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Thiamin intake, erythrocyte transketolase (*EC* 2.2.1.1) activity and total erythrocyte thiamin in adolescents

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The relationships between thiamin intake, erythrocyte transketolase (*EC* 2.2.1.1) activity coefficient (ETK-AC) and total erythrocyte thiamin were investigated in a group of adolescents (13 to 14 years old; nineteen boys, thirty-five girls). Thiamin intakes were calculated from 7 d weighed records, using food composition tables, and compared with those obtained by direct analysis of duplicate diets. Average 7 d calculated thiamin intakes were significantly lower than analysed intakes for both sexes. On an individual basis, calculated intakes ranged from 30 to 143% of corresponding analysed values. Analysed and calculated intakes were significantly correlated when expressed as mg/d; however, when expressed in terms of energy intake, the correlation was significant for males only. Thiamin intake appeared largely adequate when compared with current UK dietary recommendations (Department of Health, 1991), but the limitations of such comparisons are considered. The major food groups contributing to thiamin intake were examined and showed breakfast cereals to contribute more than 25% of dietary thiamin. A proportion of the subjects had ETK-AC values in ranges usually associated with marginal or severe thiamin deficiency. There was, however, no statistically significant relationship between erythrocyte thiamin and basal or stimulated transketolase activity, or between thiamin intake and either of the methods used to assess status. The need to re-evaluate indices of thiamin status is discussed.

Thiamin: Erythrocyte transketolase activity: Adolescence

Although such cases of vitamin B_1 (thiamin) deficiency as beriberi are rare, the Wernicke–Korsakoff syndrome (associated with excessive alcohol consumption) and biochemical evidence of thiamin deficiency continue to be prevalent within specific population groups worldwide (Davis & Icke, 1983; Thurnham, 1985; Bidlack *et al.* 1986; Haas, 1988; Tang *et al.* 1989). However, the possibility of marginal deficiency existing within an apparently healthy population is often overlooked.

The role of thiamin pyrophosphate (TPP) as an essential cofactor for metabolic enzymes is well established (Davis & Icke, 1983). More recently it has been suggested that thiamin may be intimately involved in neurological function, although the exact nature of this involvement remains unclear (Cooper & Pincus, 1978; Haas, 1988; Kim *et al.* 1990). Alterations in brain thiamin metabolism have been associated with Alzheimer's disease (Butterworth & Besnard, 1990) and effects of marginal thiamin deficiency have been reported to include anorexia, weight loss, fatigue, depression and sleep disorders, particularly in the elderly (Smidt *et al.* 1991). Effects of marginal deficiency such as these have been neglected when establishing reference nutrient intakes (RNI), which are based primarily on intakes required for the avoidance of clinical deficiency. RNI are also concluded from limited experimental evidence for groups such as adolescents, where

estimates of requirements may be complicated by periods of rapid growth (Gong & Spear, 1988; Department of Health, 1991).

Numerous studies of nutrient intake, food composition and dietary habits of adolescents and young adults are comprehensively reviewed by Bull (1988). Many of the publications mentioned include results for thiamin intake of 10–15-year-olds. However, all of these intake measurements were performed using tables of food composition, following record keeping which ranged from 24 h recalls to 14 d weighed intakes. Since investigations of the relationship between nutrient intake and measurements of status or function rely so heavily upon calculated intake values, it is important to determine the likely degree of accuracy of such data for the prediction of actual intake. One way of assessing the reliability of calculated values is to compare values computed from tables of food composition with those obtained by direct analysis of the diets consumed.

Long term urinary thiamin excretion measurements have been useful in the establishment of dietary requirements (Department of Health, 1991) but the interpretation of measurements from casual urine samples, which are strongly influenced by recent intake, is difficult, particularly in children (Sauberlich *et al.* 1979; Gibson, 1990*b*).

From 80 to 90% of total thiamin in cells is TPP (Kawasaki & Sanemori, 1985), and at present the activity of erythrocyte transketolase (ETK; *EC* 2.2.1.1), a TPP-dependent enzyme, is most frequently used as a functional index of status, since erythrocytes are among the first tissues to be affected by depletion (Brin, 1967). In thiamin deficiency the basal level of ETK activity (ETK°) is low, and an enhancement in enzyme activity after the addition of TPP *in vitro* is generally observed. Stimulated enzyme activity is expressed in terms of an activity coefficient (ETK-AC). However, prolonged deficiency induces a reduction in the apoenzyme level and both basal and stimulated ETK activities tend to fall, with no change in the TPP effect, giving rise to misleadingly 'normal' ETK-AC values (Schrijver, 1991). Thus, it has been suggested that basal enzyme activity should also be considered when assessing status. It should be noted, however, that the use of basal ETK activities, recommended for population groups with low thiamin intakes (Fidanza & Fidanza, 1974; Graudal *et al.* 1985; Nixon *et al.* 1990), makes comparisons of values difficult due to the variety of methods employed, and a range of confounding factors which influence basal activity of the enzyme (Gibson, 1990*b*).

Recently-developed HPLC procedures allowing determinations of the low concentrations of thiamin and/or its phosphate esters, particularly TPP, in blood fractions (Baines & Davies, 1988; Bailey & Finglas, 1990; Tallaksen *et al.* 1991) have great potential as sensitive indicators of thiamin status (Warnock *et al.* 1978), but the use of such measurements has yet to be firmly established.

