

**Micronutrient intake and psychological performance of schoolchildren:
consideration of the value of calculated nutrient intakes for the assessment of
micronutrient status in children**

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Although the grosser clinical manifestations of micronutrient deficiency are now rare in Western society, biochemical and functional disturbances may occur before the onset of overt deficiency states. It has been argued that, since the vast majority of subjects with apparent biochemical abnormalities do not progress to classical, overt deficiency states, these abnormalities are relatively unimportant. However, such arguments take little account of evidence linking sub-optimal intakes of several micronutrients to increased susceptibility to chronic disease; nor do they allow for the potential positive benefits for well-being and improved quality of life which are likely to be associated with optimal body function.

Recently, there has been considerable speculation about the role of marginal micronutrient deficiency in relation to optimal cognitive function in children and adolescents following the publication of four studies reporting a significant improvement in non-verbal IQ after a period of micronutrient supplementation (Benton & Roberts, 1988; Benton & Buts, 1990; Schoenthaler *et al.* 1991*a,b*). The implication of these studies is that a proportion of the subjects were not receiving a sufficient dietary supply of one or more of the micronutrients provided by the supplement, and that the correction of these dietary deficiencies resulted in improved performance in tests of intelligence. There are numerous papers in the literature implicating vitamin and mineral deficiencies in abnormal brain development and learning ability and, although the mechanisms responsible for these impairments are poorly understood, there is no doubt that micronutrients are essential for our mental well-being (Southon, 1990). However, where studies relating diet to cognitive performance do not present adequate information on the diet and nutritional status of subjects, the development of hypotheses is limited.

In those studies where dietary assessment has been performed, conclusions about dietary adequacy are based on intake values calculated from tables of food composition. The present paper considers the value of food table data for estimating micronutrient intake and micronutrient status in young, growing individuals. It presents our own data from a group of 13–14-year-old volunteers, since a search of the literature revealed no study which combined dietary assessment, from both food table calculations and direct analysis of the diet, with a range of biochemical indices of micronutrient status. Dietary intake was measured using a 7 d weighed intake method and direct analysis of duplicate diets. Micronutrient status was assessed via a range of biochemical analyses performed on fasting blood samples.

SUBJECT DESCRIPTION, DIETARY ASSESSMENT, DIETARY ANALYSIS,
BLOOD SAMPLING AND BLOOD ANALYSIS

Full details of the subjects who took part in the study, methods of dietary assessment and blood sampling are presented elsewhere (Finglas *et al.* 1992). The study was approved by the Institute's Ethics Committee.

Sixty-one subjects (13–14 years) were recruited. Seven subjects withdrew during the course of the study leaving a total of fifty-four subjects, thirty-five girls and nineteen boys. Median heights and weights were similar to the 50th percentile values for this age-group calculated by the National Centre for Health Statistics (Thomas, 1988).

Each subject kept a written, weighed record of everything consumed every 6th day for 7 weeks. On each of these days the subjects also collected duplicate portions of all food and drink consumed. The diets were stored in a domestic freezer overnight and collected the next morning. In both written record and the duplicate diets, allowance was made for waste. Dietary records were coded using McCance & Widdowson's *The Composition of Foods* (Paul & Southgate, 1978), together with appropriate Supplements (Wiles *et al.* 1980; Tan *et al.* 1985; Holland *et al.* 1988, 1989). Nutrient intakes were calculated using the Institute's computerized nutrient database. Duplicate diets were homogenized on the morning of collection. A sub-sample was taken immediately after homogenization for vitamin C analysis and further sub-samples of the homogenate were stored at -40° for future analysis.

Fasting (12 h) venous blood samples were obtained during the period of dietary assessment. Blood fractions were prepared and either analysed immediately (plasma ascorbic acid) or frozen promptly at an appropriate temperature (-40° or -196°) for subsequent analysis.

A summary of analytical techniques used for diet and blood analysis is shown in Table 1.

TRACE MINERAL AND VITAMIN INTAKES: CALCULATED *v.*
ANALYSED VALUES

Many studies use food composition data for the assessment of nutrient intakes. Since the development of hypotheses relating variations in dietary intake to functional changes relies so heavily on 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 data is to compare values computed from tables of food composition with values obtained by direct analysis of the diets consumed. In the present study, nutrient intakes were assessed for each subject using both techniques. Average analysed and calculated intakes were compared using Student's paired *t* test. Regression analysis was performed to determine the degree of association between analysed and calculated data. Data were checked for normality of distribution before statistical treatment. Tables 2 and 3 show calculated and analysed daily intakes of energy and selected micronutrients for boys and girls and presents the correlation coefficients between calculated and analysed data.

