

# Protocol and application of basal erythrocyte transketolase activity to improve assessment of thiamine status

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## Abstract

Thiamine (vitamin B1) is an essential micronutrient required as a cofactor in many metabolic processes. Clinical symptoms of thiamine deficiency are poorly defined, hence biomarkers of thiamine status are important. The erythrocyte transketolase activity coefficient (ETKac) is a sensitive measure of thiamine status, but its interpretation may be confounded where the availability of the transketolase enzyme is limited. Basal ETK activity per gram of hemoglobin provides a complementary biomarker of thiamine status; however, its measurement and calculation are poorly described. Here, we describe in detail the assessment of basal ETK activity, including the calculation of path length in microplates and the molar absorption coefficient of NADH specific to the assay, and the measurement of hemoglobin in sample hemolysates. To illustrate the application of the methods, we present ETKac and basal ETK activity from women in The Gambia and UK. In conclusion, we present a clear protocol for the measurement of basal ETK activity that will permit the harmonization of methods to improve replication between laboratories.

## KEYWORDS

beriberi, micronutrient deficiency, vitamin B1, women of reproductive age

## INTRODUCTION

Thiamine is an essential micronutrient required as a cofactor for many metabolic enzymes. Clinical symptoms of thiamine deficiency affect the cardiovascular, muscular, and nervous systems. However, symptoms are nonspecific.<sup>1</sup> Blood biomarkers of thiamine status, therefore, provide a complementary approach to diagnose acute deficiency. Furthermore, there is some evidence to suggest that chronic subclinical deficiency may have long-term consequences for cognition and gross motor skills,<sup>2-4</sup> underlining the need for a reliable and well-characterized biomarker that can provide biochemical evidence of thiamine insufficiency.<sup>2</sup>

The two most commonly assessed biomarkers of thiamine status are (1) whole blood or erythrocyte thiamine diphosphate (ThDP) concentration and (2) thiamine-dependent erythrocyte transketolase (ETK) activity, commonly expressed as the ETK activity coefficient (ETKac).<sup>2</sup> The advantages and disadvantages of each biomarker were considered recently.<sup>2</sup> ETKac is a functional marker of thiamine activity at the cellular level that reflects tissue thiamine status.<sup>5</sup> An anecdotal report suggested that in repletion of individuals with thiamine deficiency, while ThDP concentrations increased rapidly, normalization of ETKac took several weeks,<sup>6</sup> although thiamine depletion-repletion studies have shown rapid changes in ETKac.<sup>7,8</sup>

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Assessment of the ETKac requires simultaneous or consecutive measurement of basal and stimulated transketolase activity, the latter performed with the addition of exogenous ThDP. From measures of basal and stimulated activity, the ETKac is calculated and expressed as either a ratio [ETK stimulated/ETK basal], as percentage or alpha ( $\alpha$ )  $[(\text{ETK stimulated} - \text{ETK basal})/\text{ETK basal}] \times 100$  or as percent stimulation  $[(\text{ETKac} \times 100) - 100]$ , also referred to as the transketolase pyrophosphate effect (TPPE). When thiamine status is adequate, there is very little difference in activity between the basal and stimulated sample and the calculated ratio is close to 1. The greater the effect of exogenous ThDP in the stimulated sample, the higher the ratio. Although there is no international consensus on values of ETKac that indicate a deficiency, commonly quoted values are that less than 1.15 indicates sufficiency and values between 1.15 and 1.25 and more than 1.25 indicate a moderate or high risk of deficiency, respectively.<sup>2,5,9</sup>

While ETKac is a useful biomarker of thiamine status and is able to discriminate between groups, there is concern that some clinical conditions, drugs, or long-term thiamine deficiency may reduce the levels of the apoenzyme (the inactive transketolase).<sup>5,10–14</sup> In a scenario of low apoenzyme, less thiamine is required to stimulate the transketolase, and a ratio in the normal range may be obtained, regardless of the availability of thiamine. Therefore, the measurement of basal ETK activity provides an additional and complementary biomarker of thiamine status to aid the interpretation of other thiamine status indicators, providing context for the interpretation of ETKac.<sup>5,15</sup>