Further attempts to define relationships between dietary thiamin intake, various indices of status, and indicators of health and well being rely heavily on the accuracy of dietary measurements, the reliable interpretation of biochemical determinations as well as the ability to quantify the indicators of health. The present study compares thiamin intakes of apparently healthy 13–14-year-olds (a relatively understudied group of the population), as measured by two methods; a 7 d weighed dietary record, using food tables to calculate intake, and direct HPLC analysis of duplicate diets collected on the days of dietary recording. In each case intakes were measured on only one day each week for 7 consecutive weeks in order to reflect habitual intake more closely (Black *et al.* 1984). Relationships between calculated and analysed thiamin intakes and intake and biochemical indices of status (as measured by ETK°, ETK-AC and total erythrocyte thiamin) were examined and are discussed.

MATERIALS AND METHODS

Subjects

The study was subjected to ethical approval by the Institute's ethics committee before recruitment. Fifty-four subjects (thirty-five female, nineteen male), 13–14 years of age, were recruited from two local authority schools. The median (range) age, height and weight of the females and males respectively were: age 13·8 (13·3–14·3), 13·6 (13·2–14·3) years; height 1·584 (1·503–1·737), 1·616 (1·423–1·866) m; weight 51·2 (38·5–62·5), 46·7 (33·1–68·0) kg. Median heights and weights for the subjects were similar to the fiftieth percentile values calculated by the National Centre for Health Statistics for this age group (Thomas, 1988).

Measurement of intake

Each subject kept a written, weighed record of everything they ate and drank every sixth day for 7 consecutive weeks (Black *et al.* 1984). On each of these days the subjects also collected weighed duplicate portions of all food and drink consumed. A set of scales (model no. 2001; Salter Homewares Ltd., Tonbridge, Kent; maximum capacity 2 kg, accurate to 2 g), a supply of intake record sheets, a small notebook and acid-washed, plastic food and drink containers were given to each subject. An instruction booklet giving details of how to perform dietary collections and complete dietary record sheets were also provided, together with a personal timetable of recording/collection dates. In addition, a fieldworker visited each home to demonstrate the use of the dietary scales and completion of record sheets. Subjects were advised to provide as much information as possible on brand and preparation of foods and carefully note any wastage. Where appropriate, wrappers and packaging were retained as a potentially useful source of information. Instruction was given on how to record composite foods, ready meals and foods eaten outside the home. If a subject could not provide a duplicate portion of a food or drink consumed outside the home they were asked to describe it as fully as possible to allow duplicate portions to be purchased by a fieldworker and added to the duplicate diet. Catering staff at each school put aside duplicate portions for those subjects who had school meals, and these were collected and weighed by a fieldworker shortly after the meal, and appropriate recipes obtained. Subjects who took packed lunches to school prepared a duplicate lunch and later removed any items which had not been consumed before the meal was added to the day's collection.

Diet collection and preparation

The duplicate food and drink containers were kept at −18° in a domestic freezer until collection by Institute staff early the following morning, along with the dietary record sheets. The recorded information was checked in the presence of the subject to ensure that all necessary details had been provided.

The completed diets were homogenized immediately after each collection using a stainless-steel blender (R. W. Jennings Ltd., Nottingham, Notts). Weights of total homogenate and any distilled water (added to aid homogenization where necessary) were recorded. Sub-samples (100 g) were stored at −30° before thiamin analysis.

Blood collection and preparation

Fasting (12 h) venous blood samples were obtained during the period of dietary assessment. Fresh (within 1 h of collection) whole, heparinized blood (1·5 ml) was centrifuged at 8800 ***g*** for 3 min, plasma and buffy coat layer were removed and the erythrocytes were washed

three times with cold isotonic saline solution (9 g NaCl/l) before storage at −30° and subsequent determination of total erythrocyte thiamin by HPLC. Further portions of similarly-washed erythrocytes were haemolysed by dilution with distilled water (1:1, v/v) and stored in liquid N_2 for up to 2 weeks before determination of ETK° and ETK-AC.

Determination of ETK° and ETK-AC

ETK activity was determined, with and without the addition of TPP, using the method of Anderson & Nicol (1986). Following the transformation of ribose-5-phosphate (yielding glycerol-3-phosphate and sedoheptulose-7-phosphate), the assay measures the secondary enzymic consumption of NADH during the production of glycerol-3-phosphate.

Thawed, diluted erythrocyte samples were frozen and thawed a further two times using liquid N_2 and hot water (50°) to ensure complete lysis. Sub-samples (20 μl) were taken, and haemoglobin concentrations determined using a CBC5 coulter counter (Coulter Electronics, Luton, Beds). To a volume of haemolysate equal to 20 mg haemoglobin in 5 ml Quickfit™ test tubes were added 300 μl NADH–indicator enzyme (100:1 (v/v) mixture of (a) 10 mM-NADH and (b) glycerol-3-phosphate dehydrogenase (*EC* 1.1.99.5)–triose-phosphate isomerase (*EC* 5.3.1.1) suspension (GDH/TIM; 10 mg protein/ml; BCL Ltd, Lewes, East Sussex)) and a volume of 17 mM-ribose-5-phosphate sufficient to bring the final volume to 2560 μl. A reagent blank was prepared by substituting 160 μl Tris–HCl buffer (100 mM, pH 7·6) for haemolysate. The reaction tubes were stoppered, shaken, protected from light by covering with foil and incubated at 37° for 15 min before transferring portions (1·0 ml) to further tubes containing either (a) 40 μl Tris–HCl or (b) 40 μl 10 mM-TPP in Tris buffer (previously equilibrated at 37°).

