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REGULAR ARTICLE

A dose–response relationship between fish consumption and human milk DHA content among Filipino women in Cebu City, Philippines

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ABSTRACT

Aims: Human milk is the primary source of docosahexaenoic acid (DHA) for most infants, an important fatty acid for neurological development. Milk DHA is largely incorporated from the maternal diet. Little is known about whether milk DHA varies within populations with differences in maternal fish consumption. Here, we investigate this association in a sample of marginally nourished Filipino women.

Methods: Milk samples were collected during in-home interviews with 117 lactating Filipino mothers from Cebu City, Philippines, nursing infants <24 months of age. Anthropometric data and dietary recalls were also collected. Samples were analysed for total fatty acid composition using gas chromatography. Multivariate regression was used to test the association between fish consumption and milk DHA.

Results: Milk DHA showed a positive, dose–response relationship with maternal fish consumption ($p < 0.011$, $r^2 = 0.21$). Milk DHA was also positively related to protein intake, likely reflecting the association between fish and protein intake ($p < 0.009$). Unlike prior studies, parity predicted increased milk DHA ($p = 0.03$).

Conclusions: Increasing fish consumption during lactation may be a cost-effective means of maximizing DHA delivery to infants particularly in populations with marginal energy intakes during lactation. However, this must be weighed against the potential dangers of increasing exposure to fish-based pollutants.

INTRODUCTION

Long-chain, polyunsaturated fatty acids (PUFAs) are considered essential fatty acids for humans and include both the omega-3 fatty acids, such as docosahexaenoic acid (DHA) and alpha-linolenic (ALA) and the omega-6 fatty acids, linoleic acid and arachidonic acids. During infancy, DHA in particular is preferentially utilized by neurons and retinal cells (1,2), with studies reporting a positive association between early DHA intake and neural growth, cognitive function and visual acuity that persists at least through early childhood (3). DHA may contribute to the reported increases in visual acuity and IQ found in breastfed infants, as human milk, but not commercial infant formula, contains DHA (4). Clinical trials supplementing formula with DHA have reported improvements in cognitive function and retinal development in treated infants (5), and DHA-supplemented formula is now available. Human milk remains the best dietary DHA source for infants, although specific amounts of DHA are highly variable within and between populations (6,7). As humans cannot synthesize DHA but depend on conversion of ALA to DHA or preformed sources of DHA in the diet, mother's milk DHA content is strongly associated with maternal dietary DHA intakes (8).

Current recommendations for lactating women include a fish oil supplement as a source of preformed DHA (9). While considerable research has investigated the relationship between maternal fish oil dosage and milk DHA (10), minimal research has looked at augmenting dietary fish intake as a possible alternative strategy for increasing milk DHA (11). This may be particularly important for populations with limited access to supplements, which may be expensive and may be poorly tolerated by some women. Generally, past studies have focused on population-level

Key notes

- The DHA content of human milk is usually reflective of maternal dietary history.
- Here, we report a positive association between mother's self-reported fish consumption and the amount of DHA in their milk despite lower overall energy intakes.
- Promoting fish consumption among marginally nourished women may be a way of improving the DHA status of milk and mothers in populations with access to low cost cold water fish.

differences in milk DHA content and, for instance, have shown that habitually fish-consuming populations tend to have a greater percentage of total fatty acids as DHA (6,11,12). Few studies have investigated the determinants of milk DHA content among women within populations. The only example that we are aware of reported a positive association between regular fish consumption and milk DHA among a sample of Dutch women (13,14). However, similar studies have not been conducted in populations from developing economies, particularly those with lower overall nutritional intake and among whom fish may be a readily available, affordable source of preformed dietary DHA.

Here, we report the association between fish consumption and the percentage of milk fat as DHA in a large longitudinal birth cohort located in metropolitan Cebu City, Philippines, that originally enrolled more than 3000 pregnant women in 1983–84 and has since followed their offspring (17). During 09/2007–04 2008, 121 of the now-adult birth cohort members (age range 24–25) were currently breastfeeding infants <2 years of age. We collected dietary data and a human milk sample from these women for compositional analysis. This sample is characterized by relatively low energy intakes and body mass indices, but high habitual fish consumption. Prior work in this sample has reported milk macronutrient composition in this sample that is similar to that reported for other populations, including total milk fat (16). Here we build upon these findings to evaluate whether self-reported weekly portions of fish consumed predicts the contribution of DHA to milk fat in this sample.

