# EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2013. Scientific Opinion on Dietary Reference Values for vitamin C

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## SCIENTIFIC OPINION

# Scientific Opinion on Dietary Reference Values for vitamin C[1]

**EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)**[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

Following a request from the European Commission, the Panel on Dietetic Products, Nutrition and Allergies (NDA) derived Dietary Reference Values (DRVs) for vitamin C. The Panel concludes that an Average Requirement (AR) can be derived from indicators of vitamin C status, as well as a Population Reference Intake (PRI) assuming a coefficient of variation (CV) of 10 %. Several health outcomes possibly associated with vitamin C intake were also considered but data were found to be insufficient to establish DRVs. For healthy adults, the AR is determined from the quantity of vitamin C that balances metabolic vitamin C losses and allows the maintenance of an adequate body pool characterised by fasting plasma ascorbate concentrations at around 50 µmol/L. In men, an AR of 90 mg/day of vitamin C and a PRI of 110 mg/day are proposed. As no value for metabolic losses is available in women, the AR for women is extrapolated from the AR for men on the basis of differences in reference body weight, and an AR of 80 mg/day and a PRI of 95 mg/day are proposed. For infants aged 7-11 months, the Panel has decided to retain the PRI of 20 mg/day set by the SCF (1993), as no suitable evidence has emerged since the previous assessment. For children and adolescents, the ARs for vitamin C are extrapolated from the ARs for adults taking into account differences in reference body weight, and PRIs are derived, ranging from 20 mg/day for 1 to 3 year-old children, to 100 and 90 mg/day for boys and girls aged 15-17 years, respectively. For pregnant and lactating women, vitamin C intakes of 10 mg/day and of 60 mg/day in addition to the PRI of non-pregnant non-lactating women are proposed.



## KEY WORDS

vitamin C, metabolic losses, plasma ascorbate, average requirement, Dietary Reference Value, health outcomes

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[1] On request from the European Commission, Question No EFSA-Q-2011-01229, adopted on 10 October 2013.

[2] Panel members: Carlo Agostoni, Roberto Berni Canani, Susan Fairweather-Tait, Marina Heinonen, Hannu Korhonen, Sébastien La Vieille, Rosangela Marchelli, Ambroise Martin, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Sean (J.J.) Strain, Inge Tetens, Daniel Tomé, Dominique Turck and Hans Verhagen. Correspondence: nda@efsa.europa.eu

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## SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Dietary Reference Values (DRVs) for the European population, including vitamin C.

Vitamin C (L-ascorbic acid) is an enzyme cofactor for biochemical reactions catalysed by monooxygenases, dioxygenases and mixed function oxygenases. Vitamin C plays an important role in the biosynthesis of collagen, is essential for the synthesis of carnitine and catecholamines, and is also involved in the metabolism of cholesterol to bile acids. Vitamin C in aqueous solution readily scavenges reactive oxygen and nitrogen species, and is part of the antioxidant network of the body.

Gastrointestinal absorption is about 80 % for an intake of about 100 mg/day. Vitamin C is transported as the free anion ascorbate in plasma, and is distributed to all tissues. Biomarkers of body stores are related to the size and turnover of vitamin C body stores, and to the mass balance of vitamin C in the body. In this Opinion, plasma ascorbate concentration is considered as the primary indicator of body stores. The mass balance of vitamin C in the body is determined from the rate of turnover of the body pool, considering metabolic losses, urinary losses and the quantity of vitamin C required for the replacement of these losses, taking into account absorption efficiency.

Scurvy, characterised by symptoms related to connective tissue defects, occurs in adults at a plasma ascorbate concentration below 10 µmol/L and a body pool less than 300 mg, and can be prevented with an intake of 10 mg vitamin C/day. In vitamin C-depleted men, when vitamin C intake is increased to 60 to 100 mg/day, plasma ascorbate concentrations steeply increase up to a value of about 50 µmol/L, and the body pool rises to 1.0-1.5 g. When vitamin C intake is increased to above 100 mg/day, there is a progressive flattening of the curve until plasma ascorbate reaches a plateau at about 70-80 µmol/L that can be maintained only by chronic ingestion of large doses of vitamin C above 200 mg/day. Plasma ascorbate concentrations above 10 µmol/L but below 50 µmol/L are indicative of a suboptimal status with a risk of insufficiency. A plasma ascorbate concentration of 50 µmol/L is indicative of an adequate status. Urinary excretion of ascorbate is low when plasma ascorbate concentrations are low, but urinary excretion increases sharply for plasma concentrations above about 50 µmol/L, and this is assumed to reflect near-saturation of body pools.

The Average Requirement (AR) for vitamin C in healthy adults was determined from the quantity of vitamin C intake that balances metabolic vitamin C losses and maintains fasting plasma ascorbate concentrations at about 50 µmol/L. Taking a conservative approach and based on the fact that a complete set of data was only available in men, the Panel selected metabolic losses of 50 mg/day, an absorption of 80 % and a urinary excretion of 25 % of the vitamin C intake. Thus, a mean vitamin C intake of 91 mg/day (rounded to 90 mg/day) was estimated to be required to balance daily losses, and this intake represents the AR. Assuming a coefficient of variation (CV) of 10 %, a Population Reference Intake (PRI) of 110 mg/day was derived for healthy men. As no value for metabolic losses was available in women, the AR for women was extrapolated from the AR for men. Extrapolation was done by isometric scaling (linear with body weight), since vitamin C is considered to be distributed throughout the whole body, since the multi-compartment models used to calculate the metabolic losses in men consider an exchange with only one whole body tissue pool, since few sex-related differences could be observed in the pharmacokinetics of vitamin C, and since a main part of the observed differences can be explained by body weight differences between sexes. This calculation led to an AR of 78 mg/day (rounded to 80 mg/day) for women. Assuming a CV of 10 % and rounding to the closest 5, a PRI of 95 mg/day of vitamin C was derived for healthy women. Because of a scarcity of data on the influence of ageing, the Panel concluded that there were insufficient data to derive different DRVs for vitamin C for older adults compared to younger adults.

The Panel also considered several health outcomes that may be associated with vitamin C intake. The Panel decided that the available data on the effects of vitamin C intake and/or status on scurvy, blood lipids and blood pressure, common cold, and on chronic disease-related outcomes (cardiovascular

disease-related, cancer, vision-related, mortality) could not be used as criteria to derive the requirement for vitamin C.

For infants aged 7-11 months, the Panel decided to retain the PRI of 20 mg/day set by the Scientific Committee for Food (SCF, 1993), as no suitable evidence has emerged since the previous assessment. For children and adolescents, the AR for vitamin C was extrapolated from the ARs for adults taking into account differences in body weight (isometric scaling). The PRIs were derived by assuming a CV of 10 % and range from 20 mg/day for 1 to 3-year-old children, to 100 and 90 mg/day for boys and girls aged 15-17 years, respectively.

In pregnancy, plasma ascorbate concentration decreases because of haemodilution and active transfer to the fetus. For pregnant women, a vitamin C intake of 10 mg/day in addition to the PRI of non-pregnant women was proposed. In lactating women, the amount of vitamin C secreted in breast milk reflects maternal vitamin C intake rather than the infant's requirement. For women exclusively breastfeeding during the first six months *post partum*, a vitamin C intake of 60 mg/day, in addition to the PRI of non-lactating women, was proposed to cover vitamin C losses in breast milk.

The main contributors to the vitamin C intake of adults are fruits and vegetables and their juices, and potatoes. Data from dietary surveys show that average vitamin C intakes from food only in European countries range from 69 to 130 mg/day in men and from 65 to 138 mg/day in women.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on nutrient and energy intakes for the European Community[4]. The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then, new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients, these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context, EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context, EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002, the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on population reference intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance, EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically, advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;
- Protein;

[4] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

- Dietary fibre.

Following on from the first part of the task, EFSA is asked to advise on population reference intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

## ASSESSMENT

### 1. Introduction

Vitamin C (L-ascorbic acid) is a water-soluble organic compound that is needed for normal metabolic functioning of the body. Vitamin C is an essential component of the diet, as humans and other primates have lost the ability to synthesise vitamin C via the glucuronic acid pathway.

In 1993, the Scientific Committee for Food (SCF) adopted an opinion on nutrient and energy intakes for the European Community and derived for vitamin C a Lowest Threshold Intake (LTI), an Average Requirement (AR) and a Population Reference Intake (PRI) for adults. The SCF also set PRIs for infants aged 6-11 months and for children. For pregnancy and lactation, additional intakes to be added to the PRI for non-pregnant non-lactating women were proposed.