Despite its potential utility, the derivation of absolute activity has not received detailed descriptions in the literature. The basal and stimulated activities measured in the calculation of the ETKac are used exclusively for the calculation and are not sufficient, alone, to derive basal ETK activity. Additional calculations are required to determine the basal activity in units of enzyme activity as well as to express the absolute activity per unit weight of hemoglobin (Hb). Published reports of basal ETK tend to lack sufficient methodological details to allow replication<sup>16–19</sup> or use obsolete technology<sup>20,21</sup> rather than current 96-well microplate methods. Where methods are better described, assumptions used in the calculations are commonly not shown and descriptions assume a degree of understanding that may limit their reproducibility.<sup>20,22</sup> More recent reports of basal ETK activity typically lack detail on how the basal activity was measured and/or calculated.<sup>16,18,23</sup> This inhibits further advances in the field by impeding replication and initiatives to harmonize methods between laboratories.

We recently published a modern and detailed protocol describing the steps involved to measure ETKac.<sup>24</sup> Here, we expand that work and describe the equipment, analytical methods, and calculation required to determine basal ETK activity. The method described herein can be replicated with the aim to improve harmonization among laboratories. In addition, we provide example datasets from the UK and The Gambia encompassing a wide range of thiamine status.

## MATERIALS AND METHODS

### Summary of ETK activity measurement

ETK activity is a function of the conversion of NADH to NAD<sup>+</sup> in the reaction whereby dihydroxyacetone phosphate is converted to glycerol-3-phosphate (G-3-P) as part of the final steps in a set of ETK-dependent reactions. In practice, the rate of oxidation of NADH is measured by monitoring the change in absorbance ( $\Delta A$ ) at 340 nm.<sup>24</sup> In the assay, the hemolysate is added to assay reagents without (basal) and with additional ThDP (stimulated). The activity coefficient is calculated as the ratio of stimulated to basal ETK activity. The experimental details for the measurement of basal ETK activity in the context of the activity coefficient were described previously.<sup>24</sup>

In order to generate meaningful values for basal ETK activity, it is necessary to express activity in absolute units of enzyme activity relative to the Hb concentration of the diluted hemolysate used in the assay. Therefore, the following parameters are required:

- Basal ETK activity
- Path length and molar absorption coefficient (MAC) of NADH for the specific assay conditions
- Hb concentration in the hemolysate

Derivation and calculation of these parameters is explained below with a particular focus on the requirements for calculations in the 96-well microplate format. All calculations were performed in Microsoft Excel (Microsoft Excel, Microsoft Corp, USA).

### Equipment and reagents

Reagents were Sigma brand and purchased from Merck Life Science UK Ltd (Dorset, UK); details are provided in Table S1. Full details of the materials and methods for the measurement of ETKac were published previously.<sup>24</sup> Hemolysate Hb was quantified using Drabkin's reagent (Sigma-Aldrich, product code: D5941; Merck Life Science UK Limited). Control materials for Hb were purchased from Bio-Rad (Watford, UK).

Spectrophotometer readings were performed with a Jenway 7415 spectrophotometer (Cole-Palmer Scientific, St Neots, UK) and all 96-well microplate readings were performed with a Thermo Multiskan (ThermoFisher Scientific, UK). Greiner UV-Star microplates were used to measure ETK activity (product code 655801, Greiner Bio-One Ltd, Gloucestershire, UK) and standard flat-bottomed 96-well microplates to quantify Hb (Greiner product code 655101).

