National control and prevention programs for thiamine deficiency disorders

Technical Reference Materials

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List of abbreviations

DHS - Demographic and Health Survey
EA - Enumeration areas
EDTA - Ethylenediaminetetraacetic acid
ETKA - Erythrocyte transketolase activity
ETKAC - Erythrocyte transketolase activation coefficient
EWORS - Early Warning Outbreak Response System
FBS - Food balance sheets
FFQ - Food frequency questionnaire
HCES - Household consumption and expenditure surveys
HPLC - High performance liquid chromatography
LC-MS/MS - Liquid chromatography coupled to tandem mass spectrometry
LMICs - Low- and middle-income countries
LSMS - Living Standards Measurement Survey
M&E - Monitoring and evaluation
ThDP - Thiamine diphosphate
TDD - Thiamine deficiency disorders
UNIMMAP - UNICEF/WHO/UNU international multiple micronutrient preparation
WHO - World Health Organization
WRA - Women of reproductive age

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Scope

Thiamine deficiency remains a pressing public health problem in some parts of the world. Infants are particularly vulnerable as infantile beriberi, a disease caused by thiamine deficiency, can result in death within hours of clinical presentation if treatment is not provided. A few countries have initiated steps to establish control programs for thiamine deficiency disorders (TDD), but no model has been tested yet to confirm that those programs are feasible and effective. This is partly due to a lack of evidence on aspects such as diagnosis, biomarker selection and cut-offs. With support from the Bill & Melinda Gates Foundation, the Nutrition Program of the New York Academy of Sciences developed this Technical Reference to guide countries affected by TDD to establish a comprehensive TDD control program among women of reproductive age, and infants under 12 months of age. Chapter 1 provides an introduction and overview of thiamine biology. Chapter 2 focuses on TDD including a description of the problem, guidance for identification and treatment, groups at risk and an appendix with data available from selected countries. Chapter 3 provides guidance on the possible interventions that countries can implement to improve the thiamine status of their populations or target groups. Chapter 4 describes methods to a) assess the risk of thiamine deficiency using readily available, non-biomarker data; b) carry out a survey of thiamine biomarkers in target populations; and c) complement the biomarker survey with dietary intake data to understand the etiology of the problem. It also covers program design, monitoring and evaluation of a TDD control and prevention program, as well as surveillance.

Chapter 1 – Overview of thiamine biology and nutrition

1.1 - Introduction

Thiamine, also called vitamin B_1, is an essential micronutrient that has a vital role in energy metabolism and, therefore, in the growth, development and function of cells. The human body's supply of thiamine depends mostly on intake from the diet. The active form of thiamine has a short biological half-life (9-18 days), and body stores are limited, emphasizing the need for the diet to provide a regular supply of this vitamin[1,2,3].

Most (80-90%) of the approximately 30 mg of thiamine in the adult human body is in the form of thiamine diphosphate (ThDP), the main metabolically active form of thiamine. ThDP serves as an essential cofactor for enzymes involved in glucose, amino acid and lipid metabolism[1,4]; thus, a deficiency of thiamine affects key cellular metabolic processes, such as energy production, cell replication, and neural activity[1]. Thiamine circulates in the blood primarily in the erythrocytes (red blood cells) to be delivered to cells with high metabolic need (brain, liver, pancreas, heart, and muscles) and is mostly bound to the enzyme transketolase[1,2].

Whole grain cereals, meat (particularly pork), pulses, nuts and seeds and food items prepared with yeast are the richest dietary sources of thiamine. Those sources vary between industrialized countries, where fortified foods (e.g. breads and breakfast cereals) provide about 50% of the total intake of thiamine. This is in contrast to many low- and medium-income countries (LMICs), where unrefined grains and starchy roots and tubers provide 60-85% of the total dietary thiamine[1,5,6]. In LMICs thiamine fortification is not a common practice, and the consumption of monotonous diets that rely mostly on low-thiamine foods (such as polished rice and cassava) can cause thiamine deficiency and its associated disorders.

As thiamine is water soluble, a significant amount of the vitamin is lost when the cooking water is discarded. Thiamine is also heat sensitive and, therefore, can be degraded by baking, pasteurization, and boiling of foods, or when improperly stored[7]. In addition, anti-thiamine factors in food can accelerate thiamine losses. These enzymes (known as thiaminases) will inactivate thiamine and can be found in fish, shellfish, ferns, and some bacteria if they are eaten in raw or fermented foods[1]. Other foods such as ferns, tea leaves, and betel nuts that contain thiamine antagonists may reduce the bioavailability of this vitamin and cause deficiency despite the consumption of an adequate level of thiamine[1].

1.2 - Human thiamine requirements

Table 1 shows the daily Recommended Nutrient Intakes for thiamine by age group, gender, and specifications for pregnant and lactating women[8].

Table 1 - Recommended Nutrient Intakes for thiamine, by group

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	0.2 mg	0.2 mg		
7–12 months	0.3 mg	0.3 mg		
1–3 years	0.5 mg	0.5 mg		
4–6 years	0.6 mg	0.6 mg		
7-9 years	0.9 mg	0.9 mg		
10–18 years	1.2 mg	1.1 mg		
+19 years	1.2 mg	1.1 mg	1.4 mg	1.5 mg

Because of the role of this vitamin in facilitating energy utilization, thiamine requirements take into consideration energy intake, which varies according to activity levels and other factors[1]. The requirement of this vitamin is increased when carbohydrates are consumed in large amounts, which may occur with diets composed largely of polished rice and processed cassava, which are already very poor sources of thiamine. Requirements are raised during periods of increased metabolic activity (e.g. fever, muscular activity, hyperthyroidism), and during pregnancy and lactation[9]. The Recommended Nutrient Intake for thiamine during pregnancy is increased to 1.4mg/day and during lactation is 1.5mg/day. It is estimated that well-nourished lactating women transfer 0.2mg thiamine to their infants through their milk each day[8].

Thiamine toxicity is not considered a problem as there is a rapid kidney clearance of this vitamin[8], and there are no known adverse effects of high thiamine intakes. Thus, there is no tolerable upper intake level set for this vitamin[2].

1.3 - Dietary sources of thiamine

As mentioned before, the richest food sources of thiamine are whole grain cereals, yeast, meats, pulses, some nuts, and seeds. Dairy products, refined cereal grains such as polished rice and white wheat flour, and most fruits and vegetables contain very limited natural amounts of this vitamin.

Thiamine is found in the aleurone layer (the layer below the husk) of cereal grains.[1] The following figure shows the different rice grain layers, and the thiamine content that remains following their sequential removal to produce refined white rice.

Unrefined cereal grains (i.e. cereals with a high extraction rate[*]) can be a good source of thiamine, as opposed to their refined counterparts. The lower the extraction level (i.e. from whole grain to

[*] Extraction rate refers to the percentage of the grain used in producing the final product, e.g. flour. A 100% extraction flour (i.e., a high extraction flour) is a whole grain flour, milled to retain 100% of its components. White flour has a much lower extraction rate (usually 72%), as the external layers of the cereal are removed.

white cereal products), the lower the thiamine content (see figure 1 and examples of wheat flour, bread, and rice on table 2).

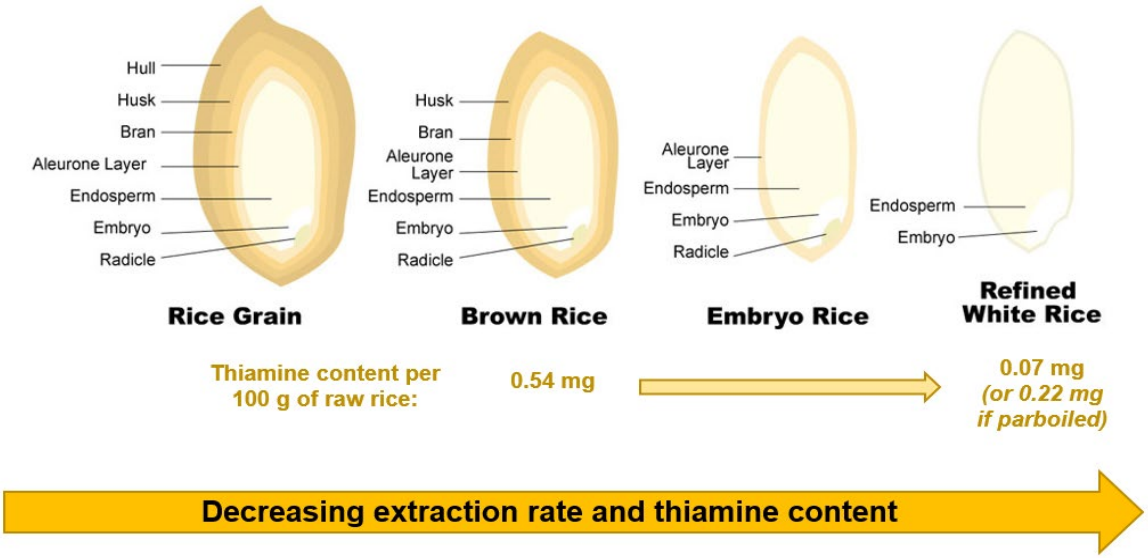


Figure 1 - The layers of the rice grain, from the whole grain to the refined grain and the associated thiamine content (adapated from Sonata Green[10]).

Polished (white) rice has a very low content of thiamine but parboiling rice before milling enables most of the thiamine to be retained in the rice grain, as it migrates into the endosperm during the process[1]. For example, white rice (unenriched, 0.07mg thiamine/100g of rice) has only one third of the thiamine content of parboiled white rice (unenriched 0.22mg thiamine/100g of rice) and only one eighth of the amount of thiamine in brown rice (unenriched, 0.54mg thiamine/100g of rice), as shown on table 2. Thiamine content of polished rice can be further reduced by precooking practices, such as repetitive rice washing and soaking.

Table 2 shows the thiamine content in a variety of foods, divided by food groups. The data were primarily compiled using the USDA Food Composition Databases[11]. Good sources of thiamine, defined as those providing at least 25% of the male adult's daily recommended intake per portion of food (i.e. >0.30mg per portion), are highlighted in green. This table can be further tailored to the dietary habits of each country, to include foods that are frequently consumed by a specific population.

Appendices 1a) and 1b) have additional food composition tables showing the thiamine content of foods that are frequently consumed in Laos[12] and Cambodia[13].

Because thiamine is heat sensitive, baking, pasteurization, or boiling of foods fortified with thiamine can reduce its content by up to 50%[7]. For example, bread has 15%–25% less thiamine than its raw ingredients, and pasteurization of milk reduces the thiamine content, which is very small to begin

with, by up to 25%[7]. This also explains the significant drop in the amount of thiamine between fresh peanuts and dry roasted peanuts (0.64mg thiamine/100g fresh peanuts vs. 0.15mg thiamine/100g

Table 2 - Thiamine content by food groups, per 100g and per typical portion size (when applicable)

Food group	Food item	Thiamine content (mg) per 100g	Thiamine content per portion	
			Food portion size (g)	Thiamine content per each portion (mg)
Cereal	Wheat flour (unenriched)			
	- White	0.08	N.A.	N.A.
	- Whole grain	0.50	N.A.	N.A.
	- Wheat germ	1.90	N.A.	N.A.
	Pita bread			
	- White, enriched	0.27	1 large (60g)	0.16
	- Whole grain, unenriched	0.34	1 large (60g)	0.20
	- White, enriched	0.60	1 large (60g)	0.36
	Rice			
	- Unenriched			
	- White, raw	0.07	1 cup (158g cooked)	0.03
	- White parboiled, raw	0.22	1 cup (158g cooked)	0.12
	- Brown, raw	0.54	1 cup (202g cooked)	0.36
	- Enriched			
	- White, raw	0.58	1 cup (158g cooked)	0.26
	Pasta (white), unenriched	0.09	1 cup (124g cooked)	0.03
	Corn (sweet, boiled)	0.09	1 cup (149g cooked)	0.14
	Cornmeal, whole-grain, yellow/white	0.39	1/4 cup (40g raw)	0.16
	Oatmeal, unenriched	0.73	1 sachet (43g)	0.30
	Sorghum, unenriched	0.33	1/4 cup (50g raw)	0.17
Meat, fish, eggs	Meats			
	- Pork, loin, lean only, raw	0.99	4oz (113g)	1.12
	- Pork, loin, lean only, roasted	1.02	3oz (85g)	0.86
	- Pork, cured (ham), lean only, canned	0.89	3oz (85g)	0.75
	- Chicken breast, meat only, roasted	0.07	3oz (85g)	0.06
	- Beef, tenderloin, lean only, cooked	0.06	3oz (85g)	0.05
	Fish			
	- Salmon, cooked	0.09	3oz (85g)	0.08
	- Sardines, canned, in oil, drained	0.08	1 can, 3.75oz (92g)	0.07
	- Tuna (yellowfin), fresh, cooked	0.13	3oz (85g)	0.12
	- Tuna, canned, in oil, drained	0.04	1 can, 6oz (170g)	0.07
	Eggs			
	- Whole, raw	0.04	1 large egg (50g)	0.02
	- Yolk, raw	0.18	1 large yolk (17g)	0.03
Pulses, nuts and seeds	Peas, boiled and drained	0.26	1 cup (160g)	0.41
	Chickpeas, boiled and drained	0.12	1 cup (164g)	0.19

	White beans, boiled and drained	0.12	1 cup (179g)	0.21
	Lentils, boiled and drained	0.17	1 cup (198g)	0.34
	Soybeans (green), boiled and drained	0.26	1 cup (180g)	0.47
	Peanuts, fresh	0.64	1oz (28.4g)	0.18
	Peanuts, dry roasted	0.15	1oz (28.4g)	0.04
	Almonds, unroasted	0.21	1oz (28.4g)	0.06
	Pistachios	0.87	1oz (28.4g)	0.25
	Walnuts	0.34	1oz (28.4g)	0.10
	Sunflower seeds	1.48	1oz (28.4g)	0.42
	Sunflower seeds, dry roasted	0.11	1oz (28.4g)	0.03
	Sesame seeds	0.79	1oz (28.4g)	0.22
Dairy products	Full fat milk, pasteurized, unenriched	0.05	1 cup (244g)	0.11
	Yoghurt, natural	0.03	6 oz (170g)	0.05
	Cheese, cow's milk	0.03	1oz (28.4g)	0.01
Vegetables	Broccoli, raw	0.07	1 cup (91g)	0.07
	Cabbage	0.06	1 cup (89g)	0.05
	Carrots	0.07	1 cup (128g)	0.08
	Tomatoes	0.04	1 cup (180g)	0.07
	Spinach	0.08	1 cup (30g)	0.02
Fruits	Orange, peeled	0.09	1 medium (130g)	0.11
	Apple, without skin	0.02	1 medium (160g)	0.03
	Banana	0.03	1 medium (118g)	0.04
	Papaya	0.02	1 cup (145g)	0.03
	Pineapple	0.08	1 cup (165g)	0.13
	Mango	0.03	1 cup (165g)	0.05
	Tamarinds, raw	0.43	1 cup (120g)	0.51
Tubers/ starchy roots	Cassava, fresh	0.09	1 cup (206g)	0.18
	Cassava, cooked/flour*[9]	0	N.A.	N.A.
	Taro, cooked	0.11	1 cup (132g)	0.14
	Potato, boiled	0.11	1 medium (136g)	0.14
	Yam	0.10	1 medium (136g)	0.13
Others	Baker's yeast (dried)	11	N.A.	N.A.
	Human milk	0.01	1 cup (246g)	0.03

N.A.: not applicable

Good sources of thiamine, defined as those providing at least 25% of the male adult's daily recommended intake per portion of food (i.e. >0.30mg per portion), are highlighted in green.

The data were primarily compiled using the USDA Food Composition Databases[14], except for the food items identified with an asterisk (*).

Chapter 2 – Thiamine deficiency

2.1 - Biomarkers for assessing thiamine status

Currently, there are two biochemical methods to assess thiamine status. First, the erythrocyte transketolase activity (ETKA) assay is a functional biomarker that measures the activity of the thiamine-dependent enzyme transketolase and is thought to provide information about tissue reserves of thiamine[9]. The assay for ETKA is performed in the absence and the presence of added thiamine and expressed as an activity coefficient, known as the erythrocyte transketolase activation coefficient (ETKAC)[9]. Second, the analysis of ThDP in the whole blood or erythrocytes is a direct biomarker of thiamine status, as it reflects the body stores of this vitamin[2].

There are other thiamine metabolites, such as free thiamine or thiamine monophosphate in the blood plasma, that reflect recent dietary intake of thiamine but do not provide information regarding the state of deficiency or the degree of depletion of tissue thiamine reserves[9]. Urinary thiamine can also be used as a biomarker but requires 24-hour urinary collection, which is not ideal in most contexts. Thus, the most commonly used biomarkers to assess thiamine status and detect thiamine deficiency are:

1) ThDP (a direct measurement biomarker) and,
2) ETKA assay (an indirect/functional measurement biomarker)[2].

The choice of the biomarker used to assess thiamine status will primarily depend on the resources of the available laboratory. Table 3 provides a comparison between ThDP and ETKA, including advantages, disadvantages, and a summary of the analytical requirements for their assays. More detailed information can be found in section 3.1.1.

2.2 - Thiamine deficiency disorders (TDD) and its consequences

Thiamine deficiency affects multiple organs and systems, resulting in a wide spectrum of clinical presentations, termed collectively “thiamine deficiency disorders” (TDD). Depending on the systems that are predominantly affected (mostly cardiovascular or nervous systems, with some gastrointestinal symptoms), TDD have been historically grouped into beriberi, which can be further divided into “wet” or edematous and “dry” or paralytic, and Wernicke’s encephalopathy or Wernicke-Korsakoff syndrome (usually associated with chronic alcoholism). However, as TDD can have a variety of clinical presentations, which tend to manifest differently in various age groups, a summary of different forms of TDD has been recently proposed[2] and is presented in Figure 2.

Table 3 - Analytical requirements for thiamine biomarkers (adapted from Whitfield et al. 2018[2])

	ThDP	ETKA
Assay Type	Direct measurement	Indirect/Functional measurement
Advantages	Biologically active vitamer and indicator of thiamine status	Functional assay of biological activity
Disadvantages	Unstable if specimen is not properly handled	Assay is not widely available
Analytical Instrument	HPLC or LC-MS/MS	UV Spectrophotometer
Specimen Type	Whole blood or washed erythrocytes *Note: if whole blood is used, either hematocrit or hemoglobin is required for interpretation.*	Washed erythrocytes
Collection Tube	Heparin or EDTA	Heparin or EDTA
Sample Processing	3x saline wash to purify erythrocytes or hemolyzed whole blood	3x saline wash to purify erythrocytes
Minimum Volume	For HPLC: 300-500 µL of erythrocytes or whole blood For LC-MS/MS: 150-250 µL of erythrocytes or whole blood	Minimum volume is 30 µL of washed erythrocytes. To aid sample handling and to allow repeat analysis a minimum of 200 µL is recommended. In practice the process is easier with larger samples; > 1 mL of whole blood is suggested.
Storage	Room temperature for a few hours, store frozen at -20°C for a few months, -80°C for several months/years	Room temperature for a few hours, store frozen at -20°C for a few weeks, -80°C for several months/years
Shipping	Dry ice or liquid nitrogen	Dry ice or liquid nitrogen
Suggested cut-offs	Whole blood: • 70-180 nmol/L: healthy range • <70 nmol/L: deficiency Erythrocytes: • 120-150 nmol/L: mild deficiency • <120 nmol/L: deficiency *Note: different cut-offs have been proposed and there is no agreement on the most adequate values.*	ETKAC values and risk of clinical thiamine deficiency • ≤1.15 (α≤15%): low risk • 1.15–1.25 (α 15% to 25%): moderate risk • >1.25 (% activation α>25%): high risk

Thiamine diphosphate (ThDP); erythrocyte transketolase activity (ETKA); erythrocyte transketolase activation coefficient (ETKAC); high performance liquid chromatography (HPLC); liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS); ethylenediaminetetraacetic acid (EDTA).

