OPINION OF THE SCIENTIFIC PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES ON A REQUEST FROM THE COMMISSION RELATED TO THE TOLERABLE UPPER INTAKE LEVEL OF VITAMIN C (L-ASCORBIC ACID, ITS CALCIUM, POTASSIUM AND SODIUM SALTS AND L-ASCORBYL-6-PALMITATE)

(REQUEST N° EFSA-Q-2003-018)

(ADOPTED ON 28 APRIL 2004)

SUMMARY

The European Food Safety Authority is asked to derive an upper level for the intake of vitamin C from food that is unlikely to pose a risk of adverse health effects.

Vitamin C is a water soluble vitamin that is an important anti-oxidant in the body. Insufficient intake results in the deficiency condition scurvy.

The vitamin is of low acute toxicity as indicated by the limited data available from studies in animals and humans. Despite the extensive use of high doses of vitamin C in some vitamin supplements, there have been few controlled studies that specifically investigated adverse effects. Overall, acute gastrointestinal intolerance (e.g., abdominal distension, flatulence, diarrhoea, transient colic) is the most clearly defined adverse effect at high intakes, but there are limited data on the dose-response relationship for adults or for groups such as children or the elderly. While there is uncertainty whether high intakes of vitamin C increase renal excretion of oxalate which could increase the risk of renal stones, an increased risk of kidney stones was not found in individuals with habitual intakes of 1.5 g/day. There are insufficient data to establish a tolerable upper intake level for vitamin C.

The available human data suggest that supplemental daily doses of vitamin C up to about 1 g, in addition to normal dietary intakes, are not associated with adverse gastrointestinal effects, but that acute gastrointestinal effects may occur at higher intakes (3-4 g/day). The absorption of vitamin C is saturated at high doses, and therefore intakes above 1 g/day would be associated with negligible increased uptake and tissue levels, but an increased risk of adverse gastrointestinal effects.

The average daily intakes reported in surveys in European countries are above the recommended daily intakes, with the 95th percentile intakes from food and supplements ranging up to about 1 g/day. These dietary intakes do not represent a cause for concern.

There has not been a systematic assessment of the safety of the long-term use of high dose vitamin C supplements.

KEY WORDS

Vitamin C, ascorbic acid, tolerable upper intake level, gastrointestinal effects, food safety.

BACKGROUND

In 2002, the European Parliament and the Council adopted Directive 2002/46/EC[1] related to food supplements containing vitamins and minerals.

In addition, and as announced in its White Paper on Food Safety, the Commission aims to put forward a proposal for harmonising legislation concerning the addition of vitamins and minerals to foods.

With a view to provide scientific support to the European Commission's legislative work in this field, the Scientific Committee on Food (SCF) issued, from October 2000 to April 2003, a series of opinions on tolerable upper intake levels of individual vitamins and minerals and safety factors in relation to their use in fortified foods and food supplements (available on the Internet at: http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).

1 - Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements. OJ L 183. 12.7.2002, p. 51.

The SCF opinions covered 22 out of the 29 nutrients, which were considered to be within their mandate for this task. The SCF did not have sufficient time to adopt opinions for the following vitamins and minerals: vitamin C, chloride, fluoride, iron, phosphorus, potassium and sodium. In addition, during the decision making process for the adoption of Directive 2000/46/EC on food supplements the Parliament requested that boron, nickel, silicon, vanadium and tin should be allowed to be used in food supplements. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the remaining 12 vitamins and minerals in accordance with the present terms of reference.

TERMS OF REFERENCE

With respect to the outstanding 12 vitamins and minerals, the European Food Safety Authority is asked 1) to review the upper levels of daily intakes that are unlikely to pose a risk of adverse health effects; 2) to provide the basis for the establishment of safety factors, where necessary, which would ensure the safety of fortified foods and food supplements containing the aforementioned nutrients.

ASSESSMENT

1. INTRODUCTION

Vitamin C (3-oxo-L-gulofuranolactone or L-threo-hex-2-enonic acid) is a 6-carbon hydroxy-lactone that is structurally related to glucose. It is a micronutrient essential to humans, primates and guinea pigs, but which is synthesised by other mammalian species from glucose and galactose. It is readily oxidised to L-dehydroascorbic acid, in which the unsaturated 2,3-dihydroxy group is replaced by a saturated 2,3-diketone function; L-dehydroascorbic acid can be reduced back to ascorbic acid.

Vitamin C is highly water soluble, and in solution can be oxidised by atmospheric oxygen to give an equilibrium mixture of ascorbic and dehydroascorbic acids. Vitamin C has important anti-oxidant properties, and protects cells against oxidative stress. Because of this general cytoprotective role, its importance has been investigated in a variety of clinical conditions, including cancer, vascular disease, and cataracts.

