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Dietary acid–base balance and intake of bone-related nutrients in Cambridge teenagers

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Objectives: To evaluate the diet of 16–18-y-old boys and girls with particular reference to intakes of nutrients believed to affect bone health and dietary acid–base balance.
Design: A 7-day food diary was completed between the months of October and December.
Setting: Cambridge, UK
Subjects: A total of 111 boys and 101 girls aged 16–18 y who were recruited into the Cambridge Bone Studies.
Main outcome measures: Mean daily intakes of foods and selected nutrients (protein, calcium, phosphorus, magnesium, potassium, vitamins C and K) were calculated. Two estimates of acid–base balance were calculated from the diet using the formulae of Remer (net acid excretion, estimated indirectly; NAE_{ind}) and Frassetto (protein/potassium ratio).
Results: Mean calcium and phosphorus intakes were above the UK Reference Nutrient intake (RNI). In all, 39% of the boys and 36% of the girls had vitamin K intakes lower than 1 μg/kg body weight/day. Calcium intake was positively correlated with all other nutrients except vitamins C and K. Boys had a significantly higher estimated net acid excretion (NAE_{ind}) than girls ($P<0.001$). Although a strong correlation ($r=0.76$, $P<0.001$) was found between the two methods, at higher acid levels a divergence was observed. A significant positive correlation was found between NAE_{ind} and the weight consumed per day of milk, cheese, meat and cereal foods and a negative correlation was found with the weight of potatoes and fruit. Diet composition is such that a lower NAE_{ind} is accompanied by a lower calcium intake.
Conclusions: The interpretation of the effects of calcium and other nutrients on bone cannot be considered in isolation from the other components of the diet. These results challenge some of the accepted perceptions about what constitutes an optimal diet for the promotion of bone health in adolescents.
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Introduction

Osteoporosis is a significant public health problem worldwide, which can result in fractures and increased morbidity and mortality in both men and women. The risk of developing osteoporosis in later life may be reduced by maximising peak bone mass (PBM) which, depending on the skeletal site, is believed to occur between late adolescence and adulthood in girls and boys (Bonjour *et al*, 1991; Anderson & Rondano, 1996; Nguyen *et al*, 2001) with over 90% of bone mineral accretion completed by 18-y of age in boys and 16 y of age in girls (Bonjour *et al*, 1994). While it is recognised that there is a strong genetic influence on PBM, there are several modifiable environmental factors of which the most important are weight-bearing activities (Bailey *et al*, 1999) and diet (Heaney *et al*, 2000; Eastell & Lambert, 2002).

Predominant emphasis has been placed on ensuring an adequate calcium intake and vitamin D status for mineralisation of the skeleton. However, there is increasing evidence supporting the direct and indirect effects of nutrients such as vitamin K (Knapen *et al*, 1989; Szulc *et al*, 1994; Luukinen *et al*, 2000), vitamin C (Gunnes & Lehmann,

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1995; Hall & Greendale, 1998; Simon & Hudes, 2001), and potassium (Tucker *et al*, 1999; New *et al*, 2000) on bone metabolism and calcium balance. Higher intakes of fruit and vegetables have been shown to be associated with higher bone mineral density (Tucker *et al*, 1999; New *et al*, 2000) and lower rates of bone loss in both men and women (Tucker *et al*, 1999). This may be due to the lower urinary calcium excretion in response to reduced acid load (Buclin *et al*, 2001). A higher dietary acid load is believed to result in bone mineral dissolution and increased bone resorption, resulting in the release of carbonate, citrate, together with calcium, sodium, potassium to buffer the acid load (Bushinsky, 2001). As a result of increased bone resorption and possibly impaired renal reabsorption, urinary calcium excretion is increased in response to dietary acid load (Barzel & Massey, 1998; Massey, 1998). The role of the skeleton in acid-base homeostasis has been discussed in detail by Barzel (1995) and New (2002).

The principal factors contributing to acid load include sulphur from the catabolism of sulphur amino acids (methionine and cysteine), which are highest in animal protein, nuts and cereals; phosphorus, which is mainly supplied by meat and dairy products and chloride. Determinants of alkali load include potassium, magnesium, sodium and calcium. Two methods for estimating dietary acid–base balance have been published and it is of interest to compare these methods. The protein/potassium ratio (Frassetto *et al*, 1998) gives an indication of the acid–base balance of the diet and has been shown to account for 71% of the variation in renal net acid excretion (Frassetto *et al*, 1998). However, this method only takes into consideration one component from each side of the acid–base equation. The method of Remer and Manz takes into account the range of nutrients previously described and it adjusts for nutrient absorption and body size (Manz *et al*, 1984). This method has also been validated against renal net acid excretion in adults and adolescents (Remer & Manz, 1994; Remer *et al*, 2003).

To achieve a greater awareness of diet composition in context of bone health of adolescents, the main objectives of this study were (1) to examine the contribution of major food groups to the intake of bone related nutrients in 16–18-y old girls and boys; (2) to compare two methods for determination of acid–base balance: an indirect estimate of net acid excretion, NAE_{ind} and the protein/potassium ratio (Remer & Manz, 1994; Frassetto *et al*, 1998); (3) and finally to investigate the associations between NAE and diet composition.