After a further 30 min incubation 100 μl portions of each sample were mixed, in 10 ml stoppered and foil-covered glass test tubes, with 4·9 ml 20 mM-phosphate buffer, pH 7·5, at 20° (prepared by adjusting 84 ml 40 mM-dibasic sodium phosphate to pH 7·5 with approximately 16 ml 40 mM-monobasic sodium phosphate and diluting to 200 ml with distilled water). The fluorescence was read using an LS-5 luminescence spectrophotometer with an autosipper attachment (Perkin Elmer, High Wycombe, Bucks) with excitation and emission wavelengths of 340 and 465 nm respectively, taking readings again at 90 min total incubation time following addition of TPP, the incubation temperature being 37° throughout. The fluorescence of NADH was linear over the range 0–24 μmol/l and calibration was performed using two levels (8 and 16 μmol/l NADH in phosphate buffer, pH 7·5) at the beginning and end of each assay run. Between-batch coefficients of variation (CV) were 4·8% for 8 μmol/l (mean 287·3, *n* 16) and 3·7% for 16 μmol/l (mean 569·2, *n* 16).

ETK activities are expressed as mU/g haemoglobin, where 1 mU = 1 nmole NADH consumed/min. Intra-assay variabilities expressed as differences of duplicate analyses from the mean were 10% (*n* 52) for basal or unstimulated ETK activity, 6·6% (*n* 52) for stimulated ETK and 12·7% (*n* 50) for ETK-AC.

$$\text{ETK-AC} = \frac{\text{ETK}^{\text{TPP}}}{\text{ETK}^{\circ}},$$

where ETK° is basal or unstimulated transketolase activity and ETK^{TPP} is transketolase activity stimulated by TPP.

Determination of total erythrocyte thiamin

The method used was that of Bailey & Finglas (1990), modified to optimize recoveries of thiamin and TPP from erythrocyte samples. The procedure is described in full below.

Samples (0·50 ml) of thawed erythrocytes were added to HCl (1·5 ml, 0·133 M) in 8 ml amber screw-capped vials (Pierce and Warriner (UK) Ltd, Chester, Cheshire) mixed, heated in a boiling waterbath for 30 min and then cooled to 37°. Sodium acetate buffer (1·8 ml, 2 M, adjusted to pH 4·5 with glacial acetic acid) and 200 μl freshly prepared enzyme suspension (taka-amylase; 100 mg/ml (*EC* 3.2.1.1; from *Aspergillus oryzae*; Serva, NY, USA) and acid phosphatase, 10 mg/ml (*EC* 3.1.3.2, Sigma, Poole, Dorset, UK) in 2 M-sodium acetate buffer, pH 4·5 as above) were added and the samples were incubated at 37° overnight with constant mixing. After cooling to room temperature the samples were centrifuged at 2000 rev./min for 10 min and the supernatants were transferred to polythene vials (Chromacol Ltd, Welwyn Garden City, Herts) and either analysed immediately or stored at −20° for up to 1 week. 'Enzyme blanks' were prepared similarly by replacing erythrocytes with 0·5 ml saline solution.

Triplicate portions (0·5 ml) of thiamin extracts were pipetted into 4 ml screw-cap amber glass vials (Pierce and Warriner (UK) Ltd.) containing 100 μl 0·1 M-HCl. Standard solutions were prepared by adding freshly prepared working standards (100 μl, 0–100 ng/ml thiamin hydrochloride in 0·1 M-HCl) to 0·5 ml portions of enzyme blank. Thiamin was oxidized to thiochrome by addition of freshly prepared oxidizing solution (0·5 ml, 0·5 mg/ml $K_3Fe(CN)_6$ in 2 M-potassium acetate buffer, adjusted to pH 4·5 with glacial acetic acid, containing 25 mM-EDTA). After mixing, the solutions were allowed to stand for 30 min at room temperature. Thiochrome was extracted by vigorous shaking by hand for 2 min with iso-butanol (0·75 ml, 2-methyl propanol, low in fluorescence, BDH Ltd, Poole, Dorset). After centrifuging at 2000 rev./min for 5 min the upper iso-butanol fraction was removed into 0·8 ml amber glass autosampler vials (Chromocol Ltd.) ready for the HPLC determination of thiochrome.

A Perkin Elmer LC 420B autosampler was used to inject iso-butanol extracts (20 μl) onto a silica analytical column (steel, 250 mm × 4 mm i.d. packed by upward displacement with Lichrosorb™ Li60 5 μm packing; Hichrom Ltd, Reading, Berks). The mobile phase was chloroform–methanol (80:20, v/v), pumped at 2·0 ml/min using an SA6410 solvent delivery system (Severn Analytical, Luton, Beds.). Thiochrome was detected fluorimetrically using an LS-5 luminescence spectrophotometer (Perkin Elmer), fitted with an 8 μl flow cell; excitation and emission wavelengths were 375 and 430 nm and slit widths 15 and 20 respectively. Spectrofluorimetric data were collected and peak areas calculated using an Autochrom™ Apex™ chromatography workstation (Severn Analytical). Thiamin concentrations were calculated by comparison of peak areas with those of oxidized standard thiamin solutions. Mean recoveries of added thiamin and TPP from erythrocyte samples were 99·8% (range 95·8–105·8%, *n* 8) and 101·2% (range 98·4–103·8%, *n* 7) respectively. Inter-assay repeatability between replicate analyses of a single pooled erythrocyte sample was 8·5% (mean 71·6ng/ml, *n* 7). Intra-assay repeatability between triplicate oxidations of single erythrocyte extracts was 2·32% (range 0·20–5·28%, *n* 13 triplicate analyses).