Vitamin C deficiency in humans leads to clinical syndromes known as scurvy in adults and Moeller-Barlow disease in children (SCF, 1993), conditions which are associated with intakes of less than 10 mg/day. Early or prescorbutic symptoms in adults include fatigue, weakness, anaemia and aching joints and muscles, while there are important effects on bone tissue in children. Later stages of deficiency are characterised by capillary fragility causing bleeding from the gums and haemorrhages, and delayed wound healing due to impaired collagen synthesis.

Advice by Linus Pauling that daily intakes of 1 g or more of vitamin C can protect against the common cold (see Miller and Hayes, 1982) was followed by other claims of beneficial effects on a variety of conditions. Because of the media attention given to these claims and the apparently low toxicity of vitamin C there has been extensive human exposure to intakes up to 10 g/day (Miller and Hayes, 1982). However despite this extensive human exposure, there are only limited data that are appropriate for use in risk assessment.

Recent reviews of vitamin C by the Food and Nutrition Board in the USA (FNB, 2000) and the Expert Group on Vitamins and Minerals in the UK (EGVM, 2003) have recommended an upper level of 2 g/day and a guidance level of 1 g/day, as supplemental intake, respectively.

2. NUTRITIONAL BACKGROUND, FUNCTION, METABOLISM AND INTAKE

Major food sources of vitamin C are plants such as citrus fruits, soft fruits and green vegetables. Animal tissues also contain vitamin C, with kidney and liver representing good sources. The amounts of vitamin C present in the food when consumed may be reduced, because it is readily lost due to dissolution in water and oxidation during cooking processes such as boiling.

Ascorbic acid is a permitted anti-oxidant additive in food, with no specified limits on the level of use. Vitamin C is present in numerous dietary supplements with manufacturer recommended daily intakes of 60-3000 mg in single vitamin preparations and 10-1000 mg/day in multi-vitamin preparations (EGVM, 2003).

In 1992, the Scientific Committee for Food (SCF, 1993) recommended a Population Reference Intake of 45 mg/day for adults, with an increase to 55 mg/day in pregnancy, and to 70 mg/day during lactation.

Vitamin C has a number of biochemical roles in the body (Basu and Dickerson, 1996). It is a strong reducing agent and antioxidant, which is important in preventing the damaging effects of free radicals. Vitamin C is an enzyme co-factor for many biochemical reactions, especially those involving oxidations, such as the synthesis of hydroxyproline from proline for collagen biosynthesis, mono-oxygenases, dioxygenases and mixed function oxygenases. It is important in the synthesis and stabilisation of neurotransmitters and carnitine, and increases the gastrointestinal absorption of non-haem iron by reducing ferric to ferrous iron (SCF, 1993).

Gastrointestinal absorption of low doses of vitamin C is efficient, and occurs in the small intestine via a sodium-dependent active transport mechanism. The extent of absorption of vitamin C is 80-90% at the usual intakes from food of 30-180 mg/day (SCF, 1993), but because the transporter is saturable, absorption efficiency gradually decreases at higher intakes (Kallner *et al*, 1979 and 1985; Hornig and Moser, 1981; Blanchard *et al*, 1997). There is a non-linear relationship between daily intake of vitamin C and plasma concentrations, with a 5-fold increase in intake from 0.5 g/day to 2.5 g/day producing only a 20% increase in plasma levels (Levine *et al*, 1996 and 1999). Ascorbyl palmitate is probably hydrolysed in the lumen of the gastrointestinal tract prior to absorption, but data defining the *in vivo* fate of this synthetic form of vitamin C have not been identified.

Ascorbic acid is widely distributed in all tissues of the body, with higher levels found in the adrenal and pituitary glands and the retina, and lower levels in kidney and muscle tissue. Vitamin C can be detected in most tissues and exists as an equilibrium mixture of ascorbic acid and dehydroascorbic acid, dependent on the redox status of the cells. Plasma and urinary vitamin C are not reliable indicators of body stores of vitamin C because they are influenced by recent dietary intake. Leukocytes contain higher concentrations of vitamin C than plasma, blood or serum (Levine *et al*, 1996), and may provide a more reliable indicator of status. A vitamin C concentration in leukocytes below 0.01 mg per 10^8 cells is generally regarded as indicative of deficiency.

Vitamin C is readily oxidised to dehydroascorbic acid, which can be reduced back to ascorbic acid or hydrolysed to diketogulonic acid and then oxidised to oxalic and threonic acid, xylose, xylonic acid and lyxonic acid (Basu and Dickerson, 1996). Some oxidation to carbon dioxide occurs at high doses, possibly due to metabolism of unabsorbed ascorbate by the intestinal microflora (Kallner *et al*, 1985). Ascorbic acid may also undergo limited conjugation with sulphate to form ascorbate-2-sulphate, which is excreted in urine. Unchanged ascorbic acid and its metabolites are excreted in the urine. Approximately 3% of a 60 mg oral dose is eliminated in the faeces. At intakes above 80-100 mg/day, most of the additional absorbed vitamin is excreted unchanged in the urine, indicating that tissue reserves are saturated at this intake level (SCF, 1993; FNB, 2000). This increasing renal elimination of ascorbic acid with increase in dose results in an inverse relationship between the elimination half-life and the dosage (Kallner *et al*, 1979) and probably arises from saturation of reabsorption from renal tubule (Blanchard *et al*, 1997).

