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Perinatal Consumption of Thiamine-Fortified Fish Sauce in Rural Cambodia
A Randomized Clinical Trial

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Editorial

Supplemental content

IMPORTANCE Infantile beriberi, a potentially fatal disease caused by thiamine deficiency, remains a public health concern in Cambodia and regions where thiamine-poor white rice is a staple food. Low maternal thiamine intake reduces breast milk thiamine concentrations, placing breastfed infants at risk of beriberi.

OBJECTIVE To determine if consumption of thiamine-fortified fish sauce yields higher erythrocyte thiamine diphosphate concentrations (eTDP) among lactating women and newborn infants and higher breast milk thiamine concentrations compared with a control sauce.

DESIGN, SETTING, AND PARTICIPANTS In this double-blind randomized clinical trial, 90 pregnant women were recruited in the Prey Veng province, Cambodia. The study took place between October 2014 and April 2015.

INTERVENTIONS Women were randomized to 1 of 3 groups (n = 30) for ad libitum fish sauce consumption for 6 months: control (no thiamine), low-concentration (2 g/L), or high-concentration (8 g/L) fish sauce.

MAIN OUTCOMES AND MEASURES Maternal eTDP was assessed at baseline (October 2014) and endline (April 2015). Secondary outcomes, breast milk thiamine concentration and infant eTDP, were measured at endline.

RESULTS Women's mean (SD) age and gestational stage were 26 (5) years and 23 (7) weeks, respectively. April 2015 eTDP was measured among 28 women (93%), 29 women (97%), and 23 women (77%) in the control, low-concentration, and high-concentration groups, respectively. In modified intent-to-treat analysis, mean baseline-adjusted endline eTDP was higher among women in the low-concentration (282nM; 95% CI, 235nM to 310nM) and high-concentration (254nM; 95% CI, 225nM to 284nM) groups compared with the control group (193nM; 95% CI, 164nM to 222M; $P < .05$); low-concentration and high-concentration groups did not differ ($P = .19$). Breast milk total thiamine concentrations were 14.4 µg/dL for the control group (95% CI, 12.3 µg/dL to 16.5 µg/dL) (to convert to nanomoles per liter, multiply by 29.6); 20.7 µg/dL for the low-concentration group (95% CI, 18.6 µg/dL to 22.7 µg/dL); and 17.7 µg/dL for the high-concentration group (95% CI, 15.6 µg/dL to 19.9 µg/dL). Mean (SD) infant age at endline was 16 (8) weeks for the control group, 17 (7) weeks for the low-concentration group, and 14 (8) for the high-concentration group. Infant eTDP was higher among those in the high-concentration group (257nM; 95% CI, 222nM to 291nM; $P < .05$) compared with the low-concentration (212nM; 95% CI, 181nM to 244nM) and control (187nM; 95% CI, 155nM to 218nM) groups.

CONCLUSIONS AND RELEVANCE Compared with women in the control group, women who consumed thiamine-fortified fish sauce through pregnancy and early lactation had higher eTDP and breast milk thiamine concentrations and their infants had higher eTDP, which was more pronounced in the high group. Thiamine-fortified fish sauce has the potential to prevent infantile beriberi in this population.

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Infantile beriberi is caused by thiamine (vitamin B_1) deficiency and typically presents among breastfed infants around 3 months of age[1-3] with a persistent hoarse cry, vomiting, anorexia, generalized edema, oliguria,[4] convulsions, and signs of heart failure.[5] Without rapid thiamine administration, infants can die within hours.[3] Mothers with poor dietary thiamine intake produce breast milk low in thiamine, placing their infants at risk of developing infantile beriberi.[2] During infancy, a period of rapid growth and development, thiamine needs are high relative to body size. Infants develop beriberi, while mothers typically remain asymptomatic.[1] Umbilical cord blood of thiamine-replete mothers has up to 3 times higher thiamine concentrations than maternal blood at birth[6] owing to preferential thiamine sequestration to the fetus during the third trimester.[7] Thus, maternal thiamine deficiency throughout pregnancy can further increase the risk of developing infantile beriberi.[2] Marginal thiamine deficiency causes apathy, fatigue, loss of appetite, and dizziness[8] and may be common among vulnerable risk groups (eg, elderly individuals)[9] and those with high-carbohydrate diets.[10]

