Folic acid, riboflavin, thiamine, and vitamin B-6 status of a group of first-time blood donors¹–³

Christine K Booth, Therese Clark, and Anne Fenn

ABSTRACT Reference intervals for long-term status measures of folate, riboflavin, thiamine, and vitamin B-6 were determined in a select group of adults. Reference subjects had no adverse medical history and did not use tobacco, alcohol, or nutritional supplements, and their diets met ≥70% of the Australian recommended dietary intake for nutrients. Red blood cell concentrations of thiamine and folate were measured by microbiological methods. Vitamin B-6 and riboflavin status were measured on the basis of the erythrocyte aspartate transaminase activity coefficient and erythrocyte glutathione reductase activity coefficient, respectively. A survey of first-time blood donors, which was conducted in Australia in 1995, revealed a significant prevalence of low red blood cell thiamine concentrations (13%) when compared with the calculated normal reference intervals. However, the most important finding in the survey was that the group of healthy, nonanemic adults (first-time blood donors) was found to have a median red blood cell folate concentration 24% below the median concentration of the carefully selected (non-supplemented) reference group. Plasma total homocysteine concentrations indicated folate deficiency in the reference group. Therefore, the 2.5th percentile cutoff for reference group red blood cell folate concentrations may have underestimated the prevalence of folate deficiency in the survey group. These data, coupled with the lack of Australian food-composition data for folate in particular, reinforce the need for monitoring nutritional status by both dietary and biochemical means. We recommend consideration of mandatory fortification of the Australian food supply with folic acid.

SUBJECTS AND METHODS

Subjects

First-time blood donors attending the Brisbane Queen Street Centre of the Red Cross Blood Transfusion Service in 1995 were invited to participate in a survey of B-group vitamin status. A smaller group was selected from staff and students of the Royal Brisbane Hospital and Queensland University of Technology for determination of normal reference intervals. The final data set included 1887 healthy adults, 111 in the reference group (53 men, 58 women) and 1776 in the survey group (851 men, 925 women). The mean age for the reference group was 39.2 y (range: 18–65 y) compared with 31.5 y for the survey group (range: 16–70 y). The age distribution of both groups was normal. There was no significant difference in the sex breakdown of

INTRODUCTION

In Australia, the fortification of food with vitamins and minerals is the subject of much debate. The Australia New Zealand Food Authority recognizes that there needs to be a sound rationale for the addition of vitamins and minerals to Australian foods and recommends that food fortification should occur only where there is an identified and proven public health and nutritional need. However, a recent inquiry by the Australia New Zealand Food Authority into vitamins and minerals highlighted the lack of Australian dietary and nutritional status data (1). Furthermore, the efficacy of food-fortification programs, such as those for thiamine (2) and folate (3), should be monitored not only by collecting data on the incidence of Wernicke-Korsakoff syndrome and neural tube defects, respectively, but also by collecting data on the nutritional status of individuals. The present work helps address this need by detailing analytic methods and normal reference intervals for folate, thiamine, riboflavin, and vitamin B-6 status in healthy adults. The prevalence of folate, thiamine, riboflavin, and vitamin B-6 deficiencies among apparently healthy adults in Queensland was documented. In each case, the method to determine long-term status rather than acute status was chosen, namely, measurement of red blood cell (RBC) total folate (4), and RBC cell total thiamine (5) concentrations, erythrocyte aspartate transaminase activation test (6), and the erythrocyte glutathione reductase activation test (7). These are measures that should not be influenced by the subject’s previous meal.

KEY WORDS Folic acid, folate, riboflavin, vitamin B-6, vitamin B-2, vitamin B-1, pyridoxine, thiamine, reference intervals, vitamin status, Australia, humans, blood donors

¹From the School of Public Health, Queensland University of Technology, Red Hill, Australia, and the Red Cross Blood Transfusion Service Queensland, Brisbane, Australia.
²Supported by a grant from Kellogg (Australia).
³Address reprint requests to CK Booth, DSTO–Defence Nutrition Research Centre, 76 George Street, Scottsdale, Tasmania 7260, Australia. E-mail: christine.boo@dsto.defence.gov.au.
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The normal reference intervals (2.5th percentile to 97.5th percentile) determined in the present study, reference intervals used
in 3 Australian hospitals, and clinically significant cutoffs from previously published reports are shown in Table 1. There were differences between the reference group and the survey group. The survey distributions for folate concentration and EASTAC differed from the reference data (Table 2 and Figure 1). In each case, the median values were significantly lower than those for the reference group (Wilcoxon test: folate, \( P = 0.0001 \); EASTAC, \( P = 0.0002 \)). However, when the proportion of survey subjects outside the normal reference intervals was considered, only the prevalence of abnormal thiamine values was significantly different from that of the reference group (Table 3). The details of the distribution of measured homocysteine values for the reference group are shown in Figure 2. The central 95% of this distribution fell within 4.4–23.6 \( \mu \text{mol/L} \). The median value was 11.0 \( \mu \text{mol/L} \).