### Sample type and preparation

Venous blood was collected into EDTA blood tubes using standard techniques. After centrifugation, plasma and buffy coat were removed and samples were frozen at  $-70^{\circ}\text{C}$  to lyse the cells, and for storage and transport. After thawing, deionized water (1:2 washed erythrocytes:

deionized water) was added to ensure cell lysis.<sup>24</sup> Lithium heparin as an anticoagulant may also be used.<sup>24</sup> To ensure adequate volume for sampling handling, the recommended volume of washed erythrocytes for the analysis of ETKac and Hb is between 200 and 500  $\mu\text{l}$ , however, the absolute minimum volume required is 90  $\mu\text{l}$ .

## Calculation of basal activity

Basal ETK activity per gram of Hb is expressed as  $\mu\text{mol}$  G-3-P produced per gram Hb per minute or U/gHb) and was calculated based on a modified version of the equation by Bayoumi and Rosalki<sup>21</sup> (Equation 1). Note that this equation includes values calculated in our laboratory that are further explained below:

$$\Delta A \times \frac{245 \times 1000}{5.64 \times 0.67 \times 30} \times \frac{1}{\text{Hb}} = \text{basal activity (in U per g Hb)} \quad (1)$$

- $\Delta A$  is the change in absorbance per minute measured at 340 nm (this is the raw basal activity measured in the ETKac assay)
- **245** is the total volume ( $\mu\text{l}$ ) of assay reagents and hemolysate per well of the 96-well microplate
- **30** is the volume ( $\mu\text{l}$ ) of hemolysate within the assay volume (a division of total volume by assay volume allows this equation to express activity in the sample component within the total assay volume only)
- **5.64** is the MAC of NADH at 340 nm in  $\text{L}^{-1} \text{mmol}^{-1} \text{cm}^{-1}$  calculated using our assay conditions (see step 5b below)
- **0.67 cm** is the calculated light path of liquid in the well (see step 5a below)
- Multiplication by **1000** converts millimoles of G-3-P produced to micromoles. This allows expression as units (U) of transketolase activity per minute<sup>25</sup>
- Hb concentration (g/L) is measured in each sample hemolysate; this concentration is divided by 10 to give the assay dilution Hb concentration (g/L). Multiplying the basal activity by 1/Hb (assay dilution) concentration gives basal activity in U/gHb or  $\mu\text{mol}$  G-3-P produced per gram enzyme activity per minute.

In practice, it is simpler if the basal activity (not corrected for Hb) is calculated first using part of Equation (1) (Equation 2):

$$\Delta A \times \frac{245 \times 1000}{5.64 \times 0.67 \times 30} = \text{basal activity (in U per 30 } \mu\text{l lysate)} \quad (2)$$

which can be further simplified to Equation (3):

$$\Delta A \times 2161.2 = \text{basal activity (in U per 30 } \mu\text{l lysate)} \quad (3)$$

## Absorbance, path length, and MAC

As stated by Beer's law, absorbance is equal to the MAC  $\times$  path length (of the light path)  $\times$  concentration. As detailed above,  $\Delta A$  is measured at 340 nm in the ETK activity assay.

Published values for MACs are based on measurements under specific conditions using a spectrophotometer and cuvettes with a

standard 1-cm path length. Therefore, it is inappropriate to use such published values where these standard experimental conditions are not met. An assay-specific MAC needs to be calculated according to the actual path length of the 96-well microplate and the assay conditions.

## Path length

Calculation of path length through a 96-well microplate is challenging as it is dependent not only on the volume of liquid in the well but also on the shape of the well and the viscosity of the mixture that affects the meniscus. It may be possible to calculate path length using the geometry of the wells but this can be difficult without knowing the exact dimensions of the well. Although this method is not recommended, it may be used as a value to check against the measured path length.

Path length in wells of a 96-well microplate was calculated by first measuring absorbance at 340 nm of freshly prepared ETK working reagent (see Supplementary Material and Ref. 24) in standard 1-cm cuvettes with a spectrophotometer.

To 12 wells of a microplate, 245  $\mu\text{l}$  of working reagent was added (equal to the total assay volume used for the ETK assay) and absorbance was measured at 340 nm with the microplate reader. The mean absorbance of the 12 wells was divided by the absorbance in the 1-cm cuvette to give a 96-well microplate path length in cm. This procedure was repeated across 3 days and the mean path length was calculated as 0.67 cm.