### 2. Definition/category

#### 2.1. Chemistry

Vitamin C is a 6-carbon hydroxy-lactone that is structurally related to glucose and has a molecular mass of 176.12 Da. Vitamin C refers to both ascorbic acid and dehydroascorbic acid. Ascorbic acid is readily oxidised to L-dehydroascorbic acid, in which the 3,4-dihydroxy groups are replaced by 3,4-diketone functions, and L-dehydroascorbic acid can be reduced back to ascorbic acid. Vitamin C in solution can be oxidised and donate electrons to oxidants, including oxygen and metal ions, to give an equilibrium mixture of ascorbic and dehydroascorbic acids.

#### 2.2. Functions of vitamin C

Vitamin C has a number of biochemical and physiological functions in the body which are largely dependent on its ability to provide reducing equivalents in various biochemical reactions (Burri and Jacob, 1997; Tsao, 1997).

##### 2.2.1. Vitamin C as cosubstrate of enzymatic reactions

Vitamin C is an enzyme cofactor for biochemical reactions catalysed by monooxygenases, dioxygenases and mixed function oxygenases. Vitamin C acts as an electron donor with a redox potential that reduces the active centre metal ion of mono- and dioxygenases and maintains metal ions in a reduced state for optimal activity of the enzyme (Burri and Jacob, 1997; Tsao, 1997).

Vitamin C plays an important role in the biosynthesis of collagen, which represents about one quarter of the total body protein and constitutes the principal protein of skin, bones, teeth, and connective tissues. Procollagen-proline dioxygenase (proline hydroxylase) and procollagen-lysine 5-dioxygenase (lysine hydroxylase), two enzymes involved in procollagen biosynthesis, require vitamin C for maximal activity (Phillips and Yeowell, 1997). Vitamin C deficiency impairs collagen synthesis.

Vitamin C is essential for the synthesis of carnitine (Hulse et al., 1978), as cofactor of two dioxygenases involved in carnitine biosynthesis (Burri and Jacob, 1997; Vaz and Wanders, 2002).

Vitamin C is also a cofactor for catecholamine biosynthesis, in particular the conversion of dopamine to noradrenaline catalysed by dopamine β-monooxygenase (Burri and Jacob, 1997).

Vitamin C is involved in the metabolism of cholesterol to bile acids via the enzyme cholesterol 7α-monooxygenase, and in steroid metabolism in the adrenal glands (Burri and Jacob, 1997; Tsao, 1997). In vitamin C deficiency, the metabolism of cholesterol to bile acids is slowed down in guinea pigs, resulting in an accumulation of cholesterol in the liver, hypercholesterolaemia and formation of cholesterol gall stones (Ginter et al., 1982).

Hydroxylation of aromatic drugs and carcinogens by hepatic cytochrome P450 is enhanced by reducing agents such as vitamin C (Tsao, 1997).

The activities of several other enzymes are also known to be dependent on vitamin C, including the mono- and dioxygenases involved in peptide amidations that are necessary for maximal activity of the hormones oxytocin, vasopressin, cholecystokinin and α-melanocyte-stimulating hormone (Cameron and Pauling, 1973), and the hydroxylation of phenylalanine in tyrosine formation (Burri and Jacob, 1997; Tsao, 1997).

### 2.2.2. Vitamin C as a reducing and antioxidant agent

Besides its ability to provide reducing equivalents for a variety of biochemical reactions, vitamin C functions physiologically as a water-soluble antioxidant acting as a free radical scavenger (Sadler et al., 1999; IOM, 2000). Vitamin C readily scavenges reactive oxygen species and reactive nitrogen species, as well as singlet oxygen and hypochlorite. The one- and two-electron oxidation products are easily regenerated by glutathione and NADH or NADPH (IOM, 2000).

Vitamin C is part of the antioxidant defence system, which is a complex network including endogenous and dietary antioxidants, antioxidant enzymes, and repair mechanisms, with mutual interactions and synergetic effects among the various components. L-ascorbic acid is able to regenerate urate, glutathione, and beta-carotene from their respective one-electron oxidation products (Edge and Truscott, 1997; Phillips and Yeowell, 1997) and α-tocopherol (vitamin E) from the α-tocopheroxyl radical produced via scavenging of lipid radicals (Bowry et al., 1995; Packer, 1997).

Vitamin C is considered to be involved in the maintenance of endothelial function possibly through its antioxidant effects (IOM, 2000). The reducing capacity of vitamin C has also been implicated in enhancing gastrointestinal absorption of dietary non-haem iron (Hallberg, 1981; Bendich and Cohen, 1990; Burri and Jacob, 1997).

### 2.2.3. Health consequences of vitamin C deficiency and excess

#### 2.2.3.1. Deficiency

A continuous lack of vitamin C in the diet causes the disease scurvy, which occurs in adults at a plasma ascorbate concentration below 10 µmol/L and a body pool less than 300 mg (Baker EM et al., 1971; Levine, 1986; Weber et al., 1996; Burri and Jacob, 1997). Scurvy is characterised by symptoms related to connective tissue defects that result from a weakening of collagenous structures. In infants, there may be important effects on bone tissue with impaired bone growth and ossification (Shenkin, 2008). In adults, scurvy is associated with tooth loss, joint pain, bone and connective tissue disorders, and poor wound healing with multiple clinical features including petechiae, bruising, and inflamed and bleeding gums. Depression, hypochondria, and mood changes are frequently associated with scurvy and may be related to deficient dopamine hydroxylation. Scurvy can be prevented with a vitamin C intake of 10 mg/day (Baker EM et al., 1971; Levine, 1986; Weber et al., 1996; Burri and Jacob, 1997).

Early or prescorbutic symptoms also include fatigue, lethargy, anaemia, aching joints, and muscle weakness (Lukaski, 2004). It has been suggested that fatigue and lethargy may be related in part to insufficient carnitine biosynthesis, and consequently deficient transport of activated long chain fatty acids into the mitochondria for energy generation (Davies et al., 1987; Jacob and Pianalto, 1997) (see Section 2.4.3.2.).

#### 2.2.3.2. Excess

EFSA did not set any Tolerable Upper Intake Level (UL) for vitamin C. The limited available data from studies in animals and humans were considered to suggest a low acute toxicity of vitamin C (Johnston, 1999; EFSA, 2004). Relationships between vitamin C intakes and adverse gastrointestinal effects or renal effects in relation to urinary excretion of oxalate were assessed, and reversible acute gastrointestinal intolerance or diarrhoea was regarded as the most clearly defined adverse effect at

high intakes (3-4 g/day). However, data on a dose-response relationship for adults (including older adults) or for children were considered to be insufficient (EFSA, 2004). Despite the extensive use of high doses of vitamin C in supplements, there were only a limited number of controlled studies that specifically investigated adverse effects.

It has been suggested that vitamin C may also exert pro-oxidant effects by reducing ferric to ferrous ion, and that this might stimulate uptake of iron from the gut as iron is absorbed in the reduced state (Wollenberg and Rummel, 1987). In addition, an increase in free iron concentration through vitamin C's ability to release ferrous ions from ferritin (Halliwell and Gutteridge, 1989) may promote the generation of free radicals through the Fenton reaction (Prousek, 2007). Whether excess vitamin C intake leads to these mechanisms *in vivo* is uncertain (Carr A and Frei B, 1999).

### 2.3. Physiology and metabolism of vitamin C

The schematic relationship between vitamin C intake, vitamin C accumulation in the body and vitamin C elimination is shown in Figure 1. The four parts of this figure will be described in the following sub-sections.