The average daily intakes in European countries are above the reference intake established by the SCF in 1993, with relatively consistent data in different countries (Table 1). The 97.5th percentiles of intake are about 5-6 times the reference intake.

3. HAZARD IDENTIFICATION

3.1. Genotoxicity

The genetic toxicology of ascorbic acid was reviewed by Shamberger (1984) at which time there was evidence for indirect mutagenic effects via the generation of oxidative damage in the presence of transition metals, and also for anti-mutagenic effects in a variety of systems.

Vitamin C would be expected to be anti-mutagenic, because of its antioxidant properties, and there are data consistent with this. For example, ascorbic acid reduces the spontaneous mutation rate in mismatch repair-defective cells (Glaab *et al*, 2001), protects against gamma-ray induced damage (Konopacka and Rzeszowska-Wolny, 2001) and reduces the activity of some genotoxic compounds (Blasiak *et al*, 2001; Nefic, 2001; Rao *et al*, 2001; Kaya *et al*, 2002; Chang *et al*, 2002), including important food-borne mutagens such as patulin (Alves *et al*, 2000) and toxins such as zearalenone and ochratoxin A (Grosse *et al*, 1997 - based on a reduction in DNA adducts measured by ^{32}P-post labelling).

Table 1. The daily intakes of vitamin C in EU countries (mg/day)

	Population	N	Method	Supplements	Mean	97.5%
Austria[a]	men + women	2488	24 h recall	Not defined	88	276
Germany[b]	men women	854 1134	7-day record	Not defined Not defined	70 83	270 282
Germany[c]	men women men women	1268 1540 240 347	Computer-assisted dietary interview	- - + +	150 151 168 156	180 176 309 285
Italy[d]	household	2734	7-day record	+	113	268
Ireland[e]	men women men women	662 717 662 717	7-day record	- - + +	81 72 116 108	212 187 588 588
Netherlands[f]	household	5958	2-day record	-	78	204
Sweden[g]	men women men women	1897 2223 770 1655	8-day record	- - + +	87 103 151 169	201 224 1056 1117
UK[h]	men women men women	833 891 833 891	7-day record	- - + +	83 (71) 81 (69) 101 (74) 112 (76)	217 205 329 473

[a] Elmadfa *et al* (1998)
[b] Heseker *et al* (1992) - median not mean value
[c] Mensink and Ströbel (1999); Mensink *et al* (2002) - values are the mean and 75th percentile
[d] Turrini (INRAN)
[e] IUNA (2001)
[f] Hulshof and Kruizinga (1999)
[g] Elmstahl *et al* (1994) - values are the median and 95th percentile
[h] Henderson *et al* (2003) - values are the mean (with the median in parentheses)

Although ascorbic acid is an antioxidant via its conversion to dehydroascorbic acid, the reversibility of the reaction can lead to the generation of reactive oxygen species via redox cycling (Ballin *et al*, 1988; Stadtman, 1991). This may be involved in some positive genotoxic effects reported with vitamin C *in vitro*, such as single strand breaks produced by sodium ascorbate (Singh, 1997), weak sister chromatid exchange (SCE) activity (Galloway and Painter, 1979; Speit *et al*, 1980; Best and McKenzie, 1988) and activity in the comet assay (Blasiak *et al*, 2000). Ascorbic acid has been reported to increase the genotoxicity of mitomycin C (Krishnaja and Sharma, 2003), cadmium chloride (Blasiak *et al*, 2000) and cobalt chloride (Kaya *et al*, 2002). The results obtained may depend on the concentrations of vitamin C studied, with protective activity at low concentrations but cytotoxicity and an increase in genotoxic activity at concentrations more than 200 μg/mL (Antunes and Takahashi, 1999). Oral and intraperitoneal doses of up to 10 g/kg in hamsters did not induce SCEs (Speit *et al*, 1980).

Positive genotoxicity results tended to occur *in vitro* when vitamin C was tested in the presence of metal ions such as iron and copper, which may be related to its reduction of the metal followed by the formation of highly reactive hydroxyl radicals via a Fenton reaction (Carr and Frei, 1999). However, DNA-reactive species can be generated by interaction between vitamin C and lipid hydroperoxide decomposition in the absence of transition metal ions (Lee *et al*, 2001); the *in vitro* concentrations used in this study were similar to those present in plasma after doses of 200 mg/day, but the toxicological significance of this observation is unclear.