Haemoglobin concentrations of fresh whole blood and of the washed erythrocyte samples and packed cell volumes of fresh whole blood samples were measured using a CBC5 coulter counter (Coulter Electronics). Thiamin concentrations were expressed as both ng/ml and nmol/l packed erythrocyte and as ng/g haemoglobin for comparison with ETK values.

HPLC determination of the thiamin content of duplicated diets

Combined 7 d duplicate diet homogenates were prepared for each subject by thawing frozen samples to +1° and adding together portions equal to 5 g/kg total intake for each individual day and mixing thoroughly. The total thiamin content of these combined

samples was determined using an application of the method of Bailey & Finglas (1990), modified in order that recoveries of thiamin from mixed diet samples were maximized. The procedure is described below.

Duplicate 10 g portions were accurately weighed into 50 ml Quickfit™ test tubes, HCl (25 ml, 0·1 M) was added and the samples were heated in a boiling waterbath for 1 h. After cooling to 37°, 5·0 ml freshly prepared enzyme solution was added (taka-amylase 10 mg/ml *EC* 3.2.1.1; from *Aspergillus oryzae*; Serva); papain (20 mg/ml, Serva), both suspended in 2 M-sodium acetate (adjusted to pH 4·5 with glacial acetic acid)). The final pH of each solution was adjusted to pH 4·5 using 3·75 M-NaOH before incubating in a shaking waterbath (BKS 300; Gallenkamp, Leicester, Leics.) at 37° overnight. The following day, after cooling to ambient temperature, the samples were made to 50 ml with 0·1 M-HCl, mixed thoroughly and centrifuged at 2000 rev./min for 10 min. Supernatants were filtered through Whatman 541 filter paper and transferred to polythene vials for either immediate HPLC analysis or storage for up to 1 week at −20°. 'Enzyme blanks' were prepared by treating 25 ml 0·1 M-HCl (containing no sample) similarly.

Duplicate samples (1·0 ml) were pipetted into 4 ml amber screw-top glass vials (Pierce and Warriner) containing HCl (100 μl, 0·1 M). Standards were prepared by adding 100 μl freshly prepared thiamin solution (0–4·0 μg/ml thiamin hydrochloride in 0·1 M-HCl) to 1·0 ml enzyme blank. Samples and standards were then oxidized by addition of 1·0 ml freshly prepared oxidizing agent (2·0 ml 100 mg/ml $K_3Fe(CN)_6$, aqueous, diluted to 50 ml with 3·75 M-NaOH). Samples were mixed and the thiochrome extracted by shaking vigorously by hand for 2 min with 1·5 ml iso-butanol. After centrifuging at 2000 rev./min for 5 min, the upper iso-butanol layer was removed into 2 ml amber autosampler vials (Chromocol Ltd). The thiochrome content was determined by HPLC using the same conditions as described earlier for the determination of total erythrocyte thiamin. Results were expressed as mg total thiamin/d.

Mean recovery of added thiamin from mixed diet samples was 98·1% (range 88·7–107·9%, *n* 30). The intra-assay variation expressed as mean percentage error between duplicate determinations was 1·26% (range 0·18–2·59%, *n* 10).

Calculation of thiamin intake from weighed dietary records

Dietary records were coded using *McCance & Widdowson's The Composition of Foods* (Paul & Southgate, 1978), together with the *Immigrant Foods Supplement* (Tan *et al.* 1985), *Additional Foods* (Wiles *et al.* 1980), *Cereals & Cereal Products* (Holland *et al.* 1988), *Milk Products and Eggs* (Holland *et al.* 1989) and unpublished compositional data (J. M. Loughridge and A. D. Walker, Agriculture and Food Research Council Institute of Food Research). Any missing food quantities were obtained using *Food Portion Sizes* (Crawley, 1988) and portion size values from previous intake studies (J. M. Loughridge and A. D. Walker, unpublished results). Total thiamin intakes (mg/d and mg/4184 kJ (1000 kcal) consumed) were calculated using the Institute of Food Research food composition database. Average 7 d intakes were expressed according to food groups by means of a spreadsheet computer program (Excel, version 2·1, 1988; Microsoft Corporation, Readmond, Washington, USA).

Analytical determination of energy intake

Bomb calorimetry was performed on freeze-dried samples of diet homogenate and metabolizable energy was estimated as follows: analysed energy (metabolizable energy)/d = (bomb energy (kJ/d) × 0·95) − (N (g/d) × 30 kJ), (Miller & Payne, 1959).

Statistical analysis

Statistical comparisons were performed using Student's paired and unpaired t tests as appropriate. The statistical analysis used is indicated in the tables of results. Relationships between analysed and calculated intakes, thiamin intake and status were examined using regression analysis. Erythrocyte thiamin values were $\log_{10}$ transformed, to normalize distribution, before performing unpaired t tests or regression analysis. All other distributions were normal.