Infantile beriberi is often viewed as a historic disease[8] but has recently been called "a forgotten disease in Asia."[11] It remains a modern public health concern with reports in the literature from Cambodia,[5,12] Laos,[13] and Burma.[3,14] However, representative prevalence data are not available, likely because nonspecific signs of this disease prevent diagnosis. In Cambodia, thiamine deficiency and beriberi stem from a lack of dietary diversity.[15] Food balance sheets indicate approximately 60% of daily calories[16] come from polished white rice, a poor source of thiamine,[1,8] and thiamine-rich foods are not typically consumed. We reported significantly lower erythrocyte thiamine diphosphate concentrations (eTDP), an indicator of thiamine status, among a representative sample of nonpregnant, nonlactating Cambodian women of childbearing age living in the rural Prey Veng province of Cambodia compared with a convenience sample of healthy, purportedly vitamin-replete Canadian women living in Vancouver, British Columbia, Canada.[17,18] Because thiamine requirements increase during pregnancy and lactation,[19] these women are possibly at an even higher risk of thiamine deficiency.

Supplementation of thiamine-deficient lactating women has been shown to improve breast milk thiamine concentrations[3,20-22]; however, supplementation is a targeted, resource-intensive intervention that relies heavily on individual compliance. Alternatively, food fortification is a passive intervention that requires little to no behavior change.[23] An estimated 90% of Cambodian individuals consume fish sauce, and this condiment is already a fortification vehicle for iron.[24] As such, thiamine fortification of fish sauce could be a sustainable, low-cost, and passive intervention[23,25] to improve the dietary thiamine intake of pregnant and lactating women and in turn their breastfed infants.

The aim of this study was to determine whether consumption of low- or high-concentration thiamine-fortified fish sauce by rural Cambodian women over 6 months during pregnancy and early lactation yielded higher eTDP compared with those consuming a control fish sauce containing no thiamine. Secondary outcomes, breast milk thiamine concentrations, and eTDP of participants' breastfed infants were also assessed in this study.

Key Points

Question Does consumption of thiamine-fortified fish sauce throughout late pregnancy and early lactation yield higher erythrocyte thiamine diphosphate concentrations among rural Cambodian mothers and their breastfed infants and higher breast milk thiamine concentrations compared with consumption of a control sauce containing no thiamine?

Findings In this double-blind randomized clinical efficacy trial, erythrocyte thiamine diphosphate concentrations were significantly higher among women consuming thiamine-fortified fish sauce and their infants than in the control group.

Meaning Thiamine-fortified fish sauce has the potential to prevent beriberi-related infant mortality in Cambodia and throughout Southeast Asia where diets are thiamine-poor and infant mortality remains high.

Methods

Study Design

This was a double-blind, 3 parallel arm, randomized clinical efficacy trial conducted among a convenience sample of 90 pregnant women in Prey Veng province, Cambodia, between October 2014 and April 2015. Eligibility criteria were as follows: 18 to 45 years old and 3 to 8 months pregnant with a singleton fetus (self-report); the female head of their household; planned to exclusively breastfeed their infant for 6 months; no history of preeclampsia, preterm delivery, or birth defects; not involved in other nongovernmental nutrition programs; not consuming thiamine-containing dietary supplements; agreement to exclusively consume the study fish sauce in their household; and no plans to leave their village for the duration of the study (6 months). Women provided written informed consent to participate. The Cambodian National Ethics Committee for Health Research and the University of British Columbia–Children's and Women's Health Centre of British Columbia Research Ethics Board approved the study. The formal trial protocols can be found in the Supplement.

Intervention: Fortified Fish Sauce

Study fish sauce was produced by Leang Leng Enterprises (Phnom Penh, Cambodia) and received Cambodian Ministry of Health Certification for Hygienic Food Production. Fish sauce was pasteurized for 90 to 120 minutes at 100°C, cooled to room temperature, then fortified as follows: low-concentration thiamine-fortified fish sauce (low, 2 g/L thiamine hydrochloride [≥98% purity], Huazhong Pharmaceutical Co); high-concentration thiamine-fortified fish sauce (high, 8 g/L thiamine hydrochloride); and control, which contained no detectable thiamine. Fortification levels were set based on previous self-reported daily fish sauce consumption among nonpregnant women in Prey Veng, Cambodia. All fish sauces were also fortified with iron as ferric sodium ethylenediaminetetraacetate (2.8 g/L; Ferrazone, Akzo Nobel Functional Chemicals BV) as per Cambodian Ministry of Planning guidelines.[24]

Fish sauce packaging was identical among the 3 groups except for a unique serial number stamp on each bottle and different colored caps for different treatment arms intended to prevent confusion among field staff and participants. Green, orange, and purple were selected for bottle caps after consultation with local women highlighted these as pleasant culturally and politically neutral colors (n = 60 women aged 18-45 years from focus group discussions in 12 villages in Prey Veng). The blinding code was known to Leang Leng Enterprises and was stored in a sealed envelope held by the principal investigator in case of emergency.

