Vitamin C intake and plasma ascorbic acid concentration in adolescents

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(*Received 8 July 1991 – Accepted 8 May 1992*)

The relationship between vitamin C intake and status was investigated in a group of adolescents (13–14 years old). Dietary intakes were assessed using a 7 d weighed dietary record method, coupled with the collection of duplicate diets. Vitamin C intakes calculated using food composition tables were compared with values obtained by direct analysis of duplicate diets. Vitamin C status was judged via measurement of plasma ascorbic acid (AA) concentration in blood samples taken after a 12–15 h fast. The relationship between calculated and analysed vitamin C intake and plasma AA concentration was examined. Average daily calculated vitamin C intakes, for the group (*n* 54) as a whole over a 7 d period, gave a good estimate of intake, as judged by prompt analysis of duplicate diets. However, analysed *v.* calculated intakes were significantly different for approximately one-third of subjects when data were examined on an individual basis. Large discrepancies between analysed and calculated values could not be accounted for on a food group basis. In all but two individuals, calculated vitamin C intake was in excess of the new reference nutrient intake (RNI, part of the new daily reference values (Department of Health and Social Security, 1991)) of 40 mg and all plasma AA concentrations were well above those used to indicate even a moderate risk of deficiency. A relationship between vitamin C intake and plasma AA was observed for both males (*n* 19) and females (*n* 35). However, the relationship was much stronger for males who showed a wider range of both intake and plasma AA values.

Vitamin C: Plasma ascorbic acid: Adolescents

Vitamin C has long been known as an essential nutrient to man. In addition to the more well-established biochemical roles for the vitamin (Bates, 1981), recently ascorbic acid (AA) has also been implicated in the immune response, allergic reactions and leucocyte function (Sauberlich, 1981), and the daily intake required to maintain optimal health remains a matter of controversy. Definition of the relationship between diet and health is an extremely complex area requiring detailed and accurate information on dietary intake and an understanding of the relationship between intake and biochemical/physiological processes which may influence the well-being of the individual. Concern for the role that vitamin C may play in degenerative diseases of aging (Frei *et al.* 1988) has stimulated the study of vitamin C intake and status, and the relationship between them, in the elderly (Jacob *et al.* 1988; Vanderjagt *et al.* 1987, 1989), but information for younger individuals is more limited, although it seems reasonable to assume that early nutrition may affect morbidity in later life.

As part of a larger study of micronutrient intake and status in adolescents, the relationship between vitamin C intake and plasma AA, as an indicator of status, was investigated. Studies of this kind rely heavily on the accuracy of dietary information obtained and the subsequent calculation of specific nutrient intake, hence detailed

consideration was given to the assessment of vitamin C intake for the group of subjects and for individuals within the group. Vitamin C intakes from 7 d weighed dietary records were calculated from food composition tables and compared with values obtained by direct analysis of duplicate diets. Since many studies rely upon food table data, direct analysis being impossible or impractical, calculated *v.* analysed vitamin C intake values were examined on a daily food group basis, as well as a combined 7 d basis, in an attempt to identify the causes of major discrepancies.

MATERIALS AND METHODS

Subjects

The study was subjected to ethical approval by the Institute Ethics Committee before recruitment.

Subjects, 13–14 years, were recruited from two local authority schools in the provincial city of Norwich, Norfolk. Sixty-one subjects were recruited. Seven subjects withdrew during the course of the study leaving a total of fifty-four subjects, thirty-five girls and nineteen boys. The median (range in parentheses) age, height and weight of the girls and boys respectively was; age 13·8 (13·3–14·3), 13·6 (13·2–14·3) years; height 1·58 (1·50–1·74), 1·62 (1·42–1·87) 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 50th 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 6th day for 7 weeks (Black *et al.* 1984) between 1 November and 21 December. On each of these days the subjects also collected duplicate portions of all food and drink consumed.

A set of Salter scales (model no. 2001; Salter Homewares Ltd, Tonbridge, Kent; maximum capacity 2 kg and accurate to 2 g), a supply of intake record sheets, a small notebook and acid-washed plastic food/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. 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 foods consumed outside the home they were asked to describe the food/meal as fully as possible. Duplicate items were then purchased by the fieldworker and added to the day's collection. Catering staff at each school put aside duplicate portions for those subjects who had school meals. These were collected and weighed shortly after the end of their dinner break and the appropriate recipes were 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 they were collected early the following day, along with the dietary record sheets. The information was checked in the presence of the subject to ensure that all the necessary detail had been provided.