RESULTS

Energy and thiamin intakes

Calculated and analysed energy and thiamin intakes are shown in Table 1. The mean energy intakes, using both calculated and analysed data, were significantly higher for boys than girls ($< 0{\cdot}001$, 22 and 26% respectively). Mean calculated and analysed thiamin intakes, expressed as mg/d, were also significantly higher for boys ($P < 0{\cdot}05$, 41 and 33% respectively) but were not significantly different when expressed as mg/4184 kJ (1000 kcal).

There was no significant difference between calculated and analysed energy intakes for girls. Calculated energy intake for the boys was approximately 4% lower than the analysed value, and this difference was significant.

Analysed thiamin intakes (mg/d and mg/4184 kJ) were significantly higher than calculated values for both sexes (mg/d; boys, $P < 0{\cdot}05$, 29% and girls, $P < 0{\cdot}001$, 42%: mg/4184 kJ; boys, $P < 0{\cdot}05$, 24% and girls, $P < 0{\cdot}001$, 40%). There was a significant correlation between calculated and analysed intakes for energy (boys r 0·91, $P < 0{\cdot}001$; girls r 0·94, $P < 0{\cdot}001$), and thiamin (mg/d) (boys r 0·59, $P < 0{\cdot}01$; girls r 0·43, $P < 0{\cdot}01$).

Fig. 1 shows the correlation for all subjects. However, when expressed as mg/4184 kJ consumed, analysed and calculated intakes of thiamin were only significantly correlated for boys (r 0·62, $P < 0{\cdot}01$). Thiamin intakes are compared with the current reference nutrient intake (RNI) of 0·4 mg/4184 kJ in Table 3.

Food groups

The contribution of various food groups to calculated thiamin intake is shown in Fig. 2. The four major food group sources of thiamin and their percentage contribution to thiamin intake were: cereals and cereal products 50·8%, vegetables 17·9%, meat and meat products 8·8%, milk and milk products 8·6%. A further breakdown of these four food groups is shown in Fig. 3. The highest single contributors to total thiamin intake were breakfast cereals (26·5%), breads (14%), potatoes (10·7%), biscuits (5·7%), pork (5·6%) and whole fresh cow's milk (4·0%).

When compared for individual subjects, mean 7 d calculated thiamin intakes ranged between 30 and 143% of the value obtained by direct analysis of diets. Twenty subjects were selected on the basis of the calculated intake being between 90 and 110% (small discrepancy, n 10) of the analysed value, or less than 50% (large discrepancy, n 10), and the diet composition was compared in terms of food groups. No food group was present in any particularly unusual amount in either of the two extreme groups.

Thiamin status

Values for ETK°, ETK-AC and total erythrocyte thiamin (expressed as ng/ml, nmol/l and ng/g haemoglobin) are shown in Table 2. Erythrocyte thiamin values were $\log_{10}$ transformed to normalize distribution, before carrying out a Students unpaired t test comparison between sexes.

Table 1. *Daily analysed and calculated energy and thiamin intakes for thirty-five girls and nineteen boys aged 13–14 years*‡

(Values are means with their standard errors for 7 d average intakes)

	Girls			Boys		
	Mean	Range	SE	Mean	Range	SE
Energy (MJ)						
Analysed§	7·36***	4·55–10·95	0·24	9·25†	6·96–12·72	0·35
Calculated‖	7·21***	4·31·11·12	0·25	8·82	5·75–12·21	0·37
Thiamin (mg)						
Analysed	1·52*†††	0·56–2·73	0·09	1·95†	0·97–4·49	0·19
Calculated	1·07*	0·56–1·80	0·05	1·51	0·75–3·55	0·16
Thiamin (mg/4184 kJ (1000 kcal))						
Analysed	0·88†††	0·41–2·51	0·06	0·88†	0·58–1·96	0·07
Calculated	0·63	0·41–0·90	0·02	0·71	0·38–1·53	0·06

Mean value was significantly different from that for boys: $*P < 0·05$, $***P < 0·001$ (Student's unpaired t test).
Mean value was significantly different from the corresponding calculated value: $\dagger P < 0·05$, $\dagger\dagger\dagger P < 0·001$ (Student's paired t test).

‡ For details of subjects and procedures, see pp. 112–116.

§ Analysed values were obtained by direct analysis of duplicate diets.

‖ Calculated values were obtained using food composition tables.

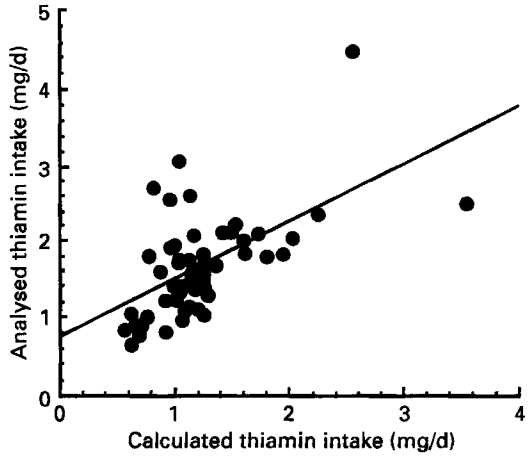

Fig. 1. Relationship between calculated and analysed thiamin intakes for fifty-four adolescents aged 13–14 years. The regression equation is $y = 0·74495 + 0·75559x$, R^2 0·341.

