British Journal of Nutrition (2004), **91**, 699–705
DOI: 10.1079/BJN20041103

Stable isotope-labelled vitamin C as a probe for vitamin C absorption by human subjects

Christopher J. Bates*, Kerry S. Jones and Leslie J. C. Bluck

MRC Human Nutrition Research, Cambridge, UK

(Received 8 September 2003 – Revised 15 January 2004 – Accepted 22 January 2004)

Factors affecting absorption of physiological doses of vitamin C in man have not been widely studied, partly because few suitable tools exist to distinguish recently absorbed vitamin C from endogenous vitamin. Stable isotope-labelled vitamin C provides such a tool. Fifteen healthy non-smoking subjects aged 26–59 years were studied. Each received 30 mg L-[1-^{13}C]ascorbic acid orally on two occasions, 3–4 weeks apart. The ascorbate was given alone or with Fe (100 mg as ferrous fumarate) or with red grape juice, which is rich in polyphenols. Blood was collected at frequent intervals for 1 h, and then each hour for a further 3 h. Total concentration of vitamin C was measured fluorometrically and its ^{13}C-isotope enrichment was measured by GC–MS after conversion to volatile trimethylsilyl esters. Peak plasma enrichment occurred within 25–50 min. No kinetic variables were significantly altered by the iron fumarate supplement. Grape juice attenuated vitamin C absorption, reaching significance at the 20 min time point. There were weak correlations between isotope enrichment and body weight or endogenous ascorbate concentration. The increment in total plasma ascorbate was smaller if calculated from isotope enrichment than from vitamin C concentration increase. The dilution pool was much larger than the plasma ascorbate pool. Further studies are needed to resolve these paradoxes. Stable isotope-labelled ascorbate is potentially useful for measurement of vitamin C absorption by human subjects.

Vitamin C: Ascorbate: Absorption: Stable isotope: Human subjects

There is little detailed information about the efficiency and kinetics of vitamin C absorption and distribution, or about the pools into which it is diluted. This is especially true for non-pharmacological doses of a few tens of milligrams. Other food components may interact either positively or negatively. Vitamin C requirements appear to vary between individuals, and may increase in situations of stress and high risk of tissue damage. It is important to study the determinants of individual variability.

The reduced form of vitamin C is absorbed in the upper ileum by a saturable energy- and Na-dependent process (Bates, 1997) involving specific vitamin C transporters, whereas the oxidised form of the vitamin, dehydroascorbate, is transported by non-specific glucose transporters (Liang *et al.* 2001). At high oral doses, passive absorption dominates active transport. For pure ascorbic acid, bioavailability is reported to be complete for 200 mg given as a single dose (Levine *et al.* 1996). For higher amounts, e.g. between 1 and 5 g, the percentage absorbed becomes progressively lower, e.g. 75 % falling to 20 % of the dose as the dose increases, the absorption being measured by urinary excretion (Hornig *et al.* 1980). When doses exceed 180 mg, a significant proportion may be degraded in the gastrointestinal tract, yielding $^{14}CO_2$ if [1-^{14}C]ascorbate is used (Kallner *et al.* 1985).

Some previous studies have used unlabelled ascorbate to measure vitamin C absorption kinetics (see Blanchard (1991) and Piotrovskij *et al.* (1993)). Other studies have used smaller doses of radioactively labelled ascorbate (Baker *et al.* 1969; Kallner *et al.* 1979, 1981). Both approaches have shortcomings: the first is insensitive in well-nourished people and the second is ethically controversial. Until recently, the use of stable isotopes has been confined to a single study that employed a difficult and time-consuming isolation of crystalline osazone products of ascorbic acid before measuring the isotope ratio (Atkins *et al.* 1964). New technology now permits the measurement of isotope ratios in ascorbate with less cumbersome preparative chemistry. It combines the advantages of labelling in distinguishing newly absorbed from endogenous ascorbate with the inherent safety of stable isotopes *in vivo*. Initial studies in our laboratory (Bluck *et al.* 1996; Izzard *et al.* 1996) have confirmed its feasibility.

The objectives of the present study were: (1) to optimise the procedure for *in vivo* measurements of stable isotope-labelled ascorbate kinetics in human subjects; (2) to undertake a preliminary exploration of the possible influence of other dietary components, namely ferrous iron and grape juice, on the kinetics of ascorbate absorption in healthy adult human subjects.

Abbreviation: AUC, area under the curve.

* **Corresponding author:** Dr Christopher J. Bates, fax + 44 1223 437515, email chris.bates@mrc-hnr.cam.ac.uk

Subjects and methods

^{13}C-labelled vitamin C (L-ascorbic acid)

The supplier of [1-^{13}C]ascorbic acid was Cambridge Isotopes Ltd, MA, USA. L-[1-^{13}C]ascorbic acid, 98–99 % isotopic purity, was used. It is one mass unit heavier than the major natural isotopic form in unlabelled ascorbic acid (molecular mass 176·13). The desiccated crystalline solid was stored at −25°C for up to 3 years without degradation.