Methods

Subjects and data collection

The subjects were from the 150 male and 144 female students who volunteered to take part in the Cambridge Bone Studies (Prentice *et al*, 2002; Stear *et al*, 2003). The female students were recruited from two Cambridge sixth-form colleges. The male students were recruited from the two sixth-form colleges, a comprehensive school and two independent schools, one of which was boarding. Seven-day food diaries were completed by 70% of the girls ($n = 101$) and 77% of the boys ($n = 111$) in the overall data set; details of these subjects are shown in Table 1. Diaries were completed by the girls between September 1996 and March 1997 and by half the boys between September 1997 and July 1998 and the other half, between October 1998 and April 1999. Portion sizes were matched against food photographs and quantities were described in household measures. They were also asked some supplementary questions about their diet such as the type of milk they usually drank, the type of fat used for cooking or spreading, whether they ate meat, and the type of water they drank.

Coding and nutrient analysis

The diet records were coded using the in-house program, DIDO (Diet In Data Out) (Price *et al*, 1995) and nutrient analysis performed using the in-house suite of programs based on the nutrient data base of McCance and Widdowson edition 5 (Holland *et al*, 1991). The vitamin K_1 content of a wide range of foods was provided by Bolton-Smith from published data (Bolton-Smith *et al*, 2000) and unpublished data (C Bolton-Smith and MJ Shearer). Intakes of sodium were calculated from food and drinks only, there was no estimate of salt used in cooking or at table. Intakes of calcium included calcium from the water drunk where it had been recorded. This was coded as Cambridge tap water unless otherwise specified.

The nutrient analysis program allowed certain related items of foods to be grouped together in order to estimate their relative contribution to total nutrient intake. Further information on group composition is given at the bottom of Table 3. The percent nutrient intake (e.g. average calcium intake from milk and cream as a percentage of total calcium intake) for each food group was calculated for each subject. The average percentage contribution for boys and girls was then determined.

Table 1 Characteristics of the boys ($n = 111$) and girls ($n = 101$)

	Boys Mean (s.d.)	*Girls* Mean (s.d.)
Age (y)	16.6 (1.6)	17.4 (0.3)
Weight (kg)	67.7 (9.7)	56.6 (7.4)
Height (m)	1.78 (0.07)	1.64 (0.07)
BMI (kg/m^2)	21.4 (2.5)	20.9 (2.3)
Occupational social class % nonmanual[a]	96	93
Smoker[b] (%)	19	23
Lacto-ovovegetarian (%)	0	19

[a]Head of household according to the Registrar General's Standard Occupational Classification.
[b]≥ 1/day.

Acid–base balance

Net acid excretion (NAE_{ind}) of the subjects was estimated indirectly from the diet by the method of Remer and Manz (Remer *et al*, 2003):

$$NAE_{ind} = (S + P + EOA) - (K + Mg)$$

where S is Sulphur calculated from the dietary protein (g/day) * 0.49; P, phosphorus (mg/day) * 0.037; K, potassium (mg/day) * 0.021; Mg, magnesium, mg/day * 0.026.

The factors take into account average intestinal absorption and convert the cations and anions into milliequivalents (mEq).

$$\text{EOA} = \text{Endogenous organic acids which were calculated from the body surface area of eachsubject using the formula}: 41 * \text{body surface area } (m^2)/1.73(m^2)$$

(Manz *et al*, 1984)

Body surface area was calculated from height and weight according to the formula:

$$\text{Weight (kg)}^{0.425} * \text{Height (cm)}^{0.725} * 0.00718$$

(DuBois and DuBois,1916)

Sodium and chlorine were not included in the calculation as salt intake was not measured and these elements tend to balance each other. Calcium was also omitted as it has the smallest impact on acid–base balance (Remer *et al*, 2003). The subjects were grouped in fifths of NAE_{ind} (both sexes combined), in order to examine the other characteristics of their diet for each fifth.

In order to rank the foods eaten according to dietary acid load, the potential renal acid load (PRAL) was calculated for 17 food groups consumed by the boys and the girls. Having analysed the nutrient intake from each of the food groups, the calculation of PRAL was the same as NAE_{ind} but endogenous organic acids was omitted (Remer & Manz, 1995).

$$\text{PRAL} = (\text{sulphur} + \text{phosphorus}) - (\text{potassium} + \text{magnesium})$$

A separate estimate of acid–base balance was arrived at using the method of Frassetto *et al* (1998), which calculates the protein/potassium ratio of the diet expressed as g/mEq.

Data analysis

Data analysis was performed using SPSS for MS Windows 10. NAE_{ind} and most nutrient intakes were found to be normally distributed with the exception of vitamins C, D and K, which were transformed into natural logs before analysis to normalise the data. Analysis of variance and simultaneous multiple regression analysis was used to examine differences between groups. The food groups chosen to go into the regression model were those that have previously been shown to have an effect on acid–base load (Remer & Manz, 1995). As a linear relationship between variables could not be assumed, Spearman rank correlation coefficients were used to investigate the association between energy adjusted nutrient intakes and NAE_{ind}. Significant differences were taken as $P < 0.05$. In order to assess the likely validity of the reported energy intakes, the intake/basal metabolic rate (EI/BMR) was calculated (Goldberg *et al*, 1991) with a ratio of less than 1.1 taken to indicate low energy reporters. In all, 12% of the boys ($n = 13$) and 8% of the girls (n = 8) were found to be potential under-reporters. The mean NAE_{ind} was not significantly different with and without under-reporters and they were included in the statistical analysis.