There was no significant difference between the sexes for mean ETK°, ETK-AC or total erythrocyte thiamin levels. The wider range of total erythrocyte thiamin values shown for girls, compared with that for boys, was due principally to one female subject with an unusually high erythrocyte thiamin; on removal of this one value the range for girls was reduced substantially and became similar to that for the boys.

The percentage of subjects with ETK-AC values usually associated with thiamin deficiency (> 1·25), or marginal deficiency (1·15–1·25; Brin, 1970) were 16·7% and 5·6% of boys, and 11·8% and 23·5% of girls respectively (Table 3). Erythrocyte thiamin

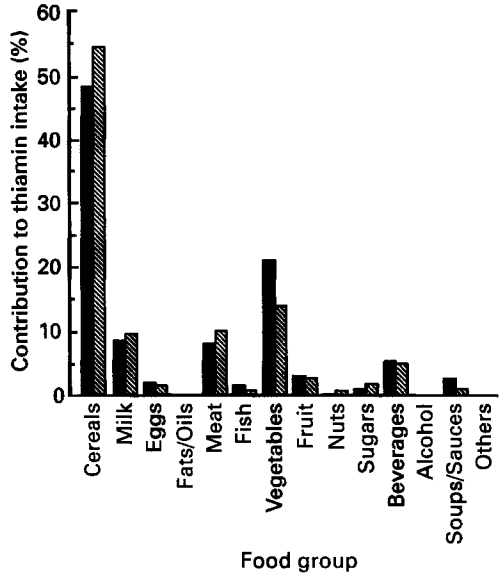

Fig. 2. Contribution of the food groups to total thiamin intake of fifty-four adolescents aged 13–14 years (■ girls, *n* 35; ▨ boys, *n* 19).

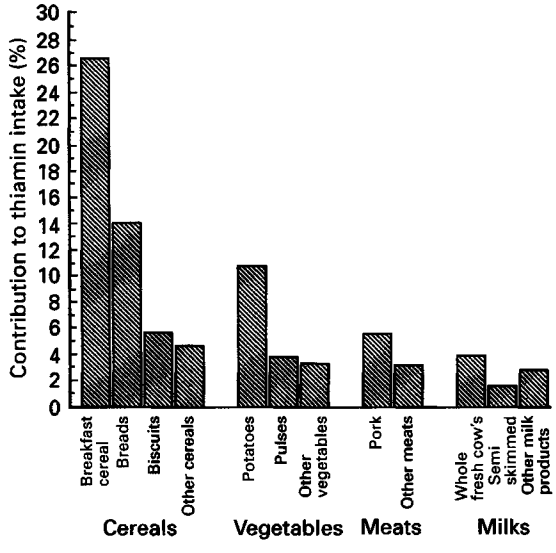

Fig. 3. Contribution of individual food types from four major food groups to total thiamin intake of fifty-four adolescents aged 13–14 years.

concentrations indicative of thiamin deficiency have as yet not been established for adolescents. Table 3 compares the values obtained in the present study with those at the bottom of the normal reference ranges for adults. There was no significant relationship between total erythrocyte thiamin and either ETK° or ETK-AC. There was a trend towards

Table 2. *Basal (ETK°), erythrocyte transketolase activity coefficient (ETK-AC), and total erythrocyte thiamin ($RBCB_1$) concentration for thirty-five girls and nineteen boys aged 13–14 years**

(Values are means with their standard errors)

	Girls			Boys		
	Mean	Range	SE	Mean	Range	SE
ETK° (mU/g Hb)	89·66	54·50–165·74	4·45	90·08	58·33–140·62	4·71
ETK-AC	1·07	0·86–1·45	0·02	1·05	0·69–1·36	0·04
$RBCB_1$ (ng/ml)†	76·50	34·09–320·40	8·14	69·53	40·39–150·34	6·06
(nmol/l)	226·8	101·0–949·9	24·1	206·1	119·7–445·7	18·0
(ng/g Hb)	22·47	10·10–91·20	2·28	20·66	11·90–44·91	1·81

Hb, haemoglobin.
* For details of subjects and procedures, see pp. 112–116.
† Erythrocyte thiamin expressed as ng/ml and nmol/l erythrocytes and as ng/g Hb for ease of comparison with other published results.

Table 3. *Percentage of adolescent subjects with low indices of thiamin status, or calculated or analysed thiamin intakes below the reference nutrient intake**

			Girls	Boys	Both sexes
ETK-AC	1·15–1·25†		23·5	5·6	17·3
	> 1·25†		11·8	16·7	13·5
$RBCB_1$	< 148·2 nmol/l (< 50 ng/ml)‡		11·8	22·2	15·4
	< 118·5 nmol/l (< 40 ng/ml)‡		8·8	0	5·8
Intake	< 0·4 mg/4184 kJ (1000 kcal)§	(Calculated)	0	11·1	3·8
		(Analysed)	2·9	0	1·9

ETK-AC, erythrocyte transketolase activity coefficient; $RBCB_1$, erythrocyte thiamin.
* For details of subjects and procedures, see pp. 112–116.
† Brin (1970).
‡ Erythrocyte thiamin concentrations indicative of risk of thiamin deficiency are as yet not established. The bottom of the normal range of adults is around 40 to 50 ng/ml (Burch *et al.* 1952; Warnock, 1978; Floridi *et al.* 1984; Baines, 1985; Van der Westhuyzen *et al.* 1988).
§ Department of Health (1991).

an inverse relationship between ETK° and erythrocyte thiamin expressed per g haemoglobin, for male subjects only, but this relationship did not reach significance ($P = 0·065$, r 0·44).