Studies with human volunteers

The volunteers were confined to healthy non-smokers, not taking high-dose vitamin C supplements and without clotting or bleeding disorders, between the ages of 18 and 60 years. Having demonstrated that [1-^{13}C]ascorbate is a suitable probe (Bluck *et al.* 2002), 30 mg [1-^{13}C]ascorbate was given orally to fifteen male and female subjects, alone or in combination with a ferrous fumarate supplement, or with a red grape juice drink (Tesco, UK; own brand) containing antioxidant polyphenol pigments. It contained 2·8 MJ and 160 g sugars/l; a standard serving was 200 ml. Each subject attended twice with a 3–4 week (minimum) interval between each visit to ensure adequate wash-out of isotope. They attended the volunteer suite of Medical Research Council Human Nutrition Research Unit (Cambridge, UK) in the early morning and were asked to avoid taking vitamin C supplements on that day. Eight of the subjects received either labelled ascorbate alone or labelled ascorbate plus Fe (100 mg as ferrous fumarate) on the first occasion and then received the other of these two alternatives on the second occasion. The other seven subjects received labelled ascorbate alone or labelled ascorbate plus 200 ml grape juice on the first occasion and the second of these two alternatives on the second occasion. Male and female subjects were randomised between the groups and the sequence of doses was also randomised between the individuals. Blood samples (4·9 ml) were collected from an indwelling butterfly cannula into Sarstedt monovettes (Sarstedt Ltd, Leicester, UK) containing heparin anticoagulant at 0, 4, 8, 12, 16, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 120, 180 and 240 min. Heparin solution was introduced into the tubing to prevent clotting between collections. The study was approved by the Cambridge Local Research Ethics Committee.

Assay of grape juice polyphenols

Grape juice polyphenols were assayed (Serafini *et al.* 1998) in order to provide an estimate of the antioxidant polyphenol content of the grape juice; this may modulate vitamin C kinetics. Tannic acid (VWR International Ltd, Lutterworth, Leics., UK; >88 % purity) was used as the calibrant, and the polyphenol content was expressed as mg/l tannic acid equivalents.

Preparation of vitamin C for analysis

Vitamin C needs to be stabilised by acidification, with chelation of metal ions and storage at a low temperature (e.g. −80°C) before assay. Acidification, chelation and removal of protein by precipitation were achieved with metaphosphoric acid (final concentration 50 g/l) for the fluorometric assay. This treatment, however, was not compatible with GC–MS, because the stabilising agent needed to be removed before derivatisation. The samples for GC–MS were therefore stabilised with TCA (100 g/l); the precipitated proteins were removed by centrifugation at 3000 ***g*** at 4°C for 10 min, and the TCA was removed by extraction with 1 ml light petroleum (b.pt. 40–60°C) before derivatisation. The petroleum-extracted samples were then shell-frozen in 2 ml GC–MS vials and lyophilised in a freeze-drier. They were then derivatised by silylation for MS as described later.

Assay of total vitamin C in plasma and other materials

The assay method for total vitamin C in plasma samples (Vuilleumier & Keck, 1989) was performed on a Roche Cobas Bio (Hoffmann-La Roche, Basel, Switzerland) centrifugal clinical analyser with a fluorescence attachment. A sample was mixed with sodium acetate to achieve a pH close to 4·5, the optimum for ascorbate oxidase activity, then ascorbate oxidase enzyme was added; after a 5 min incubation at 37°C, *o*-phenylene diamine was added to form fluorescent quinoxaline from the resulting dehydroascorbate. A fluorescence reading before addition of the final reagent provided a blank subtraction. The assay was validated by comparison with an HPLC assay: this involved separation of ascorbic acid on a C_{18} reverse-phase silica column with an ion-pairing agent, and with homocysteine in the mobile phase to prevent oxidation of the ascorbic acid, followed by quantitation with a coulometric detector. All measurements were performed in duplicate. The between-run CV of the fluorescence assay was approximately 6 % over a tenfold range of plasma ascorbate concentrations (10–100 μmol/l).

Isotope ratio measurements by GC–MS

Derivatisation of ascorbate to yield volatile trimethylsilyl derivatives was achieved by adding dry pyridine to the freeze-dried samples or standards followed by *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide containing trimethylchlorosilane (10 ml/l). This mixture was incubated in a sealed sample vial for 2 h at room temperature. GC–MS analysis was carried out on an Agilent 6890 GC interfaced with an Agilent 5973N MSD quadrupole MS in electron ionisation mode (Agilent, Stockport, Cheshire, UK). The column used was a DB-5MS 30 m × 0·25 mm (internal diameter) with a film thickness of 0·25 μm (part no. 19091S-433; Hewlett Packard, Stockport, Cheshire, UK). Column temperatures were programmed to be isothermal for 2·0 min at 75°C, then ramped at 25°C per min to 300°C, then isothermal for 1·5 min. Total run time was 12·3 min. The silylated ascorbate elutes between two large peaks of silylated glucose. The GC was fitted with the Agilent Electronic Pressure Control module, a device for the precise control of gas flow rates used to optimise the peak separations.

