instance, does not increase the DHA content of milk (Francois *et al.* 2003). DHA conversion may also be diminished by competitive inhibition from LA and *trans* FAs (TFAs) (Aitchison *et al.* 1977; Emken *et al.* 1994; van Eijsden *et al.* 2008; Gibson *et al.* 2011).

Concern over low LCPUFA availability in infancy, particularly of DHA, has prompted a wealth of research on maternal FA intake during pregnancy and lactation (Innis 2007b; Brenna & Lapillonne 2009), and considerable debate remains as to what FA intakes and milk compositions should be considered optimal (Yuhas *et al.* 2006; Smit *et al.* 2009; Uauy & Dangour 2009). Cross-cultural studies have shown that dietary intakes and percentages of DHA and total n-3 PUFA in milk are lower in populations with Westernized (i.e. industrial, agricultural) diets than in populations with traditional marine diets (Innis & Kuhnlein 1988; Koletzko *et al.* 1992; Krasevec *et al.* 2002; Yuhas *et al.* 2006; Brenna *et al.* 2007). The current ratio of n-6/n-3 PUFA in Westernized diets is estimated at 10/1 to 20/1, in contrast to ancestral estimates of 1/1 to 2/1, a result of both lower fish intake and high consumption of n-6-rich vegetable oils, processed foods and grain-fed domestic meat (Eaton 2006; Kuipers *et al.* 2010; Lindeberg 2010; Lassek & Gaulin 2012). The increasing dominance of n-6 relative to n-3 in Westernized diets may contribute to the increasing prevalence of childhood obesity (Ailhaud *et al.* 2007, 2008, Massiera *et al.* 2010), while higher intake of dietary LA relative to ALA may interfere with immune functioning (Whelan 1996) and result in lower incorporation of DHA into plasma phospholipids in infants (Sauerwald *et al.* 1996). Currently, infant formulas are modelled on breast milk compositions of US women, despite high interpopulation variability in milk LCPUFA composition, and the high LA/low n-3

LCPUFA in US milks (Gibson *et al.* 2011).It has been suggested, therefore, that standards for formula and milk FA composition should derive from populations consuming non-industrialized diets (Smit *et al.* 2002).

To date, studies of milk FA composition among traditional populations are few, and have been largely limited to coastal populations with heavy reliance on n-3-rich marine foods (e.g. the Innuit, Innis & Kuhnlein 1988), and rural African populations with low fat intake and variable access to n-3-rich foods (e.g. Prentice *et al.* 1989; Koletzko *et al.* 1991; Glew *et al.* 1995). Relatively little research on milk FA composition has been done on traditional populations consuming lacustrine resources, which likely accounted for a substantial amount of LCPUFA intake during the evolution of *Homo sapiens* (Broadhurst *et al.* 1998; Kuipers *et al.* 2005), or on forager-horticulturalists, whose diets are largely plant-based (Lindeberg 2010), but contain minimal processed foods and grain-fed meats. For extant forager-horticulturalist populations residing in and around the Amazonian Basin, freshwater fish and wild game may continue to serve as rich sources of LCPUFA.

There has also been little comparative analysis of milk FA composition between populations with divergent reproductive characteristics, such as average parity or typical duration of breastfeeding, which may also influence variation in milk LCPUFA composition. For example, maternal FA stores (Samur *et al.* 2009) may be mobilized with increasing parity (Lassek & Gaulin 2006) or weight loss (Prentice *et al.* 1989), and high parity and/or short interbirth intervals have been associated with reduced maternal and infant DHA stores (Al *et al.* 2000; Brenna & Lapillonne 2009). Milk FA composition has also been shown to vary across the first year of lactation, although trends for specific FA differ within and across studies (Harzer *et al.* 1983; Marangoni *et al.* 2000; Mitoulas *et al.* 2003). In a study of Italian mothers, milk AA and DHA percentages did not vary significantly from the first to twelfth month of lactation (Marangoni *et al.* 2000); however, little is known about variation in these FAs at later lactational stages.

We assessed milk FA composition in milk samples from indigenous Tsimane women residing in lowland Bolivia. We compared the FA composition of Tsimane milk samples with that of lactational stage-matched milk samples obtained from a Midwestern, urban US population, and analysed the additional effects of maternal age, body mass index (BMI), parity and infant age on milk FA composition in both populations.

Materials and methods

Study population: the Tsimane

The Tsimane are a high-fertility forager-horticulturalist population (most women give birth by age 18, and total fertility rate is nine children), with minimal access to modern medicine and market foods (Gurven *et al.* 2007). On average, mothers exclusively breastfeed for 3–6 months and fully wean infants 1–2 years thereafter, generally following a subsequent pregnancy or childbirth. The Tsimane diet consists primarily of freshwater fish, hunted game, and locally cultivated starches (plantains, rice, manioc) and fruit. Since 2002, researchers with the Tsimane Health and Life History Project (THLHP) have worked extensively with the Tsimane in Bolivia, providing primary medical care and collecting demographic, anthropological and biomedical data. These data and the THLHP have been summarized elsewhere (Gurven *et al.* 2007, 2008).

