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Thiamine (vitamin B1) status of boarding school students in the Southern Region of Papua New Guinea

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SUMMARY

Thiamine pyrophosphate (TPP) is the major biologically active form of thiamine (vitamin B1). This cross-sectional study assessed whole-blood thiamine pyrophosphate concentration (WBTPPC) in boarding school students in the Southern Region of Papua New Guinea. Sample size for each of the five boarding schools was calculated using the 'proportionate to population size' cluster sampling technique. The 'Clin-Rep' reagent kit was used for the extraction of thiamine pyrophosphate from whole blood. Reverse phase high performance liquid chromatography with post-column derivatization was used to determine the thiamine pyrophosphate concentration. Informed consent was obtained from 468 students, mean age 17.7 ± 1.5 years. The gender distribution of these students was 274 (58.5%) males and 194 (41.5%) females. The median and interquartile range of WBTPPC for all students was 95.41 µg/l (82.27-113.55). Severe to marginal status of thiamine deficiency was present in 6.4% of all the students. The mean WBTPPC for female students was significantly lower than that for the male students (p <0.001), with a mean difference of 14.17 µg/l (95% CI of the difference: 9.85-18.50). Severe to marginal status of thiamine deficiency was present in 9.8% of female students and 4.0% of male students. The data strongly support the need for effective implementation and monitoring of food fortification legislation in Papua New Guinea. Withdrawal of fortification or suboptimal thiamine fortification of rice and other cereal products in Papua New Guinea would have serious negative public health implications, especially among students in boarding schools.

Introduction

Thiamine pyrophosphate (TPP), the major biologically active form of thiamine (vitamin B1), is the coenzyme for oxidative decarboxylation reactions in the mitochondria (1). The daily requirement for thiamine is enhanced by heavy physical exertion, pregnancy, intermittent illness, surgery, reduced absorption of thiamine, persistent high blood alcohol levels, dysentery, diarrhoea, nausea and vomiting (1-4).

Both overt and subclinical (mild to moderate) thiamine deficiency can severely alter metabolic functions in the nervous, cardiac, respiratory and endocrine systems (1-4). Overt thiamine deficiency (both wet and dry beriberi) is rare, because most countries have implemented food fortification programs (1,2). However, in some developing countries, such as Papua New Guinea (PNG), subclinical thiamine deficiency poses a threat in communities where changes in lifestyle and eating habits have led to high-calorie malnutrition (1,2,5). Subclinical thiamine deficiency has been reported in communities where the diet

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contains polished rice as staple food, a high consumption of carbonated drinks and confectionery, betelnuts, and raw fermented fish containing high levels of anti-thiamine factors (1-4).

According to World Health Organization (WHO) criteria, a single case of properly diagnosed clinical thiamine deficiency in a population reflects a public health problem and calls for a full nutritional assessment using appropriate biochemical methods to assess the thiamine status of the population (1,2).

The criteria for assessment of thiamine status of individuals depend on the assay procedure used (1,2,6-8). The direct measurement of TPP in either erythrocytes or whole blood by high performance liquid chromatography (HPLC) is the currently recommended technique for assessment of thiamine status of individuals (6-9). This assay procedure has clear advantages over others in terms of sensitivity, reproducibility, specificity, precision and robustness (6-9). According to the recommended cut-off points, a TPP concentration below 50.85 µg/l indicates severe thiamine deficiency, whereas a TPP concentration between 50.85 and 63.56 µg/l indicates marginal thiamine deficiency (1,2,6,8,9). Several authors (6-9) have reported that it is more convenient to use whole blood for assay of TPP, because the preparation of washed erythrocytes is time-consuming and has safety implications, and accidental damage to the erythrocytes can cause loss of TPP. According to Talwar et al. (6), measurement of TPP in erythrocytes by HPLC correlated strongly with that in whole blood (r = 0.97).

A search of the published literature indicates that in PNG there has been little or no scientific study conducted to assess the thiamine status of the population (10-12). There are anecdotal reports of beriberi in the late 1950s and early 1960s in PNG (10,11). In order to prevent the incidence of micronutrient deficiencies in the population, the PNG government passed legislation banning the importation and sale of foodstuffs that are not appropriately fortified with micronutrients (13-16). According to the Pure Food Act, white rice fortified with thiamine, niacin and iron to approved levels should be sold in PNG (13-16). In 2005, the 'Rice development policy' was approved and implemented in PNG (15). The aim of this policy was to encourage local rice production among subsistence farmers, and to help boarding schools and correctional institutions become self-sufficient by producing local rice. Locally grown rice is also subject to the PNG food standards, as indicated in the Pure Food Act (14).