The completed duplicate diets were homogenized immediately after collection using a stainless-steel blender (R. W. Jennings Ltd, Nottingham, Notts). Distiller water was added to aid homogenization if necessary. The weight of water added and the weight of the homogenized diet was recorded. Sub-samples (100 g) were taken immediately for vitamin C (total ascorbate) analysis. In preliminary trials homogenized mixed diet samples, stored at −18° for 6 d, showed only 5–10% losses of total vitamin C when compared with values obtained immediately after preparation. This indicated that the sampling scheme used in the study gave realistic measurements of dietary vitamin C content.

Blood collection and preparation

Fasting (12 h) venous blood samples were obtained between 9 November and 7 December during the period of dietary assessment but not on or immediately following a day of dietary recording. Fresh (within 1 h of collection) whole heparinized blood (1·5 ml) was centrifuged at 8000 ***g*** for 4 min. The plasma was removed and stored on ice for approximately 10 min before analysis.

Vitamin C analysis

The method used was essentially that of Behrens & Madere (1987). All steps were performed under non-u.v. lighting (Gold Fluorescent; Thorn, London).

AA and dehydroascorbic acid were extracted from duplicate portions (20 g) of freshly prepared diets by homogenizing samples for 2 min in ice-cold metaphosphoric acid solution (10 ml, 85 g/l distilled water, made up fresh daily). Homogenates were made up to 100 ml with distilled water, mixed well and placed on ice. Samples (10 ml) were centrifuged at 1500 ***g*** for 10 min at 4°, filtered (Whatman no. 541), the first 1 ml or so of supernatant fraction being discarded and the remaining filtrate kept for HPLC analysis.

AA standard solutions, 0–40 μg/ml in metaphosphoric acid (8·5 g/l), were prepared freshly on each day of analysis and stored on ice before use. Portions (1 ml) of sample filtrates and standard solutions were diluted with 300 μl freshly prepared DL-homocysteine solution, 10 g/l in dipotassium phosphate buffer (2·58 M-K_2HPO_4 in distilled water, adjusted to pH 9·8 using concentrated KOH), raising the pH to 7·7 (Hughes, 1956). Samples were mixed well and left at room temperature to allow the reduction of dehydroascorbic acid to AA. After 30 min 3·7 ml metaphosphoric acid (8·5 g/l) was added to stop reduction and stabilize the ascorbic acid for the HPLC determination of total vitamin C.

Portions (300 μl) of fresh ice-cold plasma were added to equal volumes of ice-cold metaphosphoric acid (17 g/l in distilled water), mixed and centrifuged at 8800 ***g*** for 4 min. Duplicate portions (100 μl) of sample supernatant fractions and standard ascorbic acid solutions (freshly prepared, 0–10 μg/ml) were pipetted into separate amber vials together with 30 μl dipotassium phosphate buffer (2·58 M, pH 9·8) and allowed to stand at room temperature for 30 min before dilution with 370 μl metaphosphoric acid (8·5 g/l) and subsequent HPLC analysis for AA concentration.

AA in the diet and plasma samples was determined using the same HPLC system. A Shimadzu SIL-9A auto-injector (Shimadzu Corporation, Kyoto, Japan) was used to inject sample and standard solutions (20 μl) onto a 250 mm 4·6 mm i.d. Apex ODS analytical column (5 μm) (Hichrom Ltd, Reading, Berks) protected by a ODS guard column (40 μm) (Anachem Ltd, Luton, Beds). The mobile phase was prepared by mixing a sodium acetate solution (80 mM sodium acetate adjusted to pH 4·6 followed by addition of 0·150 g metaphosphoric acid and 0·129 g n-octylamine/l) and methanol (90:10, v/v). The mobile phase was pumped at a flow rate of 1 ml/min at ambient temperature using a SA3410 solvent delivery system (Severn Analytical, Luton, Beds). AA was detected electro-

chemically using an ampterometric detector (Biotech Instruments, Luton, Beds) fitted with a glassy carbon and Ag/AgCl reference electrode set at a potential of +0·6 V and range 500 nA. Data were collected using a PU4850 CCC Data Station (Pye Unicam, Cambridge, Cambs) and concentrations of AA calculated by comparison of peak areas. Detector responses to calibration standards were linear in the range 0–50 μg AA/ml. Total ascorbate contents of diets were expressed as μg/100 g homogenate and vitamin C intakes calculated as mg/d for each subject; a total of 378 duplicate diets were analysed. AA in plasma was calculated as μmol/l; a total of fifty-four plasma samples were analysed. Recoveries of added AA or dehydroascorbic acid from diet samples were 104·4 (102·4–106·9) % (*n* 7) and 101·7 (94·5–112·1) % (*n* 6) respectively, and the recovery of added AA from plasma was 100·1 (97·1–103·9) % (*n* 7). Intra-assay repeatabilities between duplicate extractions, expressed as mean percentage errors, were 0·95 ± 0·67 % (*n* 10) for total vitamin C in diet samples and 1·12 ± 0·54 % (*n* 10) for plasma AA.