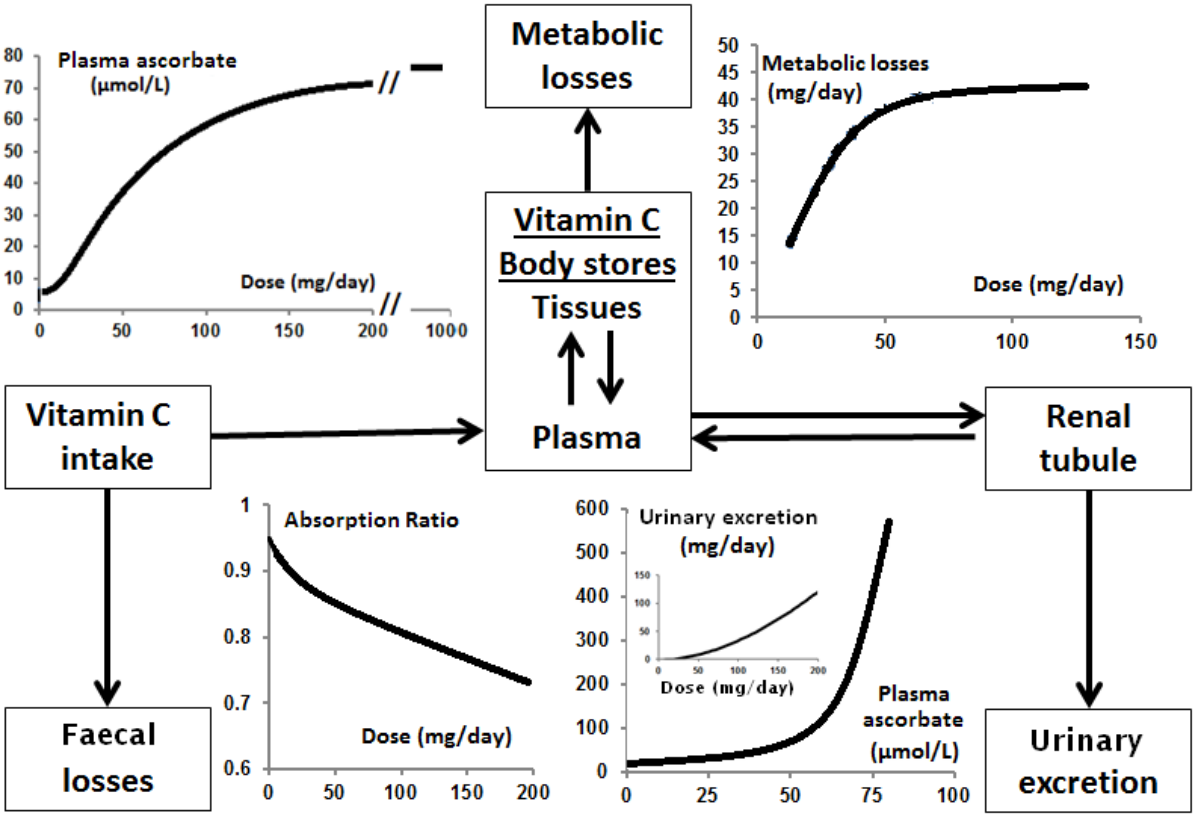


**Figure 1:** Schematic view of vitamin C kinetics in the body with the relationship between vitamin C intake, plasma concentration, body stores and urinary excretion in healthy male adults (adapted from Kallner et al. (1979); Graumlich et al. (1997)).

#### 2.3.1. Intestinal absorption

Vitamin C is absorbed in the small intestine via a sodium-dependent active transport mechanism (Tsao, 1997; Rumsey and Levine, 1998). The efficiency of intestinal absorption of vitamin C is 80-90 % at dietary intakes of 30-180 mg/day (SCF, 1993). Because the transporter is saturable, absorption efficiency gradually decreases at higher intakes (Kallner et al., 1979; Hornig and Moser, 1981; Blanchard et al., 1997; EFSA, 2004).

Two depletion-repletion studies (Baker et al., 1969; Baker EM et al., 1971) using a radio-labelled vitamin C dose were each undertaken in six healthy men of unknown age and smoking status. After the depletion phase (0-2.5 mg/day vitamin C), total vitamin C intake of the completers was increased to 4 to 66.5 mg/day (n = 4 completers) (Baker et al., 1969), or 6.5 to 130.5 mg/day (n = 5 completers) (Baker et al., 1971). Ingested radioactivity was excreted in urine and faeces except for less than 2 % of radioactivity which was expired as carbon dioxide. Faecal excreta accounted for not more than 2 % of the radioactivity excreted, thus absorption was about 98 % of intake.

In another study (Kallner et al., 1977), seven healthy non-smoking men of unknown age followed a diet low in vitamin C for nine days, were then orally supplemented with 90 mg (n = 5) or 180 mg (n = 2) of vitamin C daily for two weeks before oral administration of 30 mg (n = 5) or 60 mg (n = 2) of radio-labelled vitamin C. The vitamin C intake was then increased to four daily doses of 1 000 mg and the cumulative excretion of radioactivity in the urine was measured over a period of ten days. The average absorption was estimated to amount to 80-90 % of the labelled doses (30 mg or 60 mg).

Hornig et al. (1980) consecutively administered two daily doses of 1, 2, 3 and 4 g of vitamin C to a non-smoking man of unknown age, followed by 5 g/day of vitamin C for ten days. The mean daily urinary excretion of ascorbate was used to estimate the absorption of vitamin C, which decreased from 75 % (1 g) to about 44 % (2 g), 39 % (3 g), 28 % (4 g) and 21 % (5 g) of the ingested dose.

Using data collected over three years on healthy men (n = 50) and women (n = 25) (no age given) receiving single oral doses of vitamin C (Kübler and Gehler, 1970), i.e. 1.5 g (n = 16), 3 g (n = 29), 6 g (n = 11), or 12 g (n = 19), and for which serum ascorbate and, for some subjects (n = 25), urinary ascorbate concentrations were measured, vitamin C absorption was calculated using a pharmacokinetic model (Kübler, 1970) based on the data measured in plasma and urine. Mean absorption decreased from about 50 % for an intake of 1.5 g to about 16 % for a vitamin C intake of 12 g.

In a depletion-repletion study using a pharmacokinetic model, seven healthy men aged 20-26 years with unknown smoking status followed a diet containing 60 mg vitamin C/day for three weeks, followed by a diet with less than 5 mg/day for the entire study (Graumlich et al., 1997). The depletion phase of unknown duration was followed by a repletion phase using oral doses of 15, 30, 50, 100, 200, 500, and 1 250 mg twice daily. Each dose was applied for several days with the aim of reaching a new steady state. At that time, a one-day absorption test was performed, which involved oral administration of half of the dose given so far followed by intravenous administration of the other half of the dose. Using a multi-compartment model, the mean fractional absorption derived from oral *versus* intravenous vitamin C administration was reported to be 86 % for 15 mg/day, 85 % for 30 mg/day, 84 % for 50 mg/day, 81 % for 100 mg/day, 78 % for 200 mg/day, 75 % for 500 mg/day, and 62 % for 1 250 mg/day.

The Panel notes that data on vitamin C absorption have mainly been collected in men, that absorption is about 80 % for an intake of 100 mg/day and about 75 % for an intake of 1 g/day, and that the efficiency of absorption decreases with increasing doses.

### 2.3.2. Transport in blood and variability of plasma concentrations

In plasma, vitamin C is transported as the free anion ascorbate[5], and no specific binding protein has been identified.

When the daily vitamin C intake increases, plasma ascorbate concentration increases according to a sigmoidal profile (Sauberlich, 1975; Irwin and Hutchins, 1976; Kallner et al., 1979; Basu and Schorah, 1982; Polidori et al., 2004; Levine et al., 2011). In depleted subjects with low plasma ascorbate, when vitamin C intake is increased, plasma ascorbate concentration is characterised by a steep rise up to a value of about 50 µmol/L which is reached for a vitamin C intake between 60 and 100 mg/day (Basu

[5] In this Opinion, plasma ascorbate concentration is expressed in µmol/L. Published values expressed in another unit (e.g. mg/100 mL) have thus been converted to µmol/L.

and Schorah, 1982; Levine et al., 1996; Levine et al., 2001). When vitamin C intake is increased above 100 mg/day, there is a progressive flattening of the curve until it reaches a plateau at about 70-80 µmol/L. These high plasma concentrations can only be maintained by chronic ingestion of doses of vitamin C above 200 mg/day (Figure 1).

According to depletion-repletion studies undertaken in 15 healthy non-smoking women aged 19-27 years (Levine et al., 2001) and in seven healthy non-smoking men aged 20-26 years (Levine et al., 1996) receiving in the repletion phase increasing doses of vitamin C (30, 60, 100, 200, 400, 1 000, 2 500 mg/day, half of the dose ingested twice daily), women reach the plateau of plasma ascorbate concentration at a lower vitamin C intake than men, but the value of the plasma concentration at the plateau is similar in women and men (Levine et al., 2011). Depletion and repletion profiles of vitamin C were determined in four groups of healthy non-smoking non-institutionalised men (aged 21-28 (n = 15) or 66-74 years (n = 15)) and women (aged 20-29 (n = 14) or 65-72 years (n = 14)) (Blanchard, 1991b). Plasma ascorbate concentrations were determined upon entry into the study, once each week during a five-week period of dietary restriction of vitamin C (< 10 mg/day) and twice each week during a 2.5 to 3-week period of supplementation with 500 mg/day. Mean baseline, depleted and repleted plasma ascorbate concentrations did not differ between young and elderly subjects. Depleted plasma ascorbate concentrations did not differ between males and females, whereas mean repleted plasma ascorbate concentrations were significantly higher in women (about 95 and 102 µmol/L in young and elderly women, respectively) than in men (about 80 and 83 µmol/L in young and elderly men, respectively).