While there are food fortification legislation and regulations in place that require fortification of various foodstuffs with micronutrients, there is paucity of published data for assessing the implementation of the fortification policy in PNG. In addition, published data on the assessment of the impact and effectiveness of the fortification on target communities in PNG is scanty.

This study was prompted by the apparent lack of scientific data on the thiamine status of the population in the Southern Region of PNG.

The aim of this study was to assess the thiamine status of students in boarding schools in the Southern Region of PNG. Selection of boarding school students was based on their high vulnerability to low thiamine intake, easy accessibility, and representativeness of their age group in communities in PNG.

Subjects and Methods

Study sites

This study was carried out in the Southern Region of PNG. 5 boarding schools were selected based on easy accessibility by road, as blood samples needed to be kept at approximately +4°C in the field and during transportation from the site of collection to the Micronutrient Laboratory (MNL) in the School of Medicine and Health Sciences (SMHS), University of Papua New Guinea (UPNG), for proper storage and analysis.

Sample size

Calculation of sample size was based on a design effect of three, a relative precision of 10% and a confidence level of 95%. As there was no available information on the likely prevalence rate of thiamine deficiency in PNG, an assumed prevalence rate of 25% was used. Thus, the sample size of about 500 boarding students from the boarding schools with a total residential boarding population of 1337 students was considered sufficient for a study, with a predicted non-response rate of 20% (17).

Study design and sampling

This was a cross-sectional study using a multistage cluster sampling method for selecting the study population. The total enrolment figures for each of the selected boarding schools were obtained from the appropriate authorities in the education departments in the Southern Region of PNG (18). The enrolment figures for students in each of the classes in each boarding school were also obtained (18). The sample size for each school was calculated using the 'proportionate to population size' (PPS) cluster sampling technique (17). Each of the students in the selected boarding schools was assigned a computer-generated number and the required number of students from each school was then selected by simple random sampling, using the randomly generated number list.

All the students were between the ages of 14 and 22 years, and had been living in the boarding houses of the respective schools for at least 4 weeks before sample collection.

Collection of blood samples

About 0.5 ml of blood was collected from each student, by finger-stick, using a contact-activated single-use lancet. The blood was collected into a properly labelled EDTA-coated microtainer. Each blood sample was then kept in a cool box, protected from light and kept at 4-10ºC in the field and during transport from the field to the MNL in SMHS, UPNG. All blood samples were stored frozen at –70ºC until required for analysis.

Sample analysis and quality control

The 'Clin-Rep' reagent kit was used for the extraction of thiamine pyrophosphate from whole blood (9). All reagents used were of analytical grade and were components of the 'Clin-Rep' HPLC complete kit for assay of TPP (9). The concentration of TPP was measured using a reverse phase high performance liquid chromatography with post-column derivatization (6-9). The excitation and emission wavelengths of the HPLC detector were 376 nm and 435 nm, respectively (6-9). The HPLC operating system used in this study was the Waters Empower 2.0 software, configured for analysis of TPP in whole blood (9,19).

For internal bench quality control (QC) 'Levy-Jennings' charts for low and high TPP concentrations were prepared, and the 'Westgard' rules were used for daily monitoring of the HPLC output data throughout the period of analysis. The intra-assay coefficients of variation (CV) for the low (52.6 µg/l) and high (106.0 µg/l) concentrations were 6.6% and 4.9%, respectively. The percent recovery of TPP was 95 ± 5%.

Data analysis

Analysis of data was carried out using the Statistics Package for Social Sciences (SPSS) Version 11 for Windows. The Mann-Whitney U test, chi-squared test and t-test were used as appropriate.

Ethical clearance

Ethical clearance for this project was obtained from the Ethical and Research Grant Committee in the SMHS, UPNG, and the Medical Research Advisory Committee (MRAC), National Department of Health (MRAC No. 05/12). Permission was obtained (as required by the PNG Principal Policy Adviser of the National Health Department) from the Provincial Education Boards and the respective heads of each boarding high school. Signed informed consent was obtained from each consenting student. Blood samples were collected only from those students who returned their signed consent forms.