Acute Cardiologic Form	Aphonic Form	Pseudo Meningitic Form	Encephalopathy	Peripheral Neuropathies
• Peak prevalence in breastfed babies of 1–3 months of age • Colic • Restlessness • Anorexia • Vomiting • Edema • Cyanosis and breathlessness with signs of heart failure leading to death • Pernicious form or Shoshin Beriberi • Sudden cardiogenic shock	• Peak prevalence in 4- to 6-month-old infants • Initially hoarse cry until no sound is produced while crying • Restlessness • Edema • Breathlessness and death	• Peak prevalence in 7- to 9-month-old infants • Nystagmus (involuntary eye movement) • Muscle twitching • Bulging fontanelle • Convulsions • Unconsciousness	• Generally older children or adults but also seen in infants • Psychomotor slowing or apathy • Nystagmus or opthalmoplegia • Ataxia • Impaired consciousness • Eventually coma and death	• Older children or adults • Pain • Tingling or loss of sensation in hands and feet (peripheral neuropathy) • Muscle wasting with loss of function or paralysis of the lower extremities • Loss of ankle and knee reflexes • Cranial nerve impairment

Figure 2 - Clinical Spectrum of Thiamine Deficiency Disorders[2]

When thiamine deficiency manifests in infants (0-12 months) – also called infantile beriberi – the onset of the symptoms is often very rapid and results in death if adequate treatment is not immediately provided. This is because of the rapid growth, high energy needs and metabolic rate relative to body size, and nutrient intake at this lifestage[15,16]. Thiamine deficiency in infants is rare in the first two months of life as thiamine levels are higher in newborns due to *in utero* vitamin accumulation[17], but they fall in the third month of life, particularly if infants are breastfed by mothers who are thiamine deficient. Exclusive breastfeeding by thiamine deficient mothers places infants at highest risk of developing TDD[2], so improving the thiamine status of lactating mothers is important in preventing infantile TDD.

Thiamine deficiency in infants can present in several different forms depending on age[2,9]:

- **Acute cardiac form:**
  - Common age: 1-3 months
  - Symptoms: The infant may present with colic, irritability, anorexia (lack of appetite, with consequent refusal to breastfeed), vomiting; but also with signs of heart failure, such as cyanosis (a bluish discoloration of the skin resulting from poor circulation or inadequate oxygenation of the blood), edema, tachycardia (heart beating faster than normal while at rest), and breathlessness. Shoshin beriberi is a fulminant form of beriberi characterized by

acute cardiovascular collapse, metabolic acidosis, multi-organ-failure, no edema, and death may occur within hours.
  - Can be confused with typhoid, malaria, pneumonia, and septicemia
- **Aphonic form**
  - Common age: 4-6 months
  - Symptoms: This form of infantile beriberi causes a typical loss of voice due to partial or total paralysis of the vocal cords; infants present incessant crying, initially with hoarseness and ultimately with no sound produced ("silent cry"), irritability, edema, and breathlessness.
- **Pseudo meningitic form**
  - Common age: 7-9 months
  - Symptoms: Infants may present nystagmus (a repetitive, uncontrolled eye movement), muscle twitching, bulging fontanelle, convulsions and unconsciousness.
  - Can be confused with all forms of encephalitis and meningitis, malaria and acute vitamin A intoxication.

Thiamine deficiency <u>in older children and adults</u> can also assume different forms:

- **Encephalopathy**
  - Common age: generally older children or adults, but may be seen in infants
  - Symptoms: neurological changes affecting the central nervous system. Patients with Wernicke's encephalopathy typically present with:
    - Abnormal eye movement – nystagmus (repetitive, uncontrolled eye movement) or ophthalmoplegia (squinting where the eye does not move outwards, due to paralysis of the eye muscles)
    - Gait ataxia (inability to coordinate muscle movements, resulting in difficulty walking)
    - Cognitive impairment, impaired consciousness
    - Apathy (lack of interest, enthusiasm or concern), psychomotor slowing

    In younger children the symptoms may not include ataxia.

    Korsakoff's psychosis – the most severe form of Wernicke's encephalopathy – is characterized by a severe loss of recent memory, profound confusion and confabulation (the invention of events to cover the loss of memory).
  - Can be confused with other forms of encephalopathy, e.g. viral illness, cerebral malaria, sleeping sickness, etc.
- **Peripheral neuropathies**
  - Common age: older children or adults
  - Symptoms: The affected peripheral nervous system is manifested with tingling or loss of sensation in hands and feet (peripheral neuropathy), pain, muscle wasting with loss of function or paralysis of the lower extremities, abnormal/loss of ankle and knee reflexes.

- Can be confused with other causes of peripheral neuropathies, e.g. diabetes, hereditary disorders, inflammatory infections, auto-immune diseases, etc.

It should be noted that cardiac manifestations of thiamine deficiency ("wet beriberi"), including its fulminant form of shoshin beriberi, are also observed in adults[18,19]. Symptoms include cardiac failure, edema, pulmonary congestion with pleural effusion, in addition to lactic acidosis and cardiovascular collapse in the case of shoshin beriberi.

It is not clear why some people suffering from TDD present mostly with cardiac symptoms, while others present mostly with neurological symptoms. The diagnosis of thiamine deficiency is further complicated not only by the variable clinical presentations of TDD but also because of the presence of additional health problems (such as the comorbidities described above, under each form of thiamine deficiency), including multiple micronutrient deficiencies.

Long-term consequences of TDD

Infantile beriberi is often more fatal than Wernicke's encephalopathy. When thiamine deficiency is corrected before the development of significant brain damage, the neurological symptoms may be completely reversible. However, permanent brain damage occurs if thiamine deficiency persists[2,20]. Brain imaging techniques show similar changes in the structure of the brain between children with beriberi and adults with Wernicke's encephalopathy, such as bilateral, symmetric, hyperintensity signals in the mammillary bodies, thalamic, and periaqueductal areas[2]. The long-term consequences of thiamine deficiency in a certain period of time, or the consequences of lifelong subclinical (asymptomatic) thiamine deficiency, have not been extensively studied. However, it is possible that even asymptomatic thiamine deficiency in early life can result in long-term negative cognitive and developmental outcomes, such as marked intellectual disabilities, seizures, motor impairments, and complete heart block, based on the follow up of a cohort of Israeli children who were inadvertently fed infant formula that lacked thiamine[21].

Case definition

Because the symptoms of TDD involve a wide range of bodily functions and manifest in ways that can be easily confused with other diseases, it may only be possible to diagnose beriberi if a patient demonstrates improvement following treatment with thiamine. The currently accepted standard for diagnosing TDD in the absence of biochemical analysis is "clinical response to thiamine", also known as a thiamine therapeutic challenge. This challenge is defined by a significant improvement of symptoms within a few hours of thiamine administration.

Recently, a group of experts has proposed a new case definition for the various TDD (figure 3), which should help reduce the confusion around the diagnosis of thiamine deficiency[2]. This case definition is based on the signs and symptoms (major and minor manifestations) that are most predictive of clinical response to thiamine. Thus, figure 3 serves as a clinical guide to demonstrate when the empirical treatment with thiamine (i.e. the thiamine therapeutic challenge) should be considered,

in infants, children or adults. The thiamine therapeutic challenge is a low-cost treatment with no associated risks of toxicity, as there is no upper intake limit associated with thiamine.

Briefly, thiamine deficiency should be suspected when at least three major manifestations or the combination of at least two major and two minor manifestations are present, as described in figure 3:

- If the patient has a significant clinical improvement within 24 hours of thiamine administration, it is very likely that the patient suffered from TDD.
- If the patient has a significant clinical improvement within 72 hours of thiamine administration, it is probable that the patient suffered from TDD.

Case definitions: At least 3 major manifestations OR At least 2 major + 2 minor manifestations AND response to thiamine within 24 hours (very likely TDD) OR within 72 hours (probable TDD)

	Major Manifestations	Minor Manifestations
Infant	• Sudden heart failure between 1-6 months • Incessant cry, hoarseness, followed by loss of voice • Cyanosis and difficulty breathing • Significant liver enlargement • Bulging fontanelle • Nystagmus • Muscle twitching • Loss of consciousness • Fits (without fever)	• Reduced suckling or refusing to feed for at least 48 hours • Repetitive vomiting • Constipation • Tachycardia with warm extremities without fever (early sign)
Child or Adult	• Difficulty walking (ataxia) • Abnormal eye movements • Confusion, behavior change • Impaired consciousness, coma	• Bilateral tingling and numbness in limbs • Lethargy, apathy • Tachycardia with warm extremities • Signs of B-vitamins deficiency (e.g. angular stomatitis)

Figure 3 - An example of a proposed case definition of Thiamine Deficiency Disorders (TDD).

Treatment

The World Health Organization (WHO) suggests the following dosage of thiamine supplementation to treat individuals suffering from thiamine deficiency[9]:

- Mild deficiency states (including lactating women at risk of inadequate thiamine intakes)
  - First week: 10mg of thiamine/day, oral dose
  - Following 6 weeks: 3–5mg of thiamine/day, oral dose
- Severe deficiency states
  - *Infantile thiamine deficiency*

    - If severe heart failure, convulsions or coma occur, 25–50mg of thiamine, very slowly intravenously
    - Following week: 10mg of thiamine/day, intramuscular dose
    - Following 6 weeks: 3–5mg of thiamine/day, oral dose
- *Critically ill adults*
    - 50–100mg of thiamine, very slowly intravenously
    - Following 6 weeks: 3–5mg of thiamine/day, oral dose

Other groups have also suggested the intramuscular co-administration of magnesium and thiamine, as magnesium is a cofactor for the enzyme transketolase, proposing 1-2 mL of a 50% solution of magnesium sulfate, to correct thiamine deficiency and the frequently accompanying hypomagnesemia in some groups of patients (such as those who suffer from chronic alcoholism and malnutrition)[22].

Expected benefits

In infants with thiamine deficiency and in adults with cardiac insufficiency, the response to treatment (symptomatic relief) occurs within hours; while in cases of peripheral neuritis, there is a slower improvement over several days. The administration of the large doses of thiamine also resolves the ocular manifestations of Wernicke's syndrome promptly (within hours), but the response of the Korsakoff's psychosis is slow and incomplete for most of the patients[9]. Maternal supplementation rapidly improves the vitamin concentration in breastmilk and infant status; however, additional research is needed to establish the optimal timing, dose and duration of treatment[2].

Some population groups are more susceptible to TDD than others. The same group of experts that proposed a case definition of TDD (presented above) also identified risk factors to aid in the identification and treatment of probable cases TDD in LMIC[2]. These risk factors include:

- A high prevalence of thiamine deficiency in the population
- Humanitarian settings or those with unstable food security (e.g. conflicts or disasters, refugee/internally displaced person)
- Severe acute malnutrition
- Monotonous diet relying heavily on polished rice, refined flour or cassava
- Thiaminase consumption

The groups at risk of developing TDD are described in more detail in the next section.

2.2.1 - Groups at risk of TDD

Beriberi is still endemic in South-East Asia (and is suspected in sub-Saharan Africa), in regions where diets are monotonous, and the main source of energy comes from low-thiamine staples, such as polished rice or cassava[23]. In LMIC, the groups at risk of TDD are:

- **Infants, particularly those exclusively breastfed by thiamine deficient mothers**
  The thiamine status of a breastfed infant is closely related to the maternal intake and status (with maternal deficiency during pregnancy a likely additional risk factor for infant deficiency). Thus, infantile beriberi most often manifests during the exclusive breastfeeding period, because of the consumption of breastmilk with low levels of thiamine supplied by mothers with a low thiamine status[24]. Since WHO recommends exclusive breastfeeding during the first six months of life, the thiamine intake of the lactating mother must be adequate to prevent infantile beriberi and related morbidity and mortality.

- **Infants and children with severe acute malnutrition**
  When admitted to inpatient facilities or therapeutic feeding centers, infants (>6 months) and children with severe acute malnutrition often receive a therapeutic milk formula (F-75), which is used in the early stabilization phase of refeeding (first few days). These patients are likely to present with low or borderline thiamine reserves that can be rapidly depleted during refeeding, precipitating acute thiamine deficiency, with severe consequences in critically ill children. The thiamine content of F-75 (0.3 to 1.7mg/day depending on the total daily F75 intake with 0.85mg of thiamine/L of F-75) is significantly below the current recommendations to prevent the refeeding syndrome (100–300 mg of thiamine orally during the first three days of refeeding)[25]. Thus, in the absence of adequate thiamine supplementation, even a very cautious introduction of feedings may induce thiamine deficiency among infants and children suffering from severe acute malnutrition[25].

- **Pregnant and lactating women**
  Not only do pregnant and lactating women have increased thiamine requirements (as shown in section 1.3); but in some regions they may also follow traditional postpartum restricted diets and practices, which places them and their breastfed infants at high risk of thiamine deficiency. Reports from Laos show that more than 90% of women follow post-partum restricted diets, in which most of the avoided foods are important sources of thiamine, and drinking a traditional unsweetened herb tea (as the only beverage), resulting in a deficient intake of thiamine (96.6%), iron (92.0%), calcium (96.6%), among other nutrients[26].

- **Adults with high carbohydrate intake and submitted to heavy labor**
  A few recent reports show adult men with thiamine deficiency and beriberi outbreaks, probably because of dietary restricitons and the performance of heavy labor[18,27]. This may be explained by the low consumption of thiamine (from a diet based on high carbohydrate staple foods like polished rice and processed cassava) and the increased thiamine requirements both from the high intake of carbohydrates and from the increased physical activity[9,1].

- **Adults with intakes of anti-thiamine factors**

As described in section 1.1, there are anti-thiamine agents that can accelerate thiamine losses. These include:

- Thiaminases (heat-labile enzymes that will inactivate thiamine): found in fish, shellfish, and ferns if they are eaten raw or fermented; also found in African silkworm larvae[28] and some bacteria (Clostridium species)[1].
- Thiamine antagonists (may reduce the bioavailability of this vitamin): found in ferns, tea leaves, and betel nuts[1].

Thus, thiamine deficiency may occur despite the consumption of an adequate level of thiamine[1] in areas where it is common to drink tea, chew fermented tea leaves or betel nuts, and consume raw or fermented fish.

In higher income countries and food-secure settings, access to foods rich in thiamine (naturally occurring or fortified) ensures adequate intake of this nutrient. In these areas, TDD tend to affect mostly patient populations, such as those with chronic alcoholism in conjunction with limited food consumption, patients who are hypermetabolic and/or critically ill, patients receiving intravenous nutrition, patients suffering from chronic kidney diseases and undergoing renal dialysis, or those who have undergone a gastrectomy (partial or total surgical removal of the stomach)[8]. In these patient populations, thiamine deficiency is caused by their increased thiamine needs and/or reduced intake, absorption or utilization. Patients with long term use of diuretics (drugs used to remove water from the body for the treatment of high blood pressure and other conditions) are also at risk for thiamine deficiency, by accelerating thiamine excretion and blocking thiamine control mechanisms[29]. However, other population groups may develop TDD, for instance in cases of drought, war, or natural disaster, which may result in insufficient food intake or reliance on a monotonous diet for a long period of time.

Published data suggesting that thiamine deficiency is a potential public health problem in various locations, notably in Southeast Asia, was gathered and summarized in Appendix 2. It is possible that other countries may be affected by the same problem. To understand whether thiamine deficiency is a public health problem, it is necessary to analyze existing data or collect new data. The next chapter describes a number of interventions that may be used to improve thiamine status and chapter 4 discusses various methods for the assessment of TDD in populations.

Chapter 3 – Development of interventions to improve thiamine status

Micronutrient interventions may include fortification, supplementation, food-based approaches (including food processing and behavior change related to food procurement and consumption practices). These interventions should be accompanied by training of healthcare professionals, and education of those affected by or at risk of deficiency. While a combination of methods could be used (e.g. short-term supplementation while long term food-based solutions are implemented), fortification is likely to play a critical role in long-term TDD control programs.

Micronutrient interventions are more likely to succeed when there is a coordinated effort and effective communication among all stakeholders. For example, a successful intervention in China to tackle malnutrition and thiamine deficiency in children under 18 months of age included participatory outreach, community nutrition education (videos, pamphlets, and demonstrations about adequate "weaning food"), child growth monitoring, and thiamine distribution to new mothers right before and after delivery. This community-based action plan was supported by the city governor, government officers, maternal and child health workers, community leaders, and villagers[30].

3.1 - Food fortification

Food fortification has the potential to reach a large proportion of the population at relatively low cost. It can be a sustainable, long-term solution for countries with compatible food systems. When planning these programs, national governments and regional organizations must identify the best food vehicle and the correct level of fortification so that the correct amount of thiamine is delivered to the target groups.

Appendix 3 contains global data on thiamine fortification, according to the Global Fortification Data Exchange. It presents countries that have mandatory (n=72) and voluntary thiamine (n=14) fortification programs for wheat flour, maize flour, and rice, the year in which the fortification program was initiated, and the recommended level of thiamine. The most frequent food vehicle for fortification with thiamine is wheat flour, followed by maize flour and rice. The recommended levels of fortification vary from country to country. Other reports[31] present examples of thiamine fortification programs, such as:

- A mandatory fortification program of "vitaminized rice" in Thailand (4mg thiamine/kg of vitaminized rice)
- A voluntary fortification program of wheat flour in Vietnam (2.5mg thiamine/kg of wheat flour)
- A voluntary fortification program of processed foods in the Philippines (1/3 thiamine RDA of the target consumer/serving)

3.1.1 - Food vehicle

Table 4 presents key considerations in the identification of food vehicles for thiamine fortification.

Table 4 - Important considerations in the identification of food vehicles for thiamine fortification and setting the level of fortification[2,7,32].

Issue for consideration		Issue	Notes
Consumption pattern		The food vehicle must be consumed regularly throughout the year by a large proportion of the population at risk of thiamine deficiency	Information can be obtained from: - Individual or Household dietary intake surveys
Technical considerations	**Fortificant compound**	Thiamine hydrochloride for liquid foods. Thiamine mononitrate for dry foods	
	Stability	Possible degradation due to heat, light, oxidation, moisture, and pH	Thiamine is heat and light sensitive; is unstable in neutral and alkaline solutions and in the presence of sulfites
	Cost	Thiamine is one of the lowest cost fortificants, as it is estimated that $0.018 (USD) provides 100% of the daily thiamine requirements for a male for 1 year*	In decentralized fortification systems, costs can be significantly greater
	Organoleptic properties	The fortified food should have no changes in color, flavor, smell, or appearance; the method of preparing the food also should not be altered	Thiamine has no safety or sensory limitations
Industry structure and capacity		The number, capacity, and geographical distribution of the producers of the food vehicle need to be assessed	Ideally, the chosen food vehicles are processed at one or a few centralized facilities that produce most of national consumption needs of the food being fortified
Implementation		Costs of implementation include: the purchase of pre-mix, start-up costs of fortification equipment, staff training, and establishing a quality control and monitoring program	These costs can be decreased by co-fortifying an established vehicle. Compatibility between all the nutrients used in the co-fortification needs to be assessed.

