Thiamine pharmacokinetics in Cambodian mothers and their breastfed infants[1–3]

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ABSTRACT

Background: Thiamine deficiency is common in parts of Asia and causes beriberi. Pharmacokinetics of thiamine in deficient populations are unknown.

Objective: We characterized thiamine pharmacokinetics in Cambodian mothers and their breastfed infants.

Design: Total plasma thiamine, whole-blood thiamine diphosphate (TDP), and breast milk total thiamine were measured in 16 healthy Cambodian mothers and their infants before and after mothers received oral thiamine hydrochloride (100 mg for 5 d). Assays were also performed in 16 healthy American mothers.

Results: On day 1, Cambodian mothers were thiamine deficient, with median (range) total plasma thiamine and TDP concentrations of 2.4 nmol/L (0–4.4 nmol/L) and 58.0 nmol/L (27–98 nmol/L), respectively. After a single oral dose, the mean ± SD maximal concentration of thiamine and net area under the thiamine concentration-time curve were 73.4 ± 45.6 nmol/L and 465 ± 241 h · nmol · L^{-1}. Day 6 median maternal total plasma thiamine and TDP concentrations were normal [18.6 nmol/L (13.4–25.3 nmol/L) and 76.5 nmol/L (48–107 nmol/L), respectively; $P \leq 0.001$ compared with day 1]. Median Cambodian total breast milk thiamine concentration increased from 180 nmol/L (85–359 nmol/L) on day 1 to 403 nmol/L (314–415 nmol/L) on day 2 and 503 nmol/L (360–808 nmol/L) on day 6; the corresponding American breast milk value was 500 nmol/L (114–622 nmol/L). Median Cambodian infant total plasma thiamine and TDP concentrations increased from 3.0 nmol/L (0–7.3 nmol/L) and 38.5 nmol/L (23–57 nmol/L), respectively, on day 1 to 5.6 nmol/L (0–9.7 nmol/L) and 45.5 nmol/L (32–70 nmol/L), respectively, on day 6.

Conclusions: Thiamine-deficient Cambodian mothers effectively absorb oral thiamine, with sharp increases in breast milk thiamine concentrations, but their breastfed infants remain thiamine deficient after 5 d of maternal supplementation. Longer-term maternal supplementation may be necessary to correct thiamine deficiency in breastfed infants. This trial was registered at clinicaltrials.gov as NCT01864057.   *Am J Clin Nutr* 2013;98:839–44.

INTRODUCTION

Infantile beriberi is caused by thiamine deficiency and is common in parts of Southeast Asia (1–3). Thiamine deficiency is associated with traditional rice preparation methods, including the use of nonparboiled polished rice (from which the thiamine-rich bran has been removed) (4), and may be particularly common in nursing mothers and their infants because of in-creased dietary thiamine requirements of pregnancy and lactation (5, 6). Although the accepted cause of thiamine deficiency is dietary inadequacy, to our knowledge, pharmacokinetics of oral thiamine absorption have not been studied in Asian populations at risk of beriberi. Available pharmacokinetic data have assessed thiamine-replete whites (7, 8).

Nursing mothers in the Mesang District of Prey Veng Province, Cambodia are often thiamine deficient, and their dietary thiamine content is approximately one-half of the recommended daily intake (5). The purpose of this study was to assess the single-dose and steady state pharmacokinetics of oral thiamine administration in thiamine-deficient Cambodian mothers and their breastfed infants. We aimed to determine whether mothers effectively absorb oral thiamine and secrete it in their breast milk and whether infant thiamine concentrations improve with maternal supplementation. These data might guide beriberi prevention and treatment strategies.

After absorption, thiamine is present in plasma as thiamine and thiamine monophosphate (TMP)[4]. Thiamine diphosphate (TDP), which is a metabolically important vitamer, is present in red blood cells. HPLC allows for the quantification of these species in plasma and whole blood, respectively (9).

SUBJECTS AND METHODS

We recruited 16 healthy nursing mothers from villages near the Svai Chrum clinic in Mesang District, Prey Veng Province,

Cambodia (latitude: 11.374364°N; longitude: 105.564183°E) in January 2012. Inclusion criteria included maternal age ≥18 y and infant age between 1 and 7 mo, no maternal use of vitamin supplements in the preceding month, infant had not been administered thiamine in the past 2 mo, infant was exclusively breastfed, mother had been nursing ≥2 wk, and no acute illness was present in either the mother or child at the time of enrollment. Procedures followed were in accordance with the Helsinki Declaration. The Cambodian National Ethics Committee for Health Research approved the protocol, and all mothers gave written informed consent.