Podmore *et al* (1998a) reported that the administration of 500 mg/day of vitamin C supplements to 28 human volunteers for 6 weeks caused an increase in 8-oxoadenine but a decrease in 8-oxoguanine in the DNA of isolated lymphocytes measured by gas chromatography-mass spectrometry (GC-MS), possibly due to its pro-oxidant effect. Two letters were published as a consequence of this report, which pointed out that the dosage given would not have increased the intracellular concentrations of ascorbate in lymphocytes, and that the increased oxidation could be an artefact formed by monocytes

in the lymphocyte preparation (Levine *et al*, 1998), that artefacts were not adequately excluded, the study was not a randomised double-blind placebo controlled investigation, and the authors did not give information about smoking habits or cite previous publications (Poulsen *et al*, 1998). In their reply to these letters, Podmore *et al* (1998b) provided further support for the validity of their findings and pointed out that because 8-oxoadenine is 10-times less mutagenic than 8-oxoguanine, their results are consistent with an "overall profound protective effect".

An increase in total damage to lymphocyte DNA bases (but not in 8-hydroxyadenine or 8-hydroxyguanine) was reported in a study in which one group of 20 volunteers who were given 14 mg iron plus either 60 or 260 mg of vitamin C, but not in a second group of 18 subjects who were treated in the same way but had slightly lower pre-treatment plasma levels of ascorbic acid (Rehman *et al*, 1998). In a subsequent paper by the same research group, treatment of 20 healthy adults with ascorbate (280 mg/day), with or without supplemental iron (14 mg/day), for periods of 6 weeks in a cross-over design showed no significant rise in oxidative DNA damage as measured by GC-MS (Proteggente *et al*, 2000): significant decreases in 8-oxo-7,8-dihydroguanaine and 5-hydroxymethyl uracil were found during treatment with ascorbate and the authors concluded that there was no compelling evidence of a pro-oxidant effect resulting in DNA damage. *In vivo* administration of 1g vitamin C daily for 42 days to human volunteers did not influence the extent of endogenous DNA damage in peripheral lymphocytes measured using an Elisa technique, but reduced the extent of *ex vivo* peroxide-induced damage (Brennan *et al*, 2000). The significance of these observations is unclear and currently under further study.

Overall, the data currently available do not allow an adequate evaluation of the genotoxic potential of high intakes of vitamin C, and in particular its capacity to induce gene and chromosomal mutations. The significance of oxidative DNA damage observed *in vitro* or *in vivo* at high concentrations is unclear.

3.2. Animal toxicity data

Vitamin C has low acute toxicity. High doses of vitamin C (100 mg per 160 g animal per day) are associated with decreased growth rates in guinea pigs fed a nutritionally inadequate diet of unfortified wheat flour, but weight gain was not altered if the treated animals were fed a wheat flour diet fortified with casein (Nandi *et al*, 1973). No effects on reproductive or developmental parameters were found in guinea pigs, rats and hamsters given oral doses of up to 400 mg/kg body weight/day during pregnancy (Alleva *et al*, 1976) or in rats and mice given up to 1000 mg/kg body weight/day (Frohberg *et al*, 1973). A conditioned increase in vitamin C requirements has been reported in guinea pigs (Sorensen *et al*, 1974).

There have been a number of studies in which rats have been given high dietary concentrations of the sodium salt of ascorbic acid and the free acid in relation to the role of sodium ions in the generation of bladder hyperplasia and cancer in male rats. Using a 2-stage model of bladder carcinogenesis, in which male rats were treated with possible promoters of bladder carcinogenesis for 6 weeks, Cohen *et al* (1991) showed that sodium ascorbate at 5% in the diet (equivalent to about 2500 mg/kg body weight/day) increased the incidence of bladder cancers, but that an equimolar dietary concentration of ascorbic acid (4.44%) was inactive. In a subsequent study in which sodium ascorbate was given in the diet to rats without pre-treatment with a carcinogen, significant increases in simple, papillary and nodular hyperplasia in the urinary bladder were detected in rats fed diets containing 5% or 7% sodium ascorbate, but these effects were abolished by co-treatment with ammonium chloride which acidified the urine (Cohen *et al*, 1998); there was a small and non-significant increase in the numbers of papillomas and carcinomas in the urinary bladder at dietary levels of 5% (n=1) and 7% (n=2) compared to control (n=0) or 1% dietary level (n=0). In 1993, the JECFA concluded that similar effects produced by the sodium salt of saccharin were related to sodium-induced changes in urine volume, osmolality and pH, and were not relevant to human health (JECFA, 1993).

Dietary administration of ascorbyl palmitate at levels of 2000 ppm and 4000 ppm (equivalent to daily intakes of about 100 and 200 mg/kg body weight per day) to male rats treated with the colon carcinogen azoxymethane caused a significant reduction in the incidence (% of animals with tumours) and the multiplicities of invasive and total adenocarcinomas of the colon (Rao *et al,* 1995). In contrast, administration of 2% ascorbyl palmitate in the diet of mice (equivalent to daily intakes of about 2000 mg/kg body weight per day) did not attenuate the hyperplastic and dysplastic effects of azoxymethanol in the colon (Huang *et al*, 1992).