METHODS

Sample population

Study participants were drawn from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), a large 1-year birth cohort born in the metropolitan area of Cebu City, Philippines, from May 1, 1983 to April 30, 1984 (15). The sample included individuals living in urban, peri-urban and rural communities. In 2007–08, 132 currently lactating women from the CLHNS were identified and invited to participate in a study of mother's milk composition, breastfeeding behaviours and maternal and infant health. All agreed to participate. The present analyses were limited to offspring <24 months of age ($n = 121$). Data from four individuals were excluded based upon incomplete dietary data, low sample volumes or fatty acid profiles that suggested sample contamination (such as mastitis), resulting in a final analyses sample of 117 mother–offspring pairs. Prior to the start of the study, the study design was approved by the Institutional Review Board at Northwestern University in June 2007.

Data collection

Lactating women were interviewed at home between 6 am and 10 am. Interviews were conducted in the local language (Cebuano) by a native speaker. Each mother was asked to report her usual breastfeeding practices, health recalls for herself and her infant, and information on household composition, assets and daily activities. Dietary recalls were collected for each mother using a standard instrument for the CLHNS. Mothers were asked to list each food, by local name, they had consumed in the past 24 h. Size measures, such as spoons and measuring cups, were used to determine portion sizes. Food items were converted to standardized codes and analysed for nutritional information using a Philippines-specific nutritional calculation programme available from the Food and Nutrition Research Institute of the Philippines (17). Daily intakes of energy, macronutrients and micronutrients were calculated for each mother using known nutritional information for the dietary items after adjustment for portion size. As expected, boiled rice was the primary source of dietary energy and carbohydrates.

After the interview, maternal height, weight, mid-upper arm circumference and tricep, bicep, subscapular and suprailiac skinfold thickness measurements were collected among mothers. Height and weight were used to calculate maternal BMI, and maternal body fat was calculated using the four sites' skinfold thickness equations from Durnin and Womersely (18) consistent with prior analyses in the CLHNS. Infants weight was measured using portable baby scale (Seca 334) and length using a portable folding infantometer (Seca 417). A milk sample was collected during the interview following established protocols (19), collected at least 1 h after the previous nursing bout. Briefly, the infant was allowed to nurse for 2–3 min on either breast. Afterwards, the infant was switched to the opposite breast and the mother hand expressed an 8–10 mL sample into a sterile polypropylene container. Samples were placed in a cooler with ice packs for transport to the laboratory at the University of San Carlos where they were vortexed to ensure equal mixing before being aliquoted and frozen at −30°C. All samples were frozen within 2–3 h of collection.

Sample analyses

Frozen milk samples were shipped on dry ice to the University of Idaho where they were analysed in the McGuire laboratory. Lipid samples were analysed as described in Mosley et al. (20). Briefly, milk was extracted using a 2 : 1 chloroform/methanol ratio (21). Lipid content was determined gravimetrically and then extracted from the milk column. The extracted lipid was converted to fatty acid methyl esters (FAME) using a base-catalysed transesterification method. The FAME was suspended in hexane and analysed on a gas chromatograph (Hewlett-Packard 6890 Series with auto injector) fitted with a flame ionization detector and a 100×0.25 mm (0.2 μm film) capillary column coated with CP-Sil 88 (Chrompack, Middelburg, The Netherlands). Peaks were identified by comparison with known fatty acid standards. Response correction factors determined by the analysis of a butter oil standard with certified values (CRM 164; European Community Bureau of Reference, Brussels) are used to convert fatty acid peak area percentage to weight percentage.