Ethical approval

Written informed consent was obtained from both the subjects and their parents or guardians. Approval for the study was given by the Ethical Committee of the MRC Dunn Nutrition Unit (of which MRC Human Nutrition Research was formerly a part).

Results

Contribution of major food groups to the intake of bone-related nutrients

Table 2 shows the mean daily intake of protein, calcium, phosphorus, potassium, magnesium, and vitamins C, D and K and Table 3 shows the principal food sources. Average calcium intakes of both boys and girls were above the UK reference nutrient intake (RNI) (Department of Health, 1991) and the EU population reference nutrient (PRI) (Commission of the European Communities, 1993) with only 3% of the boys and girls having calcium intakes below the Lower Reference Nutrient Intake (LRNI). Predictably, the principal source of calcium in the diet was milk and milk products, which accounted for 42% of total intake in both the boys and girls. Cereal foods were also important contributors of calcium, 20% for the boys and 22% for the girls, due to the consumption of fortified breakfast cereals and bread. The contribution to calcium intake from water was 4.5% in boys and 6.7% in girls.

Average phosphorus intakes were higher than the RNI; only 3% of the boys and 5% of the girls had a calcium/phosphorus ratio ≥ 1. The principal sources of calcium also provided most of the phosphorus. In addition, meat and meat dishes further contributed 19% of total phosphorus in boys and 13% in girls. Potatoes contributed 7% of the phosphorus in boys and girls and beverages contributed 4.5% (boys) and 3.2% (girls).

Potassium and magnesium intakes of the boys were close to the UK RNI, but the mean intakes of the girls were below the RNI. Potatoes contributed around 25% of total potassium and 11–12% of total magnesium. The intake of sodium from foods, 4.0 g/day (boys) and 3.0 g/day (girls) was higher than the RNI (1.6 g/day) and the UK target for adult sodium intake of 2.4 g/day. (Scientific Advisory Committee on Nutrition, 2002).

Table 2 Mean daily intake of energy and nutrients of boys ($n = 111$) and girls ($n = 101$) and corresponding UK RNI values

	Boys		*Girls*	
	Mean (s.d.)	*RNI*	*Mean (s.d.)*	*RNI*
Energy (MJ)	11.7 (2.95)	11.51	9.1 (2.1)	8.83
Energy (kcal)	2790 (702)	2755	2156 (493)	2110
Protein (g/day)	93.7 (23.5)	55.2	67.6 (16.6)	45
Fat (g/day)	117.2 (34.4)		90.8 (26.9)	
Carbohydrate (g/day)	347.5 (91.6)		276 (64.6)	
Sodium (mg/day)[a]	4011 (105)	1600	3016 (78)	1600
Potassium (mg/day)	3455 (950)	3500	2675 (594)	3500
Magnesium (mg/day)	337 (97)	300	266 (63)	300
Calcium (mg/day)	1188 (442)	1000	974 (301)	800
Phosphorus (mg/day)	1591 (426)	775	1214 (298)	625
Vitamin C (mg/day)[b]	88 (53, 138)	40	82 (55, 130)	40
Vitamin D (μg/day)[b]	2.86 (1.8, 4.0)		2.25 (1.70, 3.1)	
Vitamin K (μg/day)[b]	71.5 (51.8, 99.8)		70.2 (48.6, 90.8)	
Calcium/phosphorus ratio	0.73 (0.14)		0.80 (0.13)	

[a]Sodium from food and drink only.
[b]Median and interquartile range. The intake of these nutrients was not normally distributed. RNI = Reference Nutrient Intake (Department of Health, 1991).

The intakes of vitamins were all positively skewed with some very high intakes, particularly of vitamin C, the median intake of which was double the RNI. Beverages were the highest contributors to vitamin C intakes (40%) followed by potatoes (20%) and fruit (13–14%).

Mean vitamin K intakes, expressed in relation to body weight, were close to the 'safe and adequate' recommendation of 1 μg/kg body weight/day (Department of Health, 1991), but 39% of the boys and 36% of the girls had intakes lower than this. The main source of vitamin K was green leafy vegetables, 24% (boys) and 27% (girls). Other vegetables, including green beans and peas contributed 13–14% of the vitamin K. Potatoes and potato products appeared to be a good source of vitamin K, but this was due to the fats and oils used in manufacture and cooking. Similarly, cakes and biscuits contributed 9–10% from the fats used in their manufacture.

There is no RNI for vitamin D for this age group in the UK; the intakes of this sample appear to be close to the average intake of dietary vitamin D by British adults, 3 μg/day (Department of Health, 1991) and that reported in the National Diet and Nutrition Survey of 3.3 μg/day (boys, 15–18 y) and 2.2 μg/day (girls,15–18 y) (Gregory *et al*, 2000).