3.3. Human data

Despite a number of clinical studies in which high doses of vitamin C (up to 1 g or more per day) have been given, there is a limited database on tolerability or adverse effects. A number of studies with different doses and durations have not reported adverse effects, but it is difficult to determine the significance of these because it is frequently unclear how any adverse effects were investigated (reviewed in Carr and Frei, 1999) (EGVM, 2003). In addition many studies have used a combination of vitamins and minerals, and identifying any effect of vitamin C *per se* is not possible because other parts of the treatment could mask adverse effects.

Adverse effects were not reported in recent studies in which 12 healthy adult volunteers received 500 mg/day for 8 weeks (McArdle *et al*, 2002), 19 patients with hypertension received 500 mg/day for 30 days (Duffy *et al*, 1999), 28 male smokers received 500 mg/day for 4 weeks (Aghdassi *et al*, 1999), 18 healthy male adults given 2 g/day for 6 weeks (Tofler *et al*, 2000), 130 healthy adults given 250 mg of slow release vitamin C for 3 years (Salonen *et al*, 2000), 8 adults received increasing daily doses up to a maximum of 2 g/day for 2 weeks (Johnston and Cox., 2001), 5 adults received 1 g/day for 6 months (Pullin *et al*, 2002) and 30 adults given daily doses of 500 mg for 6 weeks (Hamilton *et al*, 2000). Most of these studies had primary endpoints related to a health benefit, and assessment of adverse effects or tolerability was not a part of the study design.

A double-blind, cross-over study on the effects of daily doses of 3 g of vitamin C, combined with very high doses of nicotinamide (3 g), calcium pantothenate (1.2 g) and pyridoxine (0.6 g), in 41 children with attention deficit disorders (Haslam *et al*, 1984) reported an increase in serum transaminases. However, this cannot be assigned to the vitamin C component, because of the complex megavitamin regimen. There was little information reported on general tolerability, but 3 children did not complete the study because of excessive vomiting, abdominal discomfort or an inability to swallow the vitamin capsules and “some patients experienced nausea and vomiting during the course of treatment”.

Vitamin C was administered to 10, 269 adults aged 40-80 with coronary disease at a daily dose of 250 mg/day (in combination with 600 mg vitamin E and 20 mg β-carotene) for up to 5 years. The subjects showed good compliance and there were no significant differences in mortality or morbidity compared with a placebo group of equal size (Heart Protection Study, 2002). No effects on inflammatory markers were reported in a recent long-term multi-vitamin study (Bruunsgaard *et al*, 2003) in which 52 men aged 47-70 were treated for 3 years with a combination of 500 mg vitamin C and 182 mg of α-tocopherol daily, but it is unclear to what extent other effects, such as gastrointestinal problems, would have been recorded.

A retrospective cohort study of 994 women, of whom 277 were regular users of vitamin C supplements for up to 12 years, reported a significant increase in bone mineral density of the neck of the femur; no side effects were reported but no parameters other than bone mineral density were assessed (Morton *et al*, 2001).

Adverse effect data were not reported in a 4-year double-blind, placebo-controlled study in which patients with a history of adenoma of the large bowel were given vitamin C (1 g/day) with either vitamin E (205 patients) or vitamin E plus β-carotene (175 patients) (Greenberg *et al*, 1994). Adherence to the prescribed regimen, and information about symptoms, illnesses and hospitalisations were assessed every 6 months. The numbers of patients who withdrew from the study were similar in all groups, but 4 subjects in the two vitamin C treatment groups “stopped taking the medications because of their presumed toxicity”; no other information was provided and tolerability during the study was not reported.

No subjective side effects were reported in the study of Cook *et al* (1984) in which 17 adults were given 2 g/day with meals for 16 weeks.

No adverse effects were reported in a randomised, double-blind, placebo-controlled study in which 21 patients with coronary artery disease were given a single dose of 2 g vitamin C followed by 500 mg/day for 30 days (Gokce *et al*, 1999).

3.3.1. Gastrointestinal effects

Gastrointestinal effects are the most common adverse clinical events associated with acute, high doses of vitamin C (above 1 g daily), but these can be reduced by taking the vitamin after meals (reviewed in Miller and Hayes, 1982). The incidences of stomach pains, nausea and diarrhoea in children given 1 g/day for 3 months were similar to those in the control groups (Ludvigsson *et al*, 1977). Abdominal

distension, flatulence, diarrhoea and transient colic were reported as “fairly frequent” in a study in healthy human volunteers given daily doses which increased by 1000 mg per day each week, with adverse effects reported at doses of 3-4 g/day, although no details were given of the exact dosing regimen or the numbers of subjects studied or their age, sex or body weight (Cameron and Campbell, 1974). Two out of 15 volunteers experienced diarrhoea when consuming 10 g of vitamin C daily for 5 days in a clinical study on oxalate excretion, despite the fact that the subjects were advised to take the vitamin C tablets at mealtimes to minimise the potential for adverse gastrointestinal effects (Wandzilak *et al*, 1994).