Steady state thiamine pharmacokinetics were studied in all Cambodian subjects. Baseline fasting blood specimens were obtained on the morning of day 1 from all mothers and infants as well as maternal breast milk specimens. All mothers were administered a single tablet that contained 100 mg thiamine hydrochloride (Asian Union Laboratories Co Ltd). On the morning of days 2, 3, 4, and 5, a study nurse traveled to participants' homes and administered a 100 mg thiamine hydrochloride tablet to each mother daily. On the morning of day 6, 24 h after the last dose of oral thiamine, blood and breast milk specimens were again obtained for determination of nadir (trough) steady state thiamine concentrations. The thiamine hydrochloride tablet manufacturer conforms to Food and Drug Administration Thailand Modern Medicinal Good Manufacturing Process policies.

Eight of the Cambodian mothers also participated in a single-dose pharmacokinetic study. After the ingestion of 100 mg thiamine hydrochloride on the morning of day 1, these mothers remained fasting for another 2 h. Maternal blood specimens were obtained 0.5, 1, 2, 4, 6, and 24 h after oral thiamine administration, and a breast milk specimen was also obtained at 24 h (morning of day 2) before administration of the day 2 thiamine dose for determination of nadir (trough) thiamine concentrations after a single dose.

Blood (5 mL from mothers and 1.5 mL from infants by phlebotomy) was collected in sodium heparin tubes. Aliquots of whole blood (for TDP assay) and plasma (for thiamine and TMP assays) were frozen immediately at –20°C in a solar-powered freezer. Breast milk (4 cc) was expressed directly by mothers into a container immediately before oral thiamine administration and was also frozen immediately at −20°C. All specimens were transferred on dry ice to a −70°C freezer at the National Institute of Public Health in Phnom Penh, Cambodia, ≤2 wk of collection. Frozen specimens were batch shipped to ARUP Laboratories, Salt Lake City, UT, in a Cryoport Dry Vapor Shipper (Cryoport) at −70°C and were first thawed immediately before assay ≤3 mo of collection.

Subsequently, 16 healthy mothers who were residing in Olmsted County, MN, who had been nursing their infants ≥2 wk were also recruited. Each American mother was matched to a Cambodian mother by infant age (±2 wk for infants <2 mo of age or, otherwise, ±1 mo). All American mothers had been taking daily oral multivitamin supplements prepartum and postpartum that contained between 1.5 and 3 mg thiamine per tablet. Fasting morning blood and breast milk specimens were obtained from each mother ∼24 h after their last oral multivitamin dose, frozen immediately at −70 C°, and batch-shipped to ARUP Laboratories. No specimens were collected from American infants.

Plasma thiamine and TMP and whole-blood TDP were measured by HPLC at ARUP Laboratories as previously described (9). Reference ranges (derived from self-reported healthy American adults) were as follows: total plasma thiamine (thiamine + TMP) concentration, 8–30 nmol/L; whole-blood TDP concentration: 70–180 nmol/L. Pediatric reference ranges have not been established, but should be at least as high adult ranges (5, 10). Hemoglobin was also measured at ARUP Laboratories.

Breast milk was assayed by using the laboratory's routine thiamine method; proteins were removed by methanol precipitation before derivatization of the sample. No TDP was detected in breast milk. Thiamine compounds (thiamine and TMP) were identified by retention time and the peak shape of their corresponding fluorescent thiochromes. A comparison was made to known standard solutions of thiamine and TMP assayed using the same method. Breast-milk samples were diluted by a factor of 5 (1:5) for analysis. Total CVs for the assay measured at mean concentrations of 7.1 and 19.3 nmol/L (thiamine), 14.2 nmol/L (TMP), and 78.8 and 185.4 nmol/L (TDP) were 13.7%, 11.1%, 10.2%, 7.6%, and 6.5%, respectively. Total plasma thiamine was defined as plasma thiamine + TMP, and total breast milk thiamine was defined as breast milk thiamine + TMP.

Bioavailability of a single dose of thiamine was assessed in terms of the maximal concentration (C_{max}), the time to maximal concentration (T_{max}), and the area under the concentration-time curve (AUC) from 0 to 24 h after a single dose by subtracting the area attributable to the baseline vitamer concentration ($AUC_{0-24\ h}$). Comparisons between variables were assessed by using Wilcoxon's rank-sum test, McNemar's direct test, or Fisher's exact test as appropriate. Linear regression that predicted total breast milk thiamine was used to explore multivariate associations between maternal total plasma thiamine, TDP, and their interaction. Univariate linear regression was also used to predict infant day 6 total plasma thiamine or blood TDP with maternal blood and total breast milk thiamine variables. The SAS statistical software package version 9.3 was used for statistical analysis (SAS Institute).