Calculation of vitamin C and energy intake

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 values (J. M. Loughridge and A. Walker, AFRC Institute of Food Research) consisting of data collected from previous dietary intake studies on portion sizes, standard weight of branded foods and composition of composite dishes. Missing food quantities were obtained using *Food Portion Sizes* (Crawley, 1988). Total daily vitamin C intakes (mg/d) and energy intakes (kJ/d) were calculated using the Institute of Food Research food composition database. Average intakes of vitamin C were also expressed according to food group by means of a spreadsheet computer program (Excel, version 2.1, 1988; Microsoft Corporation, Readmond, USA).

Energy analysis

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

Statistical analysis

Before statistical comparison by unpaired *t* test or regression analysis, both analysed and calculated vitamin C intakes were $\log_{10}$-transformed to normalize distribution. Vitamin C intake data were not transformed for paired *t* tests since the differences between paired data were normally distributed.

Statistical comparisons were performed using Student's paired or unpaired *t* test, as appropriate. The statistical analysis used is indicated in each table of results. The relationship between analysed and calculated vitamin C intake ($\log_{10}$), and between vitamin C intake ($\log_{10}$) and plasma AA was established using regression analysis.

RESULTS

Vitamin C and energy intake

Energy intake (from direct analysis of duplicate diets) and vitamin C intake (both calculated and analysed) for female and male subjects are shown in Table 1. The mean analysed energy intake for males was 26 % higher ($P < 0·001$) than that for females, whilst

Table 1. *Energy intake (analysed†), fasting plasma ascorbic acid concentration (PAA) and vitamin C intake (analysed† and calculated‡) for thirty-five female and nineteen male adolescents (13–14 years)*

(Values are means with their standard errors)

		♀				♂			
		Mean	SEM	GM	Range	Mean	SEM	GM	Range
Energy (kJ/d)	Analysed	7364***	222		4548–10 950	9245	354	69	64–12 719
PAA (μmol/l)	—	77	4		37–143	81	9		39–207
Vitamin C intake (log_{10} mg/d)§: (all subjects):	Analysed	1·902*	0·025	80	43–153	2·038	0·047	109	49–246
	Calculated	1·811*	0·041	65	19–182	1·979	0·072	95	24–428
Excluding subjects who took supplements‖	Analysed	1·898*	0·026	79	43–153	2·000	0·044	100	49–211
	Calculated	1·810	0·042	65	19–182	1·912	0·061	82	24–171

GM, geometric mean.

Mean values were significantly different from those for male subjects (unpaired *t* test): * $P < 0·05$, *** $P < 0·001$.

† Obtained by direct analysis of duplicate diets.

‡ Obtained using food composition tables.

§ There was no significant difference between analysed and calculated values within sexes (paired *t* test, untransformed data; see p. 566).

‖ One female and two males who took routine supplements excluded.

the geometric means of analysed and calculated vitamin C intakes were 36 and 46% higher ($P < 0·05$) respectively in the boys; 9 and 16% higher when expressed in terms of energy intake.

Calculated and analysed vitamin C intake values for male and female subjects were not significantly different (Table 1). Mean values (with their standard errors) for analysed and calculated intakes were 84·2 (SE 4·7) and 74 (SE 6·9) mg/d respectively for girls, and 121·4 (SE 13·3) and 121·2 (SE 21·9) mg/d respectively for boys. Vitamin C intake data in Table 1 are log_{10}-transformed to normalize distributions and, therefore, geometric means are presented. There was a greater range of intake in males which was largely due to one subject who routinely took a vitamin C supplement which contributed 357 mg/d (according to product information) over and above dietary sources. Two other subjects, one male and one female, also supplemented their diet with vitamin C; the supplements providing (according to product information) an average of 12·8 and 21·4 mg vitamin C/d respectively. These supplements were included in duplicate diet collections. Intake values which exclude subjects taking supplemental sources of vitamin C are also included in Table 1.