The Tsimane dietary estimates presented later are based on community-wide time-allocation studies conducted from 2002–2003 and 2005 across seven different villages. Researchers followed randomly selected subjects from 7 am to 7 pm, recording all activities at 30-min intervals. For the present analysis, we calculated the frequency of all food and liquid items

eaten during these observations for male and female subjects aged 20 and up (319 total subjects, 145 female, 174 male, 2031 total eating observations, 93 total different food items). Following Food and Agriculture Organization specifications (2004), we estimated the average Tsimane adult daily energy requirement (DER) by multiplying estimated physical activity levels (PALs) by estimated basal metabolic ratios (BMR) for each sex and age group (age 20–29, 30–39, 40–49, 50 + years), and proportionally averaging across the individual sex/age group DERs. PAL and BMR estimates were calculated from population-wide time allocation data, and individual height and weight measurements. The average adult DER was calculated as 8687 kJ day^{-1} (2075 kcal day^{-1}) for women and 11 685 kJ day^{-1} (2834 kcal day^{-1}) for men, for an overall average of 10 278 kJ day^{-1} (2455 kcal day^{-1}).We next calculated a proportional caloric contribution of each food item by dividing the estimated average DER by the frequency of consumption per item. This caloric contribution was then weighted by the item's energetic density (kcal/100 g). The fat, protein, carbohydrate and FA content of each item was calculated from the weighted caloric contribution and published estimates obtained from the United States Department of Agriculture and international databases (Tabla de Composición de Alimentos Bolivianos 1984; INFOODS Food Composition Database for Biodiversity, version 1.0, 2010). Estimates were based on edible portions of food items only. Consumption observations did not account for cooking methods, portion sizes or portions of foods consumed (e.g. different cuts of meat or organs), and these are not factored into the nutritional estimates. When necessary, values for wild animal species and fruits were approximated from similar items.

Tsimane milk samples were collected from 37 women in a single village (population ~450) during August and September 2009 (during the dry season). All lactating women with infants under age 1 and present during the study (n = 21) were asked to participate; 20 women consented and one candidate deemed the study too time consuming and declined. An additional 17 lactating women with infants aged 1–2 later volunteered to participate or were approached for inclusion in the study, for a total of 37 subjects. All infants were singleton births; no information on birthweight was available. All subjects gave verbal consent to participate the day before and immediately prior to collection.

Milk was collected by MM between 8 am and noon in the subjects' own homes at pre-arranged times. Mothers were instructed not to feed their infants from one breast at least 1 h prior to scheduled milk collection. Mothers were administered a 24-h dietary recall, weighed (Tanita® BF-679W) and measured for height; infants were weighed (Salter Brecksell® 235-6S) and measured for length (Pediatric Infantometer). Subjects were instructed on the use of a manual breast pump (Medela Harmony™) and assisted as needed by MM. Milk was collected until completely expressed, measured for volume and manually agitated in sterile 50-mL containers, and then aliquoted on-site into four 2-mL cryotubes. Any remaining milk was given to the mother to feed to her infant or discard. Following milk collection, mothers were given a commercial, nutritional beverage and small compensatory gifts such as hair combs, soap, necklaces and clothespins.

Milk samples were stored in liquid nitrogen until transport to the Centro Nacional de Enfermedades Tropicales (CENETROP) laboratory in Santa Cruz, Bolivia, where they were stored at −20°C. Samples were later transported to the United States on dry ice and stored at −80°C.All protocols for Tsimane subject recruitment, milk collection and analysis were reviewed and approved by Tsimane community leaders and the University of California Santa Barbara Office of Research, Human Subjects Committee (ID # 09–312), and complied with research and ethical standards previously set by joint agreements between the THLHP and the indigenous Tsimane Council. The University of Pittsburgh Institutional Review Board approved all milk FA analysis protocols.

Study population: US women (Cincinnati, OH)

The comparison samples were selected from participants in a longitudinal study (2004–2007) at the Cincinnati Children's research human milk bank (RHMB). The RHMB serves as a repository where human milk and corresponding data are collected and stored for a wide range of research purposes (Geraghty *et al.* 2005). Participation in the RHMB programme is voluntary. Mothers agreed to provide milk samples and clinical data weekly for the first month, then monthly for the duration of lactation (up to 18 months post-partum); they received a nominal reimbursement and could discontinue participation at any time.

Participants for the 2004–2007 cohort were solicited in the pre-partum and immediate post-partum period through flyers at doctors' offices and other local outlets. All mothers at least 18 years of age, in good health and intending to feed their infants at least 50% breast milk for 6 months or more were eligible. A trained lactation nurse visited eligible mothers in their homes within the first week post-partum to review the protocol and obtain consent. At birth, infants must have been singletons, of at least 37 weeks gestation, weighed more than 2.5 kg, and considered to be in good health.