An observational study in 28 pregnant adolescents (n = 28) and 74 pregnant women showed that mean plasma ascorbate concentrations significantly decreased in the course of pregnancy, from about 80 µmol/L at < 14 weeks of gestation (58 samples) to about 70 µmol/L at 39-42 weeks of gestation (six samples) (Morse et al., 1975). This decrease may be related to haemodilution and active transfer to the fetus, as suggested from an *in vitro* study (Choi and Rose, 1989). A decrease in plasma ascorbate concentration was also observed in a Scottish population of pregnant women (n = 1 007), from about 64 µmol/L (median: 12 weeks of pregnancy) to 37 µmol/L at delivery (Scaife et al., 2006).

In smokers, plasma/serum ascorbate concentration is usually lower compared to non-smokers. An observational study showed that the risk of having a plasma ascorbate concentration below 11 µmol/L was significantly higher in male or female smokers compared to non-smokers (Hampl et al., 2004). Plasma ascorbate concentrations increased in subjects who quit smoking compared to smokers of at least 15 cigarettes/day for one year (Lykkesfeldt et al., 1996). A lower plasma/serum ascorbate concentration is observed in smokers even after adjustment for differences in vitamin C intakes. An observational study showed that plasma ascorbate concentrations in tobacco chewers (n = 11) and cigarette smokers (n = 23) were significantly lower than in non-smokers (n = 10), while vitamin C intake was not statistically different between groups (Giraud et al., 1995). Data collected from 459 men aged 20-60 years, including 146 non-smokers, 125 former smokers, and 188 current smokers (< 20 cigarettes/day, n = 104; ≥ 20 cigarettes/day, n = 84) showed that vitamin C intake was lower in smokers of ≥ 20 cigarettes/day compared to the other groups, and that plasma ascorbate concentration was significantly different between groups (even after adjustment for confounders including vitamin intake), being higher in non-smokers, lower in smokers of ≥ 20 cigarettes/day, but not significantly different between ex-smokers and smokers of < 20 cigarettes/day (Marangon et al., 1998).

The Panel notes that when daily vitamin C intake increases, plasma ascorbate concentration increases according to a sigmoidal profile in healthy adult men. This is characterised by a steep rise of plasma ascorbate up to a concentration of about 50 µmol/L reached for an intake between 60 and 100 mg/day, and by a progressive flattening of the curve until it reaches a plateau at about 70-80 µmol/L for higher vitamin C intakes. The Panel also notes that women reach the plateau of plasma ascorbate concentration at a lower vitamin C intake than men, but that the value of the plasma concentration at the plateau is similar in women and men, that plasma ascorbate concentration decreases during pregnancy, and that it is lower in smokers compared to non-smokers.

### 2.3.3. Distribution to tissues and metabolism

Vitamin C is distributed to all tissues. Vitamin C is efficiently transported into the cell in its reduced (ascorbate) and oxidised (dehydroascorbate) forms by active and facilitative transport systems, respectively (Tsao, 1997; Jacob, 1999; Linster and Van Schaftingen, 2007). The cellular transport of ascorbate is mediated by two active sodium-dependent cotransporters, SVCT1 and SVCT2, which show distinct tissue distribution and vary by cell type. Dehydroascorbate is transported by glucose transporters, particularly GLUT1, GLUT3 and GLUT4, and the transport is therefore not directly energetically driven, but dehydroascorbate is readily converted in the cell to ascorbate and this reductive step drives its intracellular uptake.

Per 100 g of wet tissue, higher concentrations are found in the pituitary glands (40-50 mg), the adrenal glands (30-40 mg), the eye lens (25-31 mg) and the brain (13-15 mg) than in the plasma (0.4-1.0 mg), saliva (0.07-0.09 mg), kidney (5-15 mg) and skeletal muscle tissue (up to 4 mg) (Hornig, 1975; Carr et al., 2013).

In an intervention study (Carr et al., 2013), 36 non-smoking men were randomly assigned to receive for six weeks either 0.5 or 2 kiwifruit/day. A significant correlation between the ascorbate concentration in skeletal muscle (*vastus lateralis*) measured by biopsies and fasting plasma ascorbate concentration was observed. In addition, mean muscle ascorbate concentration was significantly higher in the second plasma ascorbate quintile (21-36 µmol/L) compared to the first one (4-20 µmol/L), and in the fourth quintile (48-58 µmol/L) compared to the third one (37-47 µmol/L), but not significantly different between the second and third quintiles, nor between the fourth and the fifth (59-82 µmol/L) quintiles. The Panel notes that there was no additional increase in muscle ascorbate concentration in non-smoking men at a plasma ascorbate concentration above around 50 µmol/L.

Accumulation of vitamin C into neutrophils and lymphocytes is mediated by both low affinity and high affinity transport processes. At steady state, the relationship between vitamin C intake and ascorbate concentration in circulating cells (neutrophils, monocytes, platelets, lymphocytes) parallels that between vitamin C intake and ascorbate concentration in plasma in adult men and women (Basu and Schorah, 1982; Levine et al., 1996; Levine et al., 2001). Curves of intracellular ascorbate concentrations as a function of vitamin C doses in healthy non-smoking men (Levine et al., 1996) and women (Levine et al., 2001) showed that plateaus were reached at doses of about 100-400 mg/day in neutrophils (about 1.3 mmol/L), monocytes (about 3 mmol/L), platelets (about 3.5 mmol/L in women) and lymphocytes (about 3.5-4 mmol/L).

In mammals, the degradation of ascorbate proceeds via dehydroascorbate which is an unstable molecule, and involves spontaneous and possibly enzyme-catalysed reactions (Linster and Van Schaftingen, 2007). In the body, vitamin C is readily oxidised to dehydroascorbate, which can be reduced back to ascorbate or irreversibly hydrolysed to diketogulonic acid and then metabolised to oxalate, threonic acid, xylose, xylonic acid and lyxonic acid (Basu and Dickerson, 1996). In human subjects injected with labelled ascorbate, an average of 44 % of the total radioactivity excreted in urine was recovered as oxalate, other urinary metabolites including 2,3-diketo-l-gulonate and dehydroascorbate (Hellman and Burns, 1958), and little, if any, radioactivity was recovered as carbon dioxide. Oxidation to carbon dioxide occurs at high doses of intake, partly because of the catabolism of unabsorbed vitamin C by the intestinal microflora (Kallner et al., 1985). Ascorbate may also undergo limited conjugation with sulphate to form ascorbate-sulphate, which is excreted in the urine (Baker EM, 3rd et al., 1971).

### 2.3.4. Body stores and metabolic losses

Depending on intake, the total body pool of vitamin C in adult men varies from less than 0.3 g to around 1.5-2.0 g as derived from pharmacokinetic models (Kallner et al., 1979; Sauberlich, 1990). As for plasma and circulating cells, there is a non-linear relationship between daily vitamin C intake and body stores of vitamin C. A usual intake of vitamin C below 10 mg/day is associated at steady state with a body pool of less than 300 mg and scurvy (see Section 2.2.3.1.). When usual vitamin C intake is

increased from 10 mg/day to 60-100 mg/day, the body pool rises at steady state to 1.0-1.5 g (Baker EM et al., 1971; Kallner, 1987). An increase in vitamin C intake to above 200 mg/day induces a progressive increase in body stores that plateau at values of about 1.5-2.0 g (Kallner et al., 1979 ; Kallner, 1987). Measurement of the vitamin C body pool is complex, since the body has a wide range of inter-connected ascorbate pools, each with complex kinetic properties (Fairweather-Tait, 2011; EURRECA, online).

In the two depletion-repletion studies by Baker et al. using $^{14}$C-labelled ascorbic acid in men (see Section 2.3.1.), the depletion phase lasted for days 1-99 (Baker et al., 1969), or between day 14 and day 97 to 110 according to subject after a control phase of 13 days (Baker et al., 1971), and was followed by the repletion phase and, in one of the studies (Baker et al., 1971), by a phase of *ad libitum* diet plus supplementation of either 350 or 600 mg vitamin C/day. Individual daily metabolic losses were calculated from the slope of the urinary excretion curve of radioactivity during the depletion period following a period of adaptation to controlled levels of vitamin C intakes. Daily metabolic losses varied from 2.6 to 4.1 % (average: 3.2 ± 1.0 %, n = 5 completers) (Baker EM et al., 1971) and from 2.2 to 2.7 % ((Baker et al., 1969) reported in Baker EM et al. (1971), n = 4 completers). Pooling the individual data from these nine subjects, the average daily metabolic losses were 2.9 ± 0.6 %. The Panel notes that this would correspond to metabolic losses of 43.5 ± 9 mg/day for a saturated body pool of 1.5 g.