The correlation between mean 7 d analysed and calculated vitamin C intakes for boys was r 0·75 ($P < 0·001$; analysed vitamin C (log_{10}) = 1·07 + 0·490 calculated vitamin C (log_{10})) and for girls was r 0·72 ($P < 0·001$; analysed vitamin C (log_{10}) = 1·11 + 0·435 calculated vitamin C (log_{10})). These regressions were statistically the same for both sexes and could be expressed as a common regression r 0·76 ($P < 0·001$; analysed vitamin C (log_{10}) = 1·03 + 0·490 calculated vitamin C (log_{10}); Fig. 1). However, the association between analysed and calculated values was reduced when data were compared on a daily basis for the total recording period (r 0·41 and 0·58 for girls and boys respectively) and

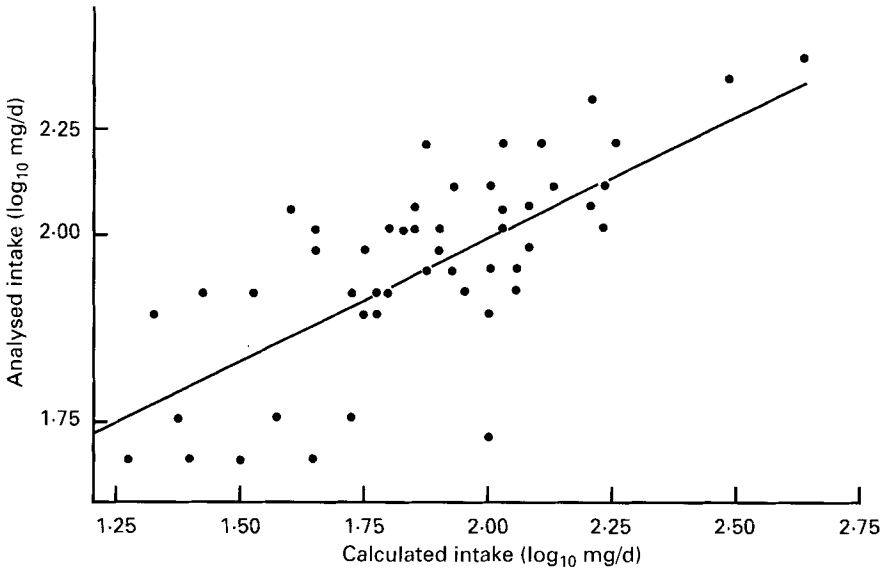

Fig. 1. Correlation between mean 7 d analysed and calculated vitamin C intake for fifty-four adolescents (13–14 years); $r = 0{\cdot}76$ ($P < 0{\cdot}001$): Data were $\log_{10}$ transformed to normalize distribution. Mean analysed vitamin C ($\log_{10}$) = $1{\cdot}03 + 0{\cdot}490$ mean calculated vitamin C ($\log_{10}$). For details of subjects and procedures, see pp. 564–566.

Table 2. *Comparison of analysed (A) and calculated (C) vitamin C intake values (within subject) over 7 d for fifty-four subjects (nineteen male, thirty-five female) and a comparison of analysed and calculated intake on each separate day (between subject)*

(Comparison of untransformed intakes by Student's paired *t* test)

	♀	♂
Within subjects†		
A = C	22	13
A < C	4	1
A > C	9	5
Between subjects‡		
A = C	6	7
A < C	0	0
A > C	1	0

* For details of subjects and procedures, see pp. 564–566.

† Values are the no. of subjects where A is equal to, less than or greater than C for 7 d of dietary assessment. Statistical comparisons made using paired *t* test at the $P < 0{\cdot}05$ significance.

‡ Values are the no. of days where A is equal to, less than or greater than C for thirty-five female and nineteen male subjects. Statistical comparisons made using paired *t* test at the $P < 0{\cdot}05$ significance level.

when compared on any one of the 7 d of recording, (*r* values ranged from 0·06 to 0·72 and 0·45 to 0·64 for girls and boys respectively). On 2 d of the 7 d the correlation between calculated and analysed intake values for the girls was not significant ($P = 0{\cdot}05$). Examination of data over the total recording period (paired *t* test) showed that for fourteen of the fifty-four subjects mean analysed intake values were significantly higher than calculated, and for five subjects mean analysed values were significantly lower than calculated (Table 2). For the group as a whole there was no statistical difference between