At all visits, the nurse measured mothers' and infants' height and weight (E-Z Carry Portable Digital Scale, Hopkins Medical Products, Baltimore, MD). Milk was collected between 10 am and noon using a hospital grade, electric breast pump (Medela, McHenry, IL), from one breast until fully expressed; mothers were asked not to feed for at least two hours prior to their scheduled sessions. Collected milk samples were stored on ice for transportation to RHMB, then aliquoted and frozen at −80°C.

For the current analysis, single samples from 37 RHMB participants whose infants' ages best approximated those of the Tsimane infants were selected. The Institutional Review Board at the Cincinnati Children's Hospital Medical Center approved all methods for consent, milk donation, questionnaire delivery, referral, and sample analysis. Dietary information on the Cincinnati mothers was unavailable for the comparative analysis; we referenced published U.S. estimates (Ervin 2004; Wright *et al.* 2004) for discussion of population differences in average dietary intake.

Milk FA analyses

Milk lipids were extracted from 100 μL of milk following established protocols (Bligh & Dyer 1959). The samples, plus 1, 2-dinonadecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Inc. Alabaster, AL) (50 mg of 19:0) used as an internal standard, were homogenized in 4 mL of methanol, 2 mL of chloroform and 1.6 mL of water. After 15 min, an additional 2 mL of chloroform and 2 mL of water were added and the samples vortexed. The lower phase was dried under nitrogen and resuspended in 1.5 mL 14% boron trifluoride methanol. The samples were then heated at 90°C for 40 min and after cooling extracted with 4.0 mL pentane and 1.5 mL water. The mixtures were then vortexed and the organic (upper) phase recovered (Morrison & Smith 1964). The extracts were dried under nitrogen and resuspended in 50 μl heptane, and 2 μL of the solution were injected into a capillary column (SP-2380, 105 m × 53 mm ID, 0.20-mm film thickness; Supelco Inc., Bellefonte, PA, USA). Individual FAs were separated with a Perkin Elmer Clarus 500 gas chromatograph (Shelton, CT, USA) equipped with a flame ionization detector. Identification of components was done by comparison of retention time with those of authentic standards (Sigma Chemical Co., St. Louis, MO, USA).The coefficients of variation between runs were: 16.8% (12:0), 4.1% (14:0); 1.9% (16:0); 1.3% (16:1n7); 2.6% (18:0), 1.4% (18:1n9), 1.9% (18:1n7), 2.3% (18:2n6), 3.0% (18:3n3), 3.0% (20:3n6), 6.3% (20:4n6), 6.0% (20:5n3), 5.5% (22:6n3). Short-chain FAs of 10 carbons or less tend to be lost during evaporation of solvents, and were not measured for the present study, which focuses on long-chain FAs at least 14

carbons in length. Drying time for the samples was minimized to limit loss of 12:0. Nevertheless, as noted earlier, the coefficient of variation% for 12:0 was higher than those reported for the other FAs. As samples from both populations were analysed concurrently, any sampling bias because of underestimation of 12:0 would not systematically affect comparisons between the two.

Statistical analyses

Percentages of specific FAs (% weight/total weight of FAs) were available for all 74 samples collected from the Tsimane and Cincinnati populations. For statistical analyses, we excluded results from mothers of one Tsimane and one Cincinnati infant aged 10 and 12 days, respectively. Although removing these infants did not affect statistical results, researchers have observed marked differences in milk FA composition between very early (7–12 days) and later lactation, and have recommended these age ranges not be compared (Harzer *et al.* 1983; Luukkainen *et al.* 1994). We also excluded results from the mothers of one Tsimane infant at very late lactation (785 days) and the closest matched Cincinnati infant (573 days); exclusion of these infants did not affect statistical results related to infant age. The final statistical analyses thus covered milk samples from 35 Tsimane mother–infant dyads (17 female and 18 male infants) and 35 Cincinnati dyads (16 female and 19 male infants).

To compare differences in infant growth between the two populations, we calculated infant WAZ (weight-for-length *z*-score), LAZ (length-for-age *z*-score), and WLZ (weight-for-length *z*-score). The *z*-scores were calculated according to the 2006 WHO international standards for breastfed infants (WHO Multicentre Growth Reference Study Group 2006). The means, standard deviations and ranges of the following descriptive characteristics were calculated for each sample population: maternal age, weight, height, BMI, parity, lactational stage, infant WAZ, infant LAZ and infant WLZ. Mean differences for each characteristic were compared with paired-sample Student's *t*-tests, with pairs matched by lactational stage. Significance levels were adjusted using the using the false discovery rate (FDR) method to correct for multiple comparisons (Benjamini & Yekutieli 2001).