Comparison of calculated and analysed energy intakes indicated that duplicate diet collections were a good reflection of 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 about 4%. There were several significant differences between

Table 1. Summary of analyses performed on duplicate diets and biochemical indices used to assess micronutrient status

Analysis	Technique	Reference
Diet		
Energy	BC	Miller & Payne (1959)
Iron	AA	} Fairweather-Tait & Wright (1990)
Zinc	AA	
Copper	AA	
Thiamin	HPLC	
Riboflavin	HPLC	Kwiatkowska <i>et al.</i> (1989)
Vitamin B ₆	HPLC	Brubacher <i>et al.</i> (1986)
Vitamin C	HPLC	Behrens & Madere (1987)
Folic acid	MA	Tamura (1990)
Blood		
Serum ferritin	KA	Boehringer-Mannheim
Plasma:		
Zn	AA	} Southon <i>et al.</i> (1989)
Cu	AA	
Folate	KA	
Vitamin B ₁₂	KA	
Ascorbate	HPLC	Behrens & Madere (1987)
Pyridoxal-5-phosphate	HPLC	Naoi & Ichinose (1988)
Erythrocyte:		
Transketolase (<i>EC</i> 2.2.1.1)	EA ⁺	Anderson & Nichol (1986)
Total thiamin	HPLC	Bailey & Finglas (1990)
Glutathione reductase (<i>EC</i> 1.6.4.2)	EA ⁺	Powers <i>et al.</i> (1983)

BC, bomb calorimetry; AA, atomic absorption; HPLC, high-performance liquid chromatography; MA, microbiological assay; KA, kit assay; EA, enzyme assay (+, basal and stimulated activity).

analysed and calculated values. For both boys and girls, folate intake calculated from tables of food composition underestimated actual intake (on the 7 d of recording) by between 43 and 47%. The relationship between calculated and analysed folate intake was poor, and was not significant for the boys. This is not unexpected since it is now known that under the conditions used for the microbiological assay of folate (used to construct most food table data) there was a lack of specificity, leading to variable underestimation of total folate in many foods (Phillips & Wright, 1983). Calculated thiamin intake was also significantly lower for both sexes than the analysed value, by between 23 and 27%, although the correlation between them was reasonable. Thus, food table data may lead to a substantial underestimation of the intake of both these micronutrients. On the other hand, calculated copper, riboflavin and vitamin B₆ intake for girls were significantly higher than the analysed value and a similar trend was observed for boys but differences just failed to reach significance at the $P < 0.05$ level. As with folate, there is some doubt about the specificity of microbiological methods used to construct food table vitamin B₆ data (Polansky *et al.* 1985). Even where average intakes assessed by food tables and direct analysis are similar, and there is a good correlation between the values, there may still be problems. Average calculated and analysed vitamin C intakes for the groups, for example, were very similar. However, when data were compared on an individual daily basis, calculated vitamin C intakes ranged between

Table 2. *Calculated and analysed average daily intakes of energy and selected micronutrients for 13–14-year-old boys†*

(Values are means with their standard errors for nineteen subjects with ranges in parentheses)

Nutrient	Calculated		Analysed		Correlation coefficient (r)‡	Statistical significance of r: P
	Mean	SE	Mean	SE		
Energy (MJ)	8.83* (5.76–12.22)	0.36	9.25 (6.96–12.72)	0.35	0.91	<0.001
Iron (mg)	14.9 (7.5–55.5)	2.4	13.2 (7.6–25.5)	1.0	0.84	<0.001
Zinc (mg)	9.3 (6.5–14.5)	0.6	9.4 (6.2–14.3)	0.5	0.89	<0.001
Copper (mg)	1.62 (0.95–2.45)	0.10	1.36 (0.78–3.41)	0.16	0.62	<0.005
Thiamin (mg)	1.51* (0.75–3.55)	0.16	1.95 (0.97–4.49)	0.19	0.59	<0.01
Riboflavin (mg)	1.97 (0.90–3.69)	0.18	1.80 (0.75–4.24)	0.18	0.76	<0.001
Vitamin B ₆ (mg)	1.25 (0.75–1.87)	0.07	1.04 (0.39–2.50)	0.11		
Vitamin C (mg)	121 (29–428)	22	121 (49–246)	13	0.78	<0.001
Folate (µg)	174*** (91–318)	14	332 (89–625)	33		

Calculated intake was significantly different from analysed intake: * $P < 0.05$, *** $P < 0.001$.