Limitations of the study

This study relies on a single morning sample, collected by hand expression between 6 am and 10 am, following the protocols of Ruel et al. (19). A single 10-mL sample of mid-milk was used as a measure of human milk fatty acid composition, as this has been demonstrated to be a reasonable estimate of full mammary evacuation (22). Daly et al., (23) have shown modest variability in milk fatty acids across a day, with long-chain, dietary-derived fatty acids such as 18 : 1 and 18 : 2 having a significant association with milk volume. A single sample collected at the beginning or middle of a nursing bout is commonly used for milk fatty acid analyses (6,24), but may result in slightly overall lower amounts of LCPUFA including DHA (25).

Statistical methods

All analyses were conducted using STATA 10.1 (StataCorp LP, College Station, TX, USA). Descriptive statistics were run for all lactating women. Mothers excluded based upon missing data did not differ from included mothers in height, weight, BMI, infant age, breastfeeding frequency or infant size; mothers excluded on the basis of infant age did not differ in weight, height or BMI from included mothers but did have, as expected, older and heavier infants (all $p > 0.000$). In the included sample, maternal descriptive statistics were also run comparing mothers with low and high milk DHA. Multivariate linear regression was used to test for an association between self-reported weekly fish consumption and milk DHA. Further models added groups of secondary predictors: infant- and maternal-level predictors (infant age, maternal BMI and parity), maternal dietary predictors (dietary energy, percentage of calories from fat and protein) and social predictors (mother's education, household assets). The final model includes only significant predictors identified in the three initial regression models. As a second measure, we calculated theoretical daily infant DHA intake assuming a standard intake of 750 mL of milk each day. Daily DHA intake was estimated from the percentage of milk fat as DHA (0.4 ± 0.19%) by total milk fat (40 ± 15 g/L). Individuals were divided into three categories of fish consumption (≤1 week, 2–3 times per week, and ≥4 per week) for comparison.

RESULTS

Mothers in this sample reported energy intakes below recommended dietary intakes for lactating women, with the majority of calories derived from carbohydrates (Table 1). The mean weekly frequency of fish consumption for this sample was 3.3 ± 2.9 portions/week, with 10% of mothers reporting fish consumption less than twice per week and an equal number reporting daily consumption. In this sample, mean daily carbohydrate intake was 225 ± 102 grams, representing nearly half of maternal calories. Fat and protein intakes were much lower, with daily intakes of less than one-fifth that of carbohydrates. The mothers in the sample had relatively low mean energy intakes (1421 ± 700 kcal) and were lean (BMI 20.2 ± 3.5), suggesting modest energy malnutrition considering the increased caloric needs during lactation. The association between fish consumption and milk DHA has not been investigated in similar populations with moderate energy restriction during lactation.

Maternal self-reported weekly fish consumption had a significant, positive association with milk DHA in an unadjusted regression model (Table 2). After adjustment for maternal dietary energy intake, calories from fat and protein, fish consumption remained a borderline significant ($p < 0.06$) predictor of milk DHA content (Table 2). Percentage of calories from fat had an inverse association

Table 1 Characteristics of the sample by DHA content of milk*

	DHA content of milk			
	All (SD)	Lower half	Upper half	p-value
Milk DHA (% total fat)	0.40 (0.19)	0.25 (0.08)	0.55 (0.15)	0.00
Milk fat (grams/100 mL)	4.0 (1.5)	4.1 (1.6)	3.9 (1.5)	0.38
Mother's age (years)	24.1 (0.30)	24.1 (0.28)	24.1 (0.28)	0.93
Mother's BMI (kg/m2)	20.2 (3.5)	20.3 (3.1)	20.0 (3.9)	0.64
Mother's weight (kg)	45.8 (8.7)	46.5 (8.3)	44.9 (9.1)	0.29
Mother's height (cm)	150.4 (5.8)	151.0 (5.6)	149.8 (6.0)	0.26
Parity (n)	2.3 (1.0)	2.2 (1.0)	2.5 (0.9)	0.17
Dietary energy (kcal)	1417 (700)	1620 (731)	1209 (602)	0.00
Percentage of calories from fat	19 (13)	23 (14)	15 (12)	0.00
Percentage of calories from protein	19 (5.0)	20 (6)	19 (5)	0.33
Frequency fish eaten (n/week)	3.3 (2.9)	3.2 (2.7)	3.5 (3.1)	0.26
Urban dwelling (%)	60.7	68	52	0.06
Mother's education (years)	9.6 (2.9)	10.0 (2.7)	9.3 (3.1)	0.20
Infant age (months)	9.6 (6.4)	8.8 (6.2)	10.5 (6.5)	0.17
Infant sex (% male)	49	55	48	0.53
Infant weight (g)	7162 (1834)	7128 (1933)	7174 (1745)	0.90
Infant birth weight (g)†	3017 (661)	3066 (730)	2964 (575)	0.43

