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

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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-\([1-\text{13C}]\)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 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 \(14\text{CO}_2\) if \([1-\text{14C}]\)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.

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Subjects and methods

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

The supplier of $[1-^{13}$C]$\text{ascorbic acid}$ was Cambridge Isotopes Ltd, MA, USA. $[1-^{13}$C]$\text{ascorbic acid}$, 98–99 % isotopic purity, was used. It is one mass unit heavier than the major natural isotope form in unlabelled ascorbic acid (molecular mass 176·13). The desiccated crystalline solid was stored at $-25^\circ$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]$\text{ascorbate}$ is a suitable probe (Black et al. 2002), 30 mg $[1-^{13}$C]$\text{ascorbate}$ 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 with a red grape juice drink (Tesco, UK; own brand) containing antioxidant polyphenol pigments. It contained 2·8 MJ (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, $\alpha$-phenylene diamine was added to form fluorescent quinoxaline from the resulting dehydrorscorbate. 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 $\mu$mol/l).

Isotope ratio measurements by GC–MS

Derivatisation of ascorbate to yield volatile trimethylsilyl derivaties 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 $\times$ 0·25 mm (internal diameter) with a film thickness of 0·25 $\mu$m (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.

Table 1. Anthropometry and baseline ascorbate

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

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.

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 ($P=0·009$) by paired t test at 20 min post-dose, and marginally significant ($P=0·04$) 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 $P<0·05$.

The $^{13}$C kinetic variables obtained when [13C]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 ($P=0·022$). Thus, the larger the pre-existing pool size, the smaller the pool enrichment for a constant magnitude of tracer
paired 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 nations of vitamin C and iron or vitamin C and grape juice.

$\text{Tracee concentration} = \text{mean concentration of tracee, i.e. native unlabelled ascorbate (\text{\mu mol/l})}$

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 ($P=0.04$); 20 min ($P=0.009$); 24 min ($P=0.04$); 28 min ($P=0.04$). 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 ($P=0.033$) and fractional enrichment ($P=0.04$), 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 ($P=0.05$). The times to reach 10, 50, 90 % and maximum slope for tracer entry were all directly related to the plasma ascorbate concentration ($P=0.02$ to $P=0.04$). This may be a function of the partial saturation of the ascorbate transporters. There was a weak inverse relation ($P=0.04$ to $P=0.05$) 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

<table>
<thead>
<tr>
<th>Index§</th>
<th>Lean body mass</th>
<th>Body weight</th>
<th>BMI</th>
<th>Body fat</th>
<th>Age</th>
<th>Gender</th>
<th>Mean ascorbate concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracer: $C_{\text{max}}$</td>
<td>$-1.6$</td>
<td>$-1.8$</td>
<td>$-0.4$</td>
<td>$+0.4$</td>
<td>$-0.2$</td>
<td>$+0.7$</td>
<td>$+1.1$</td>
</tr>
<tr>
<td>Tracee concentration</td>
<td>$+0.5$</td>
<td>$+0.1$</td>
<td>$+0.2$</td>
<td>$-0.7$</td>
<td>$-0.1$</td>
<td>$-0.5$</td>
<td>$-$</td>
</tr>
<tr>
<td>Fractional enrichment</td>
<td>$-2.4^*$</td>
<td>$-2.2^*$</td>
<td>$-0.5$</td>
<td>$+0.9$</td>
<td>$-0.2$</td>
<td>$+1.1$</td>
<td>$-0.5$</td>
</tr>
<tr>
<td>$T_{0.1}$</td>
<td>$+1.1$</td>
<td>$+0.1$</td>
<td>$-0.5$</td>
<td>$-1.6$</td>
<td>$-0.2$</td>
<td>$-1.2$</td>
<td>$+3.4^*$</td>
</tr>
<tr>
<td>$T_{0.5}$</td>
<td>$+0.3$</td>
<td>$-0.3$</td>
<td>$0.0$</td>
<td>$-0.7$</td>
<td>$0.0$</td>
<td>$-0.5$</td>
<td>$+3.5^*$</td>
</tr>
<tr>
<td>$T_{0.9}$</td>
<td>$-0.7$</td>
<td>$-0.7$</td>
<td>$+0.5$</td>
<td>$+0.3$</td>
<td>$-0.2$</td>
<td>$+0.5$</td>
<td>$+2.9^*$</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>$+1.5$</td>
<td>$+0.4$</td>
<td>$-0.7$</td>
<td>$-1.9$</td>
<td>$+0.3$</td>
<td>$-1.8$</td>
<td>$+1.9$</td>
</tr>
<tr>
<td>$T_{\text{lat}}$</td>
<td>$-0.8$</td>
<td>$-2.0$</td>
<td>$-1.7$</td>
<td>$-0.9$</td>
<td>$-1.4$</td>
<td>$+0.5$</td>
<td>$+0.8$</td>
</tr>
<tr>
<td>$T_{\text{ascend}}$</td>
<td>$-1.5$</td>
<td>$-0.9$</td>
<td>$+0.9$</td>
<td>$+1.2$</td>
<td>$-0.4$</td>
<td>$+1.4$</td>
<td>$+1.6$</td>
</tr>
</tbody>
</table>