Comparison of two estimates of acid–base balance

Table 4 shows the mean NAE_{ind} and protein/potassium ratio for the 110 boys and the 101 girls in the study. A wide range of values were found for both boys, NAE_{ind} = 33.4–114 mEq/day, and girls, NAE_{ind} = 21.8–93.2 mEq/day. Regardless of the method used, the mean dietary acid–base balance was significantly higher in the boys compared to the girls, $P<0.001$ for NAE_{ind} (67.8 mEq/day for boys *vs* 53.8 mEq/day for girls) and $P = 0.001$ for protein/potassium ratio (boys, 1.35 g/mEq/day for boys *vs* 1.25 g/mEq/day for girls). There was a significant correlation between the two methods of calculation of acid–base balance. However, at higher values of NAE_{ind} and protein/potassium ratio, there was less concordance between the two measures as shown in Figure 1. Examination of the subjects with $NAE_{ind} > 100$ (n = 8) compared to those with protein/potassium ratio > 1.38 (n = 8) showed a clear separation by body weight (mean of 75 kg compared to 58 kg). NAE_{ind} includes a factor (endogenous acid excretion) calculated from body weight and height, but when this body size factor was taken out of the calculation, the divergence still remained as the same individuals remained separated by their phosphorus intake, a mean of 2388 mg/day compared with 1307 mg/day. When the values for NAE_{ind} of all the subjects were divided into fifths, of those in the highest fifth of NAE_{ind} there was a very small group ($n = 5$) who were also in the lowest fifth of calcium intake. These had a lower consumption of milk and milk products and a higher consumption of meat and meat dishes than average, and a low consumption of fruit, but vegetable and potato intake were close to average.

Relationship between NAE and diet composition

Table 5 shows the contribution of the weights of foods eaten to the variance in NAE_{ind}. In boys and girls there was a significant positive correlation between NAE_{ind} and the weight of milk, cheese, meat and cereal foods consumed and a negative correlation with the weight of potatoes and fruit consumed. In girls, NAE_{ind} was also negatively correlated with vegetable consumption. In all, 58% of the variance in NAE_{ind} could be explained by this model in the boys and 51% in the girls.

Table 6 shows the food groups consumed ranked according to their potential renal acid load (PRAL). For both boys and girls, potatoes were the main contributor to dietary alkali load, while meat was the acidic food group most consumed

Table 3 Contribution of foods and food groups to percent intake of major bone-related nutrients in boys ($n=111$) and girls ($n=101$)

(a)	*Protein*		*Calcium*		*Phosphorus*	
	Boys Mean (s.d.)	*Girls Mean (s.d.)*	*Boys Mean (s.d.)*	*Girls Mean (s.d.)*	*Boys Mean (s.d.)*	*Girls Mean (s.d.)*
Rice and pasta	2.5 (2.0)	2.8 (2.2)	0.95 (0.94)	1.2 (1.6)	2.5 (2.4)	2.8 (2.3)
Bread	11.8 (4.9)	14.0 (5.6)	11.8 (5.5)	12 (5.3)	9.2 (4.4)	10.8 (4.7)
Breakfast cereals	3.4 (3.2)	2.6 (2.9)	1.5 (2.7)	0.82 (1.1)	4.6 (4.8)	3.3 (4.6)
Cakes, biscuits, puddings	4.9 (3.4)	7.9 (4.7)	7.2 (5.4)	9.3 (6.4)	6.6 (4.9)	10.2 (7.2)
Milk and cream	9.4 (6.4)	8.1 (6.1)	25.3 (15)	19 (13)	15.3 (9.7)	12.6 (9.0)
Cheese and yoghourt	7.9 (7.1)	12.6 (8.1)	16.8 (13)	23 (13)	9.9 (7.9)	14.1 (8.5)
Eggs and egg/cheese dishes	6.8 (6.1)	6.1 (6.4)	9.0 (9.8)	7.8 (9.0)	7.0 (6.4)	5.9 (6.2)
Spreading fats	0.3 (0.4)	0.3 (0.5)	0.23 (0.23)	0.19 (0.19)	0.32 (0.33)	0.33 (0.33)
Meat and meat dishes	33.0 (12.5)	24.2 (15.5)	4.9 (3.6)	3.3 (3.3)	18.7 (8.5)	13.5 (9.3)
Fish	4.7 (5.6)	3.5 (4.4)	1.1 (1.6)	0.78 (1.2)	3.2 (4.0)	2.3 (2.9)
Leafy vegetables	0.4 (0.4)	0.4 (0.4)	0.56 (0.72)	0.72 (0.71)	0.42 (0.47)	0.53 (0.49)
Other vegetables	3.1 (2.4)	4.6 (3.8)	2.7 (2.3)	2.9 (1.9)	3.8 (2.7)	5.0 (3.4)
Potatoes and potato products	5.4 (3.0)	5.5 (3.0)	2.0 (1.6)	1.7 (1.1)	6.5 (3.6)	6.8 (4.1)
Fruit and nuts	1.7 (2.5)	2.1 (2.0)	1.4 (1.8)	1.6 (1.6)	1.9 (2.5)	2.7 (2.3)
Sugars and confectionery	2.2 (2.1)	2.7 (1.9)	4.7 (4.7)	5.0 (3.6)	3.3 (3.1)	3.9 (2.7)
Beverages	1.5 (1.0)	1.2 (0.9)	8.1 (6.0)	9.3 (6.8)	4.5 (3.1)	3.3 (2.7)
of which water[a]			4.5 (5.5)	6.7 (7.0)		
Miscellaneous	1.5 (1.4)	1.4 (1.1)	1.7 (1.9)	1.6 (1.6)	2.2 (2.3)	2.1 (1.8)