## MAC ( $\epsilon$ )

The MAC of NADH is widely published as  $6.22 \times 10^3 \text{ M}^{-1} \text{cm}^{-1}$ ; however, this value is dependent on other variables, including assay components, pH, and temperature.<sup>26</sup> It is, therefore, recommended that a laboratory-specific value be calculated.

A solution of NADH was prepared in ETK assay buffer (see Supplementary Material and Ref. 24) and serially diluted to derive concentrations of  $0.36 \times 10^{-3}$ ,  $0.18 \times 10^{-3}$ ,  $0.09 \times 10^{-3}$  mol/L, plus a blank (0.00 mol/L), representative of NADH concentrations observed in the assay. Volumes of 245  $\mu\text{l}$  of each concentration were added into duplicate wells of a microplate and absorbance was measured at 340 nm. The mean measured absorbance for each duplicate was plotted against the concentration and a line of best fit was used to calculate the slope. The slope of the line divided by the assay path length in cm is equivalent to MAC in  $\text{M}^{-1} \text{cm}^{-1}$ . This experiment was repeated across 3 days and an average MAC was calculated. Division by 1000 gave the MAC value of  $5.64 \text{ L}^{-1} \text{mmol}^{-1} \text{cm}^{-1}$ .

## Measurement and calculation of hemolysate Hb concentration using a 96-well microplate method

Hemolysates prepared for the ETKac assay were also used to measure Hb. One vial of Drabkin's reagent was reconstituted with 1 L of

deionized water and stored in an opaque glass bottle. Sample hemolysate (10  $\mu$ l) was added to duplicate wells of a 96-well microplate. Duplicate blanks, calibrators, and controls were added to separate wells. Liquicheck Hematology-16 Control "High" (Bio-rad Watford, UK) was used as a calibrator, and Bio-rad Liquicheck Hematology-16 Control "Low" and "Normal" were used as controls. A 1-in-3 dilution in deionized water of the low control was prepared and used as an additional control. Reconstituted Drabkin's reagent (200  $\mu$ l) was added to each well. The microplate was then transferred to the plate reader, shaken at medium speed for 5 s, and read at 540 nm. The Thermo SkanIt software was used to produce a standard curve and to calculate Hb concentrations in the hemolysate samples and controls.

### Final calculation of basal ETK activity

The measured Hb hemolysate concentration from above was divided by 10 to give the assay dilution Hb concentration in g/L. For information, summary statistics of the measured concentrations of Hb in the sample hemolysates and raw basal reaction rates are reported in Table S2.

Adding Hb concentration to the simplified Equation (3) gives Equation (4):

$$\Delta A \times 2161.2 \times \frac{1}{Hb} = \text{basal activity (in U per g Hb)} \quad (4)$$

As shown in Equation (4), multiplication of the basal activity by 1/Hb (in effect dividing the basal activity by Hb concentration) gives U/gHb.

### Cut-offs and diagnosis

ETKac ratios of 1–1.14 indicate thiamine sufficiency, 1.15–1.25 indicate a moderate risk of deficiency, and >1.25 indicate a high risk of deficiency. Although none of the established biomarkers of thiamine status are reliable predictors of clinical signs of beriberi,<sup>27</sup> symptoms of beriberi have been associated with higher values of ETKac,<sup>17,28</sup> and a recent consensus report suggested that symptoms are generally associated with ETKac values over 1.4.<sup>2</sup> Cut-offs for basal ETK activity are poorly defined but a value of 0.59 U/gHb has been proposed and is derived from predictions of infantile beriberi in children.<sup>17</sup> Other values are based on population reference values, for example, 0.5 U/gHb in women aged 25–34 years.<sup>17</sup> Another study calculated a reference range of 0.57–0.83 U/gHb in 31 individuals with TPPE of  $\leq 15\%$ .<sup>15</sup>