The selective ion monitoring option of the MS instrument focused on peaks with *m*/*z* 464, 465, 466, 467 and 468, corresponding to the tetra(trimethylsilyl) unlabelled ascorbate ion and four heavier ions derived mainly from the ^{13}C and from several silicon isotopes that are present. The intensity

of each ion minus the background over a fifty scan envelope about the peak apex was used to evaluate the ion intensity. The data were further analysed by a spreadsheet method (Bluck & Coward, 1997) to determine the relative contributions of each isotope to the ascorbate content of each plasma sample. The CV for triplicate isotope ratio measurements was approximately 0·5 %. From the time course of the appearance of excess isotope in the ascorbate peaks at a series of time intervals following an oral dose of labelled ascorbate, it was possible to calculate: (1) the delay time before the dose-label first appeared; (2) the rate (at half peak) of absorption; (3) the size of the distribution pool represented by the plasma, assuming that all the dose was absorbed (or, alternatively, the percentage of the dose absorbed for any specified size of the distribution pool). Comparison of the tracer plus tracee (i.e. endogenous ascorbate) concentrations with the total ascorbate as measured by the fluorescence assay permitted further testing of the model.

Analysis of ^{13}C in expired breath

Breath samples were collected at the same time as each of the blood samples by the volunteer blowing gently through a straw into a 10 ml Exetainer (Labco Ltd, High Wycombe, Bucks., UK) until water vapour appeared on the side of the tube (approximately 10 s). The cap was replaced and the ^{13}C-enrichment of the samples was measured in an isotope ratio MS (AP2003 isotope ratio MS; Analytical Precision Ltd, Northwich, Cheshire, UK). The area under the curve (AUC) from baseline to 240 min was then calculated and was compared with the background fluctuation level. The sensitivity and precision is such that a degradation of 3 % of the dose over the period of the experiment would be detected.

Statistical methods

DataDesk statistical package for Macintosh (Data Descriptions Inc., Ithaca, NY, USA) was used for the statistical evaluations, which comprised Student's *t* test, paired *t* test, linear regression analysis and ANOVA. Data reduction from the output of the Agilent GC–MS instrument included a new spreadsheet algorithm for the calculation of isotopomer ratios (Bluck & Coward, 1997). (Isotopomers are molecules with the same structural formulas, but different relative molecular masses due to different isotopic compositions.)

Results

Table 1 summarises the anthropometric characteristics of the subjects, and their baseline ascorbate concentrations. One subject had a particularly low baseline plasma ascorbate of 11 μmol/l, which is considered to represent borderline biochemical deficiency; the remainder had concentrations ranging between 22 and 97 μmol/l, which spans the normal range of adult human populations, but none was extremely high. Likewise, their anthropometric indices were within a typical UK population range.

The polyphenol content of the red grape juice was estimated as 880 mg tannic acid equivalents/l; therefore 200 ml grape juice as drunk contained 176 mg polyphenols as tannic acid equivalents.

Table 1. Anthropometry and baseline ascorbate (Mean values and ranges)

Subjects...	Male (*n* 8)		Female (*n* 8)	
	Mean	Range	Mean	Range
Age (years)	46	27–59	44	26–59
Height (m)	1·81	1·72–1·88	1·64	1·53–1·71
Weight (kg)	81·3	70–96	73·3	54–91
BMI (kg/m^2)	24·5	22–26	27·3	20–35
Body fat (%)	21·9	16–26	37·1	24–46
Baseline plasma ascorbate (μM)	58	44–72	51	11–69

Fig. 1 shows the tracer concentration time-course curves for the two groups of subjects receiving labelled vitamin C alone, for the group receiving vitamin C plus ferrous fumarate and for the group receiving vitamin C plus grape juice. These tracer concentrations were calculated from the increment in tracer enrichment compared with zero time and the total concentration of ascorbate in the plasma, measured fluorometrically. The presence of Fe with the labelled ascorbate made no significant difference to the kinetic picture, either at any individual time point or for the overall AUC. Indeed, the mean of all AUC for vitamin C alone for all fifteen subjects was identical (within <2 %) to the mean area for the eight subjects who received vitamin C plus Fe.

For the vitamin C plus grape juice, there was a trend towards reduced absorption compared with vitamin C alone; this trend was significant ($P=0{\cdot}009$) by paired *t* test at 20 min post-dose, and marginally significant ($P=0{\cdot}04$) at 16, 24 and 28 min (Fig. 1). Overall, the AUC was not significantly different from the area for vitamin C alone, either by paired *t* test (*v.* the same subjects) or Student's *t* test (*v.* all fifteen AUC for vitamin C alone). However, a 47 % reduction was needed, given the subject numbers and observed variances, to achieve significance at $P<0{\cdot}05$.

The ^{13}C kinetic variables obtained when [^{13}C]ascorbate was given alone (i.e. without Fe or grape juice) showed no significant relationships with the subjects' ages, their anthropometric indices or their mean (baseline) ascorbate levels, apart from a weak inverse correlation between baseline ascorbate and lean body mass or body weight, and a weak direct correlation between mean (baseline) ascorbate and calculated time to 10, 50 and 90 % of maximum enrichment (Table 2). Surprisingly, there was no significant relationship between the change in baseline ascorbate between the two visits within individuals, and the change in isotope enrichment between the two visits in the same individuals (results not shown). Thus, even when the plasma ascorbate concentration changes within individuals, the ascorbate dilution pool appears not to change in parallel.