Metabolic losses were also investigated in 15 healthy non-smoking men (20-45 years) who received, during a period of three weeks, a diet devoid of vitamin C and capsules of vitamin C (1 x 30 mg/day for n = 4, 2 x 30 mg/day for n = 4, 2 x 45 mg/day for n = 3, 4 x 45 mg/day for n = 4), in order to achieve a steady state condition before oral administration of a single dose of $^{14}$C-labelled ascorbic acid (Kallner et al., 1979). A high vitamin C dosage period was then followed for one week in order to wash-out the radioactivity. The time course of radioactivity was measured in plasma and urine, and pharmacokinetic parameters were calculated. Mean plasma steady state concentrations reported for 14 subjects were about 14-66 µmol/L. Total turnover of vitamin C reflected the absorbed amount of vitamin C, and corresponded to the sum of renal losses of unmetabolised ascorbic acid and metabolic losses. The curve of metabolic losses as a function of total turnover of vitamin C showed saturation of metabolic losses at about 40 to 50 mg/day, for a total vitamin C turnover above 60 mg/day. The curve of plasma ascorbate concentration as a function of total vitamin C turnover showed that a total vitamin C turnover of about 60 mg/day corresponded to a plasma concentration of about 45-50 µmol/L.

In a depletion-repletion study in seven young men with unknown smoking status using a pharmacokinetic model (see Section 2.3.1.), maximum metabolic losses of 42 mg/day were calculated using parameters from the multi-compartment model (Graumlich et al., 1997).

In smokers, higher metabolic losses are observed as a result of increased oxidative stress (IOM, 2000). Kallner et al. (1981) compared data from 17 healthy men (aged 21-69 years) who had been chronic smokers for many years and smoked more than 20 cigarettes per day to data previously collected in non-smoking men (Kallner et al., 1979). Smokers followed the same protocol as the non-smokers, including the oral administration of capsules of vitamin C (1 x 30 mg/day for n = 4, 2 x 30 mg/day for n = 5, 2 x 45 mg/day for n = 3, 4 x 45 mg/day for n = 5). No attempts were made to estimate the smoking intensity from analyses of body fluids, but standardisation of smoking was attempted by requesting at least one cigarette before the daily blood sampling and continued smoking during the administration of the labelled ascorbate. Mean plasma steady state concentrations were about 14-89 µmol/L in smokers. The curves of the steady state plasma concentration of unlabelled ascorbate as a function of total turnover showed that higher total turnovers are required in smokers to achieve plasma concentrations comparable to those in non-smokers. Metabolic losses varied from 50.2 to 114.0 mg/day in smokers, except for one subject at 29.6 mg/day, whereas it varied from 13.7 to 47.6 mg/day in non-smokers. The curve of metabolic losses as a function of plasma steady state concentration in smokers and non-smokers showed that smokers had higher metabolic losses than non-

smokers at a corresponding plasma ascorbate concentration. Similarly, the curve of metabolic losses as a function of total turnover of vitamin C was above that obtained in non-smokers.

The Panel notes that in healthy non-smoking men aged 20-45 years, maximum metabolic losses of vitamin C were estimated to be about 40-50 mg/day, which corresponds to a plasma ascorbate concentration of about 45-50 µmol/L. The Panel also notes that no data on metabolic losses are available for women, and that smokers have higher metabolic losses compared to non-smokers at similar vitamin C intakes.

### 2.3.5. Elimination

#### 2.3.5.1. Faeces

Unabsorbed vitamin C can be found in the faeces (see Section 2.3.1.), but there is no indication that faeces constitute an important route of excretion for absorbed vitamin C.

#### 2.3.5.2. Urine

Unchanged ascorbate and its metabolites are mainly excreted in the urine. There is an increasing renal elimination of ascorbate with an increase in plasma ascorbate concentration and/or vitamin C intake. This results in an inverse relationship between the elimination half-life and the dosage because of saturation of renal tubular reabsorption (Baker EM et al., 1971; Kallner et al., 1979; Blanchard et al., 1997; Graumlich et al., 1997; Fukuwatari and Shibata, 2008; Uchida et al., 2011; Carr et al., 2012).

In 19 healthy adults (Friedman et al., 1940), the curve of the relationship between urinary ascorbate and plasma ascorbate concentration showed that urinary ascorbate excretion increases for a plasma ascorbate concentration above about 45-55 µmol/L.

In 15 healthy non-smoking men (Kallner et al., 1979) (see Section 2.3.4.), the curve of renal turnover as a function of steady state plasma ascorbate showed that the renal absorptive capacity was exceeded at plasma concentrations above about 45-50 µmol/L, with a progressive steep increase in renal excretion. Although the exact amount of ingested dietary ascorbic acid was unknown, analysis of urines for labelled ascorbic acid showed that even at low doses appreciable amounts of unmetabolised ascorbic acid (about 10 % of total urinary radioactivity as radio-labelled ascorbic acid) were excreted and this was increased to 60-80 % for higher doses. When smoking men (Kallner et al., 1981) were compared to non-smoking men (Kallner et al., 1979) (see Section 2.3.4.), the curves of renal turnover as a function of steady state plasma ascorbate concentration in smokers and non-smokers were similar.

In a depletion-repletion study in seven young men (Graumlich et al., 1997) (see Section 2.3.1.), the curve of urinary ascorbate excretion as a function of plasma ascorbate concentration showed a progressive increase above a plasma ascorbate concentration of about 50-60 µmol/L, up to about 600 mg ascorbate excretion for the highest single oral dose of 1 250 mg (i.e. at a plasma ascorbate concentration of about 80 µmol/L). There was no measurable ascorbate in urine after oral vitamin C doses of 15 or 30 mg. However, all subjects excreted ascorbate in the urine for oral doses of 100 mg or above.

In a study conducted in 60 non-smoking men aged 18–30 years consuming sequentially half, one, two and three Gold kiwifruit per day for four to six weeks each, correlation of plasma ascorbate with urinary ascorbate also showed a marked increase in urinary output at plasma ascorbate concentrations above 50-60 µmol/L (Carr et al., 2012).

According to a depletion-repletion study (Levine et al., 1996) (see Section 2.3.2.) undertaken in seven healthy non-smoking men, less than 0.4 mg/day of ascorbate appeared in urine of all volunteers after single oral vitamin C doses of 15 and 30 mg, six of seven subjects had less than 0.4 mg/day of ascorbate in urine after a single dose of 50 mg, but a single oral dose of 100 mg induced a urinary ascorbate excretion of about 25 mg. The curve of urinary excretion as a function of dose showed a

progressive increase up to about 600 mg ascorbate excretion for the highest single oral dose of 1 250 mg.

A depletion-repletion study in 15 healthy non-smoking women (Levine et al., 2001) (see Section 2.3.2.) showed that the relationship between vitamin C intake and urinary excretion followed the same profile as in men.

The Panel notes that urine is the main route of elimination of ascorbate in adults, and that at an intake of 100 mg/day approximately 25 % of the ingested dose of vitamin C is excreted in urine. The Panel also notes that ascorbate is excreted in urine for low plasma ascorbate concentrations, and that urinary ascorbate excretion increases sharply for plasma ascorbate concentrations above 45-60 µmol/L.

#### 2.3.5.3. Breast milk

Lactating women secrete vitamin C via breast milk. Vitamin C concentration in human milk reflects maternal vitamin C intake more than the infant's requirement (WHO/FAO, 2004).

Appendix 4 reports data from seven studies in Europe and the US on the mean vitamin C concentration of human milk from healthy lactating mothers not taking vitamin C supplements. Mean vitamin C concentration in human milk ranged from 35 to 90 mg/L. Mean maternal vitamin C intake reported in six studies ranged from < 80 mg/day to about 150 mg/day; mean plasma concentrations, reported in five studies, ranged from 30 to 84 µmol/L. Smoking status was reported in three studies.

Vitamin C intakes and serum ascorbate concentration were investigated in smoking (n = 16, three of them receiving vitamin C supplements) and non-smoking (n = 41, unsupplemented) women during their third trimester of pregnancy, and vitamin C concentration in their milk was also investigated. While total daily vitamin C intake and serum ascorbate concentration were not statistically different between the two groups, women smoking during lactation showed significantly lower vitamin C concentrations in both transition and mature milk as compared to non-smoking women (Ortega et al., 1998a).