*considering an overage of 40%, which is the additional amount of fortificant that must be added to compensate for losses during production, storage, food production, and distribution.

3.1.2 - Amount of fortification

The WHO and FAO "Guidelines on food fortification with micronutrients"[33] recommend using the Estimated Average Requirement (the intake level for a nutrient at which the needs of 50 percent of the population will be met) cut-point method to determine optimal levels of fortification. This approach requires knowing the target population's usual intakes of both the food vehicle and the micronutrient of interest for the fortification program. Countries thus need data on usual consumption of both potential food vehicles and thiamine in order to determine the optimal level of fortification.

Another important consideration is the loss of thiamine that occurs during the production of fortified foods. To compensate for this, overage is generally recommended for thiamine, to ensure that the fortified food delivers the targeted level of nutrients at the time it is consumed. Table 5 provides approximate overages of thiamine to be added when fortifying different foods, based on processing losses (mostly incurred in the manufacturing process, but may include losses during the cooking process). Additional overages may be necessary depending on storage time, temperature, and humidity conditions.

Table 5 - Recommended overages for thiamine and food vehicles based on losses during processing[7]

Food	Percent overage (%)
Cereals	
Wheat flour*	10
Wheat bread	20
Corn flour*	10
Corn bread	10
Corn meal***	20 - 50
Rice**	50
Pasta	40
Ready-to-eat cereals	30 - 40
Noodles	40 - 70
Cookies	10
Milk and milk products	
Pasteurized	25
Pasteurized at ultra-high temperature	50
Dry milk	20
Milk desserts	25
Fats	
Oil	-
Margarine	20
Drinks	
Juices	40 - 50
Drink mixes	10

*based on 3 months of storage at room temperature

** based on 6 months of storage at room temperature; after boiling/cooking

*** varies greatly with preparation form

Below are three examples of successful fortification of foods with thiamine, either alone or combined with other micronutrients:

1. Bread flour: Wheat flour is fortified with thiamine in many countries, and bread made with fortified wheat flour retains more than 70% of the fortified thiamine after baking. In Australia the fortification of bread flour with thiamine, which became mandatory at a level of at least 6.4mg of thiamine per kilogram in 1991, led to a 40% reduction of the incidence of Wernicke's

encephalopathy and Korsakoff's syndrome in the 5 years following the introduction of the mandatory fortification program[34,35].

2. Fish sauce: This condiment is widely used in Southeast Asia and could be considered a possible vehicle for thiamine fortification, since thiamine added after pasteurization causes no organoleptic changes. This has been tested in two studies: one conducted in a group of Cambodian mothers and their exclusively breastfed infants; another in a group of non-pregnant, non-lactating women and their children (1-5 years of age) living in rural Cambodia. In both studies the use of two fish sauces (one with a lower thiamine concentration of 2g/L and one with higher thiamine concentration of 8g/L) over a period of six months resulted in improved blood levels of thiamine and increased thiamine content of breastmilk[36,37].

   Fish and soy sauces have been used as food vehicles for iron fortification in Cambodia, Vietnam, and Thailand. Thus, co-fortifying thiamine with these products could reduce the costs of a thiamine fortification program, but it is important to understand the percentage of the target population that would be reached with this initiative by assessing their level of consumption of these products and the existence of a centralized production.

3. Rice: In populations like India, where 65% of the population consume centrally processed rice as a staple, rice is an excellent product for delivering micronutrients to a very large number of people. Fortified rice is well accepted, as it tastes and looks like unfortified rice. One example of thiamine-fortified rice is in a school feeding program. Since 2009-10, the mid-day meal program has consistently reached more than 100 million Indian school children. Subsequently, the WFP collaborated with the Department of School and Mass Education, Government of Odisha (an eastern Indian state) to integrate rice fortified with iron, zinc, vitamin A, thiamine, folic acid, and cyanocobalamin into the existing supply chain of the mid-day meal program[38]. The initiative helped reduce anemia among school children, and it showed that rice fortification is a safe and effective method to deliver micronutrients to a large proportion of school children. If governments provide fortified rice to households as well, this program could be scaled up and expanded to other population groups and other states. The proposed level of fortification with thiamine, using thiamine hydrochloride or thiamine mononitrate, was 3.5 mg/kg of rice[38].

The suggested approaches for rice fortification according to the Food Fortification Initiative are:

1. Coated rice kernels, accomplished by applying a thiamine-enriched mixture of waxes to rice kernels and mixing fortified kernels with polished rice in a ratio of 1:50−1:200; or
2. Mixing extruded, enriched, rice-shaped kernels (made by hot extrusion from a rice flour and vitamin/mineral premix) with typical polished rice in a similar ratio[2].

The major obstacle to fortifying rice with thiamine in some countries is that rice is often sourced from numerous local millers in a country or is purchased directly from small producers. This can be partly overcome if national efforts are made through the use of a rice food aid program[2]. USDA sets

the target levels of micronutrients and suggested chemical forms in fortified milled rice per gram of rice premix (or per 100 grams of finished product) for use in international food assistance programs. For thiamine the USDA suggests a level of 0.5mg per g of premix or per 100g of finished product, using thiamine mononitrate[39].

The Food Fortification Initiative helps country leaders to promote, plan, implement, and monitor fortification of industrially milled wheat flour, maize flour, and rice. Given that rice is a probable fortification modality in the affected countries, it is useful to consult their Frequently Asked Questions in rice fortification (http://www.ffinetwork.org/about/faq/faq_rice_industry.html), such as "When is rice fortification feasible?", "How much does rice fortification cost?" and "What is the shelf life of fortified rice kernels?" Important considerations such as the scale of fortification are also addressed here. For instance, rice fortification is deemed feasible "in modern mills with a production capacity of at least 5 metric tons an hour."

For countries where the food system is not compatible with the implementation of a new fortification sytem, they can consider using other existing locally fortified foods already availble in the market, such as seasoning cubes in Vietnam.

3.2 - Supplementation

Women of reproductive age (WRA), pregnant and lactating women are key target groups to reduce TDD, although depending on the context, other groups at risk of thiamine deficiency may also benefit from supplementation.

Improving the thiamine status of pregnant and lactating women through supplementation is one approach to prevent and reduce the risk of TDD in their infants. Experts recommend that in countries with reports of infantile beriberi or evidence of low biochemical thiamine status should consider[2]:

1) Adding a thiamine supplement to complement the current perinatal iron-folic acid supplementation program; or
2) The introduction of a maternal multiple micronutrient supplement containing thiamine, e.g. UNICEF/WHO/UNU international multiple micronutrient preparation (UNIMMAP); or
3) A switch from the existing standard iron-folic acid supplementation regimen in pregnancy and lactation to one including thiamine or the UNIMMAP formulation.

Adding thiamine, usually through tablets of thiamine hydrochloride, to an existing supplementation program (such as the perinatal iron-folic acid supplementation program) could be a straight-forward and cost-effective method to improve thiamine status of pregnant and lactating women and their breastfed infants. Ideally, supplements containing thiamine should be distributed to this population via antenatal and postnatal clinics. If women do not attend or cannot access these services, people who can reach them (e.g. birth attendants, community health volunteers, village chiefs, radio

announcers, other community service providers) should be educated and trained in providing advice and in directing women to local supplement sources.

3.2.1 - How, when and how much

A trial with 16 thiamine-deficient lactating Cambodian mothers, who received oral supplements of 100mg thiamine hydrochloride/day for five days, showed significant increases in the blood thiamine levels of the mothers as well as in the thiamine levels of breast milk[40]. However, blood thiamine concentrations of breastfed infants increased only modestly after five days of maternal supplementation, and most infants remained thiamine deficient. This suggests that longer-term thiamine supplementation of lactating women, probably at a dose closer to Recommended Nutrient Intake for thiamine for lactating women (1.5mg/day), may be necessary to correct and prevent thiamine deficiency in their infants. However, additional research is needed to establish the optimal dosage, timing and duration of thiamine supplementation in pregnant and lactating women. Currently, Myanmar provides thiamine supplements (10mg/day) to pregnant women and lactating women up to three months after birth and is an example that can be replicated elsewhere. However for the supplementation program to work optimally, a high proportion of pregnant and lactating women must be reached by the program and with high compliance[2].

If other population groups are deficient or at risk of thiamine deficiency, such as older infants who have started complementary feeding, alternative strategies may be used to protect these vulnerable individuals. Multiple micronutrient powders (e.g. Sprinkles), which include thiamine, are used in many countries for infants older than six months of age[2]. This form of supplementation (or in-home fortification) may help prevent TDD, although this has not yet been tested in an at-risk population.

The interaction between thiamine and other nutrients deserves some attention. Vitamin C seems to have a protective role when consumed together with thiamine[9]. Magnesium is a cofactor for the enzyme transketolase, and it has been reported that, in cases where both thiamine and magnesium deficiency coexist, the symptoms of thiamine deficiency could not be suppressed with thiamine administration until the magnesium deficiency was corrected as well[41]. Therefore, the incorporation of thiamine in micronutrient supplements that contain magnesium and/or vitamin C may be beneficial[9].

3.3 - Change in food processing and consumer behaviors

Certain eating behaviors, myths and food practices increase the risk of TDD. Formative research may be carried out to identify such issues and to develop nutritional education and behavioral change communications, on topics such as the following[9,2]:

1) Restrictive diets post-partum: Pregnant and lactating women should follow a varied diet and include daily sources of thiamine-rich foods. The consequences of food avoidances and the

typical strict post-partum food restrictions in some regions should be signaled to these populations.

2) Anti-thiamine factors: In areas where TDD are suspected, raising awareness about the anti-thiamine factors (thiaminases and thiamine antagonists) that can counteract thiamine intake is important to encourage behavior change of the associated habits (e.g. consumption of fermented tea leaves, betel nuts, African silkworm larvae or raw fish; drinking tea during meals).

3) Food diversity and increasing awareness and availability of thiamine-rich foods: Populations at risk of TDD should know about locally available thiamine rich-foods and ways to increase thiamine consumption. Whenever possible, tailored dietary advice to increase thiamine intake should be delivered. Ideally, every person seen in a healthcare facility or in the community with suspected or confirmed TDD should receive verbal and written information about the danger of thiamine deficiency, know which thiamine-rich foods are available locally and be encouraged to diversify their dietary intake, highlighting those foods in particular. For example, legumes (pulses, beans and groundnuts) are a good and affordable source of thiamine (figure 4).

   Promotion and ideally free distribution of low-cost, locally accepted types of legumes should be considered to encourage their consumption. In addition, menu planning of meals served in public services (e.g. schools, hospitals, nursing homes) should meet the thiamine requirements of those populations.

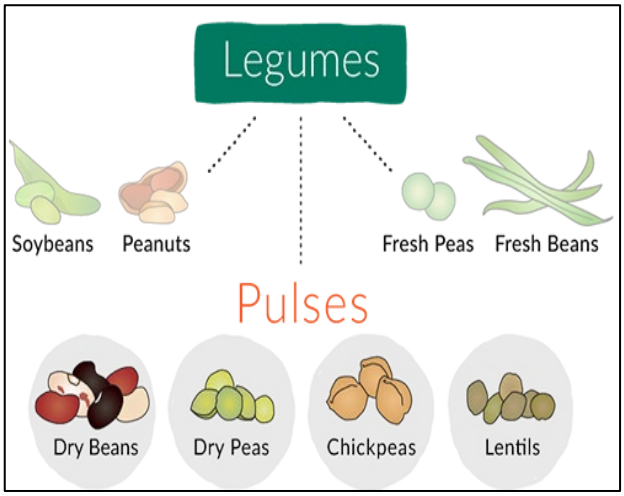


Figure 4 - Examples of legumes *(reproduced from https://pulses.org/nap/what-are-pulses/)*

4) Food processing (whole grain vs. refined): Food processing changes, particularly in the process of rice polishing, can have a significant impact on the levels of thiamine consumption. White (polished) rice tends to be more culturally accepted, and it has economic advantages because the removal of the outer bran increases the rice's shelf-life (removal of fatty acid that could go rancid in storage). However, under-milled rice and brown rice contain higher levels of thiamine. In this context, rice millers should be incentivized to remove less rice bran and consumers should

be educated about the benefits of using under-milled or brown rice to minimize thiamine losses. Concomitantly, it would be helpful to develop improved storage techniques to increase the shelf life of brown rice.

Similarly, if other cereals are consumed in the target population, the whole grain version should always be promoted because of its higher content of thiamine. For example, 200 g of whole wheat bread made of 95% extract flour (i.e. 95% of the grain is included) provides 50% of the daily thiamine requirements, while 200 g of wheat bread made of 70% extract flour (i.e. a highly milled flour where only about 70% of the grain is included) provides only 10% of the daily thiamine requirements.

5) Parboiled rice: As mentioned in section 1.3, parboiling rice before milling enables most of the thiamine to be retained in the rice grain, as it migrates into the endosperm during the process. As a result, parboiled white rice contains three times more thiamine than polished white rice, while parboiled brown rice contains four times more thiamine.

   The taste of parboiled rice is not affected, but its darker color decreases consumer acceptability. Hence, educating consumers about the benefits of using parboiled rice is essential.

6) Cooking techniques: Thiamine is water soluble and is susceptible to destruction by several factors including heat, neutral and alkaline conditions (thiamine is stable at low pH). Thus, it is important to teach the target population how to minimize the losses of this vitamin through food preparation and cooking techniques, for example, by minimizing repetitive rice washing and soaking prior to cooking; shortening the cooking time; not discarding (and reusing) the cooking rice water; choosing raw nuts and seeds instead of roasted; avoiding the use of sodium bicarbonate to green vegetables for color retention; etc.

Some educational materials for the general public ("A guide to increase thiamine intake and prevent thiamine deficiency") and for pregnant women and breastfeeding mothers explaining the importance of thiamine during pregnancy, lactation and infancy were developed and can be used to promote food processing and consumer behavior change, through healthcare professionals (including medical doctors, nurses, midwifes) and community health volunteers – see figures 7 and 8; and Appendix 4.

It should be noted that in some countries, the most vulnerable individuals, such as pregnant and breastfeeding women living in remote areas, rarely have access to or do not seek healthcare during pregnancy. It is important to find the most effective ways to reach and deliver information to these individuals. For example, in Cambodia a radio drama available in different local languages is helping Cambodian mothers to keep their babies healthy[42]. Women come together to listen to the drama and discuss what they have heard, giving them the chance to share their experiences and ask for more information. This radio show covers health related issues, such as healthy diet during

pregnancy, as well as the importance of vaccinations for children, breastfeeding, and going to the health center for health check-ups and delivering babies. This could be a good opportunity to educate pregnant and breastfeeding women about thiamine deficiency and its prevention (e.g. foods rich in thiamine, anti-thiamine factors, and appropriate cooking techniques to minimize thiamine losses).

3.4 - Education of healthcare providers and the public

Healthcare providers at all levels should be trained in providing advice on how to reduce the risk of TDD. Developing culturally-relevant education tools for healthcare providers will help them identify, treat and prevent TDD, and communicate the risks it poses to the population. Healthcare professionals (medical doctors, nurses, midwives, and others involved in the care of patients affected by or at risk of TDD) should know how to identify and treat it across different age groups. This may be achieved through:

1) Professional training, which can be delivered on admission to a new job, on regular multidisciplinary team meetings, during continuing professional development courses, in medical school curriculum
2) The use of printed posters displayed in clinical areas
3) Addition of thiamine-rich foods, and/or perinatal thiamine supplementation in the antenatal care and maternal and infant and young child feeding (MIYCF) counselling cards/flip-books
4) The use of pocket-sized handouts (if there is a pocket-sized book with general clinical guidance in place, consider adding this information to the book)
5) The inclusion of this information on internal guidelines for the hospital or clinic

Figure 5 shows an example of an educational material that can be used to train and keep healthcare professionals informed about the **identification and treatment of TDD**. It can be printed in different sizes, from a large poster to be displayed on the wall of clinical areas, to a small pocket-sized handout.

Another educational material was developed to raise awareness about infantile beriberi. It has visual representations of the common signs of infantile beriberi and provides two case studies of mother and infant thiamine deficiency (figure 6) that can be used to educate healthcare professionals and community health volunteers.

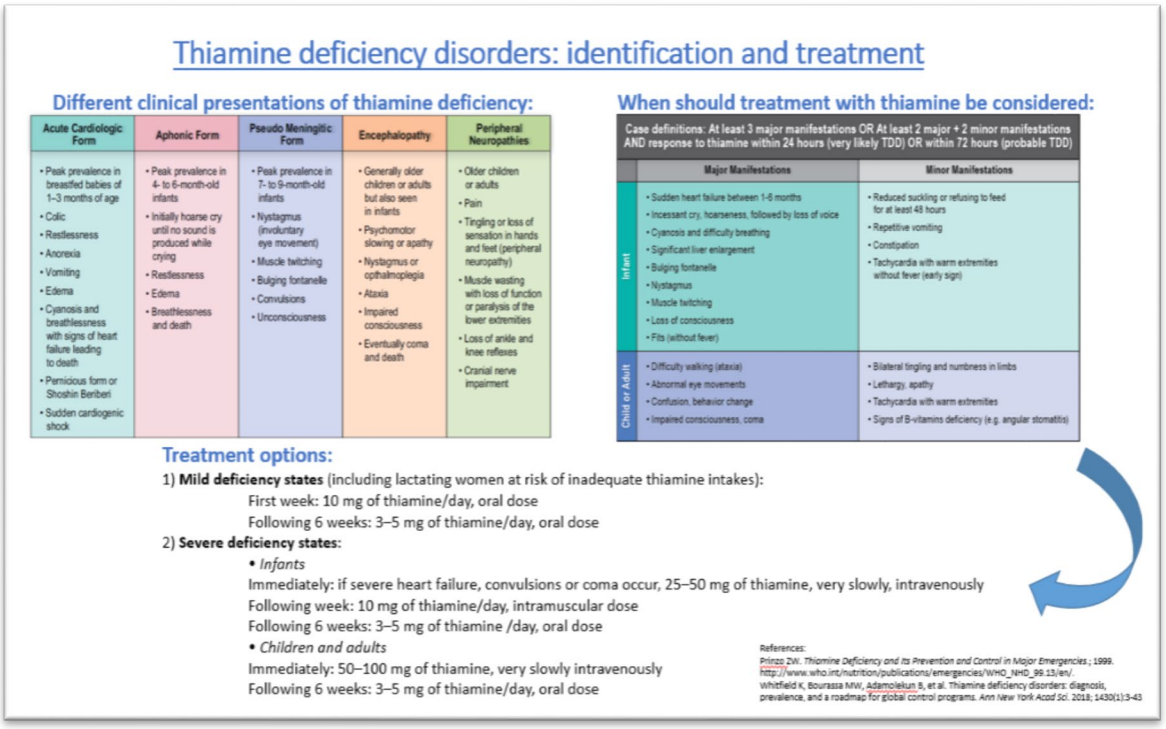


Figure 5 - Example of an educational material to healthcare professionals: “Thiamine deficiency disorders: identification and treatment.”

Figure 6 - Example of an educational material to healthcare professionals: “Infantile beriberi: clinical symptoms and case studies.”

In health facilities where the use of videos is possible, training of healthcare professionals can be supplemented with existing educational videos, such as this one: https://youtu.be/QXLj45Kt-wU

This 10-minute video was posted on behalf of Debra Coats who has been working with patients with beriberi and thiamine deficiency in Cambodia. It shows real-life examples of mothers who presented with their babies at a health clinic in Cambodia, including the symptoms (typical of infantile beriberi) and the quick recovery of these infants after administration of thiamine injection(s).