Population means, standard deviation, medians and first to third interquartile ranges were calculated for the individual percentages of each FA (% weight/ total weight) and the total FA concentration (mg mL^{-1}). Individual FA are presented as a weight percentage of total FA weight because FA concentrations are significantly affected by fat content, which varies with milk sampling conditions and nursing patterns. However, percentage contributions of FAs are unaffected by variability in milk fat content and can be reliably determined through random sampling (Koletzko *et al.* 1992). To facilitate comparison with existing literature, we additionally present the summed totals of all n-6 and n-3 FA analysed, the total n-6 and n-3 LCPUFA, and various ratios of interest. To minimize assumptions about the distribution of the FA data, population differences for specific FA percentages were compared by paired-sample Wilcoxon signed rank tests, with significance levels adjusted using the FDR method. For each population, we also computed Spearman's rank correlation coefficients between 18:0, 18:1n-9, LA, ALA, AA and DHA, adjusting significance levels by the FDR method to correct for multiple comparisons. For each population, we then computed Spearman's rank correlation coefficients between AA, DHA, maternal parity and lactational stage, with significance levels adjusted by the FDR method. All statistical analyses were conducted using Predictive Analytics Software Statistics version 18.0 (SPSS Inc., Chicago, IL, USA). Comparing all of these tests with their parametric equivalent, no inferences were changed regarding statistical significance.

Results

Descriptive statistics for the mother–infant dyads are given in Table 1. The Tsimane mothers were on average significantly younger, shorter, weighed less and had higher parity than Cincinnati mothers; Tsimane infants had significantly lower length-for-age scores than Cincinnati infants. The sample populations did not significantly differ by maternal BMI, infant age, infant weight-for-age or infant weight-for-length (Table 1).

In 24-hr dietary-recall interviews taken at milk collection, 83% of the Tsimane mothers reported eating fish at least once the day prior; 63% reported eating meat, 49% reported eating both and only one reported eating neither. From previously gathered population-wide dietary observations, we estimate that for adults aged 20 and older, the average Tsimane diet comprises 74% plant and 26% animal foods. Locally cultivated staples (rice, plantain, manioc and corn) account for 66% of total dietary energy, wild and cultivated fruits and nuts 6%, and market foods (crackers, bread, pasta, sugar) 2%. Game meat (primarily species of peccary, tapir, capybara and monkey) accounts for 17% of total dietary energy; freshwater fish 7%; and beef, poultry, and pork from free-ranging animals 2%. The Tsimane do not consume domestic milk or dairy products, and eggs account for less than 0.5% of the diet. An estimated 14% of average daily energy is derived from fat, 14% from protein and 72% from carbohydrates. Minimally, the average adult Tsimane diet contains 38 g fat per day, with 11 g saturated fat,14 g monounsaturated fat and 8 g polyunsaturated fat. Comparatively, the average US diet contains 67 g fat per day, with an estimated 33% of daily energy derived from fat, 15% from protein and 50% from carbohydrates (Wright *et al*. 2004).

Saturated, trans and monounsaturated FA composition in breast milk

Tsimane mothers had significantly higher percentages of most saturated FAs (SFA), with the exception of significantly lower 18:0, and non-significant differences in 12:0 and 14:0 (Table 2). Oleic acid (18:1n-9) was the predominant monounsaturated FA (MUFA) in both populations (90% and 75% of total MUFA in the Cincinnati and Tsimane samples, respectively), but was significantly higher among Cincinnati mothers (Table 2). Palmitoleic (16:1n-7) and vaccenic acid (18:1n-7) in Tsimane samples accounted for 17% and 8%, respectively, of total MUFA, as compared with 6% and 3% of total MUFA in Cincinnati samples. TFAs accounted for 0.6% and 1.7%, respectively, of total FA in Tsimane and Cincinnati milk, with differences largely due to significantly higher 18:1*t* in Cincinnati mothers (Table 2).

PUFA composition in breast milk

Tsimane milk samples had higher percentages of total n-3, total n-3 LCPUFA, and total n-6 LCPUFA, but Cincinnati samples showed significantly higher total n-6 (Table 3). Mean ratios of total n-6/n-3 and n-6/n-3 LCPUFA were significantly lower among the Tsimane (4/1 and 1/1, respectively) than those of Cincinnati mothers (8/1 and 3/1).Although the mean percentage of 20:4n-6 (AA) was twice as high in the Tsimane mothers (Table 2), the mean *ratio* of AA/DHA was 50% higher in the Cincinnati mothers. No significant population differences were observed in the means of 18:3n-3 (ALA), 18:3n-6 or 20:3n-6 (Table 3). Total n-6 and n-3 LCPUFA accounted for 3.5% and 1.6% of total FA among the Tsimane and Cincinnati samples, respectively; and 22% and 7% of total PUFA. In both populations, 18:2n-6 (LA) was the most predominant PUFA. Among Tsimane mothers, mean LA (10.2%) accounted for 64% of total PUFA. The mean percentage of LA among Cincinnati mothers (18.9%) was nearly twice as high and accounted for 84% of total PUFA in the North American sample (Table 2).

As shown in Table 4, EPA and DHA were significantly correlated with their precursor ALA in Tsimane mothers only. Positive correlations between metabolically distinct FAs were also observed: in Tsimane mothers, LA and oleic acid, and ALA and AA were moderately correlated, while AA was strongly correlated with EPA and DHA. LA and ALA were highly correlated among Cincinnati mothers (Table 4). In separate nonparametric correlations run for each population, neither AA or DHA was significantly correlated with maternal parity or lactational stage.