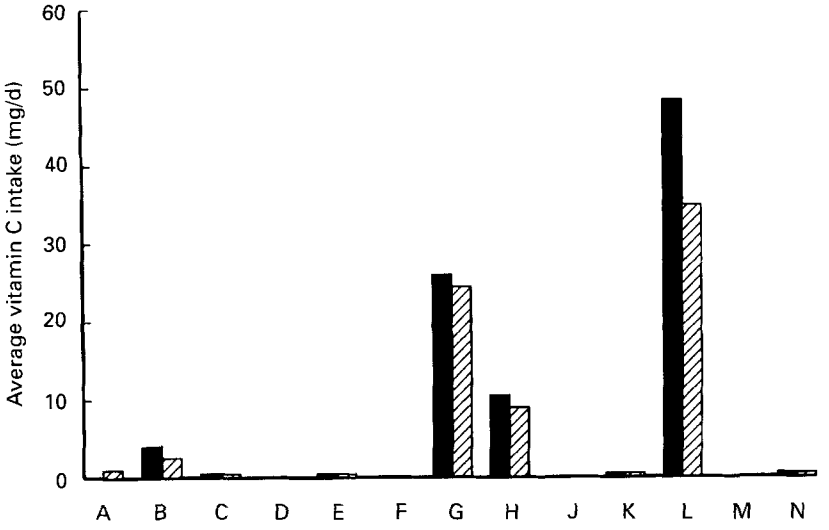

Fig. 2. Contribution of various food groups to calculated vitamin C intake for male (■; *n* 19) and female (▨; *n* 35) adolescents (13–14 years). A, cereal and cereal products; B, milk and milk products; C, eggs; D, fats and oils; E, meat and meat products; F, fish and fish products; G, vegetables; H, fruit; J, nuts; K, sugars and preserves; L, beverages; M, alcoholic beverages and N, soups, sauces and miscellaneous. For details of subjects and procedures, see pp. 564–566.

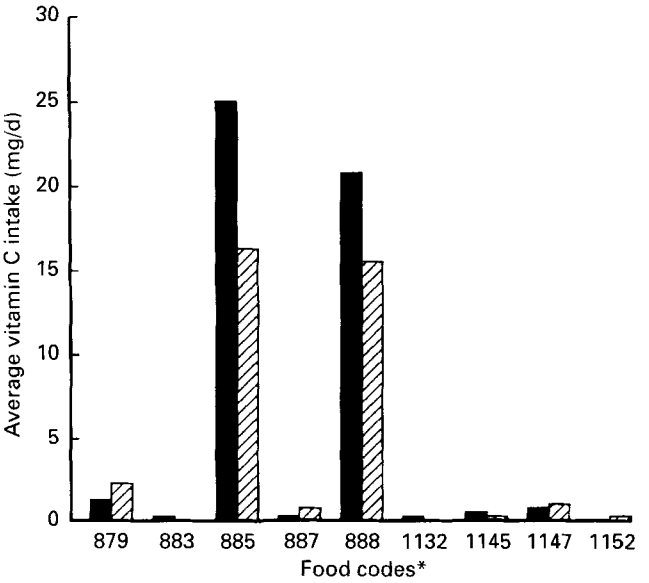

Fig. 3. Contribution of various beverages to calculated vitamin C intake for male (■; *n* 19) and female (▨; *n* 35) adolescents (13–14 years). * *McCance and Widdowson's The Composition of Foods* (Paul & Southgate, 1978). For details of subjects, see pp. 564.

Table 3. *Comparison of food group contribution to calculated vitamin C intake, and total daily vitamin C intake for forty individual days with either good agreement (discrepancy < 10%, n 20) or poor agreement (discrepancy > 350%, n 20) between analysed and calculated intake values*

(Values are means with their standard errors)

			Contribution (%)		
	Food group*	Agreement	Mean	SEM	Range
	Milk/milk products	Good	8·1	2·0	0–36·4
		Poor	7·4	1·4	0–21·0
	Vegetables	Good	32·1	5·6	0–64·2
		Poor	42·4	8·0	4·4–96·7
	Fruit	Good	26·6	7·0	0–86·1
		Poor	21·1	5·7	0–70·3
	Beverages	Good	29·1	7·7	0–87·9
		Poor	26·0	8·6	0–91·0

		Analysed			Calculated		
Vitamin C intake* (mg/d)		Mean	SEM	Range	Mean	SEM	Range
	Good	89·1	8·4	39·7–181·9	84·9	7·9	33·4–178·5
	Poor	107·0	23·7	24·1–432·6	139·0	40·6	0·4–570·1

* There were no significant differences between the group of days of good or poor agreement in either mean daily vitamin intake or food group contribution (unpaired *t* test).

analysed and calculated intakes on any individual day, but when sexes were treated separately the values for females were significantly different on 1 d of the 7 d of dietary assessment (Table 2).