Randomization

Using a predetermined master list of individual randomized identification numbers, field staff assigned identification numbers sequentially at enrollment. Women received fish sauce after their baseline study visit, and fortnightly thereafter during household visits by field staff. Because Cambodian families eat from a common pot, participants and their entire household were instructed to consume the study fish sauce ad libitum, as they normally would, throughout the 6-month study. At 1 month (November 2014), study participants attended a nutrition education workshop in their village to learn the signs, treatment, and prevention of infantile thiamine deficiency and beriberi, as well as the Cambodian Ministry of Health infant and young child feeding curriculum.[26]

Data and Biological Sample Collection

At baseline (October 6-17, 2014; mean [SD] gestation, 23 [7] weeks gestation) and endline (*t* = 6 months; April 22-29, 2015; mean [SD] age of infants, 16 [8] weeks), demographic information was collected using an interviewer-administered questionnaire in women's homes. Participants delivered during the study; field staff visited each participant within 72 hours of birth to measure the infant's length and weight using standardized techniques.

The morning following data collection, nonfasting venous blood samples were collected into evacuated edetic acid-coated tubes (Vacutainer; Becton Dickinson) from women and their infants (endline only) at a central village location. Blood samples were then transported on ice to the National Institute of Public Health laboratory in Phnom Penh within 5 hours of collection. Blood samples were centrifuged at 3000 rpm for 15 minutes at 4°C, plasma and buffy coat were removed, and erythrocytes were washed 3 times with phosphate-buffered saline (Amresco) before storage at −80°C.

Breast milk samples were collected at endline using a battery-powered single breast pump (Swing Breastpump; Medela). One full breast expression was collected from the breast that women self-identified as being more "full" (the breast not most recently emptied). Breast milk was transported along with the blood samples to National Institute of Public Health daily on ice. Total milk volume was recorded, then milk was mixed well, aliquoted into amber cryovials, and stored at −80°C. All blood and breast milk samples were shipped on dry ice to the University of British Columbia, Vancouver.

eTDP Analysis

The eTDP concentration was measured at the University of British Columbia using reverse-phase high-performance liquid chromatography with a fluorescence detector using an Agilent 1260 Infinity system with a Poroshell 120 EC-C18 column, 3 × 50 mm, 2.7 μm, protected by a Poroshell FastGuard C18 precolumn (Agilent Technologies) under amber light, according to Lu and Frank,[27] with modifications. Quantitation of eTDP was based on peak area and external standardization using thiamine diphosphate (TDP) calibration solutions (Sigma-Aldrich; standard curve range approximately 20-800nM; recoveries of low [16.8nM] and high [42.1nM] standards in deionized water were 102.5% and 93.2%, respectively). Quality controls were conducted on each run using pooled erythrocytes from 10 apparently healthy Canadian adults (interrun coefficient of variation <9%, n = 17).