Discussion

The present study demonstrates that milk FA composition differs significantly between a well-studied forager-horticulturalist population and a Westernized reference population. The differences in milk FA composition are consistent with differences in the average dietary composition of the two populations. Our discussion of dietary influences on Tsimane and Cincinnati milk FA composition draws from the population-wide Tsimane dietary estimates presented earlier and US estimates published elsewhere (Ervin 2004; Wright *et al.* 2004; Oh *et al.* 2005). These estimates are good approximations of habitual dietary intake that would influence milk FA composition from both direct intestinal absorption and body stores.

The high n-3 LCPUFA contents in Tsimane milk were expected given their regular intake of freshwater fish. As compared with mean DHA percentages reported in 84 international studies of milk FA composition (Brenna *et al.* 2007), Tsimane milk mean DHA (0.74%) ranks in the ninety-fifth percentile of means and Cincinnati milk in the seventeenth percentile. The Tsimane milk DHA percentage ranks below only mean values reported for women in the Dominican Republic (van Beusekom *et al.* 1990), Japan (Wang *et al.* 2000;Yuhas *et al.* 2006) and the Canadian Arctic (Innis & Kuhnlein 1988) (0.91–1.4%). Although the FA concentrations of fish species consumed by the Tsimane are not known, freshwater Amazonian species examined elsewhere show lower levels of DHA (18–55 mg g^{-1}) as compared with coldwater ocean species such as bluefin tuna (181 mg g^{-1}),Atlantic salmon (175 mg g^{-1}), and mackerel (100 mg g^{-1}) (Inhamuns & Franco 2008). Fish intake by the Tsimane may also be less frequent and more seasonal than that of coastal populations. While small fish may be obtained from the stream that transects the sampled Tsimane community, larger fish catches are generally obtained by making treks to neighbouring communities with larger rivers, especially during the peak dry season (May–August) when water levels are low and fishing is most productive.

Among the same international studies compiled by Brenna *et al.* (2007), the percentage of AA in Tsimane milk (1.12%) ranks highest, while that of Cincinnati (0.59%) ranks in the eighty-first percentile (Brenna *et al.* 2007). The percentage of AA was not significantly correlated with its precursor LA in either Tsimane or Cincinnati milks, suggesting AA synthesis from LA is not a limiting factor for milk AA content. Freshwater fish may also be a source of AA for the Tsimane. In a study of milk FA composition in African mothers with regular freshwater fish consumption, median milk AA content (0.52–0.70%) was higher than that of European (0.37%) and Caribbean (0.50%) mothers, with regularly consumed fish species showing higher AA content and higher AA/DHA ratios as compared with North Sea and Caribbean species (Kuipers *et al.* 2005). The high percentage of AA in Tsimane milk may also reflect their regular consumption of animal organs, including brain and liver tissue, which contain high concentrations of both AA and DHA (Cordain *et al.* 2002). Of note, AA was strongly correlated with both EPA and DHA in Tsimane milk samples (Table 4). Because of metabolic competition during synthesis from precursors (LA and ALA, respectively), an inverse relationship between AA and EPA or DHA might have been expected. The positive relationships between these LCPUFA may suggest common dietary sources.

The differences in LCPUFA composition between Tsimane and Cincinnati mothers may also reflect differences in body fat composition and parity-related mobilization of fat and FA stores (Butte & Hopkinson 1998; Lassek & Gaulin 2006). However, we observed no significant differences in maternal BMI between the two populations (Table 1). BMI and body fat percentages are also highly correlated in Tsimane adults, and body fat percentage per unit increase in BMI in Tsimane women is similar to that of US women (Gurven *et al.* in press). There was also no significant negative correlation between AA or DHA and parity in either population. However, such effects may not be expected among the relatively low-parity Cincinnati mothers, while habitual intake of AA- and DHA-rich foods may be sufficient to supply milk content in the higher parity Tsimane mothers.

We also observed no significant correlations between AA or DHA and lactational stage, which is consistent with previous studies showing no decrease in milk AA or DHA from 3 to 6 months to 1 year post-partum (Marangoni *et al.* 2000; Mitoulas *et al.* 2003), and no change in FA composition in milk trigylcerides or infant phospholipids up to 23 months of lactation (Lauber & Reinhardt 1979). Stable milk AA and DHA composition may be needed during the 2-year postnatal brain-growth spurt, when uptake of AA and DHA by the brain is maximal (Marangoni *et al.* 2000; Milligan *et al.* 2008). Prolonged breastfeeding of up to 2 years post-partum may therefore provide infants with a steady supply of AA and DHA during this critical period of brain growth and development.