RESULTS

In Cambodian subjects, the median maternal age was 24.5 y (range: 21–35 y) and median infant age was 4 mo (range: 2–6 mo); the median age of American mothers was 29.6 y (range: 24–40 y), and they were a median of 4 mo postpartum (range: 1.5–7 mo postpartum). All American mothers were white.

Baseline and day 6 concentrations of thiamine, TMP, and TDP are shown in **Table 1**. On day 1, before thiamine supplementation, median Cambodian maternal concentrations of total plasma thiamine and blood TDP were below the adult reference range, but after 5 daily doses of oral thiamine, median maternal concentrations were well within adult reference ranges. Median baseline Cambodian infant concentrations of total plasma thiamine and blood TDP were also low, and by day 6, these infant concentrations had risen only modestly, with 94% of infants having a day 6 TDP concentration below the lower limit of the adult reference range. Maternal baseline TDP concentrations did not significantly correlate with maternal hemoglobin concentrations (linear regression slope: 0.022; $P = 0.21$).

Single-dose pharmacokinetics are shown in **Figure 1** and **Table 2**. Cambodian maternal plasma thiamine concentrations

TABLE 1
Median thiamine concentrations (with ranges) in the blood and breast milk of the study subjects[1]

	Cambodian participants, day 1 (n = 16)	Cambodian participants, day 6 (n = 16)[2]	P (Cambodian day 1 compared with day 6)	American participants, day 1 (n = 16)	P (Cambodian day 1 compared with American day 1)	P (Cambodian day 6 compared with American day 1)
Maternal blood						
Thiamine (plasma, nmol/L)	2.4 (0–4.4)[3]	14.2 (9.2–18.0)	<0.001[4]	14.3 (7.8–20.7)	<0.001[5]	0.81[5]
TMP (plasma, nmol/L)	0 (0–2.6)	4.3 (2.1–8.8)	<0.001[4]	3.2 (1.3–6.7)	<0.001[5]	0.10[5]
Total plasma thiamine (nmol/L)	2.4 (0–6.9)	18.6 (13.4–25.3)	<0.001[4]	18.0 (10.4–27.3)	<0.001[5]	0.396[5]
<8 nmol/L [n (%)][6]	16 (100)	0 (0)	<0.001[7]	0 (0)	<0.001[8]	—
TDP (whole blood, nmol/L)	58.0 (27–98)	76.5 (48–107)	0.001[4]	121.7 (108–128)	<0.001[5]	<0.001[5]
<70 nmol/L [n (%)][6]	11 (69)	7 (44)	0.219[7]	0 (0)	<0.001[8]	0.007[8]
Infant blood						
Thiamine (plasma, nmol/L)	3.0 (0–7.3)	5.6 (0–9.7)	<0.001[4]	—	—	—
TMP (plasma, nmol/L)	0 (0–0)	0 (0–0)	—	—	—	—
Total plasma thiamine (nmol/L)	3.0 (0–7.3)	5.6 (0–9.7)	<0.001[4]	—	—	—
<8 nmol/L [n (%)][6]	16 (100)	11 (69)	0.40[7]	—	—	—
TDP (whole blood, nmol/L)	38.5 (23–57)	45.5 (32–70)	0.002[4]	—	—	—
<70 nmol/L [n (%)][6]	16 (100)	15 (94)	0.28[7]	—	—	—
Breast milk						
Thiamine (nmol/L)	17.2 (10–99)	123.1 (1–291)	<0.001[4]	230.8 (37–457)	<0.001[5]	0.015[5]
TMP (nmol/L)	146.3 (71–332)	376.1 (197–535)	<0.001[4]	195.0 (63–398)	0.260[5]	0.003[5]
TMP/(thiamine + TMP)	0.47 (0.36–0.48)	0.43 (0.29–0.46)	<0.001[4]	0.30 (0.14–0.44)	<0.001[5]	0.001[5]
Total thiamine (nmol/L)	179.5 (85–359)	502.7 (360–808)	<0.001[4]	500.3 (114–622)	<0.001[5]	0.395[5]