*All values are means with standard deviations given in parentheses, for the sample of 117 mothers unless otherwise indicated.
†n = 116; birth weight was unavailable for one infant.

Table 2 Regression models predicting human milk DHA as a percentage of total fatty acids§

	Unadjusted	Model 1	Model 2	Model 3	Model 4
	Unadjust B (SE)	Adjust. B (SE)	Adjust. B (SE)	Adjust. B (SE)	Adjust. B (SE)
Fish consumption	0.013 (0.006)*	0.013 (0.006)*	0.016 (0.006)†	0.016 (0.006)†	0.015 (0.006)†
Dietary energy (kcal)¶		−0.004 (0.034)			−0.004 (0.033)
Calories from fat (%)¶		−0.095 (0.023)†			−0.092 (0.023)†
Calories from protein (%)¶		0.179 (0.065)*			0.186 (0.064)‡
Body mass index (kg/m^2)			0.000 (0.005)		
Infant age (months)			0.002 (0.003)		
Parity (child number)			0.035 (0.018)		0.037 (0.017)*
Education (years)				−0.003 (0.007)	
Household assets				−0.008 (0.009)	
Lives in an urban area				0.057 (0.037)	
R^2	0.03	0.18	0.05	0.05	0.21

*p < 0.05, †p < 0.01, ‡p < 0.001.
§Five regression models testing the association between percentage of total milk fatty acids as DHA and maternal self-reported weekly fish consumption. Models were adjusted for additional predictors as described previously.
¶Dietary covariates were log-transformed for analyses, and coefficients reflect the change in a log unit of dietary energy or percentage of calories from fat or protein.

with milk DHA, and calories from protein had a positive association with milk DHA, although dietary energy intake itself was not a significant predictor in any model (Table 2). Calories from protein were positively correlated with frequency of fish consumption ($r = 0.24$, $p < 0.009$); however, there was not a significant correlation between fish consumption and dietary fat ($r = 0.02$, $p < 0.84$). Increased fish consumption may result in higher protein intakes but lower fat intakes compared with a diet where meats, such as chicken or pork, are the primary protein sources. The fish species commonly consumed in Cebu are comparatively lower in fat than these alternative sources of protein with much more of the fat in the form of LCPUFAs that can be readily incorporated into human milk.

Measures of maternal adiposity, infant age and socioeconomic status (highest completed grade and household assets scale) were also not significant predictors of milk DHA; however, parity was a significant positive predictor. Rural women had more DHA in their milk than urban women, although the differences did not reach significance ($p < 0.12$). Only weekly fish consumption, maternal parity and calories from fat and protein were significant predictors of milk DHA. After adjustment for these additional predictors, there remained a significant dose-dependent association between fish consumption and milk DHA ($r^2 = 0.21$, $p < 0.01$). In this final model, each additional portion of fish consumed per week predicted a 0.014% increase in the total fatty acids composed by DHA (Fig. 1), despite the relatively modest caloric intakes and overall lower intakes of protein and fat compared with other populations in which this relationship has been studied.

Finally, we estimated DHA transfer to the infant based upon total milk fat, % of fat as DHA, and based upon a standard volume transfer for exclusively breastfed infants. Group mean comparisons are shown in Figure 2 based on variation in weekly fish consumption by mothers. Infants of women consuming fish once a week or less had only 80% of the DHA intake of infants whose mothers consumed fish more than three times a week. Although the DHA intakes of infants whose mother's consumed fish two to three times per week and those who consuming 4+ times per week look similar, this was secondary to the reduced total milk fat among women reporting the highest weekly fish consumptions.

