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First things first: A step in the right direction for the preanalytical phase of thiamine measurements

Christine M Pfeiffer[1,2], **Zia Fazili**[1], **Ekaterina M Mineva**[1], **Phuong K Ngac**[1]

[1]The Centers for Disease Control and Prevention (CDC), National Center for Environmental Health, Division of Laboratory Sciences, Atlanta, GA

The 2018 roadmap for global control programs for thiamine deficiency disorders calls out the lack of biochemical population data on thiamine status (1). It states that "urgent public health responses are warranted in high-risk regions, considering the contribution of thiamine deficiency to infant mortality and research suggesting that even subclinical thiamine deficiency in childhood may have lifelong neurodevelopmental consequences". This is a bit of a chicken and egg dilemma, as investigators need suitable analytical methods to produce high quality biomarker data and policy makers need comparable, interpretable biomarker data to develop policies and evaluate their impact. Furthermore, analytical methods need to be paired with practical pre-analytical conditions that allow for the collection and generation of valid biological specimens. For years, the thiamine field has been hampered due to the labile physiochemical properties of thiamine compounds and the lack of simple, yet reliable analytical methods.

Thiamine, or vitamin B1, plays a critical role in carbohydrate and amino acid catabolism and gluconeogenesis (2). It is not stored in large amounts in the body and reserves can be depleted within weeks, affecting nervous and cardiovascular systems most dramatically and potentially leading to death if not corrected (2). According to dietary intake data from the 2003–2006 National Health and Nutrition Examination Survey (NHANES), most people in the United States consume the recommended amounts of thiamine (3), however, biochemical status is not directly monitored by the NHANES. Due to the role of thiamine in the metabolism of glucose, it is hypothesized that high calorie malnutrition with its excessive ingestion of simple carbohydrates, as encountered in Western populations, may be associated with thiamine deficiency (4). Globally, the case for assessing thiamine status is pressing, as low- and middle-income countries with monotonous diets and starchy, low-thiamine staples as the primary sources of energy (e.g., polished rice or cassava) experience an increased risk of thiamine deficiency (5).

Because clinical manifestations of thiamine deficiency are variable, the measurement of a suitable biomarker of thiamine status is essential. While thiamine is present in the body in its free form as well as in several phosphorylated forms, thiamine diphosphate (TDP) is the metabolically active form; it makes up 80% of total body thiamine and circulates in

[2] Address correspondence to Christine M Pfeiffer, Centers for Disease Control and Prevention, 4770 Buford Hwy, NE, MS S110-1, Atlanta, GA 30341, phone: 770-488-7926, fax: 770-488-4139, cpfeiffer@cdc.gov.

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the blood primarily in erythrocytes (6). One of the early assays to assess thiamine status is an indirect enzyme assay that measures the degree of TDP-saturation of erythrocyte transketolase, a TDP-dependent enzyme (7). While this assay provides a functional measure of thiamine status, it requires a venous blood collection and washed erythrocytes, the transketolase enzyme has limited stability, and the assay is neither standardized nor readily available (1,7). Subsequent methods based on liquid chromatography mainly use whole blood as a more practical specimen and either analyze TDP (and its phosphate esters) directly or total thiamine after enzymatic hydrolysis (8). They use predominantly fluorescence detection after derivatization to thiochrome, involving long and complex sample preparations, typically require large sample volumes, generally lack the use of an internal standard, and have limited column life due to unfavorable pH conditions (8). The wider use of mass spectrometry in research and clinical applications resulted in recent developments of highly selective, faster, and more sensitive methods for the direct measurement of underivatized TDP (and its phosphate esters) in liquid and dried whole blood (8–11).

In this issue of *The American Journal of Clinical Nutrition*, Verstraete and Stove present data from 50 healthy volunteers using a sample collection device that allows convenient volumetric absorptive microsampling (VAMS) (12). Comparing whole blood TDP measured by HPLC-MS/MS in 3 sample matrices (venous liquid, venous VAMS, and capillary VAMS), they found good agreement for all 3 comparisons with >90% of results being within ±20% of their mean; they also demonstrated that VAMS samples can be safely transported through regular mail without affecting TDP concentrations. Previously, the authors validated the use of VAMS compared to liquid blood for TDP measurement by HPLC-MS/MS and demonstrated improved TDP stability in dried blood (11). VAMS samples were stable for 1 wk at 60°C or at high humidity and for at least 1 mo at room temperature. These findings bear great relevance particularly to international low-resource settings where simplified field collection and sample processing approaches are needed to overcome issues with reluctance towards venous sampling and poor infrastructure for sample processing, frozen storage, and cold chain transport.

In recent years there has been an emerging interest to expand the application of dried blood microsampling, resulting in the novel VAMS technology as a sample collection tool for biomarker measurement in human biological samples (13–16). VAMS devices are easy to handle and collect a fixed and low blood volume by a simple finger or heel prick, eliminating the hematocrit effect and homogeneity issues known to affect dried blood spot samples. However, the technology has limitations: each microsample can only be extracted and measured once, thus requiring multiple sample collections to allow repeat analysis or different tests; furthermore, sample drying time and storage conditions are analyte-specific and need to be carefully validated.

So where does that leave us with regards to global determinations of thiamine status? The VAMS technology can help to address the pre-analytical part and may allow transport of specimens to a central laboratory for analysis, which is important in countries with limited laboratory infrastructure. For the analytical part, we still require a sophisticated laboratory with highly trained scientists, complex and expensive instrumentation, and chemicals and

supplies often difficult to procure in low-resource settings. Furthermore, this is the time to standardize procedures in order to facilitate future data interpretation and comparability across studies: a) agree on a common biomarker: TDP or total thiamine (after enzymatic hydrolysis or through summation), the latter resulting in ~20% higher concentrations; b) adopt common convention on reporting of results: express thiamine concentrations per unit of erythrocytes rather than whole blood as recently recommended (1) and akin to how red blood cell folate measurements have been handled historically; and c) introduce external quality assessment programs to provide an independent verification of laboratory measurements. Readers interested in the broader micronutrient field are invited to read a complimentary article from the Micronutrient Data Generation Initiative on increasing the availability and utilization of reliable data on micronutrient status globally also published in this issue of *The American Journal of Clinical Nutrition* (17).

Abbreviations:

TDP	thiamine diphosphate
VAMS	volumetric absorptive microsampling

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