† For details, see Finglas *et al.* (1992).

‡ Correlation coefficient between calculated and analysed data.

0.6 and 9.5 times the value obtained by direct analysis and there was a statistical difference (using a paired *t* test) between analysed and calculated intakes for approximately one-third of the subjects when they were examined individually over the 7 d of dietary assessment (Finglas *et al.* 1992). This highlights the potential unreliability of calculated data used on an individual basis. Two exceptions to this were iron and zinc, since calculated and analysed intake values compared well on an average, a daily, a group and an individual basis. Having outlined just a few of the potential problems associated with the determination of micronutrient intake from food table data, we should now consider the value of nutrient intake data alone for the prediction of nutritional status.

THE RELATIONSHIP BETWEEN INTAKE AND STATUS FOR SELECTED MICRONUTRIENTS

The implication of recent studies reporting a significant improvement in non-verbal IQ following vitamin–mineral supplementation is that dietary deficiencies are hampering neural function in children. The question of which nutrient(s) supplied by the supplements may be involved in such a response, however, has not been addressed. In those studies presenting information on dietary intake, it is tempting to assume that where the average intake of a specific micronutrient was below the recommended level, the diet

Table 3. *Calculated and analysed average daily intakes of energy and selected micronutrients for 13–14-year-old girls†*

(Values are means with their standard errors for thirty-five subjects with ranges in parentheses)

Nutrient	Calculated		Analysed		Correlation coefficient (r)‡	Statistical significance of r: P
	Mean	SE	Mean	SE		
Energy (MJ)	7.23 (4.30–11.13)	0.26	7.36 (4.54–10.95)	0.24	0.94	<0.001
Iron (mg)	9.7 (5.7–16.4)	0.5	9.8 (5.1–16.7)	0.5	0.76	<0.001
Zinc (mg)	6.8 (1.1–14.1)	0.4	6.55 (4.0–9.2)	0.26	0.89	<0.001
Copper (mg)	1.2*** (0.8–2.2)	0.05	0.9 (0.6–1.4)	0.03	0.66	<0.001
Thiamin (mg)	1.1*** (0.6–1.8)	0.05	1.5 (0.6–2.7)	0.1	0.43	<0.01
Riboflavin (mg)	1.3* (0.4–2.6)	0.08	1.2 (0.4–2.3)	0.07	0.77	<0.001
Vitamin B ₆ (mg)	1.1* (0.7–1.6)	0.04	0.7 (0.4–1.4)	0.04		
Vitamin C (mg)	75 (19–182)	7	84 (43–153)	5	0.68	<0.001
Folate (µg)	145*** (61–278)	8	252 (96–510)	19	0.42	<0.05

Calculated intake was significantly different from analysed intake: * $P < 0.05$, *** $P < 0.001$.† For details, see Finglas *et al.* (1992).

‡ Correlation coefficient between calculated and analysed data.

may be classified as 'poor' with respect to that nutrient and, conversely, where intakes are above recommended levels that the diet is 'better' (Benton & Buts, 1990). Calculated and analysed intakes obtained in the present study were compared with reference nutrient intake (RNI) values (Fig. 1); RNI being defined as an amount of nutrient that is enough, or more than enough, for about 97% of the people in a group (Department of Health, 1991). In general, average intakes for both boys and girls were around, or well in excess of, the RNI value, apart from Fe and Zn intake in girls which were approximately 66 and 75% of the recommended level respectively. On the basis of dietary intake data alone, therefore, the diet of the group could be considered adequate with respect to these micronutrients. However, information on the dietary intake of several micronutrients is at best only a rough guide to the nutritional value of the diet, and in some instances may be no guide at all (Southon *et al.* 1988).

Statistical examination, including regression analysis, was performed on calculated intakes of specific micronutrients (Fe, Zn, Cu, thiamin, riboflavin, vitamin B₆, vitamin B₁₂, folate and vitamin C) and biochemical indices of status for each of these nutrients (as outlined in Table 1) for the nineteen boys and thirty-five girls who took part in the present study. Any data which was not normally distributed was log₁₀ transformed before regression analysis. The emphasis in the present paper is on calculated rather than analysed intake data in view of the large numbers of studies which rely entirely on values calculated from tables of food composition. Table 4 presents data on the percentage of