Equally important to the identification and treatment of TDD is the **prevention** of TDD. Consequently, it is important identify the causes of thiamine deficiency in the affected patients and educate them and/or their caregivers about solutions to address these causes in a permanent and sustainable way. The correct identification and education about the cause of thiamine deficiency – such as betel nut chewing, frequent consumption of raw/fermented fish, strict dietary avoidances post-partum, or reliance on a monotonous diet – will help prevent the recurrence of TDD.

Thus, delivery of culturally relevant information to the public is recommended, both verbally and in writing and it should cover topics such as: consequences of thiamine deficiency; requirements in different population groups, good and poor sources of thiamine; special considerations during pregnancy, breastfeeding and infancy; anti-thiamine factors and food processing and preparation techniques that minimize the loss of thiamine.

Two educational materials (leaflets) were developed and can be used by healthcare professionals and community health volunteers working with individuals affected by or at risk of TDD, both in the general public (figure 7) or in the subgroups of pregnant women and lactating mothers (figure 8). The document presented in figure 7 is a resource that helps healthcare professionals and community health volunteers to remember key information needed for the education of individuals affected by TDD (with regard to increase of thiamine intake and prevention of thiamine deficiency). Figure 8 is a leaflet that can be explained and handed to pregnant women and lactating mothers affected or at risk of thiamine deficiency (on hospital discharge, in visits to community clinics, in home visits by midwives) so that they can be later consulted. These materials may also be adapted to the needs of the local populations. For example, the list of good, moderate and poor sources of thiamine can be adapted to include locally consumed foods. For this purpose, the appendices 1a) and 1b) showing the thiamine content of foods that are frequently consumed Cambodia and Laos, may be used.

Additional educational materials for the prevention of TDD may be developed, and the present document offers information that may be used for this purpose. For example, it may be helpful to create a balanced one-day (or better, a one week) menu for a young child, an adult man and a breastfeeding woman that meets the thiamine requirements of each for these target groups. If these menus are developed according to the local food habits and contain the visual representation

(in addition to the thiamine content) of each meal, they are more likely to be understood and followed by a layperson.

Figure 7 - Example of an educational material for healthcare professionals and community health volunteers working with individuals affected by or at risk of thiamine deficiency: “A guide to increase thiamine intake and prevent thiamine deficiency”.

Figure 8 - Example of an educational material for pregnant women and lactating mothers, explaining the importance of thiamine during pregnancy, breastfeeding and infancy.

Chapter 4 – Information needs for the assessment, monitoring & surveillance of TDD in the population

Knowing the prevalence of TDD in the population is preliminary to any action, and biomarker assessments are the gold standard when assessing population thiamine status. In the absence of such information, a few techniques may help determine whether TDD presents a public health risk. If the suspicion of risk is confirmed a survey should be carried out to assess the biomarker status of the target population and to determine whether TDD is indeed a public health concern. Additional information may be required if the causes and etiology of TDD need to be determined, including a survey of dietary intake and local food practices. This information can also be used to design the set of interventions, which then must be monitored for program fidelity through various means, including operations research. The set of actions to obtain, analyze, disseminate, and act upon this information is organized under the surveillance system. This Chapter covers those various aspects.

4.1 - Surrogate measures of thiamine deficiency

In the absence of biomarker data surrogate measures of TDD risk may be used, including food balance sheets, household consumption and expenditure surveys, infant mortality data, and the review of secondary evidence. These approaches are presented in more detail below.

4.1.1 - Food balance sheets (FBS)

National FBS provide information on the annual per capita availability of various food commodities for dietary consumption. This information allows estimation of the risk of inadequate intake of thiamine based on the availability of thiamine in the food supply[2] and the amounts consumed per capita per day. Limitations with this approach are that it only provides data on a national level; it does not account for local food security status nor report food consumption at the individual or household level. Also, foods not included in national production statistics (e.g. non-commercial or subsistence production) are not covered by FBS. However, FBS help to monitor trends and shortfalls in nutrient availability nationally[2] and to determine the risk of inadequate consumption of specific nutrient.

FBS data are compiled annually by FAO (http://www.fao.org/faostat/en/#data/FBS) for 185 countries and 100 food commodity groups[43]. Summary data from 2011 FBS[44] and existing food fortification programs with thiamine (according to the Global Fortification Data Exchange[45]) is presented in Appendix 5. It contains a list of countries with an average level of thiamine availability below 1.2 mg/capita/day and no thiamine fortification program (mostly Asian and sub-Saharan African countries) and a list of countries where >40% of energy comes from low-thiamine staple crops and existing thiamine fortification programs.

4.1.2 - Household consumption and expenditure surveys (HCES)

HCES are often conducted to characterize household socio-economic conditions. The World Bank's Living Standards Measurement Survey (LSMS) for instance has been applied in several countries (http://datatopics.worldbank.org/consumption/detail#datasource) and offers readily available data of this type. Aggregate household nutrient intake is approximated by multiplying the average food consumption by the corresponding nutrient values for the edible portion of the food[43]. Disaggregation by socioeconomic status and geographical area can be used to pinpoint risk groups of inadequate thiamine intake and to target interventions. While more informative than the FBS, HCES still do not account for individual food consumption or the distribution of foods among household members.

4.1.3 - Infant mortality data

Month-specific infant mortality usually decreases throughout the first six months postnatal. When infantile beriberi is common, however, infant mortality remains high or even increases to a peak at about the third month, and may again do so around the sixth to seventh month (see figure 9, from Cambodia[2]). This is because infants who are breastfed by mothers, who are themselves thiamine deficient may see their thiamine levels fall in this period. Where such trends are observed infantile beriberi should be suspected[22,9]. Countries should examine infant mortality data by month of age, for instance using Demographic and Health Survey (DHS) data, if available (https://dhsprogram.com/).

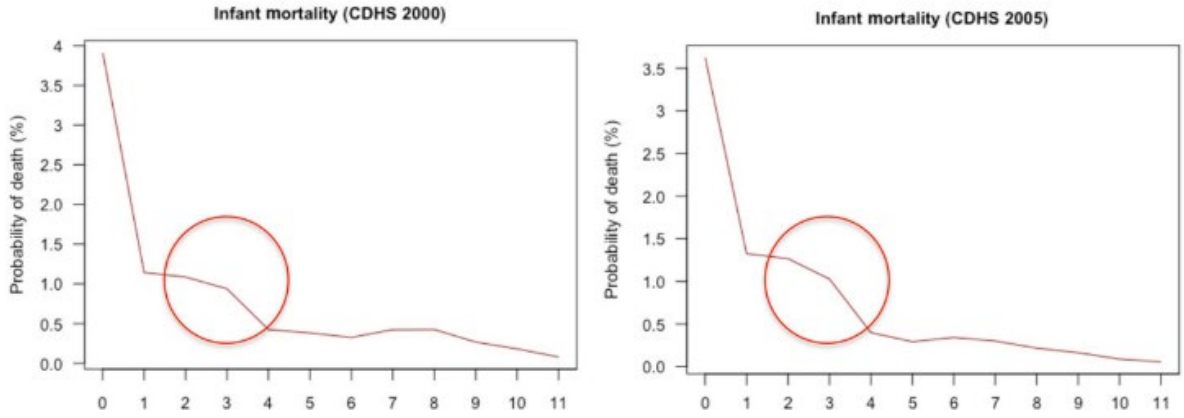


Figure 9 - Comparison of infant mortality rates by month of age in Cambodia based on data from the Cambodian Demographic and Household Surveys from 2000 and 2005[2].

4.1.4 - Other data

Other indications of risk in a population are: 1) the number of individuals with biomarker levels suggestive of deficiency, and 2) the number of individuals with clinical signs of beriberi who respond

to a thiamine therapeutic challenge[2]. The second indicator can be captured through reviews of medical records in hospitals or clinics. Countries suspecting that TDD affects their population should retrospectively review medical records or conduct prospective studies in major hospitals where TDD cases have been documented, looking for conditions associated with beriberi such as congestive heart failure and response to thiamine treatment. Two hospital-based studies from Kashmir screened their records in this way[46,47], providing valuable information on the local prevalence of TDD (see Appendix 6).

Other studies used mixed methods, such as the study conducted in Kiribati[27], which investigated an unusual and sometimes fatal illness the cause of which was later identified as thiamine deficiency. The investigation started with the evaluation of previously identified cases by interviewing medical providers and reviewing medical records, followed by the implementation of prospective surveillance in existing health facilities and the finding active cases in the community.

4.2 - Large-scale survey to assess population thiamine status

This section provides general guidance on the planning of a large-scale (regional/national) survey to assess thiamine biomarker levels in the population[48]. To understand the etiology of thiamine deficiency an optional module about dietary intake and other factors that can influence thiamine nutritional status can be added. Sampling techniques and indicators are covered below. Appendices 9 and 10 present an example of a survey instrument, which can be adjusted to the needs of each country and carried out either as standalone or as a module in a larger survey.

4.2.1 - Sampling

A representative sample of the population of interest is needed to obtain actionable information on thiamine programs. For that end, a randomized cluster survey may be indicated (although clustering may not always be needed, depending on the features of the population). In the current context the target populations are WRA (15–49 years), which will include pregnant and lactating women, and infants (<12 months). This inclusion is justified because WRA[36] and pregnant women[37] from the same population are known to have very similar blood levels of thiamine biomarkers; thus, sampling WRA will facilitate data collection. In addition to those considerations, stratification may be necessary, if it is believed that a key factor, such as residential status (urban/rural) may harbor critical differences in biomarker status. Those considerations will all affect the sample size.

Sample size

The sample size will be calculated to detect the prevalence of TDD in the target populations. The aim is to assess whether the proportion of the population that falls below the cut-off is sufficient to justify public health action. The formula for calculating the sample size for the estimation of proportion is given by[49]:

$$n = \frac{D_{est} * z^2\ 1\text{-}\alpha/2 * P_{est} * (1\text{-}P_{est})}{MOE^2}$$

where:

- D_{est} is the estimated design effect (DEFF) of the survey, *if cluster sampling is used*. The recommended values to use for key indicators can sometimes be obtained from prior studies; if not known, DEFF=2.0 is often used for randomized cluster surveys but this may lead to underestimation hence all efforts should be made to obtain a realistic DEFF.
- P_{est} represents the estimated prevalence (proportion of target group below the cutoff) at the time of the survey. A value for this can be obtained from prior local surveys if available. If no data exists, values from locations similar to that catchment area may be used. In the example below, P_{est} = .3 (30% of the target population falls below the cutoff) is used, using data from a recent study carried out in Cambodia[50].
- $z^2\ 1\text{-}\alpha/2$ is the critical value from the Normal Probability Distribution. The significance level is typically set at α = 0.05, giving a value of z_1−0.05/2 = $z_{1\text{-}0.05/2}$ = $z_{0.975}$ = 1.96.
- MOE is the margin of error. This value is typically set between 5% and 10%, but it is recommended that TDD surveys set the margin of error to 5% or MOE = 0.05.

Thus, assuming a value for P_{est} = .3, n would be

$$n = \frac{2.0 * 1.96^2 * .30\ (1 - .30)}{.05^2} = \frac{2.0 * 3.8416 * .21}{.0025} = \mathbf{646}$$

If stratification is used (e.g. to distinguish between rural and urban populations) each stratum will require the same sample size. Other elements to be considered, which may affect sample size, are Individual non-response, or inaccessible clusters: a random-generated "reserve" sample may be drawn as a precaution if it is suspected that some of the clusters are inaccessible, or that a proportion of individuals may refuse to answer the survey. For those considerations, readers may consult Stukel, 2018[49].

Sampling Design

Three-stage sampling (referring to clusters, households and individuals) can achieve representativity of the target population assuming that the sampling frame is comprehensive and up to date, and that care is taken at each stage of sampling. Precise definitions are needed to carry out this exercise, but those may vary from place to place. For instance, while a "cluster" refers to a randomly selected geographic area that contains individuals of interest, some locations may use Enumeration Areas (EA) - a geographical statistical unit that is created to support the implementation of a census and this would be preferred because a) the population for each EA may be readily available from the census; and b) EAs are usually of roughly equal size, which helps with

enumerator workload distributions. However, such resource is not always available, in which case communities may be used. A "household" consists of all people who live together (i.e., sleep) under the same roof, share cooking or housekeeping arrangements, and recognize the same lead male or female decision makers as the household head. Individuals refer to the target groups. Detailed instructions are found in Stukel, 2018[49] on three-stage cluster sampling, including how to maintain randomization at each stage and how to address weighing issues, if necessary.

Similarly, data collection location may vary by location. In general, it is preferable to collect data in the homes of sampled respondents rather than to draw the sample from individuals who come to health care clinics, as the latter may restrict respondents to those coming to the clinic only, introducing a bias in the sample. But if clinic attendance is high, then it may be more convenient to carry out data collection there. Further, non-interview activities, e.g. blood sampling or anthropometric measurements, may be simplified and the quality of the data may be improved if done in a central location such as a clinic, especially when storage and refrigeration requirements are demanding, as is the case for thiamine biomarkers. In such cases, respondents may be invited to come to that location after the interview is done, to complete data collection.

As those various points suggest, sampling involves several complex technical decisions. It is essential to obtain skilled expertise in making those decisions.

4.2.2 - Thiamine biomarkers assessment

Two biomarkers, thiamine diphosphate (ThDP) and erythrocyte transkelotase activity (ETKA) may be used to determine the prevalence and distribution of thiamine deficiency in the population. Which of the two biomarkers to choose is still under investigation as the precise relationship between the two biomarkers and how each correlates with disease manifestation is not well understood. Once a decision is made about which biomarker to use, a step-by-step guidance to assess population thiamine status using either biomarker (ThDP or ETKA) is presented in Appendix 7. This document includes instructions on how to adequately perform sample collection, preparation, storage, and analysis for each indicator.

With respect to the analytical requirements and laboratory procedures, a detailed comparison between ThDP and ETKA is presented in Appendix 8. Suitable facilities with the expertise, quality assurance mechanisms, and equipment could be trained in doing the analyses of both ThDP and ETKA methods, which could serve future surveillance programs, easing quality control issues, as well as save costs for the research project (on shipping and supplies procurement, for instance). In Asia, such facilities may exist in China, Thailand and Singapore.

4.2.3 - Dietary intake assessment

An assessment of thiamine dietary intake (including natural food sources and fortified foods) may be carried out as an optional module of the large-scale survey. When linked to the thiamine biomarkers the information from this module will help to understand the local **etiology** of TDD.

4.2.4 - Example of a large-scale survey for WRA

The full survey for WRA, which includes the biomarkers module (Appendix 9, in black) and dietary intake module (Appendix 9, in green), should collect the following information from eligible, present and consenting WRA:

Biomarkers module

Basic demographic characteristics, pregnancy and lactation status, use of nutrition supplements containing thiamine (dose and duration) to understand possible effect on thiamine blood biomarkers, symptoms of thiamine deficiency (based on the most common and self-identifiable manifestations of TDD), and information related with the blood specimen collection.

Dietary intake module

- Drugs that are regularly used, to identify those that may deplete thiamine and therefore increase the risk of thiamine deficiency (note: drug brand names need to be adapted to each country but a list of thiamine-depleting drugs is found at: *http://pennstatehershey.adam.com/content.aspx?productId=107&pid=33&qid=000718*).

- Food processing and preparation methods that increase losses of thiamine and foods that contain anti-thiamine factors. These questions will need to be adapted to the context of each country (e.g. the question about consumption of "African silkworm larvae" needs to be restricted to areas where consumption of this or equivalent foods are known), and the local names for this food needs to be identified.

- The consumption of foods fortified with thiamine (e.g. rice, fish sauce, wheat-based products, reporting the level of fortification from the label, if available). These questions will need to be adapted to the local context, depending on the thiamine fortified foods available in the country/region.

- A 24-h dietary intake recall[†], describing all the food and drinks consumed, as well as the preparation method and the amount of each item. Enumerators should be familiar with the dietary patterns of the surveyed population, have a list of foods commonly eaten by the target population and be familiar with composite dishes, their recipes and preparation methods, and

[†] "When a study aims to determine the mean intakes for a group or population, a single 24-hour recall per respondent can be carried out, especially when the sample size is sufficiently large. On the other hand, when the objective is to estimate the distribution of intakes, or to examine usual diets and correlations of individual intakes, more than one recall day per respondent is required" (*FAO. Dietary Assessment: A Resource Guide to Method Selection and Application in Low Resource Settings. Rome; 2018. http://www.fao.org/3/i9940en/I9940EN.pdf.)*

be aware of how food is served[43]. A pre-survey focus group may be used to collect the recipe ingredients for locally common dishes. During the survey, the individuals are asked about the consumed quantities of that dish and whether there was any change from the standard recipe.

- A food frequency questionnaire (FFQ) to assess the consumption of specific items over a longer time period (one month), including thiamine-rich foods (e.g. pork is distinguished from other meats), foods that contain anti-thiamine factors (e.g. raw and fermented fish is distinguished from cooked fish) and the most common low-thiamine staple foods. The foods listed in this table need to be adapted to the dietary habits of each country and to the habits of the surveyed individual by adding foods frequently consumed (but not present in the list) in the open section at the end of each food group. The last set of questions focused on the collection of information about potential food vehicles for fortification (e.g. rice, fish sauce, soy sauce, salt and bouillon cubes), such as consumption, place of purchase and producer. This list of items needs to be pre-selected, by each country, from foods that are not commonly fortified with thiamine.

4.2.5 - Example of a large-scale survey for infants

The full survey for infants, which includes the biomarkers module (Appendix 10, in black) and dietary intake module (Appendix 10, in green), should collect the following information from the child's caretaker, if consent is provided:

Biomarkers module

- Basic demographic characteristics
- Breastfeeding practices and use of formula (type and amount consumed/day, and level of thiamine fortification reported in the label)
- Use of nutrition supplements containing thiamine (including multiple micronutrient powders), dose and duration
- Information related with the blood specimen collection

Dietary intake module

- History of thiamine deficiency
- For those who have initiated complementary feeding, use of thiamine fortified foods (family foods and commercial baby foods). These questions will need to be adapted to the local context, depending on the thiamine fortified foods available in the country/region.
- For those who have initiated complementary feeding, a simple food frequency questionnaire (adapted to the food targeted age group) to help identify food items that are rarely consumed; details about food preparation methods that are important for the assessment of thiamine

intake; and to help target specific baby foods for fortification. These questions will need to be adapted to the context of each country.

Other direct methods to measure dietary intake (such as weighed food records) can be used if more precision is needed, although these tend to be more costly, labor intensive and rely heavily on respondents having good literacy and numeracy skills[43].

The proposed large-scale survey on thiamine status provides the above basic data needs for all respondent groups. The full versions of the instruments, containing a biomarkers module and an optional dietary intake module, can be found in Appendices 9 and 10 (for WRA and infants, respectively). Each organization responsible for carrying out the survey should also consider developing an enumerator training and guidance manual.

If more than 20% of the population (or population sub-group) has a thiamine biomarker concentration below the relevant cutoff, it should be considered a public health problem and large-scale intervention is recommended.