[1] TDP, thiamine diphosphate; TMP, thiamine monophosphate.
[2] After 5 daily doses of oral thiamine hydrochloride (100 mg/d).
[3] Median; range in parentheses (all such values).
[4] Signed rank test.
[5] Wilcoxon's rank-sum test.
[6] Lower limit of adult reference range.
[7] McNemar's test.
[8] Fisher's exact test.

rose quickly after oral thiamine administration. The mean $\pm$ SD maternal C_{max} for thiamine was 73.4 $\pm$ 45.6 nmol/L, and the mean maternal $AUC_{0-24 h}$ for thiamine was 465 $\pm$ 241 h · nmol · L^{-1}. After a single oral dose of thiamine, the median maternal trough (day 2) total plasma thiamine and blood TDP concentrations rose almost to the lower limit of adult reference ranges.

The baseline median Cambodian total breast milk thiamine concentration was much higher than the participants' corresponding blood concentrations but significantly lower than that shown in thiamine-replete American mothers (Table 1). After a single oral dose of thiamine, the median Cambodian trough total breast milk thiamine more than doubled, and by day 6, the median Cambodian trough total breast milk thiamine concentration was equivalent to that shown in American mothers.

In all mothers, there was a linear relation between total breast milk thiamine and both maternal total plasma thiamine and maternal blood TDP concentrations (**Figure 2**, A and B); in a multivariate analysis, there was a significant effect of TDP that modified the association between total plasma thiamine (x axis) and total breast milk thiamine (y axis), with a theoretical slope of 28 if the blood TDP concentration was 0 nmol/L and a decrease in the slope for total plasma thiamine of 0.13 for each additional unit of TDP (P = 0.046). The mean ratio of total breast milk thiamine to maternal plasma thiamine, total plasma thiamine, and blood TDP were 46.8 $\pm$ 19.5, 39.6 $\pm$ 20.4, and 5.0 $\pm$ 2.5, respectively. In Cambodian participants, the baseline

total breast milk thiamine content did not correlate with infant age, infant sex, maternal age, maternal hemoglobin concentration, or thiamine $AUC_{0-24 h}$ (P = 0.14–0.91). The ratio of TMP:(thiamine + TMP) in breast milk was inversely correlated with maternal blood TDP concentrations (Figure 2C).

In the 8 mother-infant pairs who participated in the single-dose pharmacokinetic portion of the study, maternal $AUC_{0-24 h}$ for thiamine, TMP, and TDP did not correlate with infant day 6 total
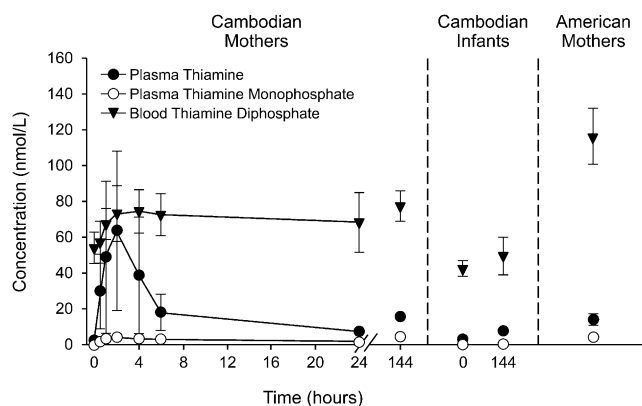

FIGURE 1. Mean ($\pm$SD) thiamine concentrations in plasma and whole blood before and after administration of oral thiamine to 8 Cambodian mothers as well as mean thiamine concentrations in their infants and matched American control mothers.

TABLE 2
Single-dose pharmacokinetics after oral administration of 100 mg thiamine hydrochloride to 8 Cambodian mothers[1]

	Plasma thiamine	Plasma TMP	Blood TDP	Breast-milk thiamine	Breast-milk TMP	Breast-milk total thiamine
Baseline value (nmol/L)	2.3 (0.0–3.6)	0.0 (0.0–0.0)	57.0 (40.0–62.0)	26.6 (9.5–99.0)	138.6 (71.4–210.8)	172.05 (84.8–245.9)
$T_{1/2}$ (h)	17.8 (9.5–32.2)	—[2]	—[2]	—	—	—
T_{max} (h)	2.0 (0.5–4.0)	2.0 (1.0–6.0)	2.0 (1.0–24.1)	—	—	—
C_{max} (nmol/L)	56.5 (19.3–144.5)	4.6 (2.6–5.9)	75.5 (57.0–105.0)	—	—	—
Median $AUC_{0-24\ h}$ (h · nmol · L^{-1})	405 (139–880)	51.8 (13.2–77.8)	300 (86–929)	—	—	—
24-h value (nmol/L)	7.6 (3.9–10.3)	1.2 (0–3.5)	68 (45–95)	201.2 (60.6–308)	225.3 (80.0–376.4)	403.0 (313.9–514.6)
Day 6 value (nmol/L)[3]	15.3 (12.0–18.0)	4.0 (2.1–7.3)	76 (48–89)	150.8 (104.6–291.3)	425.5 (197.1–535.0)	575.0 (393.9–808.3)