The Panel notes that mean vitamin C concentration of human milk from healthy mothers not taking vitamin C supplements is in the range of 35-90 mg/L.

## 2.4. Biomarkers

### 2.4.1. Biomarkers of intake

Vitamin C intake can be calculated from food consumption and the vitamin C content of foodstuffs available from food composition tables (Deharveng et al., 1999). Plasma ascorbate concentration is not a reliable indicator of vitamin C intake since the relationship between vitamin C intake and plasma concentration is sigmoidal, i.e. it is approximately linear for low vitamin C intakes but progressively reaches a plateau for plasma values above 50 µmol/L (see Section 2.3.2.).

A systematic review and meta-analysis (Dehghan et al., 2007) investigated the relationship between dietary vitamin C intakes, measured by Food Frequency Questionnaire (FFQ), dietary recalls (DR)/diary or weight record method (WR), and plasma ascorbate concentrations, from cross-sectional or validation studies published between 1960 or 1988 (according to the database considered) and 2006. Studies were excluded from the review if the dietary intake was not measured quantitatively or if the correlation between dietary intake and plasma concentration was not reported. A total of 26 studies undertaken on 26 631 non-pregnant, non-lactating adults aged 18-65 years including smokers (18 740 subjects with DR/diary methods, 6 774 with FFQ and 1 117 with WR) were considered, some of which investigated several dietary assessment methods. Mean vitamin C intakes ranged between 37 and 327.5 mg/day. Overall, there was a positive linear correlation between plasma ascorbate concentration and vitamin C intake either measured by FFQ (crude r = 0.35 for both sexes, 0.39 for females, and 0.46 for males, 18 studies), or by DR/diary (crude r = 0.46 for both sexes, 0.44

for females, and 0.36 for males, ten studies), or by WR (r = 0.39, four studies). The Panel notes that in this systematic review, vitamin C intake measured by FFQ, DR/diary or WR had a moderate relationship with plasma ascorbate concentration, and that a wide range of vitamin C intakes was considered, for which a sigmoidal relationship with plasma ascorbate was observed (Figure 1).

The Panel considers that plasma ascorbate may be used as a biomarker of vitamin C intake for low intakes, i.e. corresponding to the linear part of the sigmoidal curve of plasma ascorbate concentration as a function of vitamin C intake. The Panel also considers that for higher intakes and plasma values above 50 µmol/L, i.e. at concentrations close to the progressive flattening of the curve, plasma ascorbate concentration is of limited value as a biomarker of vitamin C intakes.

### 2.4.2. Biomarkers of body stores

Biomarkers of status are related to the mass balance of vitamin C in the body, which is determined from the rate of turnover of the body pool by taking into account metabolic losses, urinary losses and the quantity of vitamin C required for the replacement of these losses taking into account its absorption efficiency (see Sections 2.3. and 6.1.).

Plasma and leukocyte ascorbate concentrations are considered appropriate biomarkers of status (IOM, 2000; Gibson, 2005; Fairweather-Tait, 2011; Levine et al., 2011; EURRECA, online). Plasma ascorbate concentration is a valid marker of ascorbate body stores within the usual range of intakes, provided that blood samples are taken after an adequate fasting period (Read, 1987; VanderJagt et al., 1987; Levine et al., 1996). Alternatively, ascorbate concentration in cells such as leukocytes are biomarkers of ascorbate body stores independent of recent vitamin C intake. In this Opinion, plasma ascorbate, and not leukocyte concentrations, will be used as primary indicator of body stores because of the larger number of data available.

### 2.4.3. Biomarkers of function

#### 2.4.3.1. Markers of collagen metabolism

Vitamin C plays an important role in collagen metabolism. Several biomarkers of *in vivo* collagen metabolism, such as urinary excretion of hydroxyproline (Hevia et al., 1990), have been considered (IOM, 2000). Significant changes in markers of collagen turnover can be detected in severe vitamin C deficiency. However, markers of collagen turnover are not reliable markers of vitamin C stores and cannot be used to identify subjects with subclinical vitamin C deficiency (Hevia et al., 1990; IOM, 2000).

The Panel considers that markers of collagen metabolism are not a suitable criterion for deriving the requirement for vitamin C.

#### 2.4.3.2. Markers of carnitine status

Vitamin C plays an important role in carnitine synthesis (see Section 2.2.3.1.). Carnitine concentrations can be measured in urine (Davies et al., 1987; Jacob and Pianalto, 1997) and plasma (Johnston et al., 1996). Vitamin C deficiency appears to alter carnitine metabolism, and muscle carnitine is depleted in scurvy. However, neither animal nor human studies showed a consistent relationship between vitamin C stores and carnitine concentrations (Davies et al., 1987; Rebouche, 1995; Johnston et al., 1996; Jacob and Pianalto, 1997; IOM, 2000).

The Panel considers that carnitine concentrations are not a suitable criterion for deriving the requirement for vitamin C.

2.4.3.3. Markers of oxidative damage

Vitamin C plays a role in the protection of cells and molecules against oxidative damage. Oxidative damage to DNA, proteins and lipids can be measured *in vivo* using biomarkers validated for that purpose (EFSA NDA Panel, 2011).

Most of the human intervention studies available which have investigated the effects of vitamin C on reliable markers of *in vivo* oxidative damage to molecules have measured plasma or urine isoprostanes or isoprostane metabolites to assess oxidative damage to lipids. Few studies or no studies on other markers of lipid peroxidation, such as oxidised LDL particles (Van Hoydonck et al., 2004), or on markers of oxidative damage to proteins or DNA (Porkkala-Saratoho et al., 2000), are available (Heinonen et al., 2012).

The only depletion–repletion study on *in vivo* oxidative damage was conducted in hospitalised young healthy women, who were depleted with a diet containing < 5 mg/day vitamin C until plasma concentrations of ascorbate < 8 µmol/L were achieved with no signs of scurvy. Then, daily doses of 30-2 500 mg vitamin C were administered in succession until establishment of a new steady state under each dose. Plasma and urine F2-isoprostanes, as well as urine concentrations of a major metabolite of F2-isoprostanes, were unaffected by vitamin C depletion or vitamin C intake (Levine et al., 2001).

Isoprostanes have been shown to be elevated in chronic conditions associated with increased oxidative stress, such as obesity or smoking. A number of randomised, placebo-controlled intervention studies in humans have investigated the effects of vitamin C intake on isoprostanes in relation to smoking and body weight using supplemental doses of vitamin C between 500 and 1 000 mg/day. Supplementation with 500 mg/day vitamin C for two months significantly decreased urinary 8-iso-prostaglandin F2α in mostly overweight non-smoking subjects (Huang et al., 2002), and plasma F2-isoprostane concentrations in passive smokers (i.e. non-smokers for at least 12 months who had been exposed to cigarette smoke of at least one cigarette per day on at least five days/week, indoors) (Dietrich et al., 2003) and in smokers with a BMI > 26.6 kg/m$^2$, but not in smokers with a BMI < 26.6 kg/m$^2$ (Dietrich et al., 2002). Vitamin C supplementation at doses of 1 000 mg/day for two months significantly decreased plasma F2-isoprostanes in subjects with elevated values at baseline (Block et al., 2008), whereas when given for 17 days it had no effect on urinary or plasma F2-isoprostanes in smoking or non-smoking young healthy adults (Kuiper et al., 2011).

The Panel notes that the studies available suggest an effect of vitamin C supplementation at doses of 500-1 000 mg/day for two months on total lipid peroxidation in subjects with increased oxidative stress as a function of baseline values.

The Panel also notes that neither subclinical vitamin C deficiency nor subsequent supplementation with doses from 30 to 2 500 mg/day appear to affect plasma or urine isoprostanes in healthy subjects, and that isoprostanes are not reliable markers of vitamin C stores and cannot be used to identify subjects with subclinical vitamin C deficiency. Few data are available on other markers of *in vivo* oxidative damage.

The Panel considers that markers of *in vivo* oxidative damage are not a suitable criterion for deriving the requirement for vitamin C.

2.4.3.4. Markers of the function of the immune system

Leukocytes have a high vitamin C concentration that is known to decline during stress and infection as it is used to counteract the oxidative response. Vitamin C has been reported to modulate some markers of the function of the immune system such as antimicrobial cell activities, natural killer cell activities, lymphocyte proliferation, proinflammatory cytokine production, chemotaxis and delayed dermal sensitivity, generally in studies involving healthy subjects supplemented with vitamin C doses between 200 mg/day and 6 g/day. However, these markers were not considered as a suitable criterion

for deriving the requirement for vitamin C (IOM, 2000). The evidence available to the Panel does not establish that modulation of any of these markers is in itself a health outcome which could be considered as a suitable criterion for deriving a DRV for vitamin C.