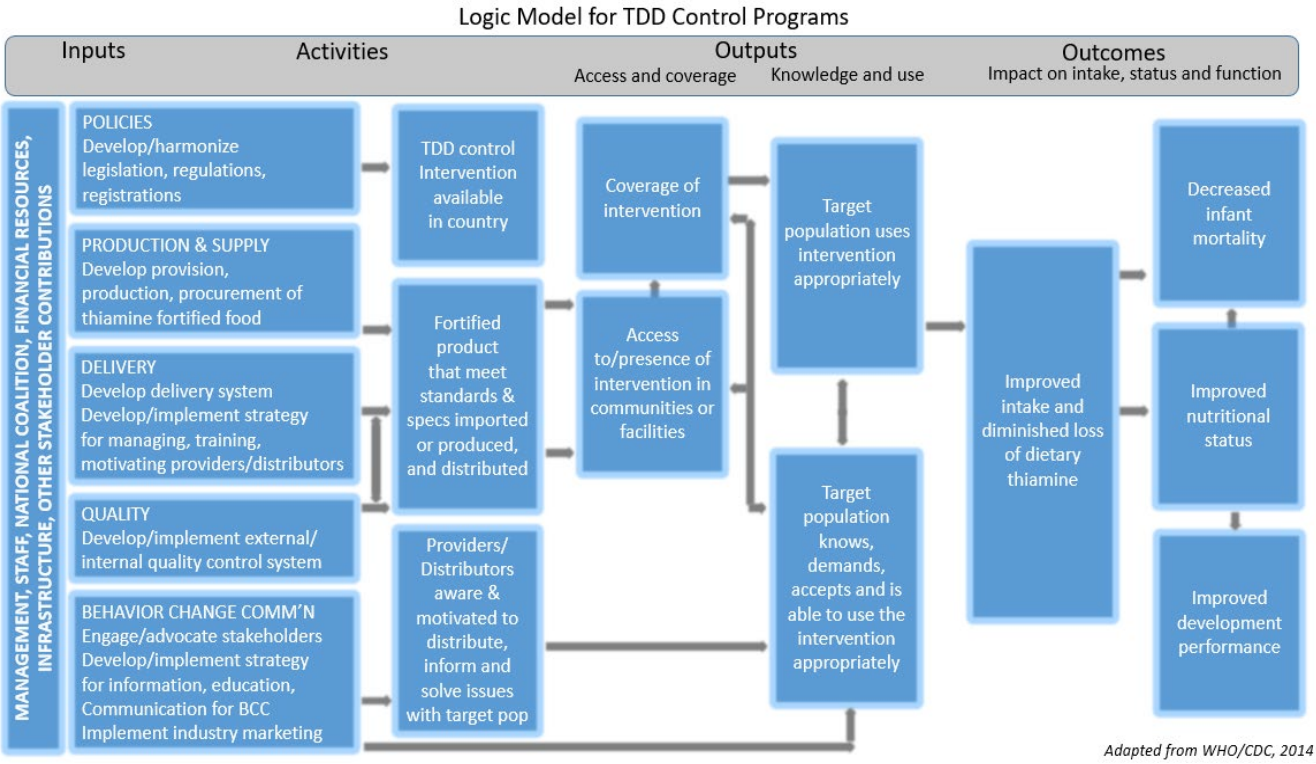
4.3 - Program design, monitoring and evaluation

Chapter 3 described the multiple components that may compose a TDD control program. Designing a TDD control and prevention program should bring all components together, so they are coherent and mutually reinforcing. The WHO/CDC logic model[51] offers a solid starting point in this regard as it specifies the high priority areas and concepts used in micronutrient interventions (Section 4.3.1); while adapting to local factors such as prevalence of TDD in the population, urgency of the response, and the food vehicle of choice (if fortification is used). Once designed and rolled out, the program should be regularly assessed to ensure proper implementation. Section 4.3.2 provides guidance on a monitoring and evaluation (M&E) approach for the TDD control and prevention program.

4.3.1 - Designing a TDD control and prevention program using the WHO/CDC logic model

A logic model helps stakeholders understand the components and activities included in the program by visually presenting the expected relationships between the resources invested (inputs), the processes taking place (activities), the direct results of those activities (outputs), and the changes that will occur in the population over time (outcomes). The clear description it provides builds a common framework and understanding among stakeholders, from policy makers to health officers to implementers; helps identify indicators for each action and their expected results; and helps communicate and advocate for the proposed actions.

To build a logic model, each component (inputs, activities, outputs, and outcomes) is described asking "what is needed to make the program work". The presumed connections between components are specified and the role of actors identified. More disaggregation may be done at a later stage, if needed. Figure 10 summarizes the general TDD Control and Prevention logic model; assuming that the "program" will combine food fortification, food supplementation and food-based approaches, the latter mostly geared at changing food consumption behaviors.



BCC = behavior change communication

Figure 10 – General logic model for thiamine deficiency disorders control and prevention programs.

4.3.2 - Monitoring and Evaluating the TDD control and prevention program

To ensure proper implementation, four types of assessment are considered, namely a) verifying that a-priori requirements are fulfilled; b) internal monitoring to assess program fidelity and whether intermediary outcomes are attained; c) impact evaluation to assess whether the actions are having the desired results on the population's thiamine status and associated disease burden; and d) process evaluation to identify stumbling blocks in the implementation process as they are discovered[‡]. The Logic Model (4.3.1) helps specify the dimensions to cover and the indicators to use in each type of assessment.

[‡] Other aspects, like formative research, are needed to appropriately design and deliver the interventions

A-priori requirements

Several of the components defined in the logic model under "inputs" need to be in place before the program starts. Below we list a list of things to ascertain before the program starts. Those are bound to differ depending on the interventions that are pursued (e.g. fortification, supplementation, food-based approaches, behavior change programs, etc.) and accordingly some elements will need to be reviewed periodically (e.g. every 6 months or yearly) (table 6).

Table 6 - Checklist of items to enable startup (frequency of verification)

COMPONENT	INDICATOR (Y/N)
Resources	Management structure and workplan in place Partnership agreements signed Funding secured (yearly) Storage facilities identified and secured (yearly) Key department officials mobilized and trained/refreshed (yearly)
Policies and regulations	Programmatic orientation determined (fortification, supplementation, etc) Legislation passed (e.g. mandatory industrial fortification) Import authorized and certificate of conformity ready (if products are brought in) Manufacturing firms certified and registered
Production & Supply (for each commodity used)	Production source ascertained for each commodity (yearly) Volume of demand known (6-monthly) Procurement system in place (yearly)
Delivery	Delivery system (transport, handling, storage) in place and effective (6-monthly) Distributors/providers trained and motivated (yearly) Supportive supervision system developed Safeguards against supply breaks devised (yearly) Staff offered intervention-specific training/refresher training (yearly) Timely reporting by all distribution centers (per national guidelines)
Quality	Quality controls defined and validated System to carry out supervision and quality control in place (6-monthly)
Social & Behavior change communications (SBCC)	Stakeholders engaged (yearly) SBCC strategy developed and validated SBCC program reviewed and staff training refreshed (yearly) Social and Behavior change communication campaign funded

Monitoring intermediary results and program fidelity

The term "fidelity" refers to the concordance of a program with the specification of the original design. Monitoring exercises are carried out to verify that implementation shows fidelity—that it is indeed rolled out as designed and that the intermediate results are attained[§]. Monitoring should be conducted regularly (e.g. yearly) to track key output and intermediate outcomes (table 7). Outputs may include product availability, supply, demand, and compliance; while intermediary outcomes may refer mostly to behaviors. Table 7 and 8 propose a list of indicators to be monitored regularly, specifying data sources, and frequency of collection/analysis. Preferably, indicators should emerge

[§] Designs should remain flexible as the information gained from process evaluations may unearth stumbling blocks that require changes in the overall design (see below) which, once validated, become part of the design.

from yearly household surveys. If this is not possible, alternate indicators based on market surveys should be used.

Table 7 - Monitoring output indicators (*all collected yearly*)

Component	Definition	Data source
Product availability	% of HHs where fortified product (FP) is found at time of survey	H Survey
	% of designated outlets where fortified product is found at time of survey	M Survey
Supply chain reliability	% of HHs that report supply chain breaks of FP in last year	H Survey
	% of designated outlets reporting FP stockouts in last year	M Survey
Demand and compliance	% of HHs that state using this fortified product exclusively	H Survey
	% of outlets that sell non-fortified version of product	M Survey
Coverage and Access	% of HHs that state the product is available locally % of HHs that state the product cost is affordable	H Survey
	% of outlets that sell at recommended price or below	M Survey

Table 8 - Monitoring intermediate outcome indicators (*all collected yearly except **)

Component	Definition	Data source
Dietary intake	% of households regularly consuming thiamine rich foods	H Survey*
	Volume of thiamine-rich food circulating in market	M Survey*
Behavior change	% of HH were lactating women follow strict food avoidances % of HHs eating foods that contain anti-thiamine factors	H Survey

H Survey: representative survey of households. M Survey: market survey of designated outlets

** Seasonality of intake may need two assessments per year (i.e. peak of wet and peak of dry seasons); seasonality may influence intake of naturally occurring thiamine and family income availability (e.g. sales of family-grown rice)*

While most of the above are assessed through the periodic administration of H and M surveys[**], opportunities to use other, existing data sources may be exploited to offer clues about intermediate indicators[48]:

- Active hospital or clinic surveillance systems may provide data on the incidence of suspected and confirmed cases of TDD affecting any age group. If available, those should be collected and reported on a regular basis, e.g. every month to a centralized system.
- Data can be also be collected at the clinic in antenatal visits and postnatal visits (for mothers and newborns) about the prescribed dosage and duration of supplementation and level of compliance.
- Where fortification programs are implemented the brand name, manufacturer, reported level of fortification, and a small sample of the food should be randomly collected from households for laboratory analysis.

[**] M Survey: representative survey of households in catchment area. W Survey: representative survey of women of reproductive age in catchment area. Low-cost statistical sampling methods using approaches like lot quality assurance sampling may be considered.

- For dietary diversification programs the proportion of the target population reaching the recommended dietary intake of thiamine and the proportion who is regularly consuming thiamine-rich foods could be queried during prenatal and postnatal visits to clinics.
- For behavior change programs the immediate knowledge acquired during behavior change communication educational programs can be assessed with small exit interviews.

Looking at this data may lead to fruitful conclusions. A good example is the link that was established between the epidemic of seasonal ataxia in Nigeria and the ingestion of the African Silkworm *Anaphe venata*, which contains thiaminases. After documenting the problem a health education program was developed and directed at hospital workers, hospitalized patients, and their relatives, to explain the etiological association of the seasonal ataxic syndrome and the larvae consumption. This program led to a 99% reduction in the annual number of hospital admissions for seasonal ataxia within two years[28]. Thus, records of TDD or symptoms before and after the delivery of health education programs to the target population group can be used to evaluate this type of intervention.

Program evaluations

Impact evaluations are meant to assess how the intervention has affected intended outcomes. Indicators of interest should be specific to all target groups, and the analysis of impact should ideally use a counterfactual (e.g. through a pre/post or a treatment/control design). The key outcomes are biochemical parameters and clinical signs of thiamine deficiency.

Table 9 – Monitoring final outcome indicators

Indicator	**Definition**	**Data source**	**Frequency**
% of WRA that are thiamine deficient	% of women 15-49 years of age that have ThDP level less than *xx* (tbd)	Biomarker survey	Bi/triennial
% of infants that are thiamine deficient	% of children <12mo with ThDP level less than *xx* (tbd)	Biomarker survey	Bi/triennial
Number of infants with clinical signs of TDD	Number of children <12mo who show clinical symptoms (tbd) of TDD over a one-year period	Hospital/clinic records	Monthly

Process evaluations

It is not unusual, especially at the start of a program, to carry out multiple process evaluations, each of them focused on a specific component of the program until implementation flows as expected. Examples of process evaluations tend to focus on aspects such as coverage, adherence, and demand—all of which are essential to program success. For instance, if demand is low a process evaluation may reveal that this is due to the population's lack of knowledge about the existence, goals, or benefits of the fortified food. Based on this information, efforts can be directed at educating the target groups and at encouraging the consumption of that food. Once those measures are taken the process evaluation may be carried out again to ensure that the action cleared the stumbling block.

Cues that a process evaluation is needed may come from various sources. Staff may report discrepancies in the way the program is executed; a monitoring assessment may show poor results on a key output or outcome indicator; supplies may not be absorbed as quickly as they should; and so on. It is therefore difficult to say in advance when and why a process evaluation will be needed; but attention should be paid, especially early on, to those aspects most crucial to the success of the intervention (access, supplies, demand, coverage, adherence, compliance[52]); and provisions should be made in the design of the program to allow for process evaluations, most usually by entrusting it to the M&E team.

4.3.3 - Surveillance

Surveillance is defined as the "ongoing, systematic collection, analysis, and interpretation of health-related data essential to the planning, implementation and evaluation of public health practice closely integrated with the dissemination of these data to those responsible for prevention and control" [53,54,††]. The six steps described in the text box below (Box 1) identify the main features and tasks of a surveillance system.

The data collected in such systems typically include demographic, socioeconomic, and clinical characteristics of the population under surveillance, data on key outcomes such as disease complications and mortality, and data on risk factors (potentially mitigating or aggravating behaviors, co-morbidity conditions, etc.). Such data can be collected from a variety of sources: as a part of population-based surveys or provided by healthcare system reporting data from populations receiving health care services (see section 4.3.2). A judicious use of medical registries (purchases, supplies consumption, etc.) can also provide information about the occurrence, type, extent, and the treatment provided for a particular disease and can be very useful for surveillance of rare conditions. Surveillance data can be used not only to monitor disease trends in caseloads over time and determine disease patterns in various populations but to guide planning and evaluation of disease control programs (e.g., determine whether prevention, screening, and treatment efforts are making a difference), help set priorities for allocating health resources and advance clinical, epidemiologic, and health services research in these disorders[54].

Once the data have been collected, it is important to have secure database systems in place along with proper data management and quality control procedures. Data should be periodically

†† Surveillance can be carried out along different modalities. Passive systems are the most common type and rely on reporting by health agents (health care providers, laboratories) of the status or pre-defined indicators (e.g. an increase in clinical presentations of a given condition). They are simple and inexpensive but are limited by the variability and completeness of reporting. Active systems involve pointed outreach by reporters to obtain or validate reports of specific health problems as provided, say, by passive reports. Syndromic systems focus on the outward signals or symptoms of a problem rather than physician-diagnosed or laboratory-confirmed illnesses. It uses information from a variety of indicators: increases in sales of specific medications, reductions in income or food insecurity that lead to dietary changes, etc. Sentinel surveillance can be active or passive and refers to the reporting of health events by health professionals who track a geographic area or a specific reporting group.

evaluated for accuracy, consistency, and completeness using standard data management procedures. Systems and procedures should be in place to protect data integrity as well as safety and security from natural disasters, computer virus attack, theft, and other threats. Once analyzed, results should be shared widely with the public and healthcare workers, public health authorities, and local and national governments and policy makers to provide the basis for designing interventions to decrease the problem. Subsequently, the effectiveness of interventions implemented in the population can be assessed by continued disease surveillance and monitoring.

For all practical purposes, the surveillance system may be conceived of as the physical embodiment of the control and prevention apparatus—the unified mechanism that collects, analyzes, and disseminates information about actions to reduce the incidence of TDD. While each country should adapt their TDD surveillance to their existing health information systems to avoid establishing parallel systems, precise steps and procedures should be established to ensure the regular compilation, analysis, and utilization of TDD-related data, possibly including modification of medical registries, specification of data pathways, identification of a responsible unit and the like.

Enabling environment and regulatory context for surveillance systems

National-based disease surveillance systems are mandated by law or regulation in some countries. Whether accountability is built into those systems where they exist must be ascertained, e.g. by specifying who must report what, how, and when; and by verifying periodically that those steps are taken. Commonly in such situations, physicians, laboratories, hospitals, clinics, and in certain instances school nurses and public health professionals are required to compile specific diseases from patient health records and to report incidence of various conditions to the local health department. Global guidance (e.g. WHO's International Health Regulation[55]) can provide models for regulatory action in this area. Also, for countries that dispose of digital health information systems, the Early Warning Outbreak Recognition System (EWORS) may offer a simple, effective tool to rapidly bring reliable and actionable outbreak data to the attention of public health authorities in the developing world. In Indonesia, for instance, twenty-nine signs and symptoms from patients with conditions compatible with infectious diseases are collected from selected provincial hospitals and analyzed daily. Data is e-mailed on a daily basis to a central data management and analysis center. Automated data analysis may be viewed at the hospital or the EWORS hub at the central level. Such a system, once made mandatory, can provide solid basis to the surveillance of TDD; and notably, some countries in Southeast Asia (e.g. Laos) are already signatory to EWORS.

Box 1 - Six main features and tasks of the surveillance system

1. Data collection: clarify the overarching goal and specific objectives of the TDD system
    - What to monitor?
    - Who will collect the data and how?
    - Who is the target population?
    - Where do we implement the system?
    - How will the data be transmitted to the person performing the analysis?
2. Data sources:
    - Reported diseases or syndromes (e.g. various presentations of TDD)
    - Electronic health records (e.g., diagnosis codes of TDD from hospital discharge data)
    - Laboratory analyses
    - Vital records (e.g., birth and death certificates)
    - Registries (e.g., use of thiamine injections, billing records)
    - Surveys
3. Data analysis
    - Determine who will analyze the data and verify the diagnosis (e.g. official diagnosis codes can include thiamine deficiency, dry beriberi, wet beriberi, among others)
    - What methodology to use
    - Frequency of data analysis
    - Other descriptive information (time, place, person) to be collected
4. Data interpretation
    - Identify the person, place, and time, to understand why it happened
    - Account for possible confounders (increased access to health care, change in reporting procedures, change in case definitions, more laboratories testing for the issue)
5. Data dissemination: feedback is crucial to improve the acceptability of the surveillance system, and cooperation from data reporters, the public and health agents
    - Specify how information will be distributed (newsletters, bulletins, surveillance summaries and reports; press releases social media).
    - Identify who needs to know (public health practitioners, clinicians and other health care providers, policy and other decision makers, community organizations, and general public).
6. Link to action: without action, the collected data is useless.
    - Action-oriented reporting: Describe the burden of disease; Monitor trends and patterns in disease, risk factors; Detect changes in disease occurrence and distribution; Provide data for programs, policies, and priorities; Evaluate prevention and control efforts.