Plasma ascorbate enrichment was inversely related to the plasma ascorbate concentration overall ($P=0{\cdot}022$). Thus, the larger the pre-existing pool size, the smaller the pool enrichment for a constant magnitude of tracer

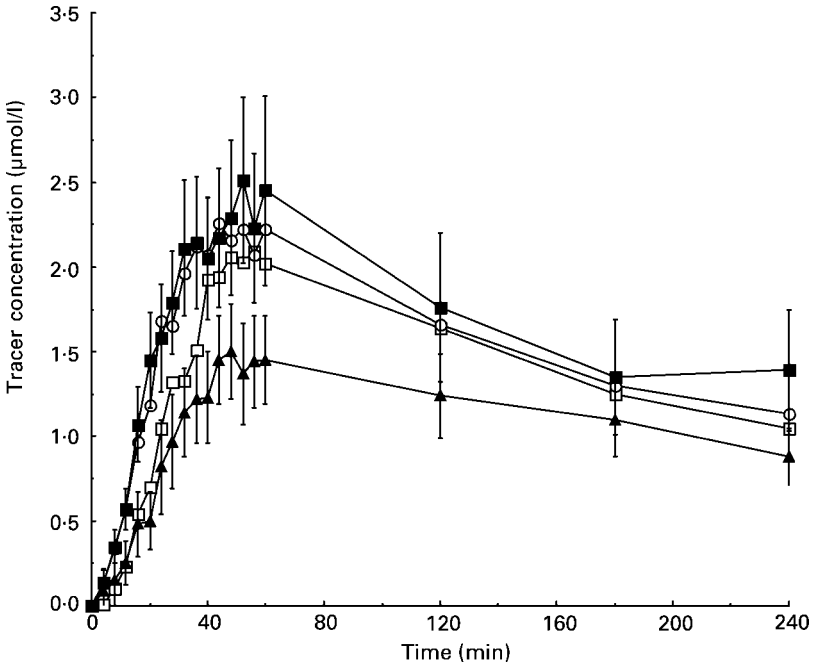

Fig. 1. Time course plots of tracer concentration following the dose of labelled vitamin C, with and without added iron or grape juice. □, Vitamin C alone (1); ○, vitamin C plus iron; ■, vitamin C alone (2); ▲, vitamin C plus grape juice. For details of subjects and procedures, see Table 1 and pp. 700–701. Tracer concentrations were calculated from the increments in ^{13}C-enrichment of the plasma ascorbate, compared to zero time (time of dosing) and from the total plasma ascorbate concentrations, measured separately by the fluorometric assay. The data for vitamin C alone (1) were obtained from the same eight subjects who provided the data for vitamin C plus iron. The data for vitamin C alone (2) were obtained from the same seven subjects who provided the data for vitamin C plus grape juice. Standard errors are shown by vertical bars for the mean values for vitamin C alone (2) and for vitamin C plus grape juice, but are omitted from the other two curves for clarity; however the magnitudes of the standard errors were similar for all four data sets. By paired *t* test, the mean tracer concentration was significantly different between the vitamin C alone (2) and the vitamin C plus grape juice curves, at the following time points: 16 min ($P=0{\cdot}04$); 20 min ($P=0{\cdot}009$); 24 min ($P=0{\cdot}04$); 28 min ($P=0{\cdot}04$). The vitamin C alone (1) and the vitamin C plus iron curves were not significantly different from each other at any of the time points depicted. None of the overall areas under the curves were significantly different from each other, either by paired *t* test for matched pairs or by Student's *t* test comparisons, between all the areas for vitamin C alone and all the areas for the combinations of vitamin C and iron or vitamin C and grape juice.

dose. Likewise, the greater the body weight, the smaller the maximum tracer concentration ($P=0{\cdot}033$) and fractional enrichment ($P=0{\cdot}04$), presumably because the greater the body weight, the larger the physical volume for dilution of the tracer. This is further reflected by the inverse relationship between tracer enrichment and lean body mass ($P=0{\cdot}05$). The times to reach 10, 50, 90 % and maximum slope for tracer entry were all directly related to the plasma ascorbate concentration ($P=0{\cdot}02$ to $P=0{\cdot}04$). This may be a function of the partial saturation of the ascorbate transporters. There was a weak inverse relation ($P=0{\cdot}04$ to $P=0{\cdot}05$) between the subjects' ages and their plasma ascorbate concentration, and a corresponding direct relationship between age and tracer

Table 2. Linear regression values for tracer and tracee kinetic indices *v.* anthropometry, age and baseline plasma ascorbate†‡ (*t* values)

Index§	Lean body mass	Body weight	BMI	Body fat	Age	Gender	Mean ascorbate concentration
Tracer: C_{max}	−1·6	−1·8	−0·4	+0·4	−0·2	+0·7	+1·1
Tracee concentration	+0·5	+0·1	+0·2	−0·7	−0·1	−0·5	–
Fractional enrichment	−2·4*	−2·2*	−0·5	+0·9	−0·2	+1·1	−0·5
$T_{0\cdot1}$	+1·1	+0·1	−0·5	−1·6	−0·2	−1·2	+3·4*
$T_{0\cdot5}$	+0·3	−0·3	0·0	−0·7	0·0	−0·5	+3·5*
$T_{0\cdot9}$	−0·7	−0·7	+0·5	+0·3	−0·2	+0·5	+2·9*
T_{max}	+1·5	+0·4	−0·7	−1·9	+0·3	−1·8	+1·9
T_{lat}	−0·8	−2·0	−1·7	−0·9	−1·4	+0·5	+0·8
T_{ascend}	−1·5	−0·9	+0·9	+1·2	−0·4	+1·4	+1·6

* $P < 0{\cdot}05$.