[1] All values are medians; ranges in parentheses. AUC, area under the concentration-time curve; C_{max}, maximal concentration; TDP, thiamine diphosphate; T_{max}, time to maximal concentration; TMP, thiamine monophosphate; $T_{1/2}$, half-life.
[2] Half-life was >24 h.
[3] Trough value after 5 daily doses of oral thiamine.

plasma thiamine or blood TDP concentrations ($P = 0.18–0.82$). Neither the day 6 total breast milk thiamine concentration nor the ratio of TMP:(thiamine + TMP) in breast milk correlated with infant day 6 total plasma thiamine or blood TDP concentrations.

DISCUSSION

Thiamine deficiency is common in parts of rural Asia. Although often clinically unapparent (2, 5), thiamine deficiency may cause beriberi and is a leading cause of infant morbidity and mortality in some populations (1, 3, 11). Thiamine deficiency is traditionally attributed to a diet low in thiamine, but the bioavailability of oral thiamine may be low even in whites (12), and pharmacokinetics of thiamine absorption and metabolism in thiamine-deficient populations have not been reported. We showed that thiamine-deficient Cambodian mothers effectively absorbed oral thiamine. Maternal blood thiamine concentrations increased appropriately with supplementation, and breast milk concentrations rapidly increased, with trough concentrations that approached control American ranges after a single dose. However, thiamine concentrations of breastfed infants increased only modestly after 5 d of maternal supplementation, and most infants remained thiamine deficient.

Single-dose pharmacokinetics in Cambodian mothers were similar to those reported for whites who received 100 mg thiamine orally. In particular, the C_{max} and $AUC_{0-24\ h}$ for thiamine and TDP equaled or exceeded comparable white values, and the T_{max} was similar (7, 8). Maternal total plasma thiamine concentrations normalized after 5 daily thiamine doses in all Cambodian mothers, and the median maternal blood TDP concentration also rose within the reference range. Total breast milk thiamine concentrations increased markedly after a single thiamine dose and were equivalent to American participants' breast milk concentrations after 5 daily doses. In contrast, infant thiamine concentrations exhibited less change. With the use of adult reference ranges, most infants remained deficient in total plasma thiamine by day 6, and only one of 16 infants achieved a normal blood TDP concentration. A likely explanation for this finding was that, although mothers received pharmacologic doses of thiamine, infants received much lower physiologic doses (a breast milk thiamine concentration of 500 nmol/L is equivalent to 0.17 mg/L).

We supplemented mothers with thiamine hydrochloride tablets. Other pharmacologic forms of thiamine may be more-efficiently absorbed (7, 13, 14), but we administered thiamine hydrochloride because it is readily available in Cambodia and appears on the WHO's model list of essential medications (15). Nonpharmacologic dietary thiamine supplementation might result in different pharmacokinetics. In one recent report, Karen refugee women who had received both micronutrient-fortified flour and thiamine mononitrate supplements had similar blood TDP concentrations to those of historical controls from the same camp who received supplements alone (16).

When we began this study, there was little information available regarding breast milk thiamine concentrations assayed by modern methods, although other investigators have since reported similar breast milk concentrations in thiamine-replete Karen refugees (17). These authors (17) also showed higher total thiamine in breast milk than blood and suggested an active transport mechanism of thiamine, in particular TMP, into breast milk. Our data extended these findings to thiamine-deficient mothers. Both total plasma thiamine and whole blood TDP concentrations were determinants of the total breast milk thiamine content, and there was a linear relation between breast milk and both plasma and blood concentrations that encompassed thiamine-deficient and -replete Cambodians as well as thiamine-replete Americans. As previously reported (17), we showed that the ratio of breast milk TMP:(thiamine + TMP) was inversely correlated with the maternal blood TDP concentration; however, this ratio did not appear to determine the responses of infants to maternal thiamine supplementation. After thiamine supplementation, there was a 7-fold increase in breast milk thiamine compared with a 2.5-fold increase in breast milk TMP, which suggested different kinetics of vitamer secretion into milk.