Appendices

Appendix 1 a) Thiamine content (mg) per 100g of edible portion of foods that are frequently consumed in Cambodia[13]

Food (in English)	Food (in Khmer)	Thiamine (mg)
Galangal, root, fresh	រំដេង	**1.10**
Peanut, groundnut, seed, whole skin, dry	សណ្តែកដី	**1.10**
Pork meat, lean, raw	សាច់ជ្រូក	**0.90**
Mungbean, seed, green, dry, raw	សណ្តែកខៀវ	**0.73**
Pork, rib, deboned, raw	សាច់ឆ្អឹងជម្ជីជ្រូក	**0.65**
Shallot, bulb, raw	ខ្ទឹមក្រហម	**0.43**
Acacia pennata, leaves	ស្ពំ	**0.37**
Tamarind, fruit, ripe, peeled	អំពិលទុំ	**0.36**
Chili, dried,	ម្ទេសក្រៀម	**0.31**
Maize (corn), white, whole grain, raw	ពោត	**0.30**
Tamarind, young pod, raw	អំពិលខ្ចី	**0.30**
Banana, flower and bud, raw, fresh	ត្រយូងចេក	0.28
Garlic, fresh, raw	ខ្ទឹមស	0.24
Leech lime, leaf, fresh	ស្លឹកក្រូចសើច	0.20
Tamarind, young leaf, fresh raw	ស្លឹកអំពិល	0.20
Winged bean, pod, green, fresh, raw	ពពាយ	0.18
Bird chili, small, fresh	ម្ទេស	0.18
Bread, wheat, white	នំប៉័ង	0.18
Fish, Mystus wolffi, raw	ត្រីកញ្ឆេះ	0.17
Yard long bean, pod, green, fresh, raw	សណ្តែកគួ	0.15
Frog, raw	កង្កែប	0.14
Water mimosa, raw	កញ្ជែត	0.13
Egg, duck, whole, raw	ពងទា	0.13
Taro, tuber, fresh, raw	មើមត្រាវ	0.12
Fish, Rasbora, raw, dried	ត្រីចង្ហា	0.12
Cake, butter	នំពងទា	0.12
Sweet potato, white, raw	ដំឡូងជ្វា	0.11
Ivy gourd, leaf, raw	ស្លឹកបាស	0.11
Mackerel, short-bodied, raw	ត្រីកាម៉ុង	0.11
Mushroom, Chinese/shiitake, dry	ផ្សិតចិន	0.10
Mushroom	ផ្សិតស	0.10
Mungbean, sprout, raw	សណ្តែកបណ្តុះ	0.10
Pumpkin, young leaf, fresh	ត្រួយល្ពៅ	0.10
Sweet basil, leaf, raw	ជីនាងវង	0.10
Rice, white, polished, raw	អង្ករ	0.09

Food (in English)	Food (in Khmer)	Thiamine (mg)
Bamboo shoot, spring variety fresh	ទំពាំង	0.08
Eggplant, purple, raw	ក្រប់វែង	0.07
Broccoli, stem, fresh	ដើមខាត់ណា	0.07
Flowering white cabbage	ស្ពៃចង្អិះ	0.07
Water spinach/Morning glory, raw	ត្រកួន	0.07
Freshwater river shrimp (Macrobrachium)	កំពិស	0.07
Tomato, fresh raw,	ប៉េងប៉ោះ	0.06
Pumpkin, raw	ល្ពៅ	0.06
Guava, white flesh, fresh,	ត្របែក	0.06
Orange, green skin, fresh, peeled	ក្រូចពោសាត់	0.06
Banana, plantain banana, ripe, peeled	ចេកអំបូង	0.06
Banana, silver bluggoe, ripe, peeled	ចេកណាំវ៉ា	0.06
Chicken, breast, raw	សាច់មាន់ សាច់ទ្រូង	0.06
Beef, shank, raw	សាច់គោ, សាច់ភ្លៅ	0.06
Radish, Chinese, white, raw	ឆៃថាវ	0.05
Gourd, sponge, fresh	នន្លោង	0.05
Spring onion, leave and stem, fresh	ស្លឹកខ្ទឹម	0.05
Carrot, peeled, raw	ការុត	0.05
Holy basil, leaf, fresh, raw	ជីម្រះព្រៅ	0.05
Banana, unripe, peeled	ចេកណាំវ៉ាខ្ចី	0.05
Sugar, granulated	ស្ករស	0.05
Pepper, black, powder, raw	ម្រេច	0.05
Ginger, root, mature, fresh	មើមខ្ចី	0.04
Cucumber, small, fresh, raw	ត្រសក់	0.04
Toddy palm, thicken sap	ស្ករត្នោត	0.04
Nile tilapia, raw	ត្រីទីឡាព្យ៉ា	0.04
Striped snake-head fish, raw	ត្រីផ្ដក់	0.04
Coconut milk, powder, instant	ខ្ទិះដូង	0.04
Noodle, rice flour, wet	នំបញ្ចក	0.04
Turmeric	រមៀត	0.03
Onion, large, fresh	ខ្ទឹមបារាំង	0.03
Papaya, unripe, peeled	ល្អុងខ្ចី	0.03
Water lily, stem, fresh raw	ព្រលិត	0.03
Climbing perch, raw	ត្រីក្រាញ់	0.03
Coconut juice, per 100ml	ទឹកដូង	0.03
Sauce, soy	ទឹកស៊ីអ៊ីវ	0.03
Gourd, wax, mixed variety	ត្រឡាច	0.02
Lime	ក្រូចឆ្មារ	0.02
Amaranth, spineless, fresh	ផ្ដឺ (ស)	0.02
Pork, skin, fried	ស្បែកជ្រូក	0.02
Striped catfish, raw	ត្រីប្រា	0.02

Food (in English)	Food (in Khmer)	Thiamine (mg)
Common silver barb, raw	ត្រីឆ្ពិន	0.02
Fish, Siamese mud carp, raw	ត្រីរៀល	0.02
Coffee, roasted	កាហ្វេ	0.02
Shrimp, fermented	កាពិ	0.01
Catfish, Gunther's walking, raw	ត្រីអណ្តែង	0.01
Black paddy crab, raw	ក្តាម	0.01
Pork, lard	ខ្លាញ់ជ្រូក	0.01
Cabbage, Chinese, salted	ស្ពៃជ្រក់	0.01
Sauce, fish	ទឹកត្រី	0.01
Oil, soybean	ប្រេងសណ្តែក	0.00
Salt, table	អំបិល	0.00
Soybean, fermented	សៀង	0.00
Lemon grass, leaf, fresh, raw	គល់ស្លឹកគ្រៃ	*0.07
Long coriander (cilantro) leaves, raw	ជីរណា	*0.07

** Corrected value*

Good sources of thiamine, defined as those providing 25% of the male adult's daily recommended intake per portion of food (i.e. 0.30mg), are highlighted in green

Appendix 1 b) Thiamine content (mg) per 100 g of edible portion of foods that are frequently consumed in Laos[12]

Food (in English)	Food (in Lao)	Thiamine (mg)
Milk powder for pregnancy, Annmum brand	Noom foun ka annmum	**2.00**
Sesame seeds, white, roasted	Maak nga khoua	**0.83**
Pork sausage, grilled	Ping sai oua moo	**0.74**
Pork, raw	Sine moo soth	**0.64**
Pork, boiled	Toom sine moo	**0.58**
Peanut boiled	Thoua din toom	**0.56**
Baby milk powder, dumex Hi Q brand	Noom foun ka Dumex Hi Q	**0.56**
Baby milk powder, lactogen brand	Noom foun ka lactogen	**0.56**
Baby food, cerelac	Ahanh deak ka Cerelac	**0.40**
Horse tamarind, young leaves	Bai ka thin soth	**0.33**
Pork, grilled	Ping sine moo	**0.32**
Chicken liver, boiled	Toom tab kai	**0.32**
Pork, liver, grilled	Ping tab moo	**0.32**
Chicken, liver, grilled	Ping tab kai	**0.32**
Pork, shredded, Chinese style	Moo foi	**0.32**
Pork, liver, raw	Tab moo dip	**0.32**
Chicken, liver, raw	Tab kai dip	**0.32**
Beef, liver, raw	Tab ngoua dip	0.28
Beef, liver, grilled	Ping tab ngoua	0.28
Beef, grilled	Ping sine ngoua	0.25
Omelet duck egg	Cheuan khai pet	0.23
Pork, spleen, raw	Mam moo dip	0.21
Cowpea, seeds, black, dried, boiled	Thoua dam toom	0.20
Milk, instant, Anmum brand	Noom kong ka annmum	0.20
Rice noodle with pork and coconut milk soup	Mee ka thi	0.20
Horse tamarind, seeds	Maak ka thin soth	0.19
Tamarind, young leaf, fresh	Bai maak kham onh	0.18
Beef internal organ barbecue	Sieb kheuang nai ngoua	0.17
Rice porridge, boiled with pork	kaho piek khao moo	0.16
Mint, leaf	Pak houm lab soth	0.15
Egg, duck, whole, boiled	kai pet toom	0.15
Chili pepper, hot, red, fresh	Maak phet soth	0.15
Ovantine, mixed with warm water	Ovantine	0.14
Wheat noodle (waiwai), instant	Mee sam let hoob	0.13
Rice noodle (mee suah)	Sen khao piek	0.13
Egg, hen, whole	Khai kai dip	0.13
Short bodied mackerel fried	Cheua pa tu	0.13

Food (in English)	Food (in Lao)	Thiamine (mg)
Duck, roasted	Ping pet	0.12
Beef ball, blanched	Look sine ngoua	0.12
Beef, lung, raw	Pod ngoua dip	0.12
Fermented rice noodle with fish soup	Khao poun nam pa	0.12
Fermented rice noodle with chicken soup	Khao poun nam kai	0.12
Cake, sponge	Khao nom khai	0.12
Bean sprouts, fresh	Thoua ngok soth	0.11
Chicken, heart	Houa chay kai dip	0.11
Short bodied mackerel, roasted	Ping pa tu	0.11
Shallot, bulb	houa boua hang	0.11
Rice, white	Khao chao	0.10
Coriander, fresh	Pak hom pome	0.10
Yard long bean, green, fresh	Maak thoua ngao soth	0.10
Soup, chicken, broth	Soup kai ka kha nor	0.10
Hairy basil, fresh	Pak e tu	0.09
Beef, dry, fried	Cheun sine ngoua hang	0.09
Nile tilapia, roasted	Ping pa nin	0.09
Egg, hen, whole, boiled	Khai kai toom	0.09
Wheat noodle (waiwai), instant, boiled with seasoning	Toom mee sam let hoob	0.09
Morning glory/Swamp cabbage, fresh	pak bong soth	0.08
Wildbetal leafbush	Pak e leuad	0.08
Beef, spleen, raw	Mam ngoua dip	0.08
Chinese cabbage blanched	Pak kaad khao louak	0.07
Mustard green, stem and leaves	Pak kaad khiew soth	0.07
Dill, fresh	Pak ce soth	0.07
Eggplant/brinjal, green, fresh	Maak kheua soth	0.07
Tomato, fresh	Maak len	0.07
Beef, blanched	Choum sine ngoua	0.07
Beef, intestine, raw	Sai ngoua dip	0.07
Garlic, fresh	Ka thiam soth	0.07
Rice noodles, topped with beef in soup	Feu gnoua	0.07
Cabbage, common, fresh	Pak ka lam pe soth	0.06
Pumpkin, mature, fresh	Maak eu	0.06
Mustard, fresh	Pak kaad soum soth	0.06
Cabbage, blanched	Pak ka lam pe louak	0.06
Spring onion, fresh	Pak houm boua soth	0.06
Beef, raw	Sine ngoua dip	0.06
Chicken, roasted	Ping kai	0.06
Beef, dried, grilled	Ping sine ngoua hang	0.06
Chicken, boiled	Toom sine kai	0.06

Food (in English)	Food (in Lao)	Thiamine (mg)
Pork, skin, raw	Nang moo dip	0.06
Coconut juice, fresh	Nam maak phao soth	0.06
Layer cake with cream, Ellse brand	Khao nom kai ka ellse	0.06
Morning glory/Swamp cabbage, blanched	Pak bong louak	0.05
Chicken, raw	Sine kai dip	0.05
Chicken, gizzard, raw	Tai kai dip	0.05
Omelet hen egg	Cheuan khai kai	0.05
Lemon grass, fresh	Houa sing khai soth	0.05
Wafers with chocolate, Shanghai brand	Khao nom ka sieng hai	0.05
Cucumber, fresh	Maak teng soth	0.04
Mustard green blanched	Pak kaad some louak	0.04
Orange, sweet, fresh	Maak kieng	0.04
Banana, ripe, yellow	Maak kouy nam souk	0.04
Beef, stomach, raw	Phoung ngoua dip	0.04
Nile tilapia fish, raw	Pa nin dip	0.04
Hen egg, fried	Cheuan khai dao	0.04
Milk UHT, Thaidenmark brand	Noom soth ka thaidenmark	0.04
Sticky rice (white), steamed	Khao niew neung	0.03
Tiliacora triandra diels	bai gna nang	0.03
Green amaranth, small, fresh	Pak huom soth	0.03
Apple, prink, fresh	Maak apple	0.03
Banana, ripe, yellow, boiled	Maak kouy nam souk toom	0.03
Pork, blood, boiled	Toom leuad moo	0.03
Onion	Houm boua gnai	0.03
Beef with bone soup	Keng du ngoua	0.03
Cracker from wheat flour, Tavan brand	Khao nom khieb ka tavan	0.03
Deep fried banana with powder	khao nom kouy cheuan	0.03
Rice steamed, white	Khao chao houng	0.02
Steamed sticky rice (white), grilled	Khao niew neung ping	0.02
Banana, flowers, fresh	Maak pee	0.02
Pak kha yeng	Pak kha yeng	0.02
Mustard green, fermented, sour	Som pak kaad	0.02
Chayote, boiled	Maak sa ver louak	0.02
Green amaranth, small, blanched	Pak huom louak	0.02
Chayote, fruit, fresh	Maak sa ver soth	0.02
Lemon, juice, fresh	Nam maak nao	0.02
Rambutan, fresh	Maak ngor	0.02
Siamese mud carp, grilled	Ping pa khao	0.02
Fermented fish, sour, fried	Cheun pa som	0.02
Yoghurt, drinking, foremost brand	Noom som ka foremost	0.02

Food (in English)	Food (in Lao)	Thiamine (mg)
Coconut milk	Ka thee maak phao	0.02
Rice Porridge, boiled with Nile tilapia fish	kaho piek khao pa nin	0.02
Rice porridge, boiled with chicken	kaho piek khao kai	0.02
Shrimp cracker from wheat flour	Khao nom kieb koung	0.02
Rice drops in sweet coconut milk	Na van lod shong	0.02
Fennel common leaves, fresh	Pak hom pae	0.01
Fermented fish, liquid	Nam pa deak	0.01
Fermented fish with bone	Pa deak niew	0.01
Soy milk, Lactasoy brand	Noom ka lactasoy	0.01
Shrimp paste	Ka pi	0.01
Fish sauce	Nam pa	0.01
Oyster sauce	Nam man hoi	0.01
Beer (USA)	Beer	0.01
Papaya salad	Tam maak houng	0.01
Pork with fat, hen egg, boiled, with sugar	Toom khem (sine moo and khai)	0.01
Porridge, white rice, boiled	kaho piek khao	0.01
Sugar, white	Nam tan	0.00
Carbonated drink	Nam ath lom	0.00

Good sources of thiamine, defined as those providing 25% of the male adult's daily recommended intake per portion of food (i.e. 0.30mg), are highlighted in green

Appendix 2) Data suggestive of thiamine deficiency from selected countries

Laos

There are several recent case reports of beriberi and suboptimal thiamine status among infants in Laos:

- A survey of 22 villages in northern Laos reported a high infant mortality rate (50 deaths among 468 live births[56]). Most of the infants (<6 months old) died between 1-3 months of age, and 17 out of the 50 deaths were suspected to be caused by infantile beriberi. Examples of thiamine deficiency, in both mother and infant, observed during the survey, are shown in **Box 2**[56]).
- In Vientiane, 13% of a cohort of 778 sick infants <1 year were found to have biochemical evidence of thiamine deficiency (despite the absence of beriberi clinical signs)[57,51]
- In a retrospective study based on reports from 51 hospitals (including central, provincial, and district hospitals), every month an average of 60 infants (0.8 per 1,000 infants) under 12 months of age and 36 adults (20 men and 16 women) were treated for thiamine deficiency. There was no seasonality associated with the caseload. In population centers like Luang Prabang, where health workers have an increased awareness of TDD, infant prevalence is highest, at an estimated 2.5 per 1,000 infants (Laos PDR Ministry of Health, unpublished data).

Box 2 - Examples of mother and infant thiamine deficiency observed during the village survey (Barennes et al, 2015)

"File n°117. Origin: Denkang village, Long district.
Mother: *28 years old, Hmong ethnic group, farmer, illiterate, 7 children, 5 deaths.*
Post-partum: *strict food avoidance after delivery: she ate only polished rice, and salt during one month.*
Symptoms: *edema of legs and arms; paresthesias, dyspnea.*
Infant: *In the age of 1 month and in good health, he died suddenly after a day with silent screams, cyanosis of the body, and inability to breastfeed. The child was anuric, no liquid stools, no fever, no cough."*

"File N°153. Origin: Nam-O village, ViengPhouka district
Mother: *25 years old, Khmu ethnic group, farmer, primary school, 2 living children and one child deceased at one month of probable meningitis.*
Post-partum*: food avoidance after delivery: she ate white rice, chicken, rarely vegetable, fish, birds, during 30 days.*
No clinical symptoms.
Infant: *2 months old. Presence of restlessness, refusal to suck, hoarseness, aphonia, and cyanosis. Symptoms appeared suddenly and severely.*
Physical examination: *dyspnea, cyanosis, tachycardia and hepatomegaly.*
Treatment: *The infant was treated by intramuscular injection of thiamine; thiamine tablets given to the mother.*
Evolution: *after 1 hour the child was able to suckle and no more cry."*

Cambodia

There are several studies suggesting that thiamine deficiency is a major public health problem affecting infants, children, their mothers, and other women of childbearing age:

- The first nationally representative biochemical thiamine status data from a country in Southeast Asia was obtained from a sub-sample of the first Cambodian national micronutrient survey, from 24 Cambodian provinces, conducted in 2014[58]. The analysis of erythrocyte ThDP data, from 719 women (15–49 years) and 761 children (6 months - 5 years), showed that, using a previously proposed – and the most conservative - cut-off of erythrocyte ThDP < 120nmol/L would classify 27% of the women, 15% of the children, and 38% of the infants subgroup (6-12 months) as thiamine deficient[50]. However, it should be noted that there is no widely accepted cut-off to define thiamine deficiency status based on erythrocyte ThDP, and other previously used cut-offs would significantly increase the thiamine prevalence rates[50].
- Multiple studies done in various regions have shown a high prevalence of infantile beriberi. In Mesang District, Prey Veng province of Cambodia, a verbal autopsy survey estimated that 45% of infants who died (mostly during their first 6 months), between January 2005 and April 2008, had beriberi, based on the clinical presentations[59]. In this same district, other cases of infantile beriberi (median age of 8 weeks) were reported in a study conducted in a Health Clinic in 2012[60]. Thiamine deficiency was also reported to be frequent in inpatient and outpatient infants in the Siem Reap province[61].
- A study that compared erythrocyte ThDP levels of women of reproductive age (non-pregnant and non-lactating), between Cambodia and Canada showed that the levels were significantly lower in the Cambodian population than the Canadian population (n = 49); within Cambodia, the levels were significantly lower in rural Prey Veng (n = 156), when compared to urban Phnom Penh (n = 146)[62] [63]. The authors who conducted this study suggest that strategies such as supplementation, fortification, and/or food-based interventions may be needed in this population, given the importance of reaching an adequate thiamine status before conception.

Myanmar

Data from a study conducted in Myanmar in 2013-2014 to understand the cause-specific mortality amongst under-fives showed that Beriberi was the second most common cause of death in infants between 28 days and 1 year of age, being most frequently reported in rural areas[64]. Aware of this public health problem, Myanmar is now providing supplements of thiamine (10mg/day) to pregnant women and lactating women up to 3 months after birth[2], although there is no data on coverage and compliance with this program. Myanmar is expected to carry out a micronutrient survey shortly that will include assessment of the prevalence of thiamine deficiency.[57]

Appendix 3) Global data on food fortification (maize flour, rice, and wheat flour) with thiamine

The following table shows the countries with existing mandatory and voluntary thiamine fortification programs of maize flour, rice, and wheat flour, including the year in which the fortification program was initiated and the recommended level of thiamine.