(b)	*Magnesium*		*Potassium*		*Sodium*	
	Boys Mean (s.d.)	*Girls Mean (s.d.)*	*Boys Mean (s.d.)*	*Girls Mean (s.d.)*	*Boys Mean (s.d.)*	*Girls Mean (s.d.)*
Rice and pasta	3.3 (2.7)	3.1 (2.6)	1.1 (0.95)	1.5 (1.6)	1.2 (2.2)	1.9 (2.5)
Bread	12.5 (7.0)	14.3 (6.5)	5.2 (3.0)	5.8 (2.6)	17.6 (7.1)	19.2 (7.0)
Breakfast cereals	6.8 (7.4)	4.7 (6.1)	3.0 (3.5)	2.2 (3.1)	6.0 (6.6)	4.8 (5.2)
Cakes, biscuits, puddings	5.7 (4.8)	8.4 (5.2)	4.3 (3.1)	6.2 (3.8)	5.7 (4.3)	9.0 (6.7)
Milk and cream	9.5 (6.5)	8.0 (6.4)	11.7 (8.2)	9.3 (7.0)	4.0 (3.1)	3.3 (3.0)
Cheese and yoghourt	3.3 (2.9)	4.4 (2.8)	2.6 (2.8)	3.3 (3.1)	5.0 (4.6)	7.3 (4.9)
Eggs and egg/cheese dishes	3.5 (3.6)	2.8 (3.0)	3.0 (3.2)	2.6 (2.8)	7.7 (7.7)	6.5 (7.1)
Spreading fats	0.1 (0.14)	0.1 (0.13)	0.16 (0.26)	0.25 (0.66)	3.6 (2.4)	3.8 (2.2)
Meat and meat dishes	11.2 (5.6)	7.7 (5.7)	14 (6.3)	9.5 (6.8)	23.9 (11.5)	16.8 (12.0)
Fish	1.9 (2.3)	1.3 (1.6)	2.0 (2.6)	1.4 (1.9)	2.4 (3.8)	1.9 (2.9)
Leafy vegetables	0.44 (0.62)	0.54 (0.58)	1.0 (1.7)	1.2 (0.99)	0.2 (0.3)	0.4 (0.5)
Other vegetables	5.3 (3.7)	6.8 (4.6)	6.8 (3.9)	9.8 (6.3)	4.7 (5.3)	5.1 (4.9)
Potatoes and potato products	12.3 (6.4)	12.3 (6.5)	24.5 (11.1)	23.8 (11.8)	4.7 (4.0)	5.3 (4.4)
Fruit and nuts	5.0 (4.9)	6.7 (5.6)	5.8 (5.1)	7.7 (6.7)	0.5 (1.0)	0.6 (1.1)
Sugars and confectionery	4.6 (4.5)	5.5 (3.9)	2.9 (2.6)	3.6 (2.5)	1.0 (1.0)	1.2 (0.8)
Beverages	12.1 (7.1)	11.7 (5.8)	8.9 (5.6)	8.9 (6.0)	2.1 (1.5)	2.5 (1.5)
Miscellaneous	2.1 (20)	2.1 (1.6)	3.2 (2.9)	2.5 (2.0)	9.7 (7.1)	10.5 (8.2)

(c)	*Vitamin C*		*Vitamin D*		*Vitamin K*	
	Boys Mean (s.d.)	*Girls Mean (s.d.)*	*Boys Mean (s.d.)*	*Girls Mean (s.d.)*	*Boys Mean (s.d.)*	*Girls Mean (s.d.)*
Rice and pasta	0.13 (0.82)	0.44 (1.1)	0.3 (1.0)	0.46 (1.4)	1.3 (3.6)	3.2 (6.4)
Bread	0 (0)	0 (0)	0.53 (2.1)	0.3 (0.78)	3.3 (3.7)	2.6 (2.1)
Breakfast cereals	0.79 (2.6)	0.82 (2.8)	15 (17)	11 (15)	1.3 (2.2)	0.94 (2.1)
Cakes, biscuits, puddings	0.84 (1.3)	1.1 (1.4)	19 (17)	23 (17)	8.5 (8.1)	10 (7.2)
Milk and cream	3.6 (4.5)	2.2 (2.1)	2.9 (4.7)	2.3 (3.9)	1.5 (2.3)	1.1 (2.1)
Cheese and yoghourt	0.33 (0.56)	0.28 (0.47)	2.7 (3.2)	3.5 (3.0)	1.6 (1.9)	2.0 (2.1)
Eggs and egg/cheese dishes	1.4 (2.1)	1.3 (1.7)	15 (15)	10 (16)	4.6 (6.0)	4.1 (5.1)
Spreading fats	0 (0)	0 (0)	28 (20)	33 (20)	5.1 (5.2)	5.0 (6.9)
Meat and meat dishes	2.5 (3.2)	1.2 (1.7)	6.0 (7.5)	4.7 (6.6)	7.6 (6.5)	4.5 (5.6)
Fish	0 (0.59)	0 (0.14)	7.3 (15)	8.4 (16)	1.1 (2.4)	0.7 (1.6)
Leafy vegetables	5.4 (7.9)	5.1 (5.4)	0 (0.2)	0 (0.24)	24 (19.6)	27 (20)
of which cooked leafy veg.					16 (19)	17 (19)
salad					11 (12)	6.8 (8.4)
Other vegetables	9.5 (8.4)	14.7 (12.5)	0 (0)	0 (0.12)	13 (8.9)	14 (8.6)
of which green peas, beans					5.5 (5.5)	6.7 (7.0)
Potatoes and potato products	20.5 (16)	19 (16.3)	1.1 (2.8)	1.5 (3.4)	12 (9.7)	8.5 (6.5)
Fruit and nuts	13.5 (17)	12.3 (12.7)	0 (0)	0 (0)	2.4 (2.9)	3.8 (4.2)