The higher percentages of LA and total TFAs in Cincinnati mothers' milks are consistent with values previously reported for US women, and likely reflect regular consumption of LA-rich vegetable oils (Brenna & Lapillonne 2009) and processed foods containing LA and hydrogenated oils (Szabo *et al.* 2007; Samur *et al.* 2009).Tsimane women generally cook with rendered animal fat, and rarely purchase or use vegetable oils. Importantly, LA and ALA compete metabolically for the enzymes used in synthesizing AA and DHA (Gibson *et al.* 2011) and, although conversion of ALA to DHA is low, replacing LA-rich oils with ALA-rich oils may increase plasma DHA levels (Brenna *et al.* 2009). We observed higher ratios of LA/ALA and AA/DHA in Cincinnati as compared with Tsimane mothers (Table 3), suggesting that high LA intake and elevated synthesis of n-6 LCPUFA metabolites may reduce DHA synthesis in the Cincinnati samples. *Trans* FA content in milk has also been shown to vary inversely with LCPUFA content (Szabo *et al.* 2007). *Trans* fat intake in the United States has dropped from 2.2% to 1.6% of total daily energy between 1980 and 1998 (Oh *et al.* 2005), and appears to have decreased even further in the last decade (Vesper *et al.* 2012). Still *trans* fat intake is likely even lower among the Tsimane, with processed market foods comprising less than 1% of daily energy in the adult Tsimane diet. Thus, in addition to DHA-rich dietary sources, the DHA status of Tsimane mothers may be favourably influenced by their lower LA/ ALA ratios and *trans* FA percentages.

It is notable that the Tsimane mothers did not significantly differ from the Cincinnati mothers in their percentages of lauric (12:0) or myristic acid (14:0) (Table 2).These medium-chain FAs are synthesized in the mammary gland, and synthesis may be increased by low-fat/high-carbohydrate diets, as has been observed for some rural African mothers (Koletzko *et al.* 1991; Kuipers *et al.* 2005). The higher percentage of 16:0 in Tsimane milk (Table 2) may similarly reflect increased synthesis, lower PUFA intake, or unaccounted-for sources of fat in the Tsimane diet. Wild game is generally lower in saturated fat than is domestic meat (Cordain *et al.* 2002), and our estimated Tsimane saturated fat intake (10.6 g day^{-1}) is less than half that of US adults (22.5 g day^{-1}) (Ervin 2004). However, our behavioural observations of foods eaten did not account for organ meat consumption or the addition of cooking fat, which may substantially increase saturated fat intake. The higher mean percentages of 15:0 and 17:0 in the Tsimane samples were also perplexing, as the Tsimane rarely consume dairy fats, which are associated with 15:0 and 17:0 in serum and

adipose tissue levels (Brevik *et al.* 2005). Future research will better quantify Tsimane saturated fat intake from animal sources.

There are several limitations to the current study. First, although most mean differences in milk FA composition between the two populations may be reasonably ascribed to differences in average dietary composition, we lack direct quantitative data on long-term dietary intakes from the subjects sampled here. We also caution that without measurements of 24-h milk energy density and yield, we cannot determine actual infant FA intake (Mitoulas *et al.* 2003; Milligan *et al.* 2008).Total FA concentrations were significantly lower and more variable in Tsimane mothers as compared with the Cincinnati mothers (Table 2), which may reflect greater variation in Tsimane mothers' parity and nursing patterns – characteristics known to substantially affect milk yield (Motil *et al.* 1997) and milk fat and FA concentrations (Koletzko *et al.* 1992). Lower milk FA concentrations may not equate to lower FA intake, however, as any differences may be balanced out across total daily milk and/or milk lipid intake (Mitoulas *et al.* 2003; Milligan *et al.* 2008). Future research is needed to better quantify and evaluate the effects of intrapopulation variation in diet, parity, body fat and lactational stage on Tsimane milk FA composition. Such research should also include longitudinal measures of average daily milk energy density and yield in order to track changes in infant FA intake over the course of prolonged lactation.

In conclusion, we have presented evidence of high LCPUFA content in milk from Tsimane mothers consuming a traditional diet of wild game, fish and cultigens. Modern diets and weaning practices are vastly different from those under which human milk FA synthesis and infant postnatal developmental patterns evolved. Milk from women practising prolonged lactation and consuming traditional diets rich in n-3 and free of industrially extracted n-6 may be more reflective of ancestral milk LCPUFA availability and thus may serve as better reference standards for FA composition in both milk and infant formula.

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Key messages

- Higher percentages of AA and DHA in milk from Tsimane women may be favourably influenced by a traditional diet of cultigens, freshwater fish and wild game.
- Although Tsimane frequently consume LA-rich plant foods such as maize, they rarely consume LA-rich vegetable oils or processed foods, which, by reducing enzymatic competition, may contribute to their elevated milk DHA.
- Higher parity was not associated with lower LCPUFA in milk.
- Milk AA and DHA did not vary with lactational age in either US or Tsimane samples.
- LCPUFA supplied by prolonged lactation may support infant requirements during the postnatal brain growth spurt.