Results

Informed consent was obtained from 468 students (response rate 84.3%). This gives a non-response rate of 15.7%, which was lower than the predicted 20% rate used in calculating the sample size. The mean ± standard deviation of age of all the students was 17.7 ± 1.5 years, the 95% confidence interval (CI) was 17.6-17.8 years, and the range was 14-22 years.

The gender distribution of the 468 students was 274 (58.5%) males and 194 (41.5%) females. The mean age of the male students was 17.9 ± 1.5 years (95% CI: 17.6 – 18.0), and the age range was 14-22 years. For the female students, the mean age was 17.4 ± 1.4 years (95% CI: 17.2-17.6) and the age range was 14-21 years. There was no statistically significant difference between the mean ages of the male and female students (p = 0.43).

The Shapiro-Wilk test indicated that the distribution curve of the whole-blood thiamine pyrophosphate concentrations (WBTPPC) for the combined study group was not normal ($p < 0.001$; df = 468), although it was normal for the males. The box plot presented in Figure 1 shows the presence of several outliers. Table 1 shows the medians and interquartile ranges, the mean and standard deviations, 95% confidence intervals, ranges and 10th centiles of the WBTPPC for all the students and for the males and females separately. The 95% confidence interval for the WBTPPC calculated after log transforming the data was 45.10-145.90 µg/l for all the students and 47.80-152.20 µg/l for the male and 47.76-133.24 µg/l for the female students.

Comparison of the WBTPPC of the male and female students using the Mann-Whitney U test indicates that the values for female students were significantly lower than those for the male students ($p < 0.001$). Further comparison of the WBTPPC in male and female students, using the t-test for equality of means, indicates a statistically significant difference ($p < 0.001$), with a mean difference of 14.17 µg/l (95% CI of the difference: 9.85-18.50).

The distribution of WBTPPC for all the students, according to the range of WBTPPC and status of thiamine nutrition, is presented in Table 2. Severe to marginal status of thiamine deficiency was present in 6.4% of all the students and was significantly higher in females than in males (9.8% vs 4.0%, p = 0.001).

Discussion

In the present study, the WHO-recommended cut-off points for TPP in either erythrocytes or whole blood, assayed by HPLC, were used to assess the thiamine status of the boarding school students (1,2,6,8,9).

Our data indicate 4.0% and 9.8% prevalence of severe to marginal status of thiamine deficiency among the male and female boarding school students,

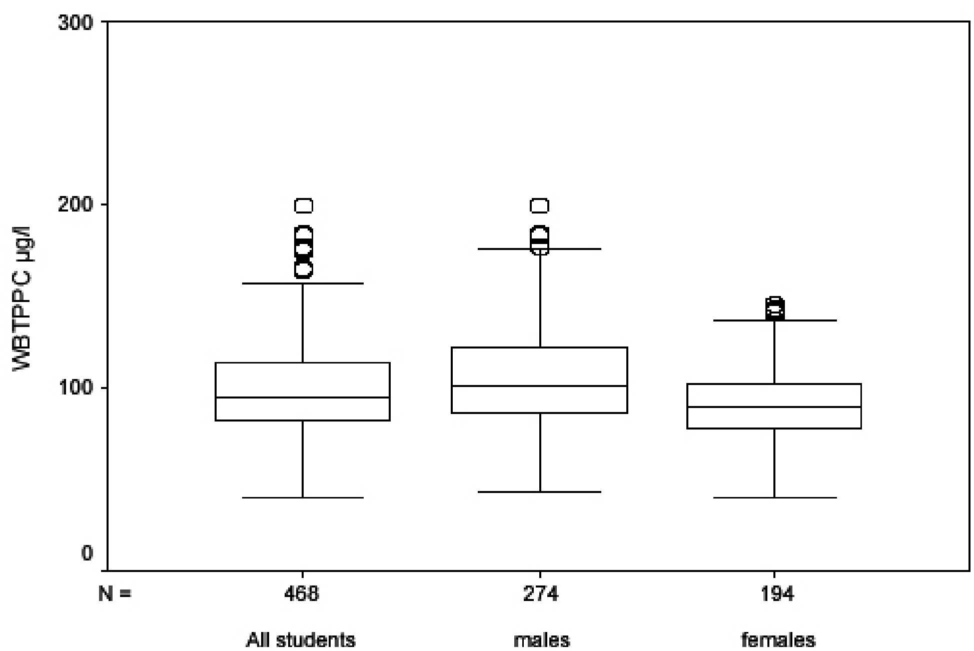

Figure 1. Box plot of whole-blood thiamine pyrophosphate concentrations (WBTPPC) for all students and for male and female students in boarding schools.