The Panel considers that markers of the function of the immune system are not a suitable criterion for deriving the requirement for vitamin C.

### 2.5. Effects of genotype

Glutathion-S-transferases (GST), which are xenobiotic-metabolising enzymes, and haptoglobin (Hp), which is a haemoglobin-binding protein, are characterised by a genetic polymorphism in humans; the null genotypes of the GSTM1 and GSTT1 genes lead to the absence of enzyme function (Yuan et al., 2012) and the Hp2-2 homozygote produces a less active protein (Cahill and El-Sohemy, 2010).

The Panel notes that plasma/serum ascorbate concentrations may be influenced by these polymorphisms, but available data are inconsistent (Langlois et al., 1997; Dusinska et al., 2001; Na et al., 2006; Cahill et al., 2009; Cahill and El-Sohemy, 2010; Block et al., 2011; Horska et al., 2011; Yuan et al., 2012).

The Panel considers that data on the effect of genotype on plasma ascorbate concentration are insufficient to be used for deriving the requirements for vitamin C according to genotype variants.

## 3. Dietary sources and intake data

### 3.1. Dietary sources

Foods with a naturally high vitamin C content are fruits such as berries, lychee, papaya, kiwi, and citrus fruits, vegetables like Brussels sprouts, cauliflower, cabbage, sweet pepper, and herbs/spices like parsley, sorrel and chives (Anses/CIQUAL, 2012). Animal tissues also contain vitamin C but in lower amounts. Kidney and liver have a higher vitamin C content than other animal-derived foods. The precise amount of vitamin C in a specific food varies according to season of harvesting, transport, shelf time prior to use, and cooking practices. When foods are processed, vitamin C may be exposed to oxygen or high temperatures, which accelerate oxidation. Because of its water solubility, vitamin C may also be lost when the cooking water is discarded.

Currently, magnesium L-ascorbate and zinc L-ascorbate may be added to food supplements[6], whereas L-ascorbic acid, sodium L-ascorbate, calcium L-ascorbate, potassium L-ascorbate, and L-ascorbyl 6-palmitate may be added to both foods[7] and food supplements[6]. The vitamin C content of infant and follow-on formulae is regulated[8].

The main contributors to the vitamin C intake of adults are fruits and vegetables and their juices, and potatoes (Serra-Majem et al., 2007; Pedersen et al., 2010; DGE, 2012) (Appendix 1).

### 3.2. Dietary intake

Typical vitamin C intakes of children and adolescents from 20 countries (Appendix 2) and of adults from 23 countries (Appendix 3) in Europe are presented. The data refer to individual-based food consumption surveys, conducted from 1989 onwards. Most studies comprise nationally representative population samples.

[6] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ L 183, 12.7.2002, p. 51.
[7] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26.
[8] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p.1.

There is a large diversity in the methodology used to assess the individual intakes of children, adolescents and adults (Appendices 2A and 3A). These differences in dietary assessment methods make comparison difficult. Age classifications may not be uniform and comparability is also hindered by differences in the food composition tables used for the conversion of food consumption data to nutrient intake data (Deharveng et al., 1999). Dietary intake data are prone to reporting errors and there is a varying degree of under-reporting in different surveys (Merten et al., 2011).

Although the differences in methodologies have an impact on the accuracy of between-country comparisons, the data presented give an overview of the vitamin C intake in a number of European countries. Most studies reported mean intakes and SD, and sometimes intake distributions. Seven surveys out of 43 reported the intakes from both food and dietary supplements.

In men, average vitamin C intakes from food only range from 69 to 130 mg/day, and in women from 65 to 138 mg/day. In adults, ranges varied from 11–65 mg/day at the lower end (2.5-25$^{th}$ percentile, without dietary supplements) to 106-1 082 mg/day (the value of 1 082 mg/day including dietary supplements) at the upper end (75-97.5$^{th}$ percentile) of the intake distributions.

In infants and young children, average vitamin C intakes from food only range from 44 to 119 mg/day. In older children (less than 14 years), average daily vitamin C intakes from food only vary between 60 and 172 mg/day in boys, and between 61 and 172 mg/day in girls. In adolescents (older than 14 years), average vitamin C intakes from food only are between 73 and 146 mg/day in males, and between 75 and 149 mg/day in females.

## 4. Overview of Dietary Reference Values and recommendations

### 4.1. Adults

D-A-CH (2013) stated that epidemiological data showed a reduction in the risk of chronic diseases, in particular cardiovascular and cancer morbidity and mortality in non-smokers with plasma ascorbate concentrations higher than 50 µmol/L, corresponding to dietary intakes of 90-100 mg/day (Carr AC and Frei B, 1999). Another criterion was saturation of immunocompetent cells, achieved with an intake of 100 mg/day (Levine et al., 1996; Levine et al., 1997b; Levine et al., 1997a). Using data on intakes required to reach plasma ascorbate concentrations of 50-75 µmol/L in male and female non-smokers (Heseker et al., 1992; Kübler, 1995), and a bioavailability from a mixed diet of at least 80 % (Levine et al., 1999), an AR of 82 mg/day and a recommended intake of 100 mg/day were derived for adults, considering a coefficient of variation (CV) of 10 %. For heavy smokers (i.e. smoking more than 20 cigarettes/day), an intake of 150 mg/day was recommended, considering reduced absorption, increased daily turnover and increased oxidative DNA damage in these subjects (Heseker et al., 1992; Loft et al., 1992; Asami et al., 1997; Prieme et al., 1998).

The Nordic countries (NNR, 2012) maintained their lower level of intake at 10 mg/day for adults, as well as their previous reference values which were based on the antioxidant activity of vitamin C and the association between a plasma ascorbate concentration of 32 µmol/L and a decreased risk of cardiovascular or cancer mortality and morbidity (unweighted mean cut-off point for a lowered risk, compared to the first quintile of plasma ascorbate, from eight studies) (Riemersma et al., 1991; Gale et al., 1995; Singh et al., 1995; Eichholzer et al., 1996; Sahyoun et al., 1996; Nyyssonen et al., 1997; Loria et al., 2000; Khaw et al., 2001). Using pharmacokinetic data (Levine et al., 1996; Levine et al., 2001), this plasma value corresponded to intakes of about 60 mg/day in men and 50 mg/day in women, which were set as the ARs. Women were considered to have a slightly lower AR than men (Olson and Hodges, 1987), but to ensure adequate iron absorption the allowance for inter-individual variation for women was assumed to be double (25 %) that for men, and the same Recommended Intake (RI) was thus set for both sexes, i.e. 75 mg/day. Cohort studies on morbidities (Boekholdt et al., 2006; Harding et al., 2008; Myint et al., 2008; Myint et al., 2011; Pfister et al., 2011) published after the last Nordic Nutrition recommendations (NNR, 2004) suggested a higher plasma ascorbate

concentration of 40-50 µmol/L as a basis to set the AR, but were considered insufficient to raise the AR. No specific recommendation was made for older adults or smokers.

WHO/FAO (2004) applied the same calculation as SCF (1993) to derive a Recommended Nutrient Intake (RNI) of 45 mg/day, considering (i) a body content of 900 mg vitamin C as it is halfway between tissue saturation (1 500 mg) and the point at which clinical signs of scurvy appear (300-400 mg), (ii) an absorption efficiency of 85 % as it is intermediate between absorption observed at low dietary doses and that seen in the intake range of 30-180 mg/day (Melethil et al., 1986; Graumlich et al., 1997), and (iii) metabolic losses of 2.9 % per day with an SD of 0.6 % (Baker EM et al., 1971). Women were considered to have a lower AR than men; however, plasma concentrations fell more rapidly in women than in men in depletion studies (Blanchard, 1991b). Thus, the same RNI was proposed for adults whatever the sex or age. The AR for vitamin C for adults was interpolated between the amount considered to protect against scurvy (10 mg/day) and the RNI of 45 mg/day, i.e. 25-30 mg/day. It was considered that there was no justification for deriving a specific RNI for smokers.