3.3.2. Renal effects

Adverse effects related to the renal system have been reported, including renal stones, renal tubular disease and oxaluria. Vitamin C consumption has been suggested to increase oxalate excretion and the risk of urinary stone formation, but the available data are both confusing and contradictory. An early report stated that there could be wide inter-subject differences in the excretion of oxalate following high doses of vitamin C (Briggs, 1976). An additional problem is that urinary oxalate can be produced from urinary ascorbic acid as an artefact of the analytical procedure, so that the validity of the analytical data depends on the extent to which this was controlled by the use of preservatives. Increased oxalate excretion would represent a risk factor for the formation of bladder stones, and there have been anecdotal case reports of kidney stones or other nephropathy (Nakamoto *et al*, 1998) in patients who have taken high daily doses of vitamin C.

Groups of 3 patients who had unilateral nephrostomy tubes after lithotripsy for renal stones were given supplemental doses of 100, 500, 1000, or 2000 mg ascorbic acid on days 2 and 3 postoperatively (Urivetzky *et al*, 1992). Urine specimens were collected from the nephrostomy catheter and also from the contralateral kidney directly into EDTA and sodium thimerosol preservative to stabilise ascorbic acid and oxalate; oxalate was measured following the removal of ascorbic acid with sodium nitrite. There was a statistically significant increase in urinary oxalate at doses of 1000 and 2000 mg. The authors estimated that there was a 6-13 mg/day increase in urinary oxalate excretion per 1000 mg/day ascorbic acid intake, and concluded that there was an increased risk of calcium oxalate renal stones.

Urinary excretion of oxalate was measured in 15 volunteers given ascorbic acid supplementation (1, 5 and 10 g/day for 5 days in a cross-over design) (Wandzilak *et al*, 1994). The 24-hour urine samples were preserved by reducing the pH to 2 by adding 20 mL of concentrated hydrochloric acid. Ascorbate was reported to be converted non-enzymatically into oxalate during analytical measurement. The study did not find an increase in urinary oxalate excretion after *ex vivo* non-enzymatic conversion of ascorbate to oxalate had been taken into account.

Supplemental vitamin C intakes of 1 g/day in 7 volunteers caused statistically significant increases in urinary excretion of oxalate (determined by an enzymatic method reported to be free from interference by ascorbic acid) (Levine *et al*, 1996). An increase in urinary oxalate excretion, measured by an enzymatic assay on urine collections stabilised with acid, was also reported in 6 subjects given 1 g of supplemental ascorbate with 2.85 litres of orange juice containing 0.62 g of ascorbate daily for 4 days, but not in the same subject given 2 g of ascorbate per day for 4 days (Liebman *et al*, 1997).

Auer *et al* (1998a) investigated the urinary excretion of oxalate in the presence and absence of EDTA preservation of the urine samples in 10 healthy male volunteers (with no history of stone formation) given 4 g of vitamin C daily for 5 days. Erroneously high oxalate concentrations were found in the absence, but not in the presence of EDTA. There was no significant increase in oxalate excretion at any stage of the protocol in EDTA preserved samples and it was concluded that large doses of vitamin C did not affect the principal risk factors associated with calcium oxalate kidney stone formation. In contrast, the same authors (Auer *et al*, 1998b) reported increased excretion of oxalate in EDTA treated urine samples from a single volunteer who took 8 g daily for a period of 8 days, at which time the study was terminated because of the detection of haematuria, which was associated with crystalluria.

A prospective study on the relationship between vitamin C intake and the risk of symptomatic kidney stones in a group of 45,251 men (Curhan *et al*, 1996) found no association with vitamin C intake in 751 cases of kidney stones. The age-adjusted relative risk for subjects with intakes of 1.5 g/day or more compared with less than 0.25g/day was 0.78 (95% confidence intervals 0.54-1.11), indicating that even if such doses do increase oxalate excretion, it is not a clinically significant effect. A similar study in a cohort of 85,557 women in whom there were 1078 incidences of kidney stones showed a relative risk of 1.06 (95% confidence intervals 0.69-1.64) for subjects with intakes of 1.5 g/day or more compared with less than 0.25 g/day (Curhan *et al*, 1999).

No significant relationships were found in an analysis of data from 5214 men and 5785 women between serum vitamin C concentrations and the prevalence of kidney stones, serum vitamin B_{12} levels, or serum ferritin levels in men, but a negative correlation with serum ferritin was found for women (Simon and Hudes, 1999).

Increased excretion of uric acid has also been reported after the ingestion of 4 g or 8 g of ascorbic acid (Stein *et al*, 1976); although the available data at lower doses are limited and conflicting, in all studies hyperuricosuria was absent at doses of less than 1 g (Levine *et al*, 1999).