Breast Milk Thiamine Analysis

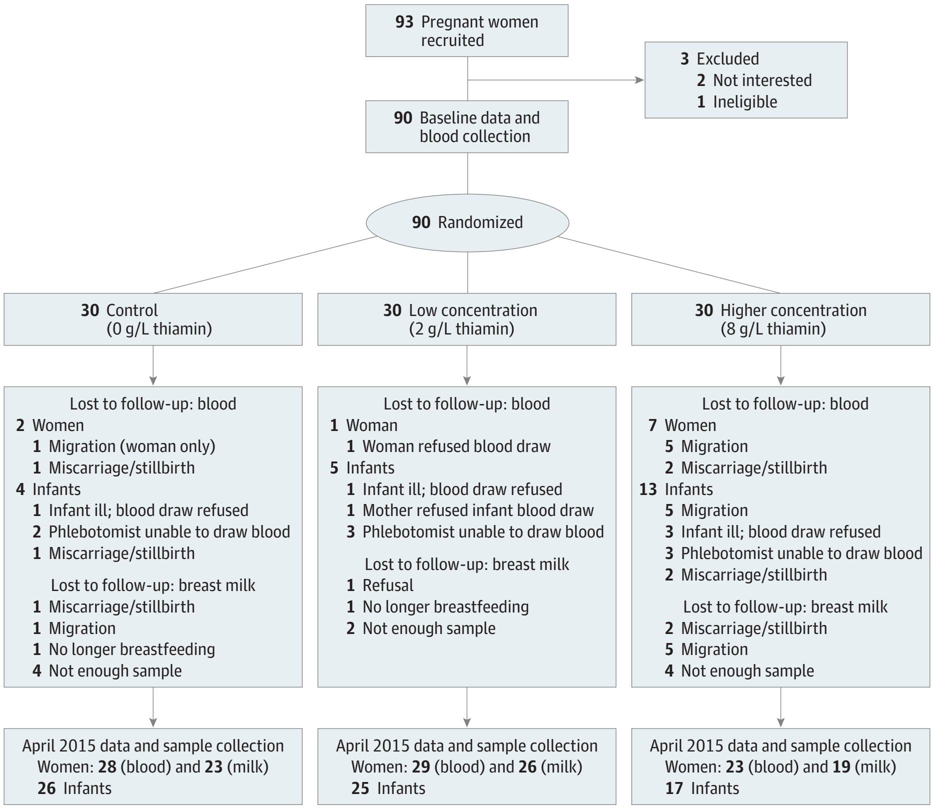
Free thiamine, thiamine monophosphate (TMP), and TDP in breast milk were analyzed at the US Department of Agriculture/ARS Western Human Nutrition Research Center, University of California, Davis, using high-performance liquid chromatography with a fluorescence detector and precolumn derivatization of the analytes to their thiochrome esters. Samples were analyzed via an Agilent 1200 series high-performance liquid chromatography with a fluorescence detector using a Phenomenex Kinetex C18 column, 150 × 4.6 mm, 5 μm, protected by a C18 precolumn, 4 × 3 mm (Phenomenex SecurityGuard); methods have been published elsewhere.[28] A pooled breast milk sample with previously established thiamine concentrations was used as an internal control. This control was measured 4 times; coefficients of variation for thiamine, TMP, and TDP were 2%, 2%, and 5%, respectively. Total thiamine was calculated from free thiamine, TMP, and TDP concentrations measured based on molecular weights: total thiamine = free thiamine + (TMP × 0.871) + (TDP × 0.707).

Statistical Analysis

We estimated a sample size of 30 mothers per group would be sufficient to detect a 30% difference in the primary outcome measure, maternal eTDP, between fortified and control groups at endline, assuming a minimum baseline of 38nM,[17] SD of 18nM,[29] 80% power, and $\alpha = .05$ using a 2-sided test. Demographic characteristics were summarized as mean (standard deviation) or number (percentage). A modified intent-to-treat approach was used to analyze maternal and infant eTDP and breast milk thiamine concentrations (3 women with stillbirths or miscarriage were excluded from analyses). Multiple imputation was used to impute missing values for maternal and infant eTDP and breast milk thiamine, TMP, and total thiamine concentrations. Multiple imputation could not be used for missing breast milk TDP values because there was too much variability and more than half of values imputed were negative; therefore, values were replaced with the mean.

General linear regression models with post hoc analysis with least significant difference adjustment for multiple comparisons were used to assess differences between the 3 treatment groups (low, high, and control) for the primary

Figure. Participant Flow and Follow-up for Women Aged 18 to 45 Years and Their Newborn Breastfed Infants in Prey Veng, Cambodia



outcome measure and maternal endline eTDP (adjusted for baseline eTDP), as well as for infant eTDP, breast milk thiamine concentrations, infant age, lactation duration, and fish sauce consumption. We reported estimated marginal means (95% confidence intervals) and predicted means of the outcome variable for the fitted models. All analyses were performed with SPSS for Macintosh, version 23.0 (IBM Corp), with a significance level of $P < .05$.

Results

Participant flow and follow-up can be found in the **Figure**. Ninety-three pregnant women in Prey Veng were screened; 90 women met the eligibility criteria, agreed to participate, and were randomized to the 3 study arms, n = 30 per group. Three participants were excluded from analyses owing to stillbirth or miscarriage. April 2015 maternal blood collection yielded n = 28, n = 29, and n = 23 for control, low, and high groups, respectively.