Table 1

Descriptive characteristics of Tsimane and Cincinnati mothers and infants*

	Tsimane (*n* = 35)		Cincinnati (*n* = 35)	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Maternal age (years)	26 ± 8.5	15–45	32 ± 4.9†	22–42
Maternal weight (kg)	55.9 ± 9.2	43.1–79.7	63.8 ± 10.1‡	50.9–95.7
Maternal height (cm)	151.1 ± 4.3	142.0–162.0	163.7 ± 5.2§	153.7–178.4
Maternal BMI (kg m^{-2})	24.5 ± 3.7	18.6–34.0	23.8 ± 3.7NS	19.7–35.1
Maternal parity	4.3 ± 2.8	1–11	1.9 ± 0.8‡	1–4
Lactational stage (weeks)	43.8 ± 23.5	6.4–82.1	43.8 ± 23.5NS	7.0–80.7
Infant WAZ	−0.9 ± 1.4	−4.1–1.8	−0.3 ± 1.1NS	−2.1–2.3
Infant LAZ	−1.4 ± 1.4	−3.5–2.1	−0.3 ± 1.0‡	−2.0–2.0
Infant WLZ	−0.1 ±1.1	−2.5 ± 2.3	−0.2 ± 1.1NS	−2.1–2.8

WAZ, weight-for-age *z*-score; LAZ, length-for-age *z*-score; WLZ, weight-for-length *z*-score; NS, non-significant; SD, standard deviation; BMI, body mass index.

*Values are means ± SD. Sample population differences were compared by paired-sample Student's *t* -tests. Significance levels are adjusted *q*-values obtained by the false discovery rate method to control for multiple comparisons

†$q < 0.05$,

‡$q < 0.01$,

§$q < 0.001$, NS.

Table 2

Mean saturated, trans, monounsaturated, and polyunsaturated fatty acid content of Tsimane and Cincinnati milk*

Fatty acid % wt/wt	Population	Mean + SD	Median	Interquartile range	Significance *q* value
Medium-chain FA					
12:0 (lauric)	Tsimane	5.72 ± 2.39	5.30	3.41	0.079
	Cincinnati	6.68 ± 2.27	6.61	2.40	
14:0 (myristic)	Tsimane	9.81 ± 4.18	9.08	6.15	0.335
	Cincinnati	8.67 ± 2.81	8.43	3.85	
Saturated FA (even)					
16:0 (palmitic)	Tsimane	24.96 ± 3.13	25.17	4.01	<0.001
	Cincinnati	20.00 ± 2.64	20.53	4.19	
18:0 (stearic)	Tsimane	5.54 ± 1.49	5.03	2.10	0.002
	Cincinnati	6.67 ± 1.51	6.49	1.73	
Saturated FA (odd)					
13:0	Tsimane	0.06 ± 0.04	0.04	0.05	0.002
	Cincinnati	0.03 ± 0.02	0.03	0.02	
15:0	Tsimane	0.43 ± 0.17	0.40	0.28	0.001
	Cincinnati	0.29 ± 0.09	0.30	0.13	
17:0 (margaric)	Tsimane	0.53 ± 0.18	0.53	0.30	<0.001
	Cincinnati	0.27 ± 0.06	0.26	0.08	
Trans FA					
16:1t	Tsimane	0.43 ± 0.12	0.42	0.13	<0.001
	Cincinnati	0.32 ± 0.04	0.33	0.05	
18:1t	Tsimane	0.21 ± 0.30	0.12	0.22	<0.001
	Cincinnati	1.23 ± 1.05	0.93	1.11	
18:2tt	Tsimane	0.00 ± 0.01	0.00	0.01	<0.001
	Cincinnati	0.11 ± 0.07	0.10	0.05	
Monounsaturated FA					
16:1n-7 (palmitoleic)	Tsimane	6.06 ± 1.72	5.70	2.27	<0.001
	Cincinnati	1.96 ± 0.61	1.91	0.92	
18:1n-7 (vaccenic)	Tsimane	2.84 ± 0.76	2.92	0.98	<0.001

Fatty acid % wt/wt	Population	Mean + SD	Median	Interquartile range	Significance *q* value
	Cincinnati	1.03 ± 0.22	1.03	0.36	
18:1n-9 (oleic)	Tsimane	27.50 ± 4.56	26.99	7.21	0.020
	Cincinnati	30.16 ± 3.44	30.39	4.58	
20:1n-9	Tsimane	0.10 ± 0.11	0.06	0.09	<0.001
	Cincinnati	0.30 ± 0.16	0.37	0.25	
24:1n-9 and 22:4n-6[†]	Tsimane	0.06 ± 0.08	0.01	0.09	0.002
	Cincinnati	0.12 ± 0.04	0.13	0.05	
n-6 Polyunsaturated FA					
18:2n-6 (LA)	Tsimane	10.23 ± 4.56	9.31	5.45	<0.001
	Cincinnati	18.88 ± 5.10	18.09	6.04	
18:3n-6 (GLA)	Tsimane	0.12 ± 0.04	0.11	0.07	0.390
	Cincinnati	0.13 ± 0.05	0.13	0.08	
20:2n-6	Tsimane	0.23 ± 0.08	0.20	0.16	0.589
	Cincinnati	0.22 ± 0.07	0.21	0.08	
20:3n-6 (DGLA)	Tsimane	0.47 ± 0.12	0.44	0.15	<0.001
	Cincinnati	0.33 ± 0.08	0.33	0.10	
20:4n-6 (AA)	Tsimane	1.06 ± 0.33	0.96	0.52	<0.001
	Cincinnati	0.55 ± 0.09	0.56	0.13	
22:5n-6 (Osbond)	Tsimane	0.21 ± 0.07	0.19	0.10	<0.001
	Cincinnati	0.05 ± 0.03	0.04	0.00	
n-3 Polyunsaturated FA					
18:3n-3 (ALA)	Tsimane	1.90 ± 0.84	1.64	1.04	0.114
	Cincinnati	1.58 ± 0.65	1.39	0.78	
20:4n-3 (ETA)	Tsimane	0.25 ± 0.18	0.17	0.23	<0.001
	Cincinnati	0.06 ± 0.03	0.05	0.03	
20:5n-3 (EPA)	Tsimane	0.20 ± 0.12	0.17	0.13	<0.001
	Cincinnati	0.06 ± 0.04	0.06	0.04	
22:5n-3 (DPA)	Tsimane	0.40 ± 0.14	0.36	0.17	<0.001
	Cincinnati	0.14 ± 0.04	0.14	0.03	
22:6n-3 (DHA)	Tsimane	0.69 ± 0.26	0.62	0.31	<0.001
	Cincinnati	0.16 ± 0.09	0.13	0.09	