### Population datasets

Samples from The Gambia were collected from 226 women of reproductive age (18–40 years old), as described previously. This was a convenience sample of women for whom biological samples were available as part of a regional population biobank in the West Kiang region of The Gambia.<sup>29,30</sup> UK samples were selected from the

National Diet and Nutrition Survey Rolling Programme (NDNS RP) dataset for Year 11 (2019–2020) and consisted of women aged 18–64 ( $n = 134$ ). The NDNS RP is a cross-sectional, nationally representative annual survey of the UK population.<sup>31,32</sup> Sample collection in each country was performed with local ethical permission and participants provided informed consent. All details of the ethical approvals, consent procedures, and blood collection methods are described elsewhere.<sup>29–32</sup> ETKac results were available from previous analyses in our laboratory.<sup>29,31</sup> Hb was measured and basal ETK activity was calculated in the same samples.

## RESULTS

Table 1 shows the ETKac and basal ETK activity for the two populations as well as the proportions of the population against the cut-offs described above. The Gambian population was at higher risk of thiamine deficiency and had a significantly higher mean ETKac and lower mean basal ETK activity compared with the UK sample (*t*-test, both  $ps < 0.0001$ ).

ETKac country distributions overlapped (Figure 1A), but the distribution was shifted right in the Gambian population sample, indicating a greater level of insufficiency (higher ETKac values) compared with the UK population sample. Distribution of basal ETK activity (Figure 1B) showed a similar pattern between the two population samples, with lower basal activities in The Gambia.

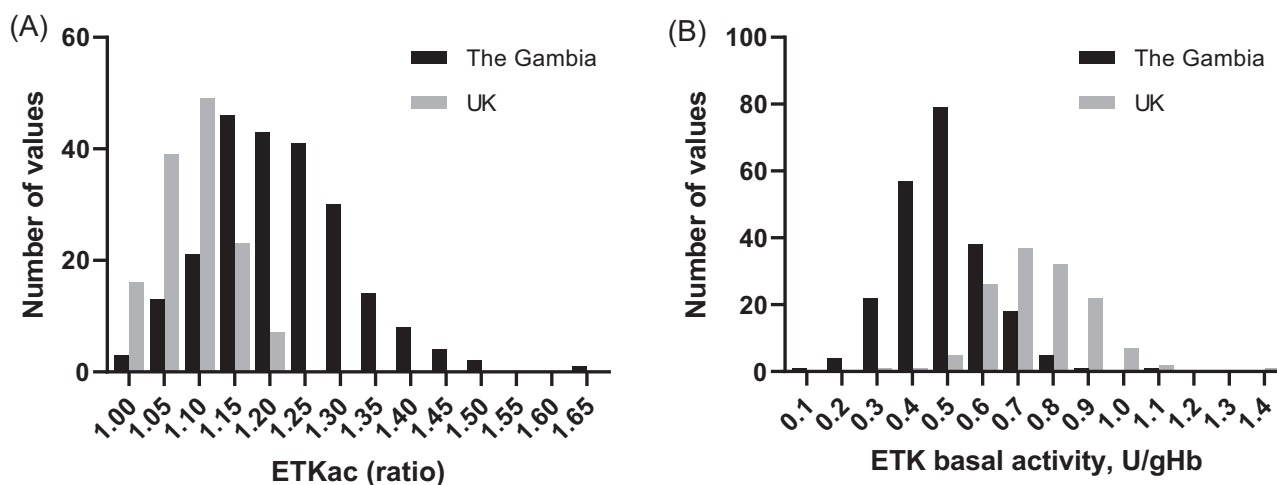
Notwithstanding uncertainties over the cut-offs, relationships between ETKac and basal ETK activity are shown in Figure 2 together with the cut-offs for sufficiency and deficiency for ETKac<sup>2</sup> and reference limits proposed for basal ETK activity.<sup>17</sup> The Gambian population sample (Figure 2A) comprises a wide range of ETKac. Of the 51 Gambian participants with an ETKac of less than 1.15, representing thiamine sufficiency, 18 (35%) had low basal ETK activity (<0.50 U/gHb). In contrast, three of 116 (2.6%) UK participants with ETKac of <1.15 had a basal ETK activity <0.50 U/gHb. Of individuals with moderate risk of deficiency (ETKac = 1.15–1.25), 49% of Gambian participants and 6% of UK participants had a basal ETK activity <0.50 U/gHb.