Our findings suggest that 5 daily doses of 100 mg thiamine hydrochloride would correct thiamine deficiency in most nursing Cambodian mothers; however, breastfed infants may remain thiamine deficient after their mothers have achieved normal thiamine concentrations. An important implication of this finding is that infants with symptomatic thiamine deficiency should receive thiamine directly; parenteral thiamine therapy rapidly increases infant blood TDP concentrations (E Keating, P Nget, S Kea, et al, unpublished observations, 2012). Because of the relatively short T_{max} of oral thiamine, twice-daily dosing should
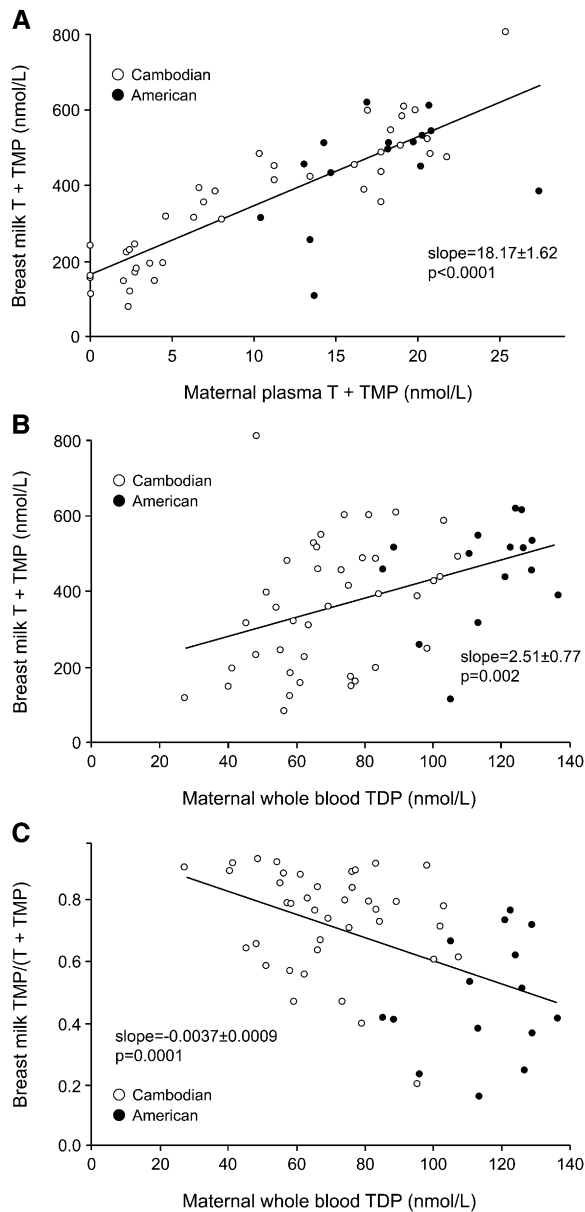
FIGURE 2. A–C: Relation between maternal blood and breast milk thiamine concentrations in 32 Cambodian and American mothers. Slopes ($\pm$SDs) were calculated by using simple linear regression, and associated P values were determined by using F tests. T, thiamine; TDP, thiamine diphosphate; TMP, thiamine monophosphate.

In conclusion, oral thiamine is efficiently absorbed by thiamine-deficient Cambodian mothers and is secreted into breast milk. Oral thiamine administration rapidly corrects maternal thiamine deficiency and increases breast milk thiamine concentrations but with modest short-term increases in breastfed infant thiamine concentrations. Infants with symptomatic thiamine deficiency should receive thiamine therapy directly. Higher maternal doses of thiamine, more frequent doses, and a longer duration of therapy may be warranted in thiamine-deficient populations.

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more rapidly correct maternal deficiency than once-daily dosing. Although the oral administration of $\leq$1500 mg thiamine hydrochloride was associated with nonlinear increases in absorption (8), doses higher than we used would likely also speed the correction of blood and breast milk concentrations in nursing mothers.

We did not assess the response to longer-term thiamine administration, which is an important limitation of this study. Longer-duration treatment would likely be of additional benefit to both mothers and infants. We did not study the pharmacokinetics of smaller maternal thiamine doses, such as those taken by our American participants. In addition, we did not assess longer-term thiamine concentrations off supplementation, and we did not measure urinary thiamine excretion.

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