Table 3 (Continued)

(c)	Vitamin C		Vitamin D		Vitamin K	
	Boys *Mean (s.d.)*	*Girls* *Mean (s.d.)*	*Boys* *Mean (s.d.)*	*Girls* *Mean (s.d.)*	*Boys* *Mean (s.d.)*	*Girls* *Mean (s.d.)*
Sugars and confectionery	0.5 (1.0)	0.56 (1.0)	0 (0)	0 (0.2)	3.0 (6.1)	1.8 (2.0)
Beverages	40 (28)	40 (27)	0 (0)	0 (0)	1.9 (4.1)	2.4 (4.5)
Miscellaneous	0.96 (1.8)	0.75 (1.1)	1.5 (2.0)	1.5 (2.4)	7.7 (6.9)	8.4 (9.8)

[a]Beverages included drinking water from the tap, which, in the Cambridge hard water area, is a significant source of calcium.
Milk includes drinks made up with milk, such as cocoa, drinking chocolate and milk shakes and milk added to tea/coffee.
Other vegetables include vegetable dishes.
Potatoes include all preparations of potatoes including chips and potato products such as crisps.
Fruit includes fresh, cooked, canned and dried.
Beverages include water, tea, coffee, fruit juices, fruit squashes, carbonated drinks, alcoholic drinks and powdered beverages without added milk/water.
Miscellaneous includes soups, sauces, spices and flavourings.

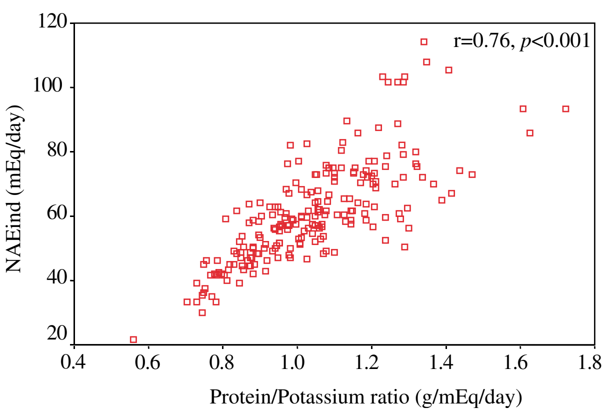

Figure 1 Comparison of estimates of acid–base balance using NAE_{ind} (Remer & Manz, 1994) and protein/potassium ratio (Frassetto *et al*, 1998).

by the boys and cheese and yoghourt were most consumed by the girls.

Nutrient inter-relationships

Table 7 shows the Spearman rank correlation coefficients between energy adjusted (nutrient/MJ) nutrient intakes and between NAE_{ind} and energy adjusted (nutrient/MJ) nutrient intakes for boys and girls together. Vitamin K intake was significantly positively correlated with intakes of potassium, magnesium and vitamin C and negatively correlated with NAE_{ind}. Calcium intake was significantly positively correlated with all nutrients and NAE_{ind} except for vitamin K and vitamin C. Sodium intake from foods was significantly positively correlated with NAE_{ind} as it was with protein and phosphorus (both of which were included in the calculation of NAE_{ind}).

Discussion

The results of this study should be considered in the context of the background of the students who volunteered to participate. They were all continuing to receive secondary education and the majority of their parents were in nonmanual occupations. Although extensive work has been carried out on the effects of calcium intake on bone mineral status in adolescents, few studies have included an evaluation of subject's usual diet. The results of this dietary assessment of 16–18-y-old adolescent boys and girls showed that, on average, their nutrient intakes met the current UK RNI. Calcium intake was significantly positively correlated with all nutrients and NAE_{ind} except for vitamin K and vitamin C. This multicollinearity is in agreement with the findings of Holbrook & Barrett-Connor (1991) in 900 men and women aged 50–79 y and indicates that the effects of dietary calcium on bone health cannot be considered in isolation.

Phosphorus intakes were higher than UK RNI, resulting in a mean calcium/phosphorus ratio lower than 1. The implications of this for bone metabolism and mineral accretion in adolescents are currently unknown. It has been suggested that a ratio of more than 1 may be associated with greater bone mineral content in young women (Teegarden *et al*, 1998) and a lower phosphorus intake would reduce $NAE_{ind.}$ However, increasing the ratio would be difficult, as the principal sources of calcium were also rich sources of phosphorus. Interestingly, less than 1% of total phosphorus intake came from soda type beverages, while 12–15% came from the milk (and cream), which also provided 19–25% of calcium intake. This contrasts with the finding that the changing food habits of teenagers in the US involve substitution of milk by phosphorus rich beverages (Calvo, 1993). However, due the high consumption of fruit juices and fortified fruit squashes by the Cambridge teenagers the greatest contribution to vitamin C intake came from beverages rather than the 'traditional' sources of fruits and vegetables.