Food groups

The contributions of various food groups to the calculated vitamin C intake of male and female subjects is shown in Fig. 2. The four major food group sources of the vitamin were milk/milk products, vegetables, fruits and beverages, with beverages contributing approximately 40–50% of the average daily intake. The two most important beverage sources of vitamin C were fresh orange juice and blackcurrant squashes (Fig. 3). Vegetable sources provided a further 25–35% of the daily vitamin C, fruits approximately 10–15% and milk products approximately 5%. Calculated vitamin C intakes ranged between 0·6 and 725% of the value obtained by direct analysis of duplicate diets when values were compared on an individual daily basis. Forty separate days of dietary recording were selected to examine the possibility that the magnitude of the discrepancy between calculated and analysed vitamin C intake was related to the food group source of the vitamin. Days of recording were chosen on the basis of a discrepancy of less than 10% (*n* 20) or greater than 350% (*n* 20). No obvious relationship between the magnitude of the discrepancy and food groups, or total amount of vitamin C, consumed that day was found (Table 3).

Vitamin C status

The mean plasma ascorbic acid concentrations for males and females were 81 and 77 μmol/l respectively. There was no significant difference between sexes but there was a larger variation in the boys (Table 1). Both analysed and calculated vitamin C intake ($\log_{10}$) were

Table 4. *Vitamin C intake values for children and adolescents from studies since 1980*

Age (years)	*n* ♀	*n* ♂	Method	Intake (mg/d) ♀	Intake (mg/d) ♂	Location	Reference
10–11	158	163	7 d weighed	41	43	Bristol, Croydon and Sheffield; England	Darke *et al.* (1980)
14–15	85	92	7 d weighed	42	50	Newcastle-upon-Tyne, England	
14–15	401	390	7 d weighed	42	49	Birmingham, England	
9–11	38	37	7 d record	84	88	Dijon, France	Boggio & Klepping (1981)
14–16	125	73	7 d record	72	88	Dijon, France	
13	137	126	24 h record	103	93	Tasmania, Australia	Woodward *et al.* (1981)
14	131	129	24 h record	91	122	Tasmania, Australia	
13–15	92	103	3 d record	101	119	Quebec, Canada	Seoane & Roberge (1983)
12	166	158	48 h recall	95	97	Helsinki, Kuopio, Oulu, Tampare and Turku; Finland	Rasanen *et al.* (1985)
10–11	814	898	7 d weighed	51	51	Great Britain	Department of Health and Social Security (1989)
12–13	45	45	3 d record	133	126	Wrexham, Wales	Benton & Roberts (1988)
11–13	(61)		7 d weighed	28	47	Dundee, Scotland	Crombie *et al.* (1990)
11–12	67	76	7 d weighed	68	67	London, England	Nelson *et al.* (1990)
13–14	35	19	7 d weighed	75	121	Norwich, England	Present study
			7 d direct analysis	84	121		

significantly correlated to plasma AA for male subjects (analysed r 0·59, $P < 0·01$; calculated r 0·63, $P < 0·01$). However, for female subjects only the correlation between analysed intake ($\log_{10}$) and plasma AA was significant ($P < 0·05$) and the r value (0·33) indicated a poorer relationship compared with male subjects.

DISCUSSION

Since 1980 there have been several reports in the literature of vitamin C intakes in children and adolescents (Table 4). In each of these studies intakes were based on calculations using food composition tables and none of them provided any information on the vitamin C status of the subjects. In view of the many studies which rely upon food composition information for the assessment of nutrient intakes, it is important to determine the likely degree of accuracy of such studies for the prediction of actual intake. This is particularly important for vitamin C, a highly labile vitamin which can vary markedly in content between different samples of the same food due to such factors as variety, cooking procedures, storage, maturity and level of fortification. It has been suggested that average values given in the food tables are not sufficiently accurate for assessing dietary intake of this vitamin (Black *et al.* 1983). Nevertheless, the practice of using food composition tables for the assessment of vitamin C intake is commonplace. In the present study a 7 d weighed record method of dietary assessment using food composition tables for the calculation of vitamin C intake was compared with direct analysis of duplicate diets. The small losses of vitamin C from samples of mixed diets, analysed immediately after preparation and again

after storage overnight and up to 6 d at −18°, suggested that the analysed values obtained in the study were realistic of the actual amounts present in the duplicate diets. The degree of discrepancy between mean 7 d and between individual daily calculated and analysed values was determined and consideration was given as to whether any particular food group was especially contributing to major discrepancies.