Baseline demographic characteristics of participants can be found in **Table 1**. Mean (SD) age of women at enrollment was 26 (5) years, most women were in their second trimester (mean [SD], 23 [7] weeks' gestation), and approximately half of women were pregnant with their first child. Most women attended 5 antenatal care visits throughout pregnancy and delivered at their local health center (**Table 2**). Mean (SD) birth length and weight were 49 (2) cm and 3.1 (0.6) kg, respectively, and only 5 infants had low birth weight (<2.5 kg). Household fish sauce consumption was similar across all 3 treatment groups: households in the low, high, and control groups consumed a mean of 15 (95% CI, 13-16), 13 (95% CI, 11-14), and 16 (95% CI, 14-17) bottles (750 mL each), respectively, during the 6-month study ($P = .07$).

Maternal baseline-adjusted endline eTDP and infant eTDP can be found in **Table 3**. Baseline-adjusted endline eTDP of women in the control group (193nM; 95% CI, 164-222nM) was lower than women in the low (282nM; 95% CI, 164-310nM; $P < .001$) and high groups (254nM; 95% CI, 225-284nM; $P = .004$); low and high groups did not differ ($P = .19$). Infants of mothers in the high group (257nM; 95% CI, 222-291nM) had higher eTDP compared with infants of mothers in the control group (187nM; 95% CI, 155-218nM; $P = .004$), but not the low group (212nM; 95% CI 181-244nM; $P = .07$). Concentrations of the 3 thiamine vitamers found in human milk, thiamine, TMP, and TDP, as well as total thiamine (calculated as the amount of thiamine from TMP, TDP, and thiamine), can be found in **Table 4**.

Discussion

To our knowledge, this is the first study that shows that perinatal consumption of a novel thiamine-fortified fish sauce for 6 months yields higher eTDP among lactating women and their breastfed infants in rural Cambodia compared with mothers consuming a control fish sauce without thiamine.

Table 1. Baseline Demographic and Biochemical Characteristics of Women Aged 18-45 Years in Prey Veng, Cambodia

Characteristic	Control (n = 28)	Low (n = 29)	High (n = 28)
Age, mean (SD), y	27 (5)	26 (5)	25 (5)
Gestation, mean (SD), wk	23 (8)	22 (7)	25 (8)
Parity, No. (%)			
Primiparous	13 (46)	16 (55)	14 (50)
Multiparous	15 (54)	13 (45)	14 (50)
Household members, mean (SD), No.	5 (1)	5 (2)	5 (1)
Education, No. (%)			
None	2 (7)	3 (10)	1 (4)
Primary	11 (39)	15 (52)	12 (43)
Lower secondary	12 (43)	8 (28)	14 (50)
Upper secondary	2 (7)	3 (10)	1 (4)
Higher education	1 (4)	NA	NA
Annual household income, mean (SD), US $[a]	1625 (1156)	1440 (1233)	1388 (1301)
Bottom 20%, No. (%)	2 (7)	3 (10)	4 (14)
Middle 60%, No. (%)	11 (39)	15 (52)	11 (39)
Top 20%, No. (%)	15 (54)	11 (38)	13 (47)
Erythrocyte thiamine diphosphate, mean (SD), nM	152 (44)	175 (62)	179 (63)

Abbreviation: NA, not applicable.

[a] Households were classified using income quintiles (2011) from Prey Veng, Cambodia.[30]

Table 2. Antenatal Care and Delivery Outcomes of Rural Cambodian Women Aged 18 to 45 Years and Characteristics of Their Newborn Infants

Characteristic	Control (n = 27)	Low (n = 28)	High (n = 23)
Antenatal, mean (SD), No.			
Antenatal care visits	5 (2)	5 (2)	5 (3)
Iron folic acid tablets	85 (19)	78 (25)	85 (23)
Deworming treatment	23 (85)	20 (71)	20 (87)
Delivery/postpartum			
Delivery location, No. (%)			
Local health center	13 (48)	17 (61)	9 (39)
District hospital	1 (4)	1 (4)	6 (26)
Provincial town hospital	3 (11)	1 (4)	2 (9)
Private clinic or hospital	10 (37)	8 (29)	6 (26)
Other: garment factory	NA	1 (4)	NA
Infant characteristics			
Female, No. (%)	14 (52)	13 (46)	9 (39)
Birth weight, mean (SD), kg[a]	3.1 (0.5)	3.0 (0.4)	3.1 (0.9)
Low birth weight (<2.5 kg), No. (%)[a]	3 (11)	1 (4)	1 (4)
Birth length, mean (SD), cm[a]	49 (2)	49 (2)	49 (3)
Age at endline, mean (SD), wk	16 (8)	17 (7)	14 (8)

Abbreviation: NA, not applicable.