Mean vitamin K intakes of the Cambridge subjects were close to 1 μg/kg/day and were comparable to intakes reported from other age groups in the UK (Price *et al*, 1996; Thane *et al*, 2002) and in the US for various age groups (Booth & Suttie, 1998). There is no current UK RNI for vitamin K but, based on requirements for hepatic synthesis of the vitamin Kdependent coagulation factors, the safe and adequate intake is 1 μg/kg body weight/day (Department of Health, 1991). More recently, the US has set an adequate intake of 125 and 90 μg/day for men and women, respectively (Institute of Medicine, 2001), to take into account the higher requirement for carboxylation of bone osteocalcin. Various studies suggest that current intakes of vitamin K in the US (Feskanich *et al*, 1999; Binkley *et al*, 2000; McKeown *et al*, 2002) and the Netherlands (Vermeer *et al*, 1995) are associated with a high proportion of under-carboxylated osteocalcin. However, it is not known whether adolescent vitamin K requirements are also higher since bone turnover is approximately 10–20 times that found in healthy adults (Szulc *et al*, 2000). Despite the fact that the Cambridge sample was an elite group in socioeconomic terms, their consumption of leafy green vegetables was very low. Low intakes of vitamin K were associated with low intakes of potassium, magnesium and vitamin C, and thus increased NAE_{ind}. At least 20% of the vitamin K intake came from fats in cakes and biscuits and, particularly in the boys' diet, potato products such as chips and crisps. It is generally recommended that consumption of these types of foods, which are generally also high in salt, should be limited as they can contribute to a high total energy intake and obesity but this might also reduce vitamin K intakes. There is also limited and conflicting evidence that the vitamin K from fats

Table 4 Mean estimates of dietary acid–base balance of boys ($n=111$) and girls ($n=101$)

	Boys *Mean (s.d.)*	*Girls* *Mean (s.d.)*	P
NAE_{ind} (mEq/day)	67.8 (15.7)	53.8 (11.6)	<0.001
Protein/potassium ratio (g/mEq/day)	1.35 (0.022)	1.24 (0.022)	<0.001

NAE_{ind} = Net Acid Excretion estimated indirectly (Remer & Manz, 1994).

Table 5 Multiple regression analysis of NAE_{ind} against weights of foods eaten

	Boys (n = 111)			*Girls (n = 101)*		
Foods or food group	β	*s.e.*	P	β	*s.e.*	P
Cereal foods	0.043	0.009	<0.001	0.059	0.009	<0.001
Liquid milk and cream	0.025	0.005	<0.001	0.016	0.006	0.011
Cheese and yoghourt	0.091	0.021	<0.001	0.081	0.019	<0.001
Meat and meat dishes	0.051	0.011	<0.001	0.057	0.014	<0.001
Potatoes	−0.082	0.012	<0.001	−0.044	0.015	0.004
Other vegetables	−0.043	0.017	0.013	−0.050	0.015	0.001
Fruit	−0.034	0.013	0.008	−0.054	0.011	<0.001

β = unstandardised coefficient; P = independent significance.
Adjusted R^2 = 0.58 (boys); 0.51 (girls). For further definition of food groups, see Table 3.

Table 6 PRAL (Mean, s.d.) of each food group and ranking according to intake for boys and girls

	Boys (n = 111)		*Girls (n = 101)*
	PRAL (mEq/day)		*PRAL (mEq/day)*
Food group	*Mean (s.d.)*	*Food group*	*Mean (s.d.)*
Potatoes	−12.77 (7.44)	Potatoes	−9.26 (5.15)
Beverages	−4.27 (3.72)	Beverages	−4.00 (3.61)
Fruit and nuts	−2.86 (3.05)	Fruit and nuts	−3.07 (3.57)
Other vegetables	−1.66 (1.50)	Other vegetables	−2.22 (1.65)
Miscellaneous	−0.45 (1.42)	Leafy vegetables	−0.34 (0.36)
Leafy vegetables	−0.29 (0.33)	Miscellaneous	−0.16 (0.80)
Fats	0.17 (0.27)	Fats	0.09 (0.24)
Sugar and confectionery	0.36 (0.70)	Sugar and confectionery	0.28 (0.58)
Breakfast cereals	1.54 (1.68)	Breakfast cereals	0.85 (1.00)
Rice and pasta	1.55 (1.28)	Rice and pasta	1.06 (0.94)
Fish	2.30 (2.63)	Fish	1.32 (1.66)
Cake, biscuits, puddings	2.51 (2.09)	Milk and cream	2.79 (2.32)
Milk and cream	4.69 (3.76)	Egg/egg & cheese dishes	3.00 (3.31)
Egg/egg & cheese dishes	4.73 (4.09)	Cakes, biscuits, puddings	3.10 (2.95)
Bread	6.15 (3.01)	Bread	5.11 (2.28)
Cheese and yoghourt	7.54 (7.59)	Meat and meat dishes	8.41 (6.16)
Meat and meat dishes	15.10 (7.21)	Cheese and yoghourt	8.54 (6.40)

For further definition of food groups, see Table 3.