Weighed dietary records and duplicate diet collections were made every 6th day over a period of 7 weeks, thus all days of the week were taken into consideration. Nelson *et al.* (1989) have suggested about 6–12 d are usually needed to obtain a reasonably precise estimate of long-term vitamin C intake since there appear to be large daily fluctuations. The method of recording at intervals over a longer period of collection was chosen in view of evidence that the accuracy of record keeping can deteriorate over two consecutive days recording and that useable records during days 5–7 are sometimes only obtained from more highly educated subjects (Gibson, 1990*a*). There is also evidence that the added burden of duplicate diet collection can result in a decrease in recorded energy intake by as much as 20% (Gibson, 1990*a*) and it was considered that seven consecutive days of collection may further reduce the accuracy of our estimate of true intake. In addition, discussion with parents and subjects indicated that compliance would be better with the chosen method of recording/collection. The potential for under-recording intake, changing diet during the period of recording and possible variation by day, week or season, and statistical considerations associated with variation in intake has been well reviewed by Bingham (1987) and Borrelli (1990) respectively. The possible extent of under-recording of total food intake in the present study was assessed using mean daily analysed energy intake values obtained in the present study and estimates of daily energy expenditure for 13–14 year olds of similar median height and weight as published in the report of a joint Food and Agriculture Organization/World Health Organization/United Nations University Expert Committee on energy and protein requirements (1985). Assuming a light level of activity (going to school, sitting, standing, moving around, social activity, washing and play), gross daily energy costs have been estimated to be 1·6 × basal metabolic rate (BMR; 6120 kJ) = 9792 kJ for boys and 1·5 × BMR (5600 kJ) = 8400 kJ for girls. Comparison of these values with analysed energy intakes obtained in the present study indicated that the energy intakes for boys and girls were 6 and 12% lower respectively, than their estimated daily energy expenditure. Using other estimates of daily energy expenditure presented by the Department of Health and Social Security (1991), similar estimates for subjects were derived (boys, 1·56 × BMR (6450 kJ/d) = 10062 kJ/d; girls, 1·48 × BMR (5420 kJ/d) = 8022 kJ/d). It appears, therefore, that there was no gross underestimation of habitual energy intake. The discrepancy would, of course, be higher if subjects had spent significant amounts of time involved in moderate and heavy activities, but it cannot be assumed that any under-recording influenced all food groups equally and no attempt was made to adjust the vitamin C intake values presented in the present paper.

The mean vitamin C intake values obtained for boys in the present study (analysed 121·4, calculated 121·2 mg/d) were at the upper end of the range (43–126 mg/d) published in several previous studies of 9–13 year olds, whilst the intake for females (analysed 84·2, calculated 74·8 mg/d) was 'middle of the range' of studies quoted (Table 4). The most important sources of vitamin C in terms of percentage contribution to intake were fresh orange juice and blackcurrant squashes which supplied in excess of 40% of the average daily vitamin C (Fig. 3).

Despite large discrepancies between analysed and calculated intake values within any one individual on any 1 d, and the fact that in some instances analysed and calculated data (for the group as a whole) on any 1 d was not significantly correlated, the overall analysed and calculated mean values for the 7 d recording/duplicate diet collection were not significantly

different from each other. There was also a reasonably good correlation between 7 d mean analysed and calculated values for both male and female subjects. It would appear, therefore, that calculated vitamin C intakes for the group of subjects assessed over 7 d, gave a good indication of actual intakes as judged by prompt direct analysis of duplicate diets. As a cautionary note, however, it should be recognised that there was a statistical difference between analysed and calculated intakes for approximately one-third of the subjects when subjects were examined individually over the 7 d of dietary assessment, indicating the potential unreliability of calculated data used on an individual basis. In addition, the relationship between the more usual method of seven consecutive days of dietary assessment and measurements made every 6th day for 7 weeks, as described in the present study, requires further investigation. It was also apparent, from consideration of percentage contribution to vitamin C intake from major food sources, that large discrepancies between analysed and calculated values could not be accounted for on a food group basis.

There is very little recent information on vitamin C status during adolescence when individuals are experiencing or are commencing a period of rapid growth and when requirements may be higher. The most commonly used methods for the assessment of vitamin C status are leucocyte and serum/plasma AA concentration. Leucocyte concentrations are considered to reflect body stores while plasma AA is more easily influenced by recent dietary intake (VanderJagt *et al.* 1989). Nevertheless, within the usual range of intakes the plasma level is generally proportional to body stores provided that blood samples are taken after an adequate fasting period (Read, 1987). In the present study plasma AA concentration measured promptly in fasting (12–15 h) blood samples was used as an index of status.