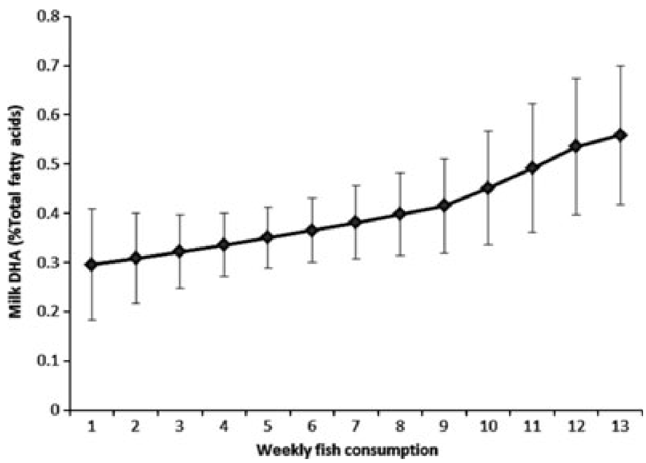

Figure 1 Milk DHA content as a percentage of total fatty acids (mean plus SEM) by weekly self-reported fish intake after adjustment for dietary predictors (energy, calories from fat and protein), parity and infant age.

DISCUSSION

Maternal fish consumption is positively associated with milk DHA content in this sample. Each additional portion of fish consumed predicted a 0.014 increase in the percentage of milk fat in the form of DHA. Fish intake was primarily a locally caught fish known as bodboron that can be either *Auchxis rochei* or *Auxis thazard* (bullet tuna/frigate

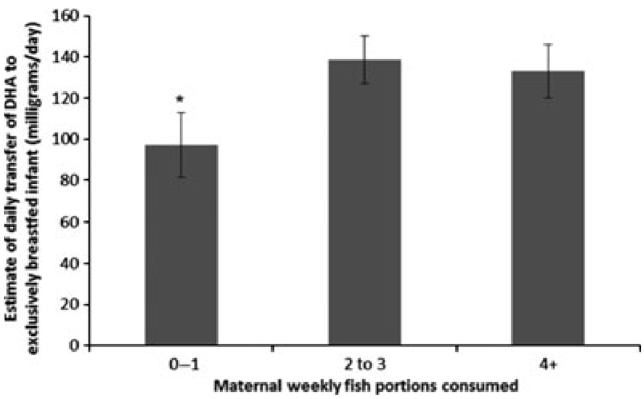

Figure 2 Estimates of daily transfer of DHA to an exclusively breastfed 6-month-old infant based upon three categories of weekly maternal fish consumption among Cebu women. Bars represent linear predictors with standard error, after adjustment for infant age, maternal parity and maternal nutritional intakes (energy, calories from fat and protein). Infants of women with fish intakes one or less times per week had significantly lower daily DHA intakes compared with infants whose mothers consumed fish two or more times each week ($p < 0.035$).

mackerel) depending on preparation and dish. Several other fish were also identified (Table S1 in Supporting Information). The most common fish women reported eating are not commercial fish; as such, limited information regarding species-specific DHA content is available. As a rough estimate, based on published reports, *A. rochei* is estimated to have approximately 20% of total fatty acids by weight as DHA (26).

Prior work on maternal fish consumption and milk DHA has been comparatively limited, with the majority of associations between fish consumption and milk DHA reported at the population level. Within populations, only a handful of studies in a relatively small number of lactating Danish women have reported a positive association between fish consumption and milk DHA (13,14). Fish intakes for the Danish women were within the ranges reported here, although portion sizes and energy intakes were likely larger. Overall, milk DHA in these women was higher than what we report at Cebu, despite similar weekly intakes, possibly reflecting differences in fish species consumed and portion sizes. For example, there is well-documented variability in DHA by fish species, with cold water fish typically having greater amounts of DHA and similar fatty acids than warm water, tropical fish.