## DISCUSSION

We have described a method to measure basal ETK activity that includes information essential to inform the final calculation. Specifically, we include information on the assessment of path length in a 96-well microplate, derivation of MAC specific to the assay conditions, and on the measurement of Hb concentration in 96-well microplates. The measurement of basal ETK activity can be integrated into a method for ETKac and, once path length and MAC are derived, only requires the additional measurement of Hb in the sample used for the ETKac assay. It is not possible to derive the sample Hb concentration from whole blood measurements, as sample Hb is dependent on the efficiency of erythrocyte washing and collection.

**TABLE 1** Erythrocyte transketolase activity coefficient (ETKac) and basal ETK activity in women in The Gambia and UK

Variable	Units	The Gambia	United Kingdom
N	<i>n</i>	226	134
Age (years)	Mean (SD)	28 (6.9)	44 (11.8)
	Range	18–40	18–64
ETKac (ratio)	Mean (SD)	1.22 (0.10)	1.09 (0.05)
	Median (95% CI)	1.21 (1.19, 1.23)	1.09 (1.08, 1.10)
	Range	1.00–1.66	1.00–1.22
ETKac < 1.15	<i>n</i> (%)	51 (23%)	116 (87%)
ETKac = 1.15–1.25	<i>n</i> (%)	98 (43%)	18 (13%)
ETKac > 1.25	<i>n</i> (%)	77 (34%)	0 (0%)
Basal ETK activity (U/gHb)	Mean (SD)	0.49 (0.13)	0.75 (0.15)
	Median (95% CI)	0.48 (0.46, 0.50)	0.75 (0.71, 0.77)
	Range	0.09–1.06	0.34–1.37
Basal ETK activity <0.5 (U/gHb)	<i>n</i> (%)	4 (3%)	127 (56%)



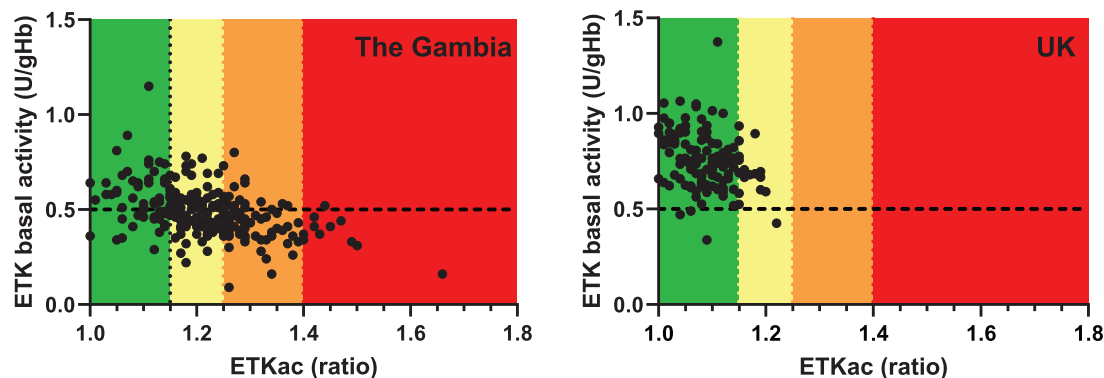
**FIGURE 1** Frequency distribution of (A) ETKac and (B) basal ETK activity in The Gambia and UK cohorts. Cut-offs for thiamine sufficiency/deficiency are: for ETKac, <1.15 indicates sufficiency, 1.15–1.25 indicates a moderate risk of deficiency, and >1.25 indicates a high risk of deficiency; for basal ETK activity, a value of <0.50 U/gHb was used based on the proposed reference range for women aged 25–34 years.<sup>17</sup> Abbreviations: ETK, erythrocyte transketolase; ETKac, erythrocyte transketolase activity coefficient.