Fatty acid % wt/wt	Population	Mean + SD	Median	Interquartile range	Significance *q* value
Total FA (mg mL^{-1})	Tsimane	11.46 ± 6.12	9.51	7.44	0.027
	Cincinnati	13.33 ± 3.66	13.54	5.79	

SD, standard deviation; FDR, false discovery rate; FA, fatty acid; LA, linoleic acid; GLA, gamma linolenic acid; DGLA, dihomogamma-linolenic acid; AA, arachidonic acid; ALA, α-linolenic acid; ETA, eicosatetraenoic acid; EPA, eicosapentaenoic acid; DPA, dipicolinic acid; DHA, docosahexaenoic acid.

*Values are mean weights (%wt: total FA weight) ± SD, medians and twenty-fifth and seventy-fifth percentile range. Population differences for each specific FA were compared with Wilcoxon paired-sample signed ranks tests, with samples matched to lactational stage. Significance values are *q*-adjusted *P*-values obtained using the FDR method to control for multiple comparisons.

†24:1n-9 and 22:4n-6 did not always separate during analysis and are reported together.

Table 3

Summary of polyunsaturated fatty acid composition in Tsimane and Cincinnati milk*

Fatty acid totals and ratios	Tsimane	Cincinnati
Total n-6	12.47%	20.58%
Total n-3	3.44%	2.00%
Total n-6/Total n-3	3.48/1	7.56/1
Total n-6 LCPUFA	1.97%	1.15%
Total n-3 LCPUFA	1.54%	0.42%
n-6 LCPUFA/n-3 LCPUFA	1.28/1	2.75/1
LA/ALA	5.38/1	11.95/1
AA/EPA	5.34/1	8.86/1
AA/DHA	1.53/1	3.43/1

LCPUFA, long-chain polyunsaturated fatty acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

* Total n-6 is the sum of mean 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6 and 22:5n-6 percentages. Total n-3 is the sum of mean 18:3n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3 percentages. Total n-6 LCPUFA is the sum of mean 20:2n-6, 20:3n-6, 20:4n-6 and 22:5n-6 percentages. Total n-3 LCPUFA is the sum of mean 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3 percentages.

Table 4

Nonparametric correlations between specific FA*

	Tsimane						
–	18:0	Oleic	LA	ALA	AA	EPA	DHA
18:0	–	−0.104^{NS}	−0.125^{NS}	0.034^{NS}	0.147^{NS}	−0.058^{NS}	0.089^{NS}
Oleic	0.105^{NS}	–	**0.558**†	−0.171^{NS}	−0.239^{NS}	−0.364^{NS}	−0.396^{NS}
LA	−0.375^{NS}	−0.237^{NS}	–	0.230^{NS}	−0.144^{NS}	−0.069^{NS}	−0.171^{NS}
ALA	−0.187^{NS}	−0.352^{NS}	**0.805**‡	–	**0.581**†	**0.806**‡	**0.607**‡
AA	0.081^{NS}	0.282^{NS}	−0.166^{NS}	−0.166^{NS}	–	**0.760**‡	**0.780**‡
EPA	0.183^{NS}	0.096^{NS}	−0.163^{NS}	−0.019^{NS}	0.356^{NS}	–	**0.809**‡
DHA	−0.126^{NS}	−0.030^{NS}	−0.066^{NS}	−0.083^{NS}	−0.061^{NS}	0.202^{NS}	–
	Cincinnati						

LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; NS, non-significant.

*Values are Spearman's rank correlation coefficients. Significance levels are adjusted *q*-values obtained by the false discovery rate method to control for multiple comparisons

$^{\dagger}q < 0.01$,

$^{\ddagger}q < 0.001$, NS).