3.3.3. Other effects

Other anecdotally reported adverse effects include metabolic acidosis and changes in prothrombin activity, but a double-blind trial in patients given 200 mg/day showed no significant effect on the incidence of thrombotic episodes (Hornig and Moser, 1981).

A low incidence of adverse effects was reported during a study in patients with multiple sclerosis who were randomised to receive either supplements providing 2 g/day vitamin C, together with 6 mg/day sodium selenite and 480 mg/day vitamin E, or placebo for 5 weeks. The patients were interviewed about side effects after 2 weeks and 4 weeks of treatment. One out of the 10 patients receiving the active supplement reported slight facial erythema at week 2, which subsequently subsided during continued treatment, one reported a peculiar urine smell and another reported an increased number of headaches; three of the 10 patients receiving the placebo reported an increased number of headaches (Mai *et al*, 1990). It cannot be determined from the data whether these were caused by the high doses of vitamin C or by the other constituents.

There is a suggestion in the literature of conditioned need-scurvy, in which scurvy-like symptoms occur soon after cessation of ingestion of high amounts of vitamin C (1 g or more per day) (Siegel *et al*, 1982). High intakes during pregnancy may result in neonatal scurvy by conditioning the offspring to require greater than the expected or recommended daily intakes, but the evidence for this is very limited (Cochrane, 1965). The reports of conditioned scurvy in humans are anecdotal and it does not represent a significant risk (Hornig and Moser, 1981).

Vitamin C increases iron uptake considerably from the gut when given to humans in single-meal studies in amounts from 25 to 1000 mg (Hallberg, 1985; Cook and Monsen, 1977). Studies of longer duration show a less marked effect (Hunt and Roughead, 2000), but even a small increase could be important in subjects with conditions such as haemochromatosis (Gerster, 1999) or in subjects heterozygous for this condition. A dose of 2 g/day vitamin C taken with meals for 16 weeks in 17 healthy volunteers, and up to 24 months in 9 subjects, had no significant effect on body iron stores (Cook *et al*, 1984); this study was limited by the small numbers of participants and their variable iron status.

Large amounts of vitamin C were reported to destroy the vitamin B_{12} content of food (Herbert and Jacob, 1974), and reduced vitamin B_{12} levels in serum were reported in 3 out of 90 individuals consuming more than 1000 mg/day of vitamin C over a minimum of 3 years (Hind, 1975). However, subsequent reports showed that these observations arose from inadequate assay methods (Newmark *et al*, 1976 and 1979), and that ascorbic acid in blood can interfere with the measurement of vitamin B_{12} (Herbert *et al*, 1978).

An increase in serum cholesterol was reported in 25 patients with atherosclerosis following treatment with 1 g vitamin C daily for 6 weeks, but not in healthy volunteers (Spittle, 1971); the authors suggested that this may have arisen due to mobilisation of arterial cholesterol deposits (which would be a benefit), but there was no direct evidence to support this. In contrast, a 10% decrease in total plasma cholesterol levels, but with no change in the cholesterol/HDL ratio, was reported in 18 healthy adult males given 2 g vitamin C daily for 6 weeks (Tofler *et al*, 2000).

There is conflicting evidence about the relationship between vitamin C intake and breast cancer. A prospective study in a large cohort (n=62,573) of postmenopausal women had found a lower risk of breast cancer in women with the highest intakes of vitamin C from food, but not from supplements (Verhoeven *et al*, 1997). However a recent nested case-control study found an increased risk of breast cancer among a cohort of postmenopausal Danish women (Nissen *et al*, 2003). A significantly increased risk was observed at intakes above 300 mg/day in comparison with intakes 60-150 mg/day. The numbers of cases and controls in the high-intake comparison were 62 and 41, respectively. When women who were taking supplemental vitamin C were excluded, the association between increasing vitamin C intake and breast cancer was weaker and no longer statistically significant.

In conclusion, the effects of vitamin C on cholesterol levels, conditioned need due to high intake, iron absorption, prothrombin time and vitamin B_{12} degradation, breast cancer and the possible pro-oxidant activity of vitamin C are not sufficiently well documented or substantiated to be used as the basis for risk assessment. There have been conflicting reports on the influence of vitamin C supplements on the presence of oxidised bases in DNA (Podmore *et al*, 1998; Rehman *et al*, 1998; Proteggente *et al*, 2000). These have been performed at relatively low doses (280 mg/day, 60 or 260 mg/day and 500 mg/day for 6 weeks respectively), and there are no data currently available at higher intakes.

4. DOSE-RESPONSE ASSESSMENT

Adequate data defining the dose-response relationships for each adverse effect described above are not available, because many studies used a single dose level only. Despite the extensive use of vitamin C supplements (up to 10 g/day) for the prevention of colds and other conditions, the tolerability of such intakes has not been subject to systematic assessment. Therefore there are few data to support the widely held view that high intakes of vitamin C are safe.

There have been a small number of studies that have investigated dose-response relationships in a controlled and scientific manner.