### DISCUSSION

The lack of reliable reference values and standardized analytic methods makes it difficult to assess vitamin status and to compare the status data reported by different surveys (22). Differences in analytic procedures and criteria for selection of the reference population can result in the calculation of differing reference intervals (Table 1). For example, supplementation with vitamin B-6 can lower the cutoff for EASTAC from < 130% to < 86% (20). The current study used strict selection criteria and reported normal reference intervals for the vitamin status of healthy subjects who consumed a diet (without nutritional supplements) that provided ≥70% of the Australian RDI for nutrients (11). This is consistent with the recommendations of the Scandinavian Committee on Reference Values (23).

In clinical practice, it is usual to compare an observed patient’s value with the corresponding normal reference interval (the central 95% of the reference population). Such a comparison conveys information about the similarity of the patient’s values to the given set of reference values. This contrasts with clinical decision cutoffs, which are based on the analysis of data from several population groups (healthy persons and patients with relevant diseases). Except for EASTAC, the normal reference intervals determined by the present study were consistent with clinical cutoffs described in the literature (Table 1).

The values of EASTAC that correspond with either optimal body stores of vitamin B-6 or to borderline deficiency are not well established. Gibson (24) referred to the > 50% clinical cutoff proposed by Sauberlich et al (25), and Leklem (19) suggested a clinical cutoff of > 80%. The different assay conditions used by laboratories may explain the lack of consensus regarding cutoffs for EASTAC. The cutoff proposed by Sauberlich et al (25) was revised to 130% by Bayouni and Rosalki (20) and confirmed by Mount et al (12). These authors optimized the substrate concentrations used in the automated assay. The assay conditions described by the latter authors were used in the present study. When the cutoff of > 80% was used, > 70% of both the reference and survey groups had abnormal EASTAC results. The literature suggests that such a high prevalence of vitamin B-6 deficiency in healthy subjects is unlikely. In fact, the reference group had a significantly higher median EASTAC value than the survey group, which indicates better vitamin B-6 status in the survey subjects (Table 2).

Possible folate, thiamine, vitamin B-6, and riboflavin deficiencies were identified in first-time blood donors (Table 3). For folate, vitamin B-6, and riboflavin, the prevalence of abnormal values was not significantly different from that of the reference group and the blood donors were therefore considered to have a low risk of these deficiencies.

First-time blood donors in the survey group could be at risk of thiamine deficiency. A significant proportion (13%) of the sur-

### TABLE 1

Reference intervals for thiamine, folate, vitamin B-6, and riboflavin status measures

<table>
<thead>
<tr>
<th>Vitamin Measure</th>
<th>Reference Range</th>
<th>Other Laboratories</th>
<th>Suggested Clinical Cutoff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell folate (nmol/L)</td>
<td>220–1100</td>
<td>290–1800(^2)</td>
<td>&lt; 253 (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>360–1450(^3)</td>
<td></td>
</tr>
<tr>
<td>Red cell thiamine (nmol/L)</td>
<td>200–520</td>
<td>190–400(^4)</td>
<td>&lt; 190 (18)</td>
</tr>
<tr>
<td>EASTAC (%)</td>
<td>&lt; 190</td>
<td>&lt; 120(^2)</td>
<td>&gt; 80 (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt; 130 (20)</td>
<td></td>
</tr>
<tr>
<td>EGRAC (%)</td>
<td>&lt; 35</td>
<td>&lt; 66(^2)</td>
<td>&gt; 40 (21)</td>
</tr>
</tbody>
</table>

1\(^{st}\) Except for folate, which was measured with an immunologic method at Royal Brisbane Hospital, assay methods were those used in the present study. Reference numbers in parentheses.

2\(^{nd}\) Royal Brisbane Hospital, Pathology Department, Brisbane, Australia.

3\(^{rd}\) Princess Alexandra Hospital, Brisbane, Australia.

4\(^{th}\) Royal Perth Hospital, Perth, Australia.

### TABLE 2

Distribution of each of the vitamin measurements for the reference and survey groups

<table>
<thead>
<tr>
<th>Vitamin Measure</th>
<th>Reference Group</th>
<th>Survey Group</th>
<th>Kolmogorov-Smirnov Test (( P ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folate (nmol/L)</td>
<td>Median 615</td>
<td>Median 468</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>2.5th percentile 216</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97.5th percentile 1100</td>
<td>1010</td>
<td></td>
</tr>
<tr>
<td>Thiamine (nmol/L)</td>
<td>Median 315</td>
<td>Median 308</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td></td>
<td>2.5th percentile 200</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97.5th percentile 520</td>
<td>734</td>
<td></td>
</tr>
<tr>
<td>EASTAC (%)</td>
<td>Median 121</td>
<td>Median 101</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>2.5th percentile 18.6</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97.5th percentile 212</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td>EGRAC (%)</td>
<td>Median 14.7</td>
<td>Median 15.1</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td></td>
<td>2.5th percentile 0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97.5th percentile 47.6</td>
<td>53.5</td>
<td></td>
</tr>
</tbody>
</table>