Source: Global Fortification Data Exchange (updated October 1, 2018)[65]

Country	Year	Mandatory or voluntary	Recommended Nutrient Level	Average Value (mg/kg)
Maize Flour				
Burundi	2015	Voluntary	6.5 +/- 2.9 mg/kg recommended factory level, 3.6 mg/kg minimum regulatory level, 9.4 mg/kg maximum regulatory level, addition of this nutrient is optional	6.5
Costa Rica	1999	Mandatory	minimum level of 4.0 mg/kg	4
Dominican Republic	2009	Voluntary	minimum level of 6.2 mg/100 g, we assumed the units were expressed per kg	6.2
El Salvador	2009	Mandatory	minimum level of 6.1 mg/kg	6.1
Guatemala	2016	Mandatory	2.5 mg/kg (average content to add)	2.5
Kenya	2012	Mandatory	4.0 +/- 2.0 mg/kg recommended factory level, 1.5 to 6.0 mg/kg (regulatory requirements)	4
Malawi	2011	Mandatory	3.6 - 9.4 mg/kg (regulatory requirements)	4.5
Mexico	2005	Mandatory	5 mg/kg	5
Mozambique	2012	Voluntary	5 mg/kg is the minimum addition level, no safety limit set, addition of this nutrient is optional	5
Nigeria	2014	Mandatory	6.0 mg/kg	6
Rwanda	2010	Voluntary	6.5 +/- 2.9 mg/kg recommended factory level, 3.0 mg/kg minimum regulatory level, no maximum level set	6.5
South Africa	2008	Mandatory	2.1875 mg/kg	2.19
Uganda	2006	Mandatory	0.2 mg/100 g minimum required level, no tolerable maximum set, for low-extraction maize flour	2
United States of America	2017	Mandatory	It contains in each pound not less than 2.0 milligrams (mg) and not more than 3.0 mg of Thiamine	5.5
Venezuela, Bolivarian Republic of	1996	Mandatory	0.31 mg/ 100 g average addition level, 0.20 mg/100 g minimum addition level, 0.50 mg/100 g maximum addition level	3.1
Zimbabwe		Mandatory	minimum level of 4.5 mg/kg	4.5

Rice				
Costa Rica	2006	Mandatory	5.3 mg/kg	5.3
India	2016	Voluntary	3.5 mg/kg minimum required level, addition of this nutrient is optional	3.5
Nicaragua	2014	Mandatory	5 mg/kg	5
Panama	2009	Mandatory	5 mg/kg	5
Papua New Guinea	2007	Mandatory	0.5 mg/100 g	5
United States of America	2017	Mandatory	each pound of the rice contains not less than 2.0 milligrams (mg) and not more than 4.0 mg of Thiamine	6.6
Venezuela, Bolivarian Republic of	1993	Voluntary	1.0 mg/100 g	10
Wheat Flour				
Antigua and Barbuda	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Argentina	2002	Mandatory	6.3 mg/kg	6.3
Australia	2014	Mandatory	not less than 6.4 mg/kg	6.4
Bahamas	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Barbados	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Belize	1998	Mandatory	6.0 mg/kg	6
Bolivia, Plurinational State of	2011	Mandatory	minimum level of 4.4 mg/kg	4.4
Burundi	2015	Mandatory	9.8 +/- 4.4 mg/kg recommended factory level, 4.6 mg/kg minimum regulatory level, 14.2 mg/kg maximum regulatory level	9.8
Canada	2016	Mandatory	0.64 mg/100 g	6.4
Chile	2014	Mandatory	6.3 mg/kg	6.3
China, People's Republic of	2012	Voluntary	3 - 5 mg/kg	4
Colombia	1996	Mandatory	6 mg/kg	6
Costa Rica	2001	Mandatory	5.4 mg/kg	5.4
Cuba	2012	Mandatory	5 - 6 mg/kg	5.5
Dominica	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Dominican Republic	2009	Mandatory	minimum level of 6.2 mg/kg	6.2
Ecuador	2011	Mandatory	2.2 - 5.8 mg/kg	4
El Salvador	2007	Mandatory	minimum level of 6.2 mg/kg	6.2
Ethiopia	2017	Voluntary	9 mg/kg, recommended factory level	9
Fiji	2009	Mandatory	6.0 mg/kg	6

Ghana	2006	Mandatory	8.4 mg/kg +/- 10%	8.4
Grenada	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Guatemala	2001	Mandatory	4.0 - 6 mg/kg	5
Guinea	2006	Mandatory	4.05 g/ton	4.05
Guyana	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Haiti	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Honduras	2007	Mandatory	6.2 mg/kg	6.2
India	2016	Voluntary	minimum level of 3.5 mg/kg for Atta flour, addition of this nutrient is optional	3.5
Indonesia	2009	Mandatory	minimum level of 2.5 mg/kg	2.5
Jamaica	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Kazakhstan	2008	Mandatory	1.8 - 3.6 mg/kg	2.7
Kenya	2012	Mandatory	5.0 to 15 mg/kg (regulatory requirement)	10
Kiribati	2014	Mandatory	6.0 mg/kg	6
Kuwait	2006	Mandatory	minimum level of 6.38 mg/kg	6.38
Liberia	2016	Mandatory	5.1 - 13.3 mg/kg	8.5
Malawi	2011	Mandatory	9.0 mg/kg recommended factory level, 5.4 to 14.2 mg/kg regulatory requirements	9
Malaysia	2017	Voluntary	not less than 0.42 mg thiamine/100 g flour	4.2
Mexico	2009	Mandatory	5 mg/kg	5
Morocco	2006	Mandatory	4.5 g/ton	4.5
Mozambique	2012	Voluntary	5 mg/kg is the minimum addition level, no safety limit set, addition of this nutrient is optional	5
Nicaragua	2007	Mandatory	minimum level of 6.2 mg/kg	6.2
Nigeria	2014	Mandatory	6.0 mg/kg	6
Palestine, State of	2010	Mandatory	2.9 mg/kg average addition level, minimum level of 2.0 mg/kg, no maximum tolerance level set	2.9
Panama	2003	Mandatory	minimum level of 6.2 mg/kg	6.2
Paraguay	2002	Mandatory	minimum level of 4.50 mg/kg	4.5
Peru	2006	Mandatory	minimum level of 5 mg/kg	5
Qatar	2006	Voluntary	minimum level of 6.38 mg/kg	6.38
Saint Kitts and Nevis	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Saint Lucia	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Saint Vincent and the Grenadines	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Saudi Arabia	2006	Mandatory	minimum level of 6.38 mg/kg	6.38

Sierra Leone	2010	Voluntary	8.4 mg/kg +/- 10%	8.4
Solomon Islands	2010	Mandatory	6.0 mg/kg	6
South Africa	2008	Mandatory	1.9444 mg/kg	1.94
Suriname	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Tanzania, United Republic of	2010	Mandatory	5 to 15 mg/kg	10
Trinidad and Tobago	1992	Mandatory	not less than 0.44 mg/100 g, not more than 0.77 mg/100 g	6.05
Uganda	2016	Mandatory	9.8 +/- 4.4 mg/kg recommended factory level, 4.6 mg/kg minimum regulatory level, no maximum regulatory level set	9.8
United Arab Emirates	2006	Voluntary	minimum level of 6.38 mg/kg	6.38
United Kingdom of Great Britain and Northern Ireland	1998	Mandatory	not less than 0.24 mg/100 g	2.4
United States of America	2017	Mandatory	it contains in each pound 2.9 milligrams of thiamine	6.38
Venezuela, Bolivarian Republic of	2001	Mandatory	1.5 average addition level, minimum level of 1.2 mg/kg, 1.8 mg/kg maximum tolerance level	1.5
Zimbabwe	2016	Mandatory	9.0 mg/kg	9

Appendix 4) Examples of educational materials (available in PDF)

- For healthcare professionals:
  - "Thiamine deficiency disorders: identification and treatment"
  - "Infantile beriberi: clinical symptoms and case studies"

  - "A guide to increase thiamine intake and prevent thiamine deficiency". Includes:
    - Consequences of thiamine deficiency
    - Good food sources of thiamine
    - Moderate and poor food sources of thiamine
    - Recommended intake of thiamine/day
    - Pregnancy, breastfeeding and infancy
    - Anti-thiamine factors
    - How to reduce thiamine losses through food processing and preparation

- For pregnant women and lactating mothers, explaining the importance of thiamine during pregnancy, breastfeeding and infancy

Appendix 5) Data suggestive of thiamine deficiency worldwide, based on the analysis of food balance sheets (2011)[66] and global food fortification data[65]

Countries with average level of thiamine availability below 1.2 mg/capita/day and no thiamine fortification program in place

Country	Energy, kcal/capita/day	Thiamine availability, without fortification (mg/capita/day)
Somalia	1691	0.67
Sri Lanka	2486	0.72
Bangladesh	2431	0.78
Tajikistan	2100	0.78
Mongolia	2445	0.87
Cambodia	2404	0.91
Guinea-Bissau	2303	0.92
Malaysia	2839	0.93
Botswana	2260	0.96
Thailand	2755	1
Mauritius	3045	1.03
Brunei Darussalam	2900	1.04
Lao PDR	2354	1.07
Japan	2712	1.09
DPR of Korea	2103	1.13
Swaziland	2266	1.13
Gambia	2848	1.14
Myanmar	2527	1.15
Sao Tome and Principe	2669	1.15
Libya	3195	1.19

Countries with >50% of energy provided by low-thiamine staple crops

Rice

Country	% of energy from rice	Listed by the Global Fortification Data Exchange for flour, maize or rice fortification with thiamine
Bangladesh	70.8	No fortification program
Cambodia	63.2	No fortification program
Lao PDR	61	No fortification program
Viet Nam	51.7	No fortification program
Madagascar	50.3	No fortification program
Indonesia	48.4	Mandatory fortification for wheat flour
Myanmar	46.3	No fortification program
Philippines	44.9	No fortification program
Sri Lanka	41.9	No fortification program
Liberia	40.5	Mandatory fortification for wheat flour
Thailand	40.3	No fortification program

Maize

Country	% of energy from maize	Listed by the Global Fortification Data Exchange for flour, maize or rice fortification with thiamine
Lesotho	56	No fortification program
Zambia	50.2	No fortification program
Malawi	48.9	Mandatory fortification for wheat flour
Zimbabwe	40.3	Mandatory fortification for wheat flour

Wheat

Country	% of energy from wheat	Listed by the Global Fortification Data Exchange for flour, maize or rice fortification with thiamine
Afghanistan	65.7	No fortification program
Azerbaijan	53.5	No fortification program
Turkmenistan	50.8	No fortification program
Uzbekistan	49.8	Mandatory fortification for wheat flour
Tajikistan	48.7	No fortification program
Tunisia	48.6	No fortification program
Iraq	46.9	No fortification program
Algeria	44.3	No fortification program
Georgia	42.9	No fortification program
Yemen	41.9	No fortification program
Morocco	41.3	Mandatory fortification for wheat flour
Mongolia	40.3	No fortification program

Note: *the staple food that provides a significant contribution to the total amount of available energy/capita/day in the listed countries is usually not fortified with thiamine. For example, Indonesia has 48.4% of the energy coming from rice but the thiamine mandatory fortification policy is for wheat.*

Appendix 6) Examples of retrospective and prospective hospital-based studies from Kashmir informing the local prevalence of TDD

The following studies provide examples of both prospective and retrospective hospital-based studies from Kashmir, which included infants and adults, and provided valuable information on the prevalence of local TDD:

- A retrospective hospital-based study with adult patients (Shah et al, 2017)[46]:
  This study, published in 2017, is a retrospective analysis of 50 nonalcoholic patients admitted with a diagnosis of Wernicke's encephalopathy in the Neurology Department of a large hospital in Kashmir, in Northern India (a region with reported incidence of TDD), between June 2011 to December 2015. Two of these 50 patients were pregnant women and 2 were postpartum. The collected data were reviewed for analysis of the demographic features, clinical presentation, management and outcome of the included patients. The clinical diagnosis of Wernicke's encephalopathy followed official guidelines, which requires two of the following four signs: dietary deficiencies, eye signs, cerebellar dysfunction and either an altered mental state or mild memory impairment. Furthermore, associated brain magnetic resonance imaging findings (performed during the acute phase, in 20 patients) were also considered as supportive evidence for diagnosis, showing both typical and atypical lesions, but there were no data on biochemical thiamine status (analysis not performed). All patients initially received intravenous thiamine (300 mg to 600 mg/day) for 5–10 days followed by oral maintenance, and 49 of the 50 patients showed partial or complete response to intravenous administration of thiamine. It was observed that all patients consumed polished rice as the major component of their diets (and washed it before cooking it) and regularly consumed green tea and bread made of highly polished flour.
  The authors concluded: *"The results of the study indicate that the disorder is a significant health problem in this community. Majority of the patients did not have any major disease known to be associated with Wernicke's encephalopathy such as cancer, gastrointestinal surgery, renal or psychiatric illness, or prolonged starvation. Besides, the cohort did not have other features of malnutrition. This indicates that it is the diet that is selectively deficient in thiamine which is mainly responsible for this disorder in our studied patients".*

- A prospective hospital-based study with infants (Bhat et al., 2017[47]):
  This prospective study, also conducted in Kashmir, included infants (1-6 months old) who were exclusively breastfed with acute onset encephalopathy associated with opthalmoplegia and nystagmus, admitted in the Department of Pediatrics of a large hospital in the city of Srinagar, between November 2014 and October 2015. Exclusion criteria were patients with fever, cerebrospinal fluid pleocytosis, positive blood cultures, elevated acute phase reactants, septic focus, and dyselectrolytemia. All patients were evaluated as per unit protocol including a complete septic and metabolic assessment, including the levels of whole blood ThDP. Brain magnetic resonance imaging and cranial ultrasonography were also performed. Data collected

included demographic details (name, age, sex, consanguinity, etc.), anthropometric measurements, diet history (feeding history of the infant and the mother's dietary history) and other relevant medical history (history of sibling deaths or neurological diseases, developmental history, and birth details). In addition, data regarding the treatments received by the study population and overall mortality were collected. During the 1-year study period, 50 patients were included in this study, of which 90% belonged to the lower socioeconomic class and 80% of their mothers followed traditional food avoidance practices and thoroughly washed polished rice. The authors have empirically administered 100 mg/day of intravenous thiamine in normal saline for 30 minutes (to which the majority of the patients showed a rapid response) and an oral supplement containing 10 mg thiamine/day after discharge. A proportion of the patients (10%) died, and these patients had the most severe symptoms and presented to the hospital after 24 hours of the onset of symptoms. Authors considered thiamine deficiency a major public health concern because it contributes to a significant percentage to infant mortality in Kashmir.

Appendix 7) Step-by-step guidance to assess population thiamine status with thiamine biomarkers: thiamine diphosphate (ThDP); and erythrocyte transketolase activity (ETKA)

1) Identify appropriate population group, calculate sample size, and select a representative sample of the population (more information in section 4.2)

2) Obtain ethical approval and informed consent

3) Data collection - as per the large-scale survey, but the minimum required data are:
   - Participant/survey ID; date of birth; sex; date
   - Biomarker to be analyzed
     - erythrocyte ThDP, or
     - whole blood ThDP (+ hematocrit or hemoglobin; explanation in step 8, below), or
     - ETKA

4) Sample collection (from skilled phlebotomists)
   - Clean subject's skin with alcohol at site of the antecubital vein
   - Restrict occlusion of subject's arm with tourniquet for < 1 minute
   - Draw blood and collect it into heparin or EDTA blood collection tubes

5) Sample preparation
   For analysis of erythrocytes ThDP and ETKA:
   - 3 cycles of washing with isotonic saline solution (0.9% NaCl), centrifugation, removal of supernatant and the top few mm of cells, resuspension in saline.
   - The washed cells, without supernatant, are frozen at -70^{o}C or colder and are osmotically lysed after thawing by re-suspending in water prior to analysis

   For analysis of whole blood ThDP: no additional steps are required

6) Sample storage
   - Protected from light and stored cold:
     - room temperature: a few hours
     - at -20°C: a few months
     - at -80°C: several months/years
   - If shipping is required, use dry ice or liquid nitrogen

7) Sample analysis
   For HPLC analysis of whole blood and erythrocyte ThDP:
   - Thaw samples in a dark room (or with amber light) and keep on ice until analyzed.
   - Precipitate proteins with TCA on ice
   - Spin down and transfer supernatant to fresh tube

- Wash twice with water-saturated methyl-tert-butyl ether to remove lipid-soluble components
- Aliquots of standards, blank and sample solutions are derivatized with methanol freshly prepared potassium ferricyanide and NaOH and filtered before analysis
- Samples are run on an HPLC with a fluorescence detector using an excitation wavelength of 375 nm and an emission wavelength of 435 nm.

For analysis of ETKA:

- Lyse washed erythrocytes with water;
- Pre-incubate lysate with ThDP ("activated") or buffer ("basal")
- Perform enzyme reaction with ribose-5-phosphate as substrate, coupled to triose phosphate isomerase (TPI) and glycerol dehydrogenase (GDH) with NADH as cofactor in a UV-transparent 96-well microplate and read using a UV-vis spectrophotometer at 37°C, at an absorbance of 340nm.
- Calculate ratio of activated to basal activity (linear reaction rate)

8) Data analysis and suggested* cut-offs
   - Whole blood ThDP: Data must be normalized to RBC volume or hemoglobin concentrations. Best practice would be to present both the measured ThDP (nmol/L whole blood) and the ThDP normalized to hematocrit (nmol/L RBCs) or hemoglobin (nmol per gram hemoglobin)
     - Healthy Range: 70-180 nmol/L
     - Deficiency: <70 nmol/L
   - Erythrocyte ThDP:
     - 120-150 nmol/L: mild deficiency
     - <120 nmol/L: deficiency
   - Erythrocyte transketolase assay
     - Erythrocyte transketolase activation coefficient (ETKAC), i.e. ETKA with added ThDP / baseline ETKA, should be reported, and is sometimes expressed as the percentage activation α:
       - Low risk of deficiency: ≤1.15 (α≤15%)
       - Moderate risk of deficiency: 1.15–1.25 (α15%-25%)
       - High risk of deficiency: >1.25 (% activation α>25%)
     - Alternatively, report basal ETKA per unit mass of hemoglobin. Due to the limited availability of reference ranges for basal ETK activity, the following cut-offs may be used to define thiamine deficiency:
       - Infants: ≤0.59 micromoles/min/gHb
       - Females aged 4–18y and 19–24y: ≤0.57 micromoles/min/gHb
       - Females aged 25–34y: ≤0.50 micromoles/min/gHb
       - Females aged 35–49y: ≤0.47 micromoles/min/gHb

**Different cut-offs have been proposed and there is no agreement on the most adequate values.*

Appendix 8) Comparison between the analytical requirements of ThDP and ETKA[2]

	ThDP	ETKA
Sample Type	- Requires venous blood collected into EDTA- or heparin-containing specimen tubes, and can be measured in erythrocytes or in whole blood - Whole blood ThDP requires less sample processing - For erythrocyte ThDP analysis, the cells must be washed with isotonic saline solution (see the details on the right)	- Requires venous blood collection; washed, anticoagulated (heparin or EDTA) erythrocytes are used for this assay - Erythrocytes are washed three times with isotonic saline solution (0.9% NaCl) to avoid osmotic damage to the cells
Sample Storage	Thiamine is stable for a few hours refrigerated; for a few months stored frozen at -20°C; and several months stored frozen at -80°C.[67]	Washed erythrocytes, without supernatant, should be stored in at -80°C, but can be stored in a refrigerator for a few hours prior to freezing.
Analytical Techniques	Can be measured directly using one of two liquid chromatography techniques: - High performance liquid chromatography (HPLC) with either pre- or post-column derivatization coupled with fluorescence detection[68]: samples are prepared by removal of proteins and derivatization to produce fluorescent thiochrome compounds that are separated on a reverse phase analytical column, then detected and quantified.[67] - Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)[69]: allows sensitive and selective measurement of underivatized thiamine diphosphate.	Measured with a UV-vis spectrophotometer at an absorbance of 340 nm in a UV-transparent 96-well microplate. All wells must be held at the same temperature, 37°C, and all reagents must be in excess throughout the temperature equilibration and reading phases to ensure linearity.
Minimum Volume	- For HPLC: 300-500µL of erythrocytes or whole blood - For LC-MS/MS: 150-250µL of erythrocytes or whole blood	Minimum volume is 30 µL of washed erythrocytes. To aid sample handling and to allow repeat analysis a minimum of 200µL is recommended. In practice the process is easier with larger samples, > 1mL of whole blood is suggested.