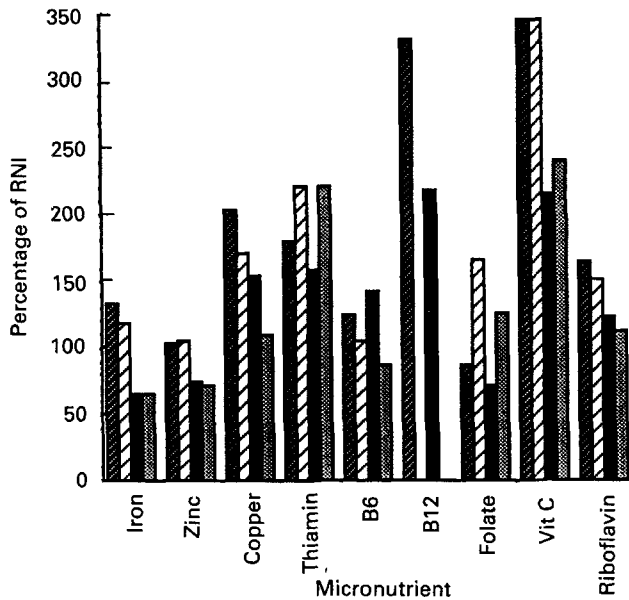


Fig. 1. Comparison of average, daily calculated and analysed intakes of selected micronutrients for a group of 13–14-year-old boys ((■), calculated; (▨), analysed; n 19) and girls ((■), calculated; (▩), analysed; n 35) with reference nutrient intake (RNI) values (Department of Health, 1991). For details of subjects and procedures, see Finglas *et al.* (1992).

subjects with low biochemical indices of status and the proportion of these subjects, and of all subjects, whose average daily calculated intake was below the RNI. Data relating to analysed intake values are also included in Table 4 for comparison. Reference limits and cut-off points for many biochemical indices are still a matter of debate, particularly for children (Gibson, 1990). The values shown in Table 4 do not necessarily indicate a deficiency state but are generally accepted as being at the lower end of the normal distribution. (Gibson, 1990). For several nutrients (Cu, thiamin, riboflavin (boys only), vitamin B₆ (girls only)) a substantial number of the subjects showed 'low' indices of status but none of them consumed less than the RNI on the basis of calculated intakes. Of the other nutrients examined, only with Fe was it observed that all the girls with a low index of status also consumed, on average, less than the RNI. However, the average daily calculated Fe intake of 97% of all the girls in the study was below the RNI, whilst only 21% of them had a low serum ferritin. Of the nine nutrients examined, a significant correlation between calculated intake and status was found only for vitamin C (r 0.63, $P < 0.001$, boys only) and riboflavin (r 0.33, $P < 0.05$, girls only).

It is recognized that some biochemical indices, although routinely used, are not particularly sensitive, and may be influenced by confounding factors unrelated to the dietary intake of the specific nutrient under investigation. However, even where the biochemical measurement performed is known to provide a very good indicator of status in an apparently healthy population, a relationship between total intake and status is not always found. A prime example of this is Fe. In most individuals the concentration of serum ferritin parallels the total amount of storage Fe and serum ferritin values of < 10 $\mu\text{g/l}$ for children up to 14 years are almost always indicative of a depletion of Fe stores.

Table 4. Percentage of 13–14-year-old boys (n 19) and girls (n 35)* with 'low' indices of status for selected micronutrients, and the percentage of subjects with average, daily, calculated and analysed intake below the reference nutrient intake (RNI) value (Department of Health, 1991)

Index	Value indicative† of 'low' status	Percentage of subjects with 'low' index (SWLI)		Percentage SWLI with calculated (analysed in parentheses) intake below RNI		Percentage of all subjects with calculated (analysed in parentheses) intake below RNI	
		Boys	Girls	Boys	Girls	Boys	Girls
Iron:							
Serum ferritin	<10 µg/l	11	21	50 (50)	100 (100)	42 (26)	97 (100)
Zinc:							
Plasma	<10.7 µmol/l	0	0	—	—	58 (53)	91 (97)
Copper:							
Plasma	<11 µmol/l	29	62	0 (0)	0 (52)	0 (0)	3 (46)
Thiamin:							
ETK-Ac*	>1.14	22	32	0 (0)	0 (0)	11 (0)	0 (0)
Riboflavin:							
EGR-Ac*	>1.20	37	31	0 (14)	36 (36)	16 (26)	49 (26)
Vitamin B ₆ :							
Plasma pyridoxal-5-phosphate	<50.8 nmol/l	50	27	11 (78)	0 (78)	5 (58)	0 (82)
Vitamin B ₁₂ :							
Plasma	<74 pmol/l	0	0	—	—	0	6
Folate:							
Plasma	<6.8 nmol/l	0	0	—	—	74 (17)	91 (38)
Vitamin C:							
Plasma ascorbate	<17 µmol/l	0	0	—	—	16 (0)	14 (0)

ETK-Ac, EGR-Ac, erythrocyte transketolase (*EC* 2.2.1.1) and glutathione reductase (*EC* 1.6.4.2) activity coefficients (Ac) respectively, where

$$\text{Ac} = \frac{\text{enzyme activity with added coenzyme}}{\text{enzyme activity without added coenzyme}}$$

* For details, see Finglas *et al.* (1992).