* $P < 0.05$.

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

‡ $T_{\text{ascend}}$: time between $T_{\text{lat}}$ and $T_{\text{max}}$.

§ $T_{0.1}$, time to 10 % of maximum tracer concentration; $T_{0.5}$, time to 50 % of maximum tracer concentration; $T_{0.9}$, time to 90 % of maximum tracer concentration; $T_{\text{max}}$, time to maximum tracer concentration (estimated from intercept of linear ascending slope and the horizontal maximum concentration); $T_{\text{lat}}$, time of latency (estimated from intercept of the linear ascending slope and the horizontal latency line); $T_{\text{ascend}}$, time between $T_{\text{lat}}$ and $T_{\text{max}}$. For details of subjects and procedures, see Table 1 and pp. 700–701.
enrichment; this is consistent with the known decline in ascorbate stores (ascorbate retention) with increasing age. There was a marginally higher value for \( T_{\text{ascend}} \) in female than in male subjects.

Comparison of the key kinetic indices, \( C_{\text{max}} \) 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: \( P=0.7 \) and \( P=0.15 \) for \( C_{\text{max}} \) (Fe and grape juice treatments respectively) and \( P=0.8 \) and \( P=0.15 \) for tracer enrichment (Fe and grape juice respectively). None of these treatment effects was significant at \( P<0.05 \).

The \( ^{13} \text{C} \) enrichment of \( \text{CO}_2 \) in expired air was below the minimum measurable value for all subjects, and even in the presence of Fe, \(<3\% \) of the dose was converted to \( ^{13} \text{CO}_2 \). 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 \( \mu \)mol/l) was about twice the ascorbate concentration increment that was estimated from the change in tracer:tracee ratios (about 2 \( \mu \)mol/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 \( ^{1-14} \text{C} \)) ascorbate and its degradation products after ingesting labelled ascorbate.
4. Plasma and/or urine kinetics after ingesting stable isotopically-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 \([1-^{14} \text{C} ]\)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 \([1-^{14} \text{C} ]\)ascorbate also measured turnover (Kallner et al. 1977a,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 \([1-^{14} \text{C} ]\)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 \( ^{14} \text{C} \)-labelled vitamin C was extensively degraded, yielding up to 35% of its \( ^{14} \text{C} \) label as \( ^{14} \text{CO}_2 \).

**Stable isotope dose kinetics.** Atkins et al. (1964) gave 50 mg \([1-^{13} \text{C} ]\)ascorbic acid to adult male subjects and collected urine for 50 d. Urinary ascorbate and oxalate were isolated by fractional crystallisation and \( ^{13} \text{C} \)-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 \( ^{13} \text{C} \)-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. 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. 1999), 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 [13C]ascorbate dose of 30 mg, the ingested dose ought in theory to be distributed into a pool of 15 × 30 = 450 mg ascorbate. However, the observed mean increase in plasma ascorbate according to the [13C] 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 13C-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, myricetin, 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 [13C]ascorbate dose to 13CO2 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.

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References