In Cebu, milk DHA content was lower than reported for other habitually fish-consuming populations from the Pacific region, but was in line with prior reports from the Caribbean and other populations (6,7). In addition, the level of DHA reported at Cebu was lower than that described previously in several samples of women living in other areas of the Philippines. Two recent studies of women in Manila reported mean DHA as a percentage of total fat of $0.65 \pm 0.49\%$ ($n = 100$) and $0.74 \pm 0.05\%$ ($n = 54$), higher than the $0.40 \pm 0.19\%$ that we found in Cebu (27,28). Reported nutritional intakes were greater among the women studied by Tiangson et al. (27), which might have resulted in greater maternal DHA intakes in these women. Comparative dietary data were not available for Yuhas et al. (28). In addition, methodological differences in collection technique may have influenced results, as da Cunha et al., (25) recently demonstrated small but significant increases in DHA from fore to hind milk that had not been previously reported for other fatty acids (15). This study collected mid-feed samples, while prior studies collected full mammary expression (28) and hind milk samples (27). Although protocol differences between studies make it challenging to compare absolute values between populations, the uniform sampling protocol used across all women in the present study will help ensure within-study comparability as reflected in the significant relationships with fish consumption that we document. Additionally, it has been suggested that maternal genetic variation at the FAD3 locus may explain some of the differences in milk LCPUFA content, particularly in relationship to fish intake (29). However, it is unlikely that these genetic differences explain the variability in milk fatty acids seen within this sample.

Compared with other studied populations, the overall energy, fat and protein intakes for this sample are quite low (Table 1). The lower energy intakes in this sample were paired with relatively low intakes of protein and fat as well. Dietary protein had a positive association with milk DHA and dietary fat an inverse association. While these findings seem counterintuitive, they may in fact be indexing fish consumption. Dietary protein, but not fat, is positively associated with fish consumption in this sample and is also independent of dietary fat intake. These associations provide additional support for a beneficial effect of increased fish consumption on milk DHA content. In addition to the protocol differences noted previously, one possible contributor to the lower milk DHA reported at Cebu compared with other habitually fish-consuming populations may be the overall lower dietary intakes and nutritional status of these mothers. For instance, portion size of fish, independent of frequency, was relatively small, which could contribute to the low milk DHA relative to fish intake.

At present, there are no established recommendations for DHA intake by infants (8). Koletzko et al., (1) suggest that a minimum of 0.2–0.3% of total fat should be in the form of DHA. Almost all mothers at Cebu, including those with low fish consumption (<2 times weekly), produced milk with this minimum level of DHA. However, maternal fish consumption is associated with dose-dependent improvements in estimated infant DHA intake, which, as shown in randomized, controlled clinical trials of formula fortification, is modestly beneficial to infant visual and cognitive development. At Cebu, the percentage of total milk fat as DHA increased with frequency of fish consumption, suggesting potential benefits to offspring, and women with higher fish consumption were estimated to transfer more DHA to their exclusively breastfed infants. However, because women with the highest fish intakes also produced milk with modestly lower total milk fat content, estimates for total DHA transfer to a breastfed infant did not increase further at the highest levels of fish consumption. The increase in milk fat predicted by maternal consumption of fish suggests that this is an effective way of increasing DHA availability to infants and possibly promoting infant cognitive and visual development. These potential benefits must be weighed against the risk of increasing dietary mercury, as many of the optimal sources of DHA (salmon, tuna) may also have high levels of mercury. Frigate tuna, and other 'lower' quality tunas, as consumed by this sample and frequently used as canned tuna in the United States (30), appear to have lower mercury levels than higher grade (so-called 'sushi grade' tuna) and may be a way of ameliorating risks of mercury exposure while increasing preformed DHA intake.

CONCLUSIONS

In this sample of lactating Filipino women, we report a positive association between maternal fish consumption as measured by weekly fish intake and human milk DHA content. Compared with fish oil supplements, promoting increases in fish consumption may be a cost-effective method of improving milk DHA and adequate essential fatty acid delivery in support of offspring cognitive development in populations in which supplementation with fish oil- or other DHA-rich sources is not available or cost-effective. Prior investigations of fish consumption and milk DHA had been largely limited to well-nourished samples of European women. Similar findings here suggest that the positive associations between dietary fish intake and milk DHA may apply widely across populations varying widely in diet and nutritional sufficiency.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Species of fish commonly consumed by mothers in this sample.