TABLE 1

WHOLE-BLOOD THIAMINE PYROSPHOSPHATE CONCENTRATION (WBTPPC) FOR ALL STUDENTS AND FOR MALE AND FEMALE STUDENTS IN BOARDING SCHOOLS

	All students (468)	Male (274)	Female (194)
Median (µg/l)	95.41	101.89	90.01
Interquartile range (µg/l)	82.27-113.55	86.56-121.57	77.45-103.03
Mean (µg/l)	98.80	104.67	90.50
Standard deviation	25.21	26.10	21.37
95% CI (µg/l)	45.10-145.90	47.80-152.20	47.76-133.24
Range (µg/l)	39.62-199.75	42.39-199.75	39.62-145.70
10th centile (µg/l)	69.40	73.16	63.90

TABLE 2

DISTRIBUTION OF WHOLE-BLOOD THIAMINE PYROPHOSPHATE CONCENTRATION (WBTPPC) OF BOARDING SCHOOL STUDENTS ACCORDING TO RANGE OF WBTPPC AND STATUS OF THIAMINE NUTRITION

*Range of WBTPPC (µg/l)	*Status of thiamine nutrition	All students (468)	Male (274)	Female (194)
		Percent distribution (N)		
<50.85	Severe deficiency	1.3 (6)	0.4 (1)	2.6 (5)
50.85-63.56	Marginal deficiency	5.1 (24)	3.6 (10)	7.2 (14)
>63.56	Normal status	93.6 (438)	96.0 (263)	90.2 (175)

*Reference 1 and 2

respectively. These values were lower than the 18.2% and 16.6% prevalence of severe to marginal status of thiamine deficiency recently reported among boys and girls in Taiwan (20) and the 22.0% and 32.0% prevalence of low thiamine status reported for boys and girls, respectively, in British schools in the early 1990s (21).

The 10th centile WBTPPC for the male boarding school students (73.16 µg/l) was significantly higher ($p = 0.01$) than the corresponding value (63.90 µg/l) for the female students. This indicates relatively poor thiamine status among the female compared with the male students. This result contradicts the findings reported by Talwar et al. (6), Shaw et al. (20) and Bovet et al. (22), but supports the findings by Bailey et al. (23).

Some of the factors that can cause low WBTPPC in adolescents and youths include long-term marginal dietary intake of thiamine, poor dietary choices, reduced energy intake, and/or inappropriate type, duration and intensity of physical exercise (6, 20-22). Thus, the difference in the thiamine status of the male and female boarding school students in our study may be due to a combination of these factors. In addition, although the male and female students in the boarding schools consume meals mainly prepared in the schools, teenage girls tend to 'watch their weight' and may consume less food than their male counterparts, which may lead to inadequate intake of thiamine.

From the public health point of view, our data indicate that a significant number of female boarding school students are at risk of developing marginal thiamine deficiency, because those with a WBTPPC below the 10th centile (63.9 µg/l) are very close to the lower limit of normal (63.56 µg/l). Marginal and severe thiamine deficiency may occur in the event of stress, increased physical activity and increased energy output during exercise, or increased caloric intake without a corresponding increase in the intake of thiamine (2,3,20-22).

There is a need for program planners to ensure that appropriate nutrition and health education, and information and awareness campaigns on the significance of dietary intake of thiamine and other micronutrients are carried out in the various boarding schools in the Southern Region of PNG. Our findings strongly indicate the need for continued effective implementation of the food fortification legislation in PNG.

Conclusions

Our data indicate marginal and severe thiamine deficiency among a significant proportion of students in boarding schools. Females are at greater risk of developing thiamine deficiency than males.

Continued fortification of rice and other cereals with thiamine is strongly recommended. Any change to the current nutrition legislation that would lower the levels of thiamine fortification could significantly increase the prevalence of thiamine deficiency among the high-risk groups in the communities for whom rice and other cereals are staple foods.

Public education and awareness programs outlining the benefits of consuming foods rich in thiamine and other micronutrients should be carried out in all the boarding schools in PNG.

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