A linear relationship between dietary vitamin C intake and plasma AA concentration has been found previously in the elderly over the concentration range 17–80 μmol/l (Newton *et al.* 1983; Thurnham, 1985). Plasma AA tended to plateau at about 80 μmol/l in this age-group, corresponding to an intake of about 100 mg/d. In adult populations generally it is reported that serum concentrations rarely exceed 80 μmol/l because the renal clearance of the vitamin rises sharply with daily intakes greater than 100 mg/d (Friedman *et al.* 1940). In the present study of 13–14 year olds, twenty-five of the fifty-four subjects had plasma AA concentrations of 81–90 μmol/l, six subjects 91–95 μmol/l, three subjects approximately 100 μmol/l and one subject approximately 200 μmol/l. These plasma AA values corresponded to mean analysed intakes of 77, 90, 100 and 246 mg/d respectively. The plasma AA concentrations for 13–14 year olds obtained in the present study were much higher than those obtained in several other studies of 12–18 year olds performed in the 1940s and presented in a thorough review of the area by Irwin & Hutchins (1976). In these early studies plasma AA values of approximately 50 μmol/l for an intake of about 80 mg/d are quoted for this age-group. The reason for this difference is unclear but it is possible that the prompt analysis, coupled with the use of more recently developed sensitive analytical techniques, has resulted in improved measurement of the vitamin compared with techniques employed in earlier investigations. In addition, plasma AA concentrations obtained in our laboratory for healthy adult (20–64 years, n 46; mean plasma AA 50 (range 3–93) μmol/l) and elderly (64–74 years, n 74; mean plasma AA 40 (range 4–89) μmol/l) populations (S. Southon, unpublished results) indicated that the high values obtained for the adolescents in the present study were not an artifact due to methodological factors.

The subject with a plasma AA concentration of 200 μmol/l and an average daily intake of 246 mg/d was the only subject who supplemented their diet with a significant amount of vitamin C. Individuals who routinely consumed supplements were not excluded from the study population since we were interested in determining the relationship between habitual intake of the nutrient and status, as judged by plasma AA levels. The supplement was taken

daily (morning) and, as stated previously, the subject fasted for 12–15 h overnight before a blood sample was taken. It may not necessarily be true, therefore, that plasma AA levels cannot be used to identify persons regularly consuming excessive amounts of the vitamin (Gibson, 1990*b*), at least in the case of younger individuals where there may be physiological differences in the metabolism of vitamin C.

Further evidence for a difference in vitamin C metabolism in younger individuals is indicated by the lack of a sex difference in plasma AA concentration. Adult women appear to have higher plasma AA concentrations than men on similar intakes, but such sex differences are not apparent before adolescence (Dodds, 1969) and indeed the plasma AA concentrations of boys and girls in the present study were not significantly different. However, mean analysed or calculated vitamin C intake was significantly lower in the female subjects whilst plasma AA values were similar to the males. This may be indicative of the emergence of the sex difference which becomes more apparent in later life.

There was also a possible sex difference relating to the relationship between vitamin C intake and status in the present study. There was a significant relationship between both calculated and analysed intakes and plasma AA in males but in females there was no relationship to calculated intake and only a very poor relationship to analysed intake. The most likely explanation for this possible difference is the wider range of intakes and plasma AA values obtained for males, particularly at the upper end of the range, whilst for either sex there were few low intake or plasma AA values. It is generally assumed that the relationship between vitamin C intake and status reaches a plateau at an intake of approximately 100 mg/d and a fasting plasma AA of approximately 80 μmol/l. It appears from the present study, however, that in certain individuals habitual intakes in excess of this amount may result in a further increase in plasma AA concentration. This is supported by a study performed by Schorah *et al.* (1981) which showed that a daily supplement of 1 g vitamin C given to elderly subjects for 60 d could increase the fasting plasma AA concentration up to 151 μmol/l.

In conclusion, the present study indicated that vitamin C intakes for groups calculated from food composition tables gives a reasonable estimate of actual intake, as judged by prompt analysis of duplicate diets. However, findings presented here clearly demonstrate the unreliability of calculated values used on an individual basis. In general, the adolescents taking part in the present study had vitamin C intakes well in excess of the current RNI of 40 mg (Department of Health and Social Security, 1991) and status, as judged by plasma AA concentration, was extremely good and was well above the value of 11–22 μmol/l used to indicate moderate risk of deficiency (Gibson, 1990*b*). A stronger relationship between vitamin C intake and plasma AA was observed in males, although this could be a misleading mathematical artifact as both intake and status values showed a wider range compared with females.

This work is supported by the UK Department of Education & Science (DES), the UK Ministry of Agriculture, Fisheries and Food (MAFF) and the Vitamin Forum. The authors thank Heather Sarsby, Zoe Piper, Wendy Lee, Jane Lumb and Joanne Belsten for their invaluable assistance with this study and Dr Julia Leach for taking blood samples from the subjects. Special thanks also to the subjects themselves, their parents, teachers and the catering staff at the two schools.

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Printed in Great Britain