Measurement and calculation of the basal ETK activity has been described previously. In early studies dating back four decades or more, sufficient detail was provided to follow the method and repeat the protocol.<sup>20,21,33,34</sup> More recently, typical reporting of the methods is scant or points to previously published manuscripts that may or may not provide detail or provide incomplete method details to allow replication of the method.<sup>16–19,35</sup> In addition, no previously published method provides details of the steps required to calculate basal ETK activity using the 96-well microplate method.

If a laboratory measures ETKac, then the additional calculation of basal ETK activity is relatively straightforward if the methods outlined here are followed. Experiments to calculate values for path length and MAC in a specific laboratory only need to be performed once. The assay

to measure Hb in 96-well microplates is straightforward, inexpensive, and quick. Although the method to measure ETKac and basal ETK activity has multiple steps, it requires relatively little in the way of costly equipment and associated specialist, technical knowledge. For this reason, it is particularly suited to resource-limited countries where there is often evidence of thiamine deficiency.

A set of commonly used ETKac cut-offs to define thiamine deficiency are based on studies conducted in different patient groups.<sup>27,36,37</sup> In contrast, thiamine deficiency cut-offs for basal ETK activity are not well described. Soukaloun et al. used receiver operator curve analysis to determine a basal ETK activity cut-off of 0.59 U/gHb and reported good sensitivity and specificity to determine the risk of clinical infantile beriberi (75% [48–93%] and 85% [66–96%],



**FIGURE 2** Relationship between ETKac and basal ETK activity (U/gHb) in The Gambia (left) and UK (right). Shaded areas indicate commonly used cut-offs for thiamine sufficiency (green), moderate risk of deficiency (yellow), high risk of deficiency (orange), and ETKac purportedly associated with beriberi (red).<sup>2</sup> The horizontal dashed line indicates a previously proposed but unconfirmed lower limit of the proposed reference range (0.50 U/gHb) for UK women aged 25–34 years.<sup>17</sup> Abbreviations: ETK, erythrocyte transketolase; ETKac, erythrocyte transketolase activity coefficient.

respectively). This was in contrast to the use of ETKac cut-offs that showed no discrimination between cases and controls.<sup>17</sup> The cut-off value of 0.59 has since been used in other studies,<sup>18,23,35</sup> but remains unsubstantiated. Soukaloun et al.<sup>17</sup> also reported reference ranges of between 0.47 and 0.57 U/gHb for children and women aged between 4 and 49 years and derived from UK NDNS data.<sup>17</sup> However, the validity of these cut-offs and reference ranges and their suitability across different populations, age, and sex groups has not yet been tested. Basal ETK activity is reported to be more closely related to ThDP than ETKac,<sup>18</sup> possibly because of the potential ambiguity in activity coefficients when apoenzyme levels are low, and was observed to better predict infantile beriberi than ETKac.<sup>17</sup> Other studies have reported that ETKac provides a more robust and sensitive marker of thiamine status.<sup>8</sup> The question of whether basal ETK activity or the ETKac better reflects thiamine status is not resolved and may depend on the population under study. What is evident is that basal ETK activity is useful for the interpretation of ETKac in the context of variable apoenzyme concentrations.