Neither calculated nor analysed thiamin intakes, within the range measured, were significantly correlated with any of the thiamin status variables measured.

DISCUSSION

Energy and thiamin intakes

Comparison of calculated and analysed energy intakes indicated that duplicate diet collections, in the present study, were a good reflection of the foods weighed and recorded in the dietary diary. Calculated energy intake for the boys was significantly lower than the

analysed value, but the difference was only in the order of 4%. Consideration of the energy intake of this group of subjects is presented elsewhere (Finglas *et al.* 1992).

Although average calculated and analysed thiamin intakes were significantly correlated for the group as a whole, no relationship between intake values, expressed per unit energy, was found for girls. In addition, mean 7 d analysed intakes (mg/d and mg/4184 kJ) were significantly higher than calculated values for both sexes. It is also important to note that calculated thiamin intakes for individual subjects varied between 30 and 143% of the analysed values. The reason for this difference is uncertain, but it is possible that in some cases actual foods eaten differed considerably from those analysed for the compilation of food tables. It is also likely that the use of recently developed, sensitive HPLC techniques, such as that presented in this paper, has resulted in improved measurement of the vitamin in foods and mixed diets, in comparison with those methods used to compile the food tables. No particular food group appeared to be the cause of errors between calculated and analysed intakes. Thus, thiamin intakes calculated from tables of food composition may lead to a variable, and often substantial, underestimation of actual intake in population groups. The method of collecting and weighing intakes every sixth day for 7 weeks was chosen in view of the evidence that accuracy of record keeping deteriorates over consecutive days of recording and that the extra burden of collecting duplicate diets can result in a decrease in recorded energy intake of as much as 20% (Gibson, 1990*a*) as discussed further elsewhere (Finglas *et al.* 1992).

The mean analysed thiamin intakes found in this study fall at the highest end of the range reported in other studies of 13–15 year olds (Bull, 1988; boys 0·9–2·14 mg/d, girls 0·8–1·54 mg/d) and, using both analysed and calculated intake data, only three subjects were found to consume less than the UK RNI of 0·4 mg thiamin/4184 kJ (Department of Health, 1991). However, the mean thiamin intake for the group was well above the RNI which, for children, has been determined from limited experimental evidence. Bearing in mind also that requirements differ considerably between individuals, a subject's nutriture cannot be determined by such comparisons. If 0·8 mg/d is considered an absolute minimum thiamin intake, regardless of energy consumption (Horwitt, 1986), eight subjects were found to consume on average less than this daily allowance on the basis of calculated intake data, but only one subject on the basis of analysed data. Nevertheless, a proportion of both boys and girls in the present study could be classified as having poor thiamin status on the basis of ETK-AC (17%) and total erythrocyte thiamin values (6%). It is interesting to note that the two largest single contributors of thiamin to the diets of these subjects were breakfast cereals and breads, virtually all of which are fortified with thiamin during production under current UK policies. The bioavailability of thiamin hydrochloride (used to fortify foods) compared with that of endogenous food thiamin has been investigated; however, results appear conflicting depending on the status index used (Vinson *et al.* 1989; Finglas, 1993).

ETK activity

In the present study ETK-AC values indicated that 13·5% of subjects (three males, four females) were at high risk of thiamin deficiency and 17·3% (one male, eight females) were at moderate risk, according to the criteria of Brin (1970). All of these subjects had thiamin intakes above the RNI. ETK activity determination is a popular method for the measurement of thiamin status; however, very few papers give values specifically for the adolescent age group. Where values are available (Reinken *et al.* 1979; Marktl *et al.* 1982; Widhalm *et al.* 1986; Gans & Harper, 1991), a significant proportion of the adolescent population studied also had ETK-AC values indicative of abnormally low thiamin status, although the criteria for establishing normal-, moderate- and high-risk individuals vary

slightly between authors. The apparently high incidence of poor thiamin status found in this and other studies, for population groups previously supposed to be healthy, poses several questions. Are ETK-AC values adequate predictors of risk of less severe thiamin deficiency and if so are adult reference ranges correct or applicable to younger individuals? Do current RNI values underestimate the requirements of adolescents for this vitamin?

In the present study, although analytical variability of fluorescence measurements was low, small changes in fluorescence with time led to relatively large variability in both basal transketolase activities and ETK-AC values (expressed as differences of duplicate analyses from means: fluorescence 1·8%, basal ETK 10%, ETK-AC 12·7%). Such large variability, if also experienced by other workers, throws doubt on the reliability of the transketolase assay for routine determination of thiamin status. Establishment of RNI which take into account such non-specific and subjective criteria as loss of appetite, sleep disturbances, inactivity, malaise, irritability, forgetfulness and depression would be difficult to achieve. However, the work of Smidt *et al.* (1991) with the elderly suggests that this task is not impossible.