1\(^{st}\) EASTAC, erythrocyte aspartate transaminase activity coefficient, which was calculated as the stimulated enzyme activity minus the unstimulated activity divided by the unstimulated activity multiplied by 100; EGRAC, erythrocyte glutathione reductase activity coefficient.
vey group had low RBC thiamine concentrations. Thiamine has been considered to be one of the marginally adequate nutrients in the Australian diet. Certainly, poor thiamine status has been identified in homeless people (26) and a high incidence of Wernicke-Korsakoff syndrome has been recorded (27). It was believed that thiamine enrichment of flour to the concentration of 6.4 mg/kg, which commenced in 1991, would alleviate the problem of thiamine status in the Australian population (28). The present finding suggests that further monitoring of the fortification program is needed.

Possibly the most important finding in the survey concerns folate status. Inadequate dietary intake of folate has been found to increase the risk of spina bifida and other neural tube defects (29) and the evidence is convincing that an increased intake of folate can prevent most neural tube defect cases. The official recommendation for Australian women at conception and for 4 wk thereafter is to consume 400 \( \mu \)g folate/d (30). Homocysteine, which has been identified as an independent risk factor for cardiovascular disease, is inversely related to folate status. Moderately elevated homocysteine concentrations may be the first biochemical marker of insufficient intracellular folate.

There is evidence that strongly suggests that the 2.5th percentile cutoff underestimates folate deficiency (31). A more appropriate cutoff for RBC folate in the population might be determined by using plasma total homocysteine as a functional measure of folate status and studies of the prevention of neural tube defects. Recent studies suggest that the cutoff for a healthy plasma homocysteine concentration (ie, minimal risk for coronary artery disease) may fall below 10–15 \( \mu \)mol/L (31–33). In the present study, 6.8% of first-time blood donors (\( n = 97 \)) fell below the 2.5th percentile reference value for RBC folate of 220 nmol/L. This is clearly an underestimate of folate deficiency because it is likely that a large number of the reference subjects were folate deficient (15% of the reference group had homocysteine values >15 \( \mu \)mol/L and 55% of the reference group had homocysteine values >10 \( \mu \)mol/L; Figure 2). Furthermore, the median RBC folate concentration in first-time blood donors would need to be increased by 31% to have a median equal to that of the reference group (Table 2).

The Australian RDI for folate for men and women is 200 \( \mu \)g (11). The folate content of Australian foods, and consequently an estimation of the availability of folate in the Australian food supply, has not been described. Surveys, which have used several international food-composition tables, indicate that Australian...
women are at risk of poor folate intake, particularly during pregnancy (34, 35). The recent National Nutrition Survey (36) estimated the mean daily intake of folate by adult males to be 307 μg and by adult females to be 233 μg. This is considerably less than the daily folate intake of 350 μg estimated to be needed to maintain normal plasma homocysteine concentrations (37). The recent voluntary addition of folic acid to the Australian food supply (38), if widely adopted by the food industry, was expected to dramatically reduce the proportion of women with inadequate intake of folate (34). In light of the data presented here, this assumption appears optimistic. The 30% difference in median RBC folate concentration between a carefully selected reference group and healthy first-time blood donors could justify calling for mandatory folic acid fortification of food. The argument for mandatory folic acid fortification is boosted by the fact that, to date, it has been supplemental folic acid rather than dietary folate that has been proven to prevent neural tube defects. Presumably, this is because of the increased bioavailability of folic acid compared with the various forms of folate found in foods.

The finding of vitamin deficiency in apparently healthy Australian adults was unexpected because Australian dietary intake data suggest that this is a group at low risk for nutritional deficiency (39). However, previous work has shown that the use of food-composition data may lead to substantial over- or underestimation of the intake of several micronutrients and that the calculated amount of a specific micronutrient consumed did not adequately predict status as measured by several biochemical indexes (40). Furthermore, nutritional status is dynamic and changes with dietary and lifestyle habits and physiologic state. For example, alcohol intake, cigarette smoking, use of oral contraceptives, and exercise training can increase the requirements for specific vitamins (41–43).

Biochemical evidence of thiamine, vitamin B-6, and folate deficiencies has been found in homeless people (26, 44) and in an Aboriginal community (45). This is the first survey to suggest that thiamine and folate deficiencies are prevalent in an apparently healthy population of Australian adults.

We conclude that the prevalence of specific vitamin deficiencies in apparently healthy Australian adults may be greater than suggested by national dietary intake data. We recommend ongoing monitoring of the thiamine and folic acid fortification programs. We recommend mandatory fortification of the Australian food supply with folic acid and that biochemical assessment of vitamin status, which can be performed reliably and more cheaply than food-consumption surveys, should be considered as a viable alternative or addition to future nutrition surveys in Australia.

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REFERENCES