† See Gibson (1990).

Fe status, however, cannot be predicted by calculating the total intake of Fe because of the marked influence of food source and dietary mix on Fe absorption (Southon *et al.* 1988). Fe has been selected for special mention because of the growing number of studies reporting behavioural differences between Fe-deficient and non-deficient infants and schoolchildren (Hallberg, 1991). Fe, thiamin and Zn deficiency in humans have all been associated with reduced cognitive performance and are known to have important roles in neural function (Southon, 1990). Assessment of nutritional adequacy with respect to these nutrients is, therefore, particularly important in studies of the influence of micronutrient supplementation on mental development and intellectual performance. As indicated by the present study, however, measurement of total intake for these three nutrients does not necessarily provide a guide to status. The estimation of Zn status is notoriously difficult, but reasonable biochemical indices of Fe and thiamin status are

Table 5. Comparison of biochemical indices of status, measured at an initial time-point and 16 weeks later, for 13–14-year-old boys and girls†

(Values are means of differences between time-points with their standard errors)

Index	Boys			Girls		
	n	Mean	SE	n	Mean	SE
Height (mm)	9	3.4	3.3	20	4.1*	2
Weight (kg)	9	0.24	0.24	20	0.48	0.42
Serum ferritin (µg/l)	9	0.09	2.94	19	1.49	2.19
Plasma zinc (µmol/l)	6	-0.76	0.46	19	1.51	0.88
Plasma copper (µmol/l)	5	1.56	1.78	19	3.76*	0.53
Plasma folate (nmol/l)	8	0.66	4.36	19	0.09	2.05
Plasma vitamin B ₁₂ (pmol/l)	8	178	82	19	161**	41
Plasma ascorbate (µmol/l)	7	-11.6	6.5	19	-17.3***	4.2
Plasma pyridoxal-5-phosphate (nmol/l)	8	13*	5	17	9.6*	3.7
ETK-Ac	8	-0.13	0.07	18	0.04	0.02
Erythrocyte thiamin (nmol/l)	8	33.2	41.8	19	-30.6*	11.6
EGR-Ac	8	0.05	0.09	19	0.08	0.03

ETK-Ac, EGR-Ac, erythrocyte transketolase (EC 2.2.1.1) and glutathione reductase (EC 1.6.4.1) activity coefficient (Ac) respectively, where

$$\text{Ac} = \frac{\text{enzyme activity with added coenzyme}}{\text{enzyme activity without added coenzyme}}$$

Difference between time-points was significant: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For details, see Finglas *et al.* (1992).

available and should be included, along with as many other indices of micronutrient status as is practicable, in future investigations of diet–mental performance interactions.

MICRONUTRIENT STATUS: TIME DEPENDENCY

Table 5 shows values for a range of biochemical indices of micronutrient status, measured in a small number of subjects at an initial time-point and 16 weeks later. Blood samples at each time-point were taken after a 12 h fast and at a similar time in the morning to minimize diurnal variation. The subjects were not taking any dietary supplements and reported that there had been no major changes in their dietary habits. The effect of time was statistically examined by paired *t* test; differences between paired data were normally distributed.

There was very little difference between the two time-points in the values obtained for the boys; apart from an apparent improvement in pyridoxine status. For girls, however, there were significant differences for several biochemical indices. Cu, vitamin B₁₂ and vitamin B₆ status appeared to have improved, whilst vitamin C, riboflavin and thiamin status, as judged by erythrocyte thiamin concentration, appeared to have declined. These changes in the female subjects was accompanied by a small but significant increase in height.

Relationships between nutrient intake and nutritional status are not static but alter with dietary habits and physiological state. Young children and adolescents, in particular, experience several stages of rapid growth and development which may influence

their dietary requirements and micronutrient status over a relatively short period. The data presented in Table 5, although from only a small number of subjects, indicate that it is important in investigations of the functional significance of micronutrient supplementation to provide information on changes in status over the course of the study; not only for the test group but also for control and placebo groups. Measured improvements in the intellectual performance of children could then be assessed in relation to any changes in nutritional status, which may occur irrespective of the administration of dietary supplements.

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