The mean basal transketolase activity found in this study of 13–14-year-olds (approximately 90 mU/g haemoglobin) was substantially lower than that found for adults in a study performed by Anderson & Nichol (1986). Unfortunately, most other studies of transketolase activity in juveniles have not reported basal levels. However, the very nature of ETK assay procedures, whereby the absolute transketolase activity depends upon individual laboratory conditions with respect to several variables, makes between-laboratory comparisons extremely difficult. This presents a major problem in the use of this measurement as an index of thiamin nutriture. The age dependence of ETK and ETK-AC is discussed by Reinken *et al.* (1979) and Rooprai *et al.* (1990); unfortunately ages between 14 and 18 years are not considered.

No significant relationship was found in the present study between ETK-AC and ETK° despite a number of abnormally high percentage TPP effects (> 25%). This is contrary to findings by Nixon *et al.* (1990), who observed in groups of thiamin-deficient patients that variation in TPP effect was due only to variation in ETK°. They found a stronger correlation between ETK° and activated ETK, and suggested that calculation of TPP effect is without value for individuals with low thiamin status.

Total erythrocyte thiamin

It has been suggested that erythrocyte TPP levels may be a preferred method of assessing status, since studies with thiamin-deficient rats showed that erythrocyte thiamin concentration fell before any changes in ETK activity occurred (Warnock *et al.* 1978). In the present study the mean erythrocyte thiamin concentration for 13–14-year-olds was 219·7 nmol/l (74·1 ng/ml). This value is lower than those found for apparently healthy adult and elderly populations: 275·7 nmol/l (93 ng/ml) and 346·9 nmol/l (117 ng/ml) respectively (O'Rourke *et al.* 1990), but similar to that found for healthy adults from the Norwich area, 227·1 nmol/l (76·6 ng/ml, *n* 36, A. L. Bailey, S. Southon, A. J. A. Wright and P. M. Finglas, unpublished results). Other workers (Nishi *et al.* 1984; van Der Westhuyzen *et al.* 1988) have found low blood thiamin levels in adolescents in comparison with adult populations in South Africa and Japan respectively; there appear to be no other UK values for the adolescent age group. Age-dependent changes in thiamin levels for infants and children are discussed by Wyatt *et al.* (1991) who reported that whole-blood total thiamin decreases in the first year of life and then remains relatively stable until the age of 6 years, but little is known about levels in later childhood and adolescence. In the present study, approximately 12% of the girls and 22% of the boys were classified as having a 'low' erythrocyte thiamin concentration, that is below 148·2 nmol/l (50 ng/ml)

which is at the bottom of the normal range for adults (Table 3). However, reference limits and cut-off points for this index of status, particularly for children, have as yet not been established. Furthermore, comparisons of data are complicated by authors' differing preferences for either erythrocyte or whole-blood determinations of either total thiamin or individual phosphate esters. There is a need, as with many of the other micronutrients, to evaluate such biochemical indices in relation to functional indices of health, particularly in relation to 'marginal' rather than severely deficient intakes. In the absence of such information it is difficult to interpret the significance of biochemical values in the UK situation where extremely low intakes of vitamin are not usually encountered.

Correlations between indices of status and intake

Erythrocyte thiamin was not significantly correlated with ETK° or ETK-AC, contrary to the findings of Warnock *et al.* (1978), Baines & Davies (1988), Fidanza *et al.* (1989) and Takeuchi *et al.* (1989). It would appear that this lack of correlation is indicative of biological or analytical variability of either or both of these indices, since both total erythrocyte thiamin and ETK-AC are each essentially measurements of erythrocyte TPP. van Dokkum *et al.* (1990) observed a large variation in blood thiamin, urinary thiamin excretion and transketolase activity in subjects with fixed thiamin intakes. They suggested that single determinations of any of these indices were insufficient for an evaluation of thiamin status.

During thiamin depletion, changes in erythrocyte TPP concentration occur before alterations in transketolase activity (Warnock *et al.* 1978), thus total erythrocyte thiamin may be a more sensitive indicator of status than transketolase activity which would be maintained until intakes were excessively low. However, in the present study neither ETK°, ETK-AC or erythrocyte thiamin were significantly correlated with thiamin intake (either calculated or analysed values), confirming findings by Herbeth *et al.* (1985), Fidanza *et al.* (1989) and Gans & Harper (1991). This lack of correlation could be explained by large variability in status indices, particularly the analytical variability experienced in ETK results, in combination with possible differences in bioavailability from various food sources (van den Berg *et al.* 1994) and the relatively limited range of thiamin intakes measured in the present study. It is also possible that the intakes measured did not reflect adequately the subjects' habitual intake. Significant correlations have been found between ETK° and intake (Fidanza & Fidanza, 1974) but the population studied were of known low thiamin intake and at high risk of deficiency, in which case the relationship between intake and status might be expected to be more pronounced, whereas at higher intakes it is possible for status to have plateaued. It is clear that great care must be exercised when attempting to interpret thiamin intake or status measurements.

Conclusions

Our study indicates the current unreliability of food table values for the estimation of thiamin intake in individuals and population groups. Thiamin intakes, calculated or measured by direct HPLC analysis of foods, have not been found to correlate with ETK° or ETK-AC values or total erythrocyte thiamin concentrations in the adolescent group studied. It is shown that there is a need to re-evaluate biochemical indices of thiamin status and to determine the relationships between these and more sensitive functional indices of health, particularly in apparently healthy populations where marginal deficiency may be a problem.

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