[a] Low-concentration group, n = 27.

These results are similar to previous studies showing that maternal thiamine supplementation resulted in higher breast milk thiamine concentrations. Prentice and colleagues[20] reported a significant increase in breast milk thiamine concentration from 16.0 μg/dL to 22.0 μg/dL ($P < .001$; to convert to nanomoles per liter, multiply by 29.6) among Gambian women consuming a supplemental food containing 1.36 mg of thiamine daily for 1 year. Similarly, breast milk thiamine of 5 thiamine-deficient Indian women increased from 10.9 μg/dL to 26.8 μg/dL after daily micronutrient supplementation of increasing doses (0.2 mg/d to 20 mg/d) over 8 months.[21]

While eTDP was significantly higher among women consuming fortified fish sauce compared with women in the control group, there was no significant difference between women in the low and high groups. This finding suggests that consumption of fish sauce fortified at or even below 2 g/L is sufficient to establish maternal thiamine adequacy. However, eTDP was significantly higher among infants of mothers in the high group (257nM; 95% CI, 222-291nM) compared with the control group (187nM; 95% CI, 155-218nM; $P = .004$), but not among infants of mothers in the low group (212nM; 95% CI, 181-244nM) compared with the control group ($P = .26$),

Table 3. Maternal and Infant eTDP at Endline (6 Months) by Treatment Arm[a]

Group	No.	eTDP, Estimated Marginal Means (95% CI), nM	*P* Value[b]		
			Control vs Low	Control vs High	Low vs High
Mothers					
Control	28	193 (164-222)	<.001	.004	.19
Low	30	282 (235-310)			
High	29	254 (225-284)			
Infants					
Control	28	187 (155-218)	.26	.004	.07
Low	30	212 (181-244)			
High	29	257 (222-291)			

Abbreviation: eTDP, erythrocyte thiamine diphosphate concentrations.

[a] General linear regression models using modified intent-to-treat analysis (missing data points: mothers, n = 7 [control, n = 1; low, n = 1; high, n = 5] and infants, n = 19 [control, n = 3; low, n = 5; high, n = 11], imputed using multiple imputation). Maternal model was adjusted for baseline eTDP.

[b] Post hoc analysis (with least significant difference adjustment for multiple comparisons) that assessed pairwise differences between low, high, and control groups.

Table 4. Thiamine Concentrations in Mature Human Milk From 1 Full Breast Expression by Rural Cambodian Mothers Aged 18 to 45 Years and Estimated Daily Total Thiamine Intake of Infants Fed This Milk[a]

Thiamine Form	Estimated Marginal Means (95% CI)		
	Control (n = 28)	Low (n = 30)	High (n = 29)
Thiamine, μg/L	23 (10-37)	60 (48-72)	50 (37-63)
TMP, μg/L	135 (118-153)	164 (147-181)	142 (123-160)
TDP, μg/L	4 (2-6)	5 (3-7)	6 (4-7)
Total thiamine, μg/L[b]	144 (123-165)	207 (186-227)	177 (156-199)
Estimated infant intake, μg/d[c]	110 (93-126)	160 (144-176)	135 (118-152)

Abbreviations: TDP, thiamine diphosphate concentrations; TMP, thiamine monophosphate.

SI conversion factor: To convert thiamine to nanomoles per liter, multiply by 29.6.

[a] General linear regression models assessed breast milk thiamine concentrations among women in low, high, and control groups using modified intent-to-treat analysis (missing data points: mothers, n = 19 [control, n = 6; low, n = 4; high, n = 9] imputed using multiple imputation, except for TDP, where missing values were replaced with mean).

[b] Total thiamine calculated as free thiamine + (TMP × 0.871) + (TDP × 0.707).

[c] Estimated infant thiamine intake calculated based on daily breast milk consumption of exclusively breastfed infants in developing countries: 0 to 2 months, 714 mL/d; 2 to 5 months, 784 mL/d; 6 to 8 months, 776 mL/d.[31]

suggesting that higher maternal dietary thiamine intake may be required to ensure adequate transfer of thiamine to the infant during early lactation.