† For details of subjects and procedures, see Table 1 and pp. 700–701.

‡ *n* 15, df 13; gender: male 1, female 2.

§ Tracer: C_{max}, maximum concentration of tracer [^{13}C] ascorbate in plasma (μmol/l); tracee concentration, mean concentration of tracee, i.e. native unlabelled ascorbate (μmol/l); fractional enrichment, maximum tracer:tracee ratio; $T_{0\cdot1}$, time to 10 % of maximum tracer concentration; $T_{0\cdot5}$, time to 50 % of maximum tracer concentration; $T_{0\cdot9}$, time to 90 % of maximum tracer concentration; T_{max}, time to maximum tracer concentration (estimated from intercept of linear ascending slope and the horizontal maximum concentration); T_{lat}, time of latency (estimated from intercept of the linear ascending slope and the horizontal latency line); T_{ascend}, time between T_{lat} and T_{max}.

enrichment; this is consistent with the known decline in ascorbate stores (ascorbate retention) with increasing age. There was a marginally higher value for 'T_{ascend}' in female than in male subjects.

Comparison of the key kinetic indices, C_{max} and fractional enrichment, between the group receiving ascorbate alone and the same group receiving ascorbate plus Fe, or receiving ascorbate plus grape juice, failed to reveal any significant treatment-related effects. Thus, the P values for paired t tests between treatments (7 df for the Fe treatment and 6 df for the grape juice treatment) were: $P=0{\cdot}7$ and $P=0{\cdot}15$ for C_{max} (Fe and grape juice treatments respectively) and $P=0{\cdot}8$ and $P=0{\cdot}15$ for tracer enrichment (Fe and grape juice respectively). None of these treatment effects was significant at $P<0{\cdot}05$.

The ^{13}C enrichment of CO_2 in expired air was below the minimum measurable level for all subjects, and even in the presence of Fe, <3 % of the dose was converted to $^{13}CO_2$. Therefore, the extent of any ascorbate oxidation in the gut before absorption was undetectable, under all the conditions tested, including the ferrous iron co-ingestion.

The estimated increment in plasma ascorbate that was based on the total ascorbate concentration change as measured fluorometrically (about 4 μmol/l) was about twice the ascorbate concentration increment that was estimated from the change in tracer:tracee ratios (about 2 μmol/l), a difference that was highly significant. This implies that in addition to the amount of ascorbate that is provided by the ingested tracer, an approximately equal amount of unlabelled ascorbate is also appearing simultaneously in the plasma from another (presumably endogenous) source.

Discussion

Previous approaches

Several approaches have been used to explore ascorbate kinetics in man:

(1) Plasma or urine ascorbate concentration AUC and related kinetic variables, after a single dose of unlabelled ascorbic acid.
(2) Depletion and repletion for several weeks in a 'metabolic ward', measuring body ascorbate pool size, turnover rate and efficiency of use of different food sources of ascorbate during repletion.
(3) Plasma or urine kinetics of radioactive (usually 1-^{14}C) ascorbate and its degradation products after ingesting labelled ascorbate.
(4) Plasma and/or urine kinetics after ingesting stable isotope-labelled ascorbate.

Unlabelled dose kinetics. Levine *et al.* (1996) measured the AUC in plasma after oral or intravenous doses of 200, 500 and 1250 mg ascorbic acid. Bioavailability was complete for the 200 mg dose, but much lower for the higher doses. However, the kinetics of urinary excretion may differ between oral and intravenous doses.

After a 500 mg oral dose of ascorbic acid, Blanchard *et al.* (1990; Blanchard, 1991) calculated kinetic variables. The distribution space was 22 to 44 litres, considerably greater than plasma plus other extracellular volumes. An even larger dilution volume (81–87 litres) was calculated by Zetler *et al.* (1976). Bioavailability estimates ranged from 79 % of a dose of 200 mg ascorbic acid to 45 % at 400 mg, 23 % at 1000 mg and 13 % at 2500 mg. From plasma responses to single ascorbate doses between 200 and 2500 mg, Blanchard *et al.* (1997) concluded that direct AUC comparison of an oral with an intravenous dose tends to overestimate ascorbate availability.

Piotrovskij *et al.* (1993) estimated kinetic variables and pool sizes for a three-compartment model. The rapid dilution pool was similar in size to the plasma pool (about 11 litres), whereas the steady state pool was much larger (about 178 litres). Assuming complete absorption of a 60 mg dose, only 22 % of a 500 mg dose was absorbed.

Most studies with unlabelled vitamin C have used 200–2500 mg per dose, much higher than the physiological range.

Depletion–repletion studies with unlabelled ascorbate. Mangels *et al.* (1993) depleted and then repleted subjects with vitamin C in food, without or with an Fe supplement (20 mg as ferrous fumarate). No differences were observed between the different repletion protocols, except for raw broccoli, where repletion was slightly slower.

A long-term repletion protocol by Graumlich *et al.* (1997) provided 30, 60, 100, 200, 400, 1000 and 1250 mg ascorbate/d for 114–179 d. Using a five-compartment model, the 'central' compartment had a volume (11–12 litres) similar to the extracellular space (about 15 litres). The calculated ascorbate availability was 89 % for the lowest dose and 47 % for the highest. Before repletion, the 'tissue' compartment was about 8 mmol (1400 mg). At a daily intake of 200 mg, 72 % was absorbed, of which 71 % was excreted in the urine and 29 % was metabolised.