Table 7 Spearman rank correlation between nutrients[a] and NAE_{ind} for boys and girls combined

		Ca (mg/MJ)	*P (mg/MJ)*	*K (mg/MJ)*	*Mg (mg/MJ)*	*Vit C (mg/MJ)*	*Vit D (µg /MJ)*	*Vit K (µg /MJ)*	*Prot. (g/MJ)*	*Na (mg/MJ)*	NAE_{ind}
Calcium (mg/MJ)	r_s	1.00	**0.76**	**0.23**	**0.51**	0.06	**0.19**	0.08	**0.30**	**0.19**	**0.25**
	P		<0.001	<0.001	<0.001	0.37	0.005	0.25	<0.001	0.01	<0.001
Phosphorus (mg/MJ)	r_s		1.00	**0.42**	**0.65**	0.01	0.09	0.13	**0.64**	**0.28**	**0.39**
	P			<0.001	<0.001	0.90	0.20	0.06	<0.001	<0.001	<0.001
Potassium (mg/MJ)	r_s			1.00	**0.61**	**0.26**	−0.10	**0.29**	**0.29**	0.06	**-0.39**
	P				<0.001	<0.001	0.14	<0.001	<0.001	0.38	<0.001
Magnesium (mg/MJ)	r_s				1.00	**0.16**	0.04	**0.27**	**0.33**	0.13	-0.10
	P					0.02	0.57	<0.001	<0.001	0.07	0.15
Vitamin C (mg/MJ)	r_s					1.00	−0.02	**0.24**	-0.05	**-0.17**	**-0.28**
	P						0.77	<0.001	0.50	0.01	<0.001
Vitamin D (µg /MJ)	r_s						1.00	**0.19**	-0.08	0.12	0.08
	P							0.005	0.28	0.09	0.23
Vitamin K (µg /MJ)	r_s							1.00	0.03	0.04	**-0.21**
	P								0.66	0.56	0.002
Protein (g/MJ)	r_s								1.00	**0.35**	**0.52**
	P									<0.001	<0.001
Sodium (mg/MJ)	r_s									1.00	**0.25**
	P										<0.001
NAE_{ind} (mEq)	r_s										1.00
	P										

r_s = Spearman's rank correlation coefficient (rho). r_s values in bold are significant; $P < 0.05$.
P = two-tailed significance.
NAE_{ind} = Net Acid Excretion estimated indirectly (Remer & Manz, 1994).
[a]All nutrients are expressed relative to total energy consumed and vitamins C,D,K are log transformed.

is better absorbed than that from vegetables (Gijsbers *et al*, 1996; Booth *et al*, 1999).

A wide range of acid–base balance values were found using both the Remer and Frassetto methods, that is, NAE_{ind} and protein/potassium ratio, respectively. These results have shown that the Frassetto method may underestimate the acid load of those with larger body size and high phosphorus intakes. The lowest NAE_{ind} was found in girls with a very high fruit and vegetable consumption and little or no meat consumption. However, there were others with low NAE_{ind} who only consumed chips, baked beans, crisps, chocolate, peanuts and lager, all contributing to a high intake of potassium and thus a lower acid load. Examination of these diets indicates that a low acid load is not necessarily associated with current concepts of a healthy diet (a diet low in fat and providing 5 servings of fruit and vegetables). In this sample, NAE_{ind} was positively related to sodium intake from foods. Higher sodium intake has been shown to increase urinary calcium excretion in adults and children (Shortt & Flynn, 1990; Matkovic *et al*, 1995); therefore, it is possible that the combined effects of a high salt intake, a low calcium intake and a high NAE_{ind} may compromise optimal bone mineral accretion.

NAE_{ind} was positively related to phosphorus intake, as would be expected, and nearly half of the phosphorus was derived from meat and dairy products (two subjects with high NAE_{ind} ate no meat but consumed very large quantities of cheese). Since dairy products were also the main source of calcium, a reduction in these foods might be considered to be counter-productive (although it is not known whether a more alkali diet reduces calcium requirement). Care may need to be taken in interpreting the apparent benefits of a low NAE_{ind} for bone health, in that subjects with a low NAE_{ind} generally had little or no meat intake and those with a high NAE_{ind} had a high consumption of milk products. Interpretation of the effects of NAE_{ind} on bone should be coupled with an evaluation of overall diet and nutrient intake.

In the wider population of teenagers and adults, those following a weight loss regime that advocated high protein, low carbohydrate diets (Reddy *et al*, 2002) could be at increased risk of incurring a negative effect on bone mineral, particularly since such diets often do not include potatoes that are an important factor for lowering NAE_{ind}.

Although most of the CABS subjects were living at home, at this age adolescents are reaching a stage of some independence in relation to diet. The diets of these teenagers were characteristic of the modern Western diet being high in protein, fat, sodium and phosphorus. Average calcium intakes were above the UK RNI, but there were several subjects whose diet was characterised by a low calcium and a high sodium content combined with a high acid load. More research is needed to determine whether such a dietary profile compromises achievement of optimal peak bone mass. The findings of this study indicate that it is important to consider overall diet quality when interpreting the effects of single nutrients or NAE_{ind} on bone. Further analysis on the effects of nutrient intake and dietary patterns on bone mineral status will shed light on the relative importance of these findings.

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