One of the limitations of the method overall, partly due to the higher concentration of thiamine in leukocytes,<sup>5</sup> is the requirement for washed red blood cells. Whole blood has been considered as a simple, alternative sample type since its use removes the need for saline washes immediately after sample collection and prior to sample freezing. Limited evidence to date suggests differences in transketolase activity but similar ETKac,<sup>38,39</sup> but further work is required to demonstrate the utility of whole blood and to better understand the effects of raised leukocyte count.<sup>40</sup> A handful of studies have examined the stability of the transketolase enzyme and the ETKac result under various conditions in whole blood and hemolysates (reviewed in Ref. 24). In general, storage up to 24 h under refrigerated conditions or longer-term storage at  $-70^{\circ}\text{C}$  does not appear to affect ETKac results.<sup>2,24</sup> However, additional studies confirming the stability and reproducibility of both basal ETK activity and ETKac measurements are warranted.

An additional limitation is our understanding about clinical or nutrition-related conditions that may affect levels of the apoenzyme and the basal ETK activity. Levels of apoenzyme may be affected in different conditions and may be lower in diabetics and higher in pernicious anemia and severe illness.<sup>16,41</sup> Animal studies have suggested that chronic thiamine deficiency may cause low apoenzyme levels.<sup>41</sup> Observation of a relationship between transketolase activity and age was observed in children<sup>42</sup> but not adults.<sup>16</sup> Different forms of transketolase may also influence the measurement of enzyme activity.<sup>43</sup> Although these studies provide some information on potential factors that may affect the transketolase enzyme, the evidence base is small and further work is necessary to confirm the findings.

Data from the UK and The Gambia show the contrasting thiamine status of these two populations. Values for Gambian ETKac data were published previously,<sup>29</sup> and, as may be expected based on ETKac, mean basal ETK activity was lower in The Gambia than in the UK. Notwithstanding the fact that the cut-off for basal ETK activity is based on limited data and may not be equally applicable in these populations; a large proportion of women judged to be thiamine sufficient on the basis of ETKac cut-offs had low basal ETK activity. The appropriateness of these cut-offs and the implications for health require further enquiry. Previous studies from different countries have reported similar basal ETK activities to those reported here, with study means/medians generally ranging between  $\sim 0.5$  and  $\sim 1$  U/gHb. Mothers in Laos had a mean basal ETK activity of 0.58 U/gHb.<sup>17</sup> A number of other studies performed in Laos reported values in malaria-infected patients<sup>18,19</sup> and in infants with<sup>17</sup> and without<sup>17,44</sup> clinical signs of thiamine deficiency. Other clinical populations studied include diabetics and alcoholics in Poland<sup>16</sup> and, in the UK, patients with chronic fatigue syndrome<sup>45</sup> and alcohol withdrawal syndrome.<sup>23</sup> Study designs vary and do not always include healthy control groups. Bailey et al. studied apparently healthy adolescents in the UK and reported a mean basal ETK activity of 0.90 U/gHb.<sup>22</sup> As discussed, comparison between studies is hindered by variability in methods and/or lack of method detail.

## CONCLUSION

We present a method to measure and calculate basal ETK activity. This will allow laboratories to introduce and/or harmonize protocols and review assay performance to facilitate collaboration between laboratories for the measurement of thiamine status.

Harmonization of methods will allow researchers to more readily combine datasets to better understand the utility of basal ETK activity as a complementary marker of thiamine status. Harmonization is a necessary precursor to bridging the gap between defining cut-offs for deficiency and understanding the nutritional (i.e., thiamine deficiency) and the broader etiology of thiamine-related disorders. Furthermore, the measurement of transketolase activities in erythrocytes and other tissues allows the exploration of the role of thiamine and the effectiveness of thiamine analogs as clinical treatments, for example, in renal disease<sup>46</sup> and dementia.<sup>47</sup> A long-term goal of this effort toward international harmonization is to develop a standard reference sample.

## AUTHOR CONTRIBUTIONS

K.S.J. and D.A.P. conceptualized the study and designed the experiments with the input of M.W.B, C.C., and A. K. K.S.J and D.A.P wrote the manuscript. D.A.P. performed the experiments. All authors contributed to the writing of the manuscript and have reviewed it.

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## COMPETING INTERESTS

The authors declare no competing interests.

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## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/nyas.14962>

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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