Radioactive dose kinetics. Controlled depletion studies using [1-^{14}C]ascorbate indicated an initial ascorbate body pool size of about 1500 mg (Baker *et al.* 1969). At zero intake, the ascorbate declined at about 2·6 % per d.

Studies with [1-^{14}C]ascorbate also measured turnover (Kallner *et al.* 1977*a*,*b*, 1979, 1981) and this was increased in smokers. Body pool sizes were 800 mg at an intake of 30 mg/d, rising to 1850 mg at an intake of 180 mg/d. An absorption efficiency of 80–90 % was estimated for 30–60 mg [1-^{14}C]ascorbic acid. Hornig *et al.* (1980) found only 75 % absorption for a 1 g dose, falling to 21 % for a 5 g dose. At doses of 1–4 g, Kallner *et al.* (1985) found that 1-^{14}C-labelled vitamin C was extensively degraded, yielding up to 35 % of its 1-^{14}C label as $^{14}CO_2$.

Stable isotope dose kinetics. Atkins *et al.* (1964) gave 50 mg [1-^{13}C]ascorbic acid to adult male subjects and collected urine for 50 d. Urinary ascorbate and oxalate were isolated by fractional crystallisation and ^{13}C-enrichments were measured by combustion isotope-ratio MS. The calculated ascorbate body pool was 47–51 mg/kg body weight, equivalent to a 3 g body pool. Turnover was about 1·25 mg/kg per d and half-life was about 27 d. Intake from food unfortunately was not recorded.

Studies in our laboratory (Izzard *et al.* 1996; Bluck *et al.* 2002) showed that GC–MS of silyl esters accurately estimates ^{13}C-enrichment of plasma ascorbate, even after physiological doses of ascorbate (30–60 mg). In three subjects ascorbate distribution volumes were 32, 33 and 53 litres.

Vitamin C–food interactions

Most studies have failed to detect differences in availability between ascorbate given alone and that present in food: e.g. baked potatoes (Clayton & Folsom, 1940), raw cabbage and canned tomato juice (Clayton & Borden, 1943), orange juice (Hawley *et al.* 1936; Pelletier & Keith, 1974; Nelson *et al.* 1975; Johnston & Luo, 1994), raspberries (Todhunter & Fatzer, 1940), and papayas and guavas (Hartzler, 1945).

However, Jones & Hughes (1984) and Vinson & Bose (1988) reported higher absorption of vitamin C from foods rich in polyphenols (blackcurrant or citrus extract) than in those without. Hopper & Ayres (1950), Timberlake (1960), Clegg & Morton (1968), Harper *et al.* (1969) and Miller (1998) found evidence that polyphenols in fruits or fruit extracts protect ascorbate against oxidative destruction. In contrast, however, Song *et al.* (2002) reported a non-competitive inhibition of ascorbate transport by the polyphenol quercetin, in a cell culture model.

Greatly increased body Fe stores resulted in accelerated oxidative catabolism of ascorbic acid in Africa (Lynch *et al.* 1967; Hankes *et al.* 1974). Fe–vitamin C supplements were thought to damage DNA (Rehman *et al.* 1998), but Yang *et al.* (1999) found no lipid damage by vitamin C plus Fe supplements.

Conclusions

In the present study, absorbed ascorbate was rapidly diluted into a pool that was much larger than the plasma pool. Within subjects, the percentage change in plasma ascorbate enrichment was unrelated to the change in ascorbate concentration in the plasma pool. A dose of 30 mg (170 μmol) ascorbate diluted into a plasma volume of about 2·5 litres ought to increase the concentration by 70 μmol/l, instead of only 2 or 4 μmol/l, as observed in our present study. Therefore, the ascorbate dilution pool must be very much greater than the plasma, or even the plasma plus erythrocyte pool.

The observed mean increase in plasma ascorbate by the fluorometric assay was about 4 μmol/l. With a plasma ascorbate of 60 μmol/l and a [^{13}C]ascorbate dose of 30 mg, the ingested dose ought in theory to be distributed in a pool of $15 \times 30 = 450$ mg ascorbate. However, the observed mean increase in plasma ascorbate according to the ^{13}C enrichment calculation was only about half of this, i.e. about 2 μmol/l, suggesting a dilution pool of about 900 mg. This discrepancy might be explicable if the dose of labelled ascorbate carried with it some unlabelled ascorbate from the stomach into the upper ileum. Alternatively, if the plasma pool were more elastic than that of the tissues, and there is a very rapid exchange between the plasma pool and that of the easily accessible tissues, the ^{13}C-label may equilibrate completely and rapidly, but the increment in total ascorbate concentration in plasma may be greater than the increment in the tissues.

Our present study sought interactions during absorption between vitamin C and ferrous iron, or between vitamin C and a polyphenol-rich drink of red grape juice, which contains anthocyanins, flavanols and flavonols, including quercetin, myrecetin, catechin and rutin (Frankel *et al.* 1998; Lapidot *et al.* 1998).