	ThDP	ETKA
Quality Control and Standards	External standard: a 1mM stock solution of ThDP should be prepared in 0.1M HCl and stored at -80°C. Working standard solutions should be prepared fresh by dilution the stock solution to concentrations of 0, 25, 50, 100, 200, 400, 600nmol/L. Internal standards: to account for losses during sample preparation, while desirable, these are rarely used due to the lack of appropriate thiamine standards useful for HPLC/fluorescence methods. Isotopically-labeled internal standards are available for LC-MS/MS methods. Calibrators and QC material (as lyophilised whole blood) are available (e.g. from Chromsystems (Germany) and Recipe (Germany)). The Royal College of Pathologists of Australasia (RCPA) runs a quality assurance program (QAP) for thiamine.	The assay does not require a calibrant. Quality control specimens should be prepared from bulk samples from single donors and stored at -70°C; the between-assay coefficient of variation (SD*100/mean) of controls in the "adequate status" range is typically 3 to 5%.
Data Analysis and Presentation	- Erythrocyte ThDP concentrations should be reported in nmol/L red blood cells (RBCs). - In whole blood ThDP analysis, there is a need to normalize to RBC volume or hemoglobin concentrations.[70] Best practice would be to present both the measured ThDP (nmol/L whole blood) and the ThDP normalized to hematocrit (nmol/L RBCs) or hemoglobin (nmol per gram hemoglobin). This will ensure that data from different studies are comparable and allow hematocrit or hemoglobin normalized whole blood data to be directly compared with erythrocyte ThDP concentrations.	- ETK activity is expressed in terms of the rate of decrease of absorbance at 340 nm, corrected for any changes in the reagent blank. - The ratio of the absorbance in the presence and absence of exogenous ThDP gives the ETK Activation Coefficient (ETKAC), i.e. ETK activity with added ThDP / baseline ETK activity. - ETKAC should be reported and is sometimes expressed as the percentage activation α. - Alternatively, basal ETK activity per unit mass of hemoglobin (micromoles/min/gHb) may be reported.

	ThDP	ETKA
Suggested* Cut-offs *Note: different cut-offs have been proposed and there is no agreement on the most adequate values.*	Whole blood: - 70-180 nmol/L: healthy range - <70 nmol/L: deficiency Erythrocytes[71]: - 120-150 nmol/L: mild deficiency - <120 nmol/L: deficiency	ETKAC values and risk of clinical thiamine deficiency: - ≤1.15 (α≤15%): low risk - 1.15–1.25 (α15%-25%): moderate risk - >1.25 (% activation α>25%): high risk Alternatively, the proposed cut-offs for thiamine deficiency using basal ETKA (in micromoles/min/gHb) are[15]: - Infants: ≤0.59 - Females aged 4–18y: ≤0.57 - Females aged 19–24y: ≤0.57 - Females aged 25–34y: ≤0.50 - Females aged 35–49y: ≤0.47
Challenges and Limitations	Pre-analytical: - Specimens should be protected from light and frozen at -80°C. - Specimens of whole blood must be frozen to ensure lysis of erythrocytes. Analytical: - Chromatographic separation of thiamine metabolites is necessary for analysis by HPLC with optical detection. This is not necessary for determination by mass spectrometry. - HPLC requires derivatization of the thiamine species, which is not required for LC MS/MS. - Analytical methods have not been standardized. Considerable variation has been observed between laboratories.[72]	Pre-analytical: - As freezing causes erythrocyte lysis, the erythrocyte washing must be completed prior to freezing. - Fresh-frozen specimens must be used, freeze thaw cycles can diminish the transketolase activity. Multiple aliquots should be prepared and stored in the event that a sample needs to be re-analyzed. Analytical: - Maintaining uniform temperature across the plate is required for each enzyme assay procedure - The assay can be difficult to standardize, and inter-assay precision can be poor without careful analytical procedures. Interpretational: ETKA can be influenced by factors other than ThDP concentration, such as age, genetics, and variability in binding of the apoenzyme.[73]

Thiamine diphosphate (ThDP); erythrocyte transketolase activation coefficient (ETKAC); ethylenediaminetetraacetic acid (EDTA); high performance liquid chromatography (HPLC); liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)

Appendix 9) Large-scale survey on thiamine status for women of reproductive age

Large-scale survey on thiamine status: Biomarkers module for women of reproductive age (W)

W1. a) Survey ID: Cluster number __ __ __ __ **b)** Household number __ __ __ __ __ **c)** Individual number (if available) __ __ __ __ __ __	**W2.** Date *(DD/MM/YYYY):*
W3. Interviewer name:	**P4.** ID:
W5. Date of birth of respondent *(DD/MM/YYYY):*	**W 6.** Consent obtained: 1. No ☐ 2. Yes ☐ *If "No", stop here and thank the participant for her time.*
Interviewer notes: *use this space to record notes about the interview with this woman, such as call-back times, number of attempts to re-visit, reasons for incomplete interview, etc.* _____ _____ _____ _____	

Pregnancy & lactation, use of nutrition supplements and symptoms of thiamine deficiency

W7. Are you pregnant? 1. No ☐ *(go to **P9**)* 2. Yes ☐ (*go to **P8***)

W8. Do you know how many weeks you have been pregnant? 1. No ☐ 2. Yes ☐ *If "Yes",* **P8 a)** _____ weeks

W9. Do you have children? 1. No ☐ 2. Yes ☐ *If "No", go to the next question.*

If yes, W9 a) How many? *(number)* _____

W9 b) Are you breastfeeding any of your children? 1. No ☐ 2. Yes ☐

W9 c) If you breastfed other children, when did you stop breastfeeding your last child?

date: ____/_____(MM/YYYY)

W10. Nutrition supplements containing thiamine

Are you currently taking any vitamin supplements? 1. No ☐ 2. Yes ☐

If yes, ask to see the bottle/box and check the label to confirm whether it contains thiamine (also known as vitamin B1).

If yes, **W10 a)** amount of thiamine: _____mg/day; **W10 b)** since (date):___/____/_____(DD/MM/YYYY)

W11. Symptoms of thiamine deficiency

Did you feel any of these symptoms at least in the past 24h?

W11 a) tingling or loss of sensation on hands or feet 1. No ☐ 2. Yes ☐

W11 b) muscle problems: muscle weakness starting with feet, muscle loss, difficulty in walking 1. No ☐ 2. Yes ☐

W11 c) swollen feet or legs 1. No ☐ 2. Yes ☐

Blood specimen collection

W11. Blood drawn? 1. No ☐ 2. Yes ☐

If "No", record the reason below, stop here and thank the participant for her time.

W11 a) If yes, date: _____ *(DD/MM/YYYY)* time: _____ *(HH:MM)*

W11 b) If yes, biomarker to be analyzed:

☐ 1. Erythrocyte ThDP ☐ 2. Whole blood ThDP ☐ 3. ETKA Tube ID:_____

Notes: *use this space to record notes about problems associated with blood collection, samples processing issues, storage conditions, etc.*

_____

_____

_____

_____

_____

Large-scale survey on thiamine status: Dietary intake module for women of reproductive age (optional)

Medications that can deplete thiamine

W12. Are you taking any medications? 1. No ☐ 2. Yes ☐

If "No", go to the next question. If "Yes", interviewer says: I'd like to see the list of medications (you can show me the boxes/bottles) that you are regularly taking (>3months), so I can identify those that reduce levels of thiamine in your body.

Anticonvulsant Medications

W12 a) Phenytoin (brand name: ……) 1. No ☐ 2. Yes ☐

Cardiovascular Medications

W12 b) Digoxin (brand name: ……) 1. No ☐ 2. Yes ☐

Diuretics

W12 c) Bumetanide (brand name: ……) 1. No ☐ 2. Yes ☐

W12 d) Ethacrynic Acid (brand name: ……) 1. No ☐ 2. Yes ☐

W12 e) Furosemide (brand name: ……) 1. No ☐ 2. Yes ☐

W12 f) Torsemide (brand name: ……) 1. No ☐ 2. Yes ☐

Food habits related to thiamine deficiency and food fortification

Food habits

W13. If you drink tea, do you usually drink it during a meal? 1. ☐ No 2. ☐ Yes

W14. Do you chew fermented tea leaves or betel nuts? 1. ☐ No 2. ☐ Yes

W15. Do you consume African silkworm larvae? 1. ☐ No 2. ☐ Yes

W16. Do you consume raw or fermented fish (paste)? 1. ☐ No 2. ☐ Yes

W17. Do you soak or wash the rice before cooking it? 1. ☐ No 2. ☐ Yes

W18. Do you eat parboiled rice? 1. ☐ No 2. ☐ Yes

W19. If pregnant or breastfeeding: do you avoid eating certain foods? 1. ☐ No 2. ☐ Yes

If yes, W19 a) If yes: which foods?_____

If yes, W19 b) Why? _____

Food fortification

Interviewer shows pictures of thiamine fortified foods consumed locally and asks:

W20 a) Do you use any of these foods, which are fortified with thiamine? *(examples below)*

☐ 1. Rice ☐ 2. Fish sauce ☐ 3. Wheat product (flour, bread, pasta) ☐ 4. Other: _____

If yes, see the label of the food and, if fortification with thiamine is reported, record the amount of thiamine per unit (as described in the label) e.g. 1mg of thiamine per 100g of wheat flour

W20 b) Level of fortification: ____mg thiamine/____g (or mL) of _____ *(product and brand)*

W21 c) Level of fortification: ____mg thiamine/____g (or mL) of _____ *(product and brand)*

W21. Dietary assessment: 24-h recall

Interviewer says: ***Now I'd like to ask you to describe everything that you ate or drank yesterday during the day or night, whether you ate it at home or anywhere else. Please tell us for each meal consumed: the time, place, name of food or drink, preparation method (e.g. roasted, raw, fried) and amount (e.g. using household measures, such as slices, cups, teaspoons, etc.). Let's start with the first food or drink consumed yesterday.***

Did you have anything to eat or drink when you woke up, for breakfast? If yes, at which time? Where were you? What did you eat or drink? How was it cooked or prepared? How much/how many slices/spoons? Anything else?

Repeat the questions for the remaining meals.

Food and drinks	**Preparation method (if possible)**	**Amount (in household measures)**
Breakfast **Time:** **Place:**		

Mid-morning snack **Time:** **Place:**		
Lunch **Time:** **Place:**		
Mid-afternoon snack **Time:** **Place:**		
Dinner **Time:** **Place:**		
Night snack **Time:** **Place:**		

W22. Dietary assessment: food frequency questionnaire

Now I'd like to ask you how frequently you eat a list of approximately 45 foods, from a frequency of never to less than once a month, to everyday. How often during 1 week have you eaten the following foods?

	Food	1. Every day (7x/week)	2. 4-6 x/ week	3. 2-3 x/ week	4. 1x / week	5. 1-3 x/ month	6. Never or less than 1x/month
SEAFOOD	1. Fresh raw fish						
	2. Dry raw fish						
	3. Fermented fish						
	4. Cooked fish						
	5. Shellfish						
	6. Other seafood (name): _____						
MEAT	7. Beef (not tinned)						
	8. Pork meat (not tinned)						
	9. Chicken meat (not tinned)						
	10. Liver of animal (name): _____						
	11. Other meat (name): _____						
TINNED FOOD	12. Tinned fish						
	13. Tinned pork						
	14. Tinned beef						
	15. Other tinned food: _____						
DRINKS	16. Tea						
	17. Fruit juice: _____						
	18. Other soft drink: _____						
	19. Beer						
	20. Other alcoholic drink: _____						
CEREALS/ STARCHY ROOTS	21. Rice (circle the most consumed: white or brown or parboiled)						
	22. Cassava						
	23. Taro						
	24. Bread (circle: white or whole grain)						
	25. Pasta/noodle						
	26. Potatoes						
	27. Other cereals /starchy roots: _____						
FRUIT/ VEG	28. Orange						
	29. Pineapple						
	30. Tamarinds						
	31. Other fruit/veg.: _____						
PULSES	32. Soybeans						
	33. Chickpeas						
	34. Lentils						
	35. Beans						
	36. Other pulses:_____						
NUTS	37. Seeds: sesame						
	38. Other seeds (name): _____						
	39. Nuts: peanuts						
	40. Other nuts (name): _____						
	44. Other:_____						
CON DIME	41. Fish sauce						
	42. Soy sauce						
	43. Bouillon cubes						

	Food	1. Every day (7x/week)	2. 4-6 x/ week	3. 2-3 x/ week	4. 1x / week	5. 1-3 x/ month	6. Never or less than 1x/month
	44. Salt						
	45. Other:_____						

W23. Potential food vehicles for fortification

A list of foods that are not fortified with thiamine but could be potential fortification vehicles is selected (examples below). Interviewer says: now I'd like to know where you buy and who produces a few foods (e.g. rice and condiments)

Product	***Place of purchase*** *(e.g. supermarket; local market)*	***Producer (or brand)*** *(e.g. local producer; "Maggi")*
Rice		
Salt		
Bouillon cubes		
Fish sauce		
Soy sauce		

Appendix 10) Large-scale survey on thiamine status for infants

Large-scale survey on thiamine status: Biomarker module for infants (aged <12 months) (I)

I1. a) Survey ID: Cluster number __ __ __ __ **b)** Household number __ __ __ __ __ **c)** Individual number (if available) __ __ __ __ __ __	**I2.** Date *(DD/MM/YYYY):*
I3. Interviewer name:	**I4.** ID:
I5. Date of infant birth *(DD/MM/YYYY):*	**I6.** Consent obtained: 1. No ☐ 2. Yes ☐ *If "No", stop here and thank the participant for her/his time.* *If "Yes", record relationship between infant and the person who will answer the survey on behalf of the infant:* ***I6 a) From:*** *1. Mother ☐ 2. Father ☐ 3. Grandparent ☐ 4. Other ☐* _____
Interviewer notes: *use this space to record notes about the interview with this infant's carer, such as call-back times, number of attempts to re-visit, reasons for incomplete interview, etc.* _____ _____ _____	

Breastfeeding history and use of nutrition supplements

I7. Has the baby ever been breastfed? 1. No ☐ 2. Yes ☐ If yes, **I7 a)** for how long? _____ months **I8.** Is the baby still breastfed? 1. No ☐ 2. Yes ☐ If yes, **I8 a)** exclusively? 1. No ☐ 2. Yes ☐ *If yes, go to question I10.* **I9.** Is the infant receiving infant formula? 1. No ☐ 2. Yes ☐ *If yes, ask to see the container of formula and check the label to collect the level of thiamine* **I9 a)** Name of the formula? _____ **I9 b)** Amount (mL) of prepared formula the infant consumes/day: _____ **I9 c)** Thiamine content/100mL of prepared formula: _____mg
I10. Nutrition supplements containing thiamine Is the baby currently taking any vitamin supplements, including multiple micronutrient powders? 1. No ☐ 2. Yes ☐ *If yes, ask to see the bottle/box and check the label to confirm whether it contains thiamine (also known as vitamin B1).* If yes, **P10 a)** amount of thiamine: _____mg/day; **P10 b)** since (date):___/____/_____(DD/MM/YYYY)

Blood specimen collection

I11. Blood drawn? 1. No ☐ 2. Yes ☐ *If "No", go to the next section.*

I11a) If yes, date: _____ time: _____

I11b) If yes, biomarker to be analyzed:

- ☐ 1. erythrocytes ThDP Tube ID : _____
- ☐ 2. whole blood ThDP
- ☐ 3. ETKA

Notes: *use this space to record notes about problems associated with blood collection, samples processing issues, storage conditions, etc.*

_____

_____

Large-scale survey on thiamine status: Dietary intake module for infants (optional)

History of thiamine deficiency and complementary feeding

I12. Has the baby ever been diagnosed or treated for thiamine deficiency, or beriberi?

1. No ☐ 2. Yes ☐ **I12 a)** If yes, age of diagnosis: _____ month(s)

I13. Has the complementary feeding been initiated, i.e. is the baby eating other foods in addition to milk?

1. No ☐ 2. Yes ☐ **I13 a)** If yes, continue to next section. If not, the survey is completed.

Fortified "family foods" and "baby foods" (commercial foods developed specifically for infants)

I14 a) *Interviewer shows pictures of thiamine fortified foods consumed locally by adults and infants and asks:*

Does the baby eats any of these foods, which are fortified with thiamine? *(examples below, infant formula does not count)*

If yes, ask to see the label of the "family foods" (such as rice, fish sauce, wheat flour) and the "commercial baby foods" (e.g. instant cereal, baby biscuits) and record the amount of thiamine per unit as described in the label, e.g. 2mg of thiamine per 100g of Cerelac infant cereal

Family foods: ☐ 1. Rice ☐ 2. Fish sauce ☐ 3. Wheat product (flour, bread, pasta)

Baby foods: ☐ 4. Baby cereal ☐ 5. Baby biscuits ☐ 6. Other: _____

I14 b) Level of fortification: ____mg thiamine/____g of _____ *(product and brand)*

I14 c) Level of fortification: ____mg thiamine/____g of _____ *(product and brand)*

I14 d) Level of fortification: ____mg thiamine/____g of _____ *(product and brand)*

I14 e) Level of fortification: ____mg thiamine/____g of _____ *(product and brand)*

I16. Dietary assessment: food frequency questionnaire (infants, <12 M):

Now I'd like to ask you how frequently your baby eats a list of approximately 30 foods, from a frequency of never to less than once a month, to everyday. How often during the past month have your baby eaten the following foods?

	Food	1. Every day (7x/week)	2. 4-6 x/ week	3. 2-3 x/ week	4. 1x / week	5. 1-3 x/ month	6. Never or less than 1x/month
SOLIDS	1. Rice (circle: white or brown or parboiled)						
	2. Cassava						
	3. Pasta/noodles						
	4. Bread/crackers						
	5. Cooked cereals (e.g. oatmeal)						
	6. Commercial baby cereal (brand):_____						
	7. Commercial baby biscuits (brand):_____						
	8. Commercial snack foods (brand):_____						
	9. Crisps / savory snacks (brand):_____						
	10. Meat (pork)						
	11. Meat (liver)						
	12. Meat other: _____						
	13. Fish (name): _____						
	14. Eggs						
	15. Pulses (name): _____						
	16. Fruits (name): _____						
	17. Fruits (name): _____						
	18. Vegetables (name): _____						
	19. Vegetables (name): _____						
	20. Yoghurt or cheese:						
	21. Soup (made of_____)						
	22. Ready meal (made of_____)						
	23. Other (name): _____						
LIQUIDS	24. Water						
	25. Tea						
	26. Milk (cow)						
	27. Tinned or powered milk (brand): _____						
	28. Fruit/vegetable juice (name): _____						
	29. Soft drink (brand): _____						
	30. Other liquid (name): _____						

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