4.1. Gastrointestinal effects

Two out of 15 volunteers experienced diarrhoea when consuming 10 g of vitamin C daily for 5 days (Wandzilak *et al*, 1994). In a study in healthy human volunteers given increasing doses of vitamin C, abdominal distension, flatulence, diarrhoea and transient colic were reported as “fairly frequent” at doses of 3-4 g daily (Cameron and Campbell, 1974). Lower intakes appear to be tolerated without gastrointestinal effects since no subjective side effects were reported in 17 adults given 2 g/day for 16 weeks (Cook *et al*, 1984). The Miller and Hayes (1982) review concluded that doses greater than 1 g/day could result in adverse gastrointestinal effects. The data of Ludvigsson *et al* (1977) indicate that 1 g/day would not produce adverse gastrointestinal effects in children.

4.2. Renal effects

A review of the early investigational studies on the relationship between ascorbic acid intake and oxalate excretion (Hornig and Moser, 1981) concluded that there were methodological problems with many of the studies.

A statistically significant increase in urinary oxalate excretion was reported in groups of 3 patients with calcium oxalate renal stones given 1 g or 2 g of supplemental ascorbic acid daily. Precautions were taken to prevent artefactual formation of oxalic acid by collection of intrarenal urine specimens from a catheter into EDTA and sodium thimerosol preservative (Urivetzky *et al*, 1992).

The more extensive cross-over study by Wandzilak *et al* (1994) in which 15 subjects were given 1000, 5000 and 10,000 mg vitamin C each for 5 days reported no increase in oxalate excretion after correction for non-enzymatic *ex vivo* formation. However the data are difficult to interpret because of the highly acidic preservative used.

The two studies by Auer *et al* (1998a and b) indicate no increase in the urinary excretion of oxalate in 10 healthy male volunteers (with no history of stone formation) given 4 g of vitamin C daily for 5 days, but a marked increase associated with haematuria and crystalluria in a single individual who took 8 g daily for a period of 8 days.

In summary, one study reported that high intakes of vitamin C (1 or 2 g per day) increased the urinary excretion of oxalic acid in patients with renal stones, but this was not found in studies in healthy volunteers. Data from the cohort studies (Curhan *et al*, 1996 and 1999) show that intakes of 1.5 g/day do not increase the risk of kidney stone formation.

CONCLUSIONS AND RECOMMENDATIONS

1. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

The vitamin is of low acute toxicity as indicated by the limited data available from studies in animals and humans. Despite the extensive use of high doses of vitamin C in some vitamin supplements, there

have been few controlled studies that specifically investigated adverse effects. Based on the limited data, acute gastrointestinal intolerance is the most clearly defined adverse effect at high intakes, but there are limited data on the dose-response relationship for adults or for groups such as children or the elderly. There are insufficient data to establish a tolerable upper intake level for vitamin C.

2. RISK CHARACTERISATION

The available human data suggest that supplemental daily doses of vitamin C up to about 1 g in addition to normal dietary intakes are not associated with adverse gastrointestinal effects, but that acute gastrointestinal effects may occur at higher intakes (3-4 g/day). While there is uncertainty whether high intakes of vitamin C increase renal excretion of oxalate, which could increase the risk of renal stones, an increased risk of kidney stones was not found in individuals with habitual intakes of 1.5 g/day. The absorption of vitamin C is saturated at high doses, and therefore intakes above 1 g/day would be associated with negligible increased uptake and tissue levels, but an increased risk of adverse gastrointestinal effects. There are no data on the gastrointestinal absorption or tolerability of esterified forms of vitamin C, such as ascorbyl palmitate, but such esters might be expected to show similar properties, and therefore this conclusion applies to these forms as well as ascorbic acid and its salts.

The average daily intakes reported in surveys in European countries (Table 1) are above the Population Reference Intake, with the 95th percentile intake from food and supplements ranging up to about 1 g/day. These dietary intakes do not represent a cause for concern.

There has not been a systematic assessment of the safety of the long-term use of high dose vitamin C supplements.

3. RECOMMENDATIONS FOR FURTHER WORK

Any future studies on possible benefits of high intakes of vitamin C should investigate the nature and incidence of adverse effects. Very few data are available on esterified forms of vitamin C, such as ascorbyl palmitate, and these forms should be included in future studies.

The potential for vitamin C to induce gene or chromosomal mutations *in vivo* in humans at high doses (1 g or more) should be investigated especially pro-oxidant effects on DNA bases, using sensitive methods, because there are inadequate data to ensure the safety of long-term high-dose intakes.

Subgroups of the population at increased risk have not been investigated; individuals who are predisposed to gastrointestinal problems, kidney stones or who are unable to regulate iron absorption, due to haemochromatosis or thalassaemia, should be included in future studies on the possible beneficial and adverse effects of vitamin C.

The conflicting evidence about vitamin C intake and breast cancer is noted and no conclusion is possible at this time. The possible association warrants further research to clarify any relationship for both dietary sources and vitamin C supplements.

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