100 mg ferrous iron had no significant effect on the extent of degradation of the [^{13}C]ascorbate dose to $^{13}CO_2$ or on ascorbate absorption kinetics. Ascorbate is known to enhance Fe absorption, probably by keeping it reduced and by complexing it. Even a small amount of ascorbate can improve Fe absorption (Diaz *et al.* 2003). However, this interaction does not enhance ascorbate absorption, possibly because ascorbate is not subject to the same transport restrictions that apply to Fe.

Grape juice did not have a significant overall effect on the AUC of tracer concentration curve in our present study, where a 47 % change would have been significant. A transient inhibitory effect of the grape juice was observed, reaching significance around 20 min after dosing.

Further kinetic studies with stable isotope-labelled ascorbate are needed to clarify the physical identity of the ascorbate dilution pool, and the paradox in the magnitude of the apparent change in this pool after dosing. In public health terms, however, the most important conclusion from the present study is that vitamin C in foods or in nutrient supplements is unlikely to be rendered significantly less available, or to be substantially degraded, even if substantial quantities of ferrous iron salts are ingested at the same time.

Acknowledgements

This work was supported in part by the Food Standards Agency under their Optimal Nutrition Programme. The analytical procedures were developed by Dr G. S. McLeod; the vitamin C and polyphenol analyses were performed by Ms Filomena Liuni and Ms Amelia Ireland, and the volunteer procedures, including the dosing and phlebotomy, were performed by Ms Sarah Jones and Ms Lorraine Cooney. We also wish to thank the volunteers for their cooperation.

References

Atkins GL, Dean BM, Griffin WJ & Watts RWE (1964) Quantitative aspects of ascorbic acid metabolism in man. *J Biol Chem* **239**, 2975–2980.

Baker EM, Hodges RE, Hood J, Sauberlich HE & March SC (1969) Metabolism of ascorbic-1-14C acid in experimental human scurvy. *Am J Clin Nutr* **22**, 549–558.

Bates CJ (1997) Bioavailability of vitamin C. *Eur J Clin Nutr* **51**, S28–S33.

Blanchard J (1991) Effects of gender on vitamin C pharmacokinetics in man. *J Am Coll Nutr* **10**, 453–459.

Blanchard J, Conrad KA, Mead RA & Garry PJ (1990) Vitamin C disposition in young and elderly men. *Am J Clin Nutr* **51**, 837–845.

Blanchard J, Tozer TT & Rowland M (1997) Pharmacokinetic perspectives on megadoses of ascorbic acid. *Am J Clin Nutr* **66**, 1165–1171.

Bluck LJC & Coward WA (1997) Peak measurement in gas chromatographic/mass spectrometric isotope studies. *J Mass Spectrom* **32**, 1212–1218.

Bluck LJC, Izzard AP & Bates CJ (1996) Measurement of ascorbic acid kinetics in man using stable isotopes and gas chromatography/mass spectrometry. *J Mass Spectrom* **31**, 741–748.

Bluck LJC, Jones KS & Bates CJ (2002) ^{2}H and ^{13}C labelled tracers compared for kinetic studies of ascorbic acid metabolism in man – a factor analytical approach. *Rapid Commun Mass Spectrom* **16**, 879–883.

Clayton MM & Borden RA (1943) The availability for human nutrition of the vitamin C in raw cabbage and home-canned tomato juice. *J Nutr* **25**, 349–369.

Clayton MM & Folsom MT (1940) A method for the study of the availability for human nutrition of the vitamin C in foods, with an application to the study of the potato. *J Home Econ* **32**, 390–395.

Clegg KM & Morton AD (1968) The phenolic compounds of blackcurrant juice and their protective effects on ascorbic acid. II. The stability of ascorbic acid in model systems containing some of the phenolic compounds associated with blackcurrant juice. *J Fd Technol* **3**, 277–284.

Diaz M, Rosado JL, Allen LH & Garcia OP (2003) The efficacy of a local ascorbic acid-rich food in improving iron absorption from Mexican diets: a field study using stable isotopes. *Am J Clin Nutr* **78**, 436–440.

Frankel EN, Bosanek CA, Meyer AS, Silliman K & Kirk LL (1998) Commercial grape juices inhibit the in vitro oxidation of human low-density lipoproteins. *J Agric Food Chem* **46**, 834–838.

Graumlich JF, Ludden TM, Conry-Cantilena C, Cantilena LR, Wang Y & Levine M (1997) Pharmacokinetic model of ascorbic acid in healthy male volunteers during depletion and repletion. *Pharmaceut Res* **14**, 1133–1139.

Hankes LV, Jansen CR & Schmaeler M (1974) Ascorbic acid catabolism in Bantu with haemosiderosis (scurvy). *Biochem Med* **9**, 244–255.

Harper KA, Morton AD & Rolfe EJ (1969) The phenolic compounds of blackcurrant juice and their protective effects on ascorbic acid. III. The mechanisms of ascorbic acid oxidation and its inhibition by flavonoids. *J Fd Technol* **4**, 255–267.

Hartzler ER (1945) The availability of ascorbic acid in papayas and guavas. *J Nutr* **30**, 355–365.

Hawley EE, Stephens DJ & Anderson G (1936) The excretion of vitamin C in normal individuals following a comparable quantitative administration in the form of orange juice, cevitamic acid by mouth and cevitamic acid intravenously. *J Nutr* **11**, 135–145.

Hopper FC & Ayres AD (1950) The enzymatic degradation of ascorbic acid. Part I – The inhibition of the enzymatic oxidation of ascorbic acid by substances occurring in blackcurrants. *J Sci Fd Agric* **1**, 5–8.