This study had several strengths, including both antenatal and postpartum consumption of fish sauce, frequent fortnightly follow-ups with participants, and biological sample collection from mothers (both blood and breast milk) as well as their breastfed infants. While we collected a full breast expression from the breast that mothers self-reported as not most recently emptied, we did not have information on the last feed, and this 1 expression is not necessarily representative of usual milk.

No infants in this study showed clinical symptoms of infantile beriberi; however, a lack of clear biomarker cutoffs hinders assessment of biochemical thiamine adequacy. The Institute of Medicine used observed thiamine concentrations of 21.0 μg/dL in milk produced by well-nourished mothers[32,33] to set the adequate intake (AI; a guideline for dietary nutrient intake) of thiamine for infants aged 0 to 6 months at 200 μg/d.[19] Using estimated daily breast milk intakes for exclusively breastfed infants in developing countries,[31] we predicted daily thiamine intake of infants in our study (Table 4). Only 9 infants (low, n = 7; high, n = 2) would have consumed at least 200 μg/d. Curiously, a 2012 study of thiamine concentrations in mature milk (≥2 weeks) collected from women globally (including the United States) highlighted wide variation in breast milk thiamine concentrations by region,[34] and none achieved the AI.[19,31] This is consistent with a 2015 study in Malawi reporting that only 50% of antiretroviral-treated mothers living with human immunodeficiency virus who had been consuming thiamine-containing dietary supplements for 6 months produced breast milk that would meet the infant thiamine AI.[35] We recognize that AIs are developed when there is not sufficient evidence available to establish an estimated average requirement and recommended dietary allowance and are expected to meet or exceed the needs of individuals in that age group.[19] As such, thiamine intakes lower than the AI of 200 μg/d could be sufficient and may not impose risk of infantile beriberi. Actually, the AI was rounded up from 160 μg/d,[19] but even when using a cutoff of 160 μg/d, only 18 infants (low, n = 14 and high, n = 4) would have had sufficient thiamine intake. The current infant thiamine AI was set based on data from only 24 American women in 1980.[32,33] Improvements in analytical techniques for quantification of thiamine in biological samples during the past 35 years[36] may explain some of the discrepancy between the AI cutoff and values published within the last 5 years; as such, thiamine AIs for infants aged 0 to 6 months should be revisited.

The amount of thiamine in breast milk is known to increase with lactation duration[34]; therefore, the age of the child likely influences breast milk thiamine concentration. In our study, infant age was not associated with total thiamine in breast milk but approached significance (r = .22; P = .07; children aged 3-28 weeks at endline data collection).

The main limitation of our study is a lack of interpretive criteria for normal or healthy eTDP for women because cutoffs in the literature vary widely: greater than 70nM,[37] greater than 140nM,[38] and greater than 148nM.[39] Without cutoffs, it is more difficult to quantify the fortification level required for adequate maternal status to prevent infantile beriberi. However, it is well established that adequate maternal thiamine intake throughout pregnancy and lactation allows for thiamine sequestration in utero[7] and production of thiamine-replete breast milk,[2,19] preventing infantile thiamine deficiency and beriberi among breastfed infants. Herein, we showed that consumption of thiamine-fortified fish sauce, a condiment consumed by most Cambodians,[24] for 6 months throughout late pregnancy and early lactation was associated with maternal eTDP and breast milk thiamine and in turn infant eTDP. This intervention is facilitated by existing fortification infrastructure within existing factories because fish sauce has already been successfully fortified with iron in Cambodia and Vietnam.[24] Therefore, fish sauce could be a simple and sustainable vehicle for thiamine fortification throughout Southeast Asia, and there is potential for the addition of other micronutrients as well. Furthermore, fortification is a population-wide intervention, so thiamine-fortified fish sauce could improve thiamine intake of all consumers, potentially preventing beriberi outbreaks[40] and improving thiamine status of women[17,18] before conception.

Conclusions

Perinatal consumption of thiamine-fortified fish sauce for 6 months resulted in improved eTDP and breast milk thiamine concentrations among lactating women in rural Cambodia and in turn eTDP among their breastfed infants. Thiamine-fortified fish sauce has the potential to prevent infantile beriberi in this population. However, more research designed to enable a large, pragmatic randomized clinical trial is required to address some of the limitations of this efficacy trial, in particular, optimizing the level of fortification and acquiring clinical diagnoses of infantile beriberi.

ARTICLE INFORMATION

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