Hornig D, Vuilleumier J-P & Hartmann D (1980) Absorption of large, single, oral intakes of ascorbic acid. *Int J Vit Nutr Res* **50**, 309–314.

Izzard AP, Bluck LJC & Bates CJ (1996) Measurement of stable isotope [13-C] ascorbate using gas chromatography with mass spectrometry (GCMS). *Proc Nutr Soc* **55**, 88A.

Johnston CS & Luo B (1994) Comparison of the absorption and excretion of three commercially available sources of vitamin C. *J Am Diet Assoc* **94**, 779–781.

Jones E & Hughes RE (1984) The influence of bioflavonoids on the absorption of vitamin C. *IRCS Med Sci* **12**, 320.

Kallner A, Hartmann D & Hornig D (1977*a*) Determination of bodypool size and turnover rate of ascorbic acid in man. *Nutr Metab* **21**, 31–35.

Kallner A, Hartmann D & Hornig D (1977*b*) On the absorption of ascorbic acid in man. *Int J Vit Nutr Res* **47**, 383–388.

Kallner A, Hartmann D & Hornig D (1979) Steady-state turnover and body pool of ascorbic acid in man. *Am J Clin Nutr* **32**, 530–539.

Kallner A, Hartmann D & Hornig D (1981) On the requirements of ascorbic acid in man: steady-state turnover and body pool in smokers. *Am J Clin Nutr* **34**, 1347–1355.

Kallner A, Hornig D & Pellikka R (1985) Formation of carbon dioxide from ascorbate in man. *Am J Clin Nutr* **41**, 609–613.

Lapidot T, Harel S, Granit R & Kanner J (1998) Bioavailability of red wine anthocyanins as detected in human urine. *J Agric Food Chem* **46**, 4297–4302.

Levine M, Conry-Cantilena C, Wang Y, *et al.* (1996) Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc Natl Acad Sci USA* **93**, 3704–3709.

Liang W-J, Johnson D & Jarvis SM (2001) Vitamin C transport systems of mammalian cells. *Mol Membr Biol* **18**, 87–95.

Lynch SR, Seftel HC, Torrance JD, Charlton RW & Bothwell TH (1967) Accelerated oxidative catabolism of ascorbic acid in siderotic Bantu. *Am J Clin Nutr* **20**, 641–647.

Mangels AR, Block G, Frey CM, Patterson BH, Taylor PR, Norkus EP & Levander OA (1993) The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. *J Nutr* **123**, 1054–1061.

Miller NJ (1998) Flavonoids and phenylpropanols as contributors to the antioxidant activity of fruit juices. In *Flavonoids in Health and Disease* pp. 387–403 [CA Rice-Evans and L Packer, editors]. New York: Marcel Dekker.

Nelson EW, Streiff RR & Cerda JJ (1975) Comparative bioavailability of folate and vitamin C from a synthetic and a natural source. *Am J Clin Nutr* **28**, 1014–1019.

Pelletier O & Keith MO (1974) Bioavailability of synthetic and natural ascorbic acid. *J Am Diet Assoc* **64**, 271–275.

Piotrovskij VK, Kallay Z, Gajdos M, Gerykova M & Trnovec T (1993) The use of a nonlinear absorption model in the study of ascorbic acid bioavailability in man. *Biopharm Drug Dispos* **14**, 429–442.

Rehman A, Collis CS, Yang M, Kelly M, Diplock AT, Halliwell B & Rice-Evans C (1998) The effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. *Biochem Biophys Res Commun* **246**, 293–298.

Serafini M, Maiani G & Ferro-Luzzi A (1998) Alcohol-free red wine enhances plasma antioxidant capacity in humans. *J Nutr* **128**, 1003–1007.

Song J, Kwon O, Chen S, Daruwala R, Eck P, Park JB & Levine M (2002) Flavonoid inhibition of sodium-dependent vitamin C transporter 1 (SVCT1) and glucose transporter isoform 2 (GLUT2), intestinal transporters for vitamin C and glucose. *J Biol Chem* **277**, 15252–15260.

Timberlake CF (1960) Metallic components of fruit juices IV. Oxidation and stability of ascorbic acid in blackcurrant juice. *J Sci Fd Agric* **11**, 268–273.

Todhunter EN & Fatzer AS (1940) A comparison of the utilisation by college women of equivalent amounts of ascorbic acid (vitamin C) in red raspberries and in crystalline form. *J Nutr* **19**, 121–130.

Vinson JA & Bose P (1988) Comparative bioavailability to humans of ascorbic acid alone or in a citrus extract. *Am J Clin Nutr* **48**, 601–604.

Vuilleumier JP & Keck E (1989) Fluorometric assay of vitamin C in biological materials, using a centrifugal analyser with fluorescence attachment. *J Micronutr Anal* **5**, 25–34.

Yang M, Collis CS, Kelly M, Diplock AT & Rice-Evans C (1999) Do iron and vitamin C co-supplementation influence platelet function or LDL oxidizability in healthy volunteers? *Eur J Clin Nutr* **53**, 367–374.

Zetler G, Seidel G, Siegers C-P & Iven H (1976) Pharmacokinetics of ascorbic acid in man. *Eur J Clin Pharmacol* **10**, 273–282.