Afssa (2001) considered the curve relating the median plasma ascorbate concentrations to increments of 25 mg in intake, derived from three 24-hour dietary recalls and one plasma ascorbate determination in subjects of a French cohort study (SU.VI.MAX, n = 2 509 women aged 35-60 years and 3 116 men aged 40-65 years at inclusion). Plasma concentrations first increased according to intakes, then reached steady state values of 64 ± 1 µmol/L in women, and 56 ± 3 µmol/L in men. A sub-group was then selected of subjects whose plasma ascorbate concentrations were equal to those values ± 5 %, i.e. in the range of 53-59 µmol/L for men (n = 257) and 61-67 µmol/L for women (n = 443). Their normalised mean intakes were 85.4 and 85.9 mg/day for men and women, respectively. Adding to these values twice a theoretical SD of 15 %, the Population Reference Intake (PRI) was set at 110 mg/day for both sexes. Considering plasma ascorbate concentrations of subjects from the SU.VI.MAX cohort, as well as subjects smoking more than ten cigarettes/day and whose intake distribution was similar, Afssa recommended that smokers ingest 20 % more vitamin C per day (i.e. 130 mg/day). A reference value of 120 mg/day was set for adults aged 75 years and older, based on considerations related to supplemental vitamin C intake in older people and immunity, cardiovascular risk, cancer risk, and cognition.

The US Institute of Medicine (IOM, 2000) stated that in order to set requirements, there were no human data to derive a dose-response relationship between vitamin C intake and *in vivo* antioxidant protection, but that antioxidant protection and neutrophil ascorbate concentrations are correlated (Anderson and Lukey, 1987). Using the limited data on plasma and neutrophil concentrations and urinary excretion during vitamin C depletion-repletion in healthy men (Levine et al., 1996), IOM selected the value of 80 % of maximal neutrophil ascorbate concentration (i.e. 1 mmol/L), which was equivalent to a vitamin C intake of about 75 mg/day considered to be sufficient to maintain near-maximal neutrophil concentrations with minimal urinary losses, and set this value as the Estimated Average Requirement (EAR) for men. Because of the lack of data, the EAR for women (60 mg/day) was extrapolated from the value for men, and the quotient between the reference body weight of women and that of men (adapted from NHANES III 1988-1994). A Recommended Dietary Allowance (RDA) was derived by adding to the EAR twice an assumed CV of 10 % (assumption based on the variation in basal metabolic rate and the variation estimated for the protein requirement in adults (Garby and Lammert, 1984; FAO/WHO/UNU, 1985)). The RDAs were thus 90 mg/day for men (19-50 years) and 75 mg/day (rounded value) for women of the same age. No consistent differences in the absorption or metabolism of ascorbic acid due to ageing were demonstrated, and therefore the RDA set for adults aged more than 50 years was the same as for adults aged less than 50 years. Because of higher metabolic losses of vitamin C, smokers were considered to require an additional 35 mg/day of vitamin C over that needed by non-smokers (Kallner et al., 1979; Kallner et al., 1981). Passive smokers as well as people under excessive physical and emotional stress were also considered, but without setting specific values.

The SCF (1993) considered a total body pool of 900 mg vitamin C (providing reserves for periods of low intake or high needs), multiplied by metabolic losses of 2.9 % (Baker EM et al., 1971) (with two

SDs of 0.6 % to set the PRI), and divided by an absorption efficiency of 85 %. Thus, an AR of 30 mg/day and a PRI of 45 mg/day were derived for adults (rounded value). A similar calculation targeting the maintenance of a body pool of 600 mg yielded an LTI of 12 mg/day. The PRI for older adults was considered to be the same as for younger subjects.

The Netherlands Food and Nutrition Council (1992) considered that 45 to 60 mg vitamin C/day were necessary to maintain the body pool in adults (estimated to be 1 500 mg), and that the requirement would thus be between 50 and 65 mg/day considering an absorption efficiency of 80-90 % (Baker et al., 1969; Baker EM et al., 1971; Hodges et al., 1971; Kallner et al., 1977, 1979; Kallner et al., 1982). Hence, a reference value of 70 mg/day was estimated for adults, assuming an AR of 58 mg/day and adding to it a 20 % safety margin for variation, based on depletion-repletion studies (Baker et al., 1969; Baker EM et al., 1971; Hodges et al., 1971).

The UK COMA (DH, 1991) focused on the sigmoidal relationship between vitamin C intake and plasma ascorbate concentrations (Bates et al., 1979; Basu and Schorah, 1982). Measurable amounts of ascorbate were found to begin to circulate in the plasma of most people at an intake of 40 mg/day, which was set as the Reference Nutrient Intake for both sexes, and also for older adults because of a lack of data. The EAR of 25 mg/day was interpolated between that value and the Lower Reference Nutrient Intake (LRNI) of 10 mg/day for adults (based on scurvy prevention). Because of increased losses of vitamin C, it was considered that the intake of smokers would need to be higher by up to 80 mg/day compared to that of non-smokers (Kallner et al., 1981; Smith and Hodges, 1987).

An overview of Dietary Reference Values for vitamin C for adults from various authorities is given in Table 1. In addition, some organisations presented some considerations regarding smokers, and proposed for this population an additional daily intake of 35 mg (IOM, 2000), 50 mg (D-A-CH, 2013), 80 mg (DH, 1991) or 20 % (Afssa, 2001).

**Table 1:** Overview of Dietary Reference Values for vitamin C for adults

| | D-A-CH (2013) | NNR (2012) | WHO/FAO (2004) | Afssa (2001) | IOM (2000) | SCF (1993) | NL (1992) | DH (1991) |
|---|---|---|---|---|---|---|---|---|
| **Age** (years) | ≥ 19 | ≥ 18 | ≥ 19 | 20-74 | ≥ 19 | ≥ 18 | ≥ 19 | ≥ 19 |
| **PRI** | | | | | | | | |
| **Men** (mg/day) | 100 | 75 | 45 | 110 | 90 | 45 | 70 | 40 |
| **Women** (mg/day) | 100 | 75 | 45 | 110 | 75 | 45 | 70 | 40 |
| **Age** (years) | | | | ≥ 75 | | | | |
| **PRI** | | | | | | | | |
| **Men** (mg/day) | | | | 120 | | | | |
| **Women** (mg/day) | | | | 120 | | | | |

NL: The Netherlands

### 4.2. Infants and children

D-A-CH (2013) estimated the Adequate Intake (AI) for infants from birth to four months considering an average vitamin C concentration in human milk of 65 mg/L (Souci et al., 2008) and an average milk intake of 0.75 L/day. The values for older children were interpolated between this AI and the reference value for non-smoking adults.

The Nordic countries (NNR, 2012) extrapolated ARs for children (< 14 years) from adult ARs, using an assumed growth factor of 1.3 for children aged less than two years (i.e. reference values of 20 mg/day for 6-11 months and 25 mg/day for 12-23 months) and a factor of 1.15 for children aged 2-13 years. The ARs were then multiplied by 1.25 to derive RIs.

WHO/FAO (2004) concluded that the amount of vitamin C in human milk reflects maternal dietary vitamin C intake rather than the infant's needs. The RNI was set to 25 mg/day for infants from birth to six months and increased gradually for older children.

For children, Afssa (2001) tested several parameters such as energy expenditure or square height to derive values either from the vitamin C intake of breast-fed infants between six months and one year, or from the PRI for adults. Estimates from both methods were similar for girls but not for boys, and those derived initially for girls were chosen for both sexes considering the lack of difference in requirement according to sex in infants and adults.

For infants aged 7-12 months, the IOM (2000) set an AI of 50 mg/day, considering a vitamin C concentration of 45 mg/L in human milk and a milk consumption of 0.6 L/day (Dewey et al., 1984; Salmenpera, 1984) corresponding to an intake of 27 mg/day from human milk at nine months of lactation, to which an intake of 22 mg/day from solid foods was added (Montalto et al., 1985). An alternative method based on extrapolation from the AI for breast-fed infants aged less than six months and the quotient between the reference body weights of older and younger infants (adapted from NHANES III 1988-1994) provided the same value. For older children, the EARs were estimated from those for adults using the quotient between their reference body weights (adapted from NHANES III 1988-1994). RDAs were derived by adding twice an assumed CV of 10 %, because of the unknown SD of the requirement for vitamin C.

Because of the lack of data, the SCF (1993) set the PRI for infants aged 6-11 months at 20 mg/day, mentioning that it corresponds to about three times the intake known to prevent scurvy in infants. The PRIs for older children were increased gradually to those for adults.

The mean intake of vitamin C through human milk was considered as the reference value for infants by the Netherlands Food and Nutrition Council (1992) and the UK COMA (DH, 1991), i.e., respectively, 35 mg/day (Thomas et al., 1979; Olson and Hodges, 1987) and 25 mg/day (Bates and Prentice, 1988). For children, the values were either interpolated between the Dutch reference values for infants and for adults, or scaled down from the UK adult values.

An overview of Dietary Reference Values for vitamin C for children is presented in Table 2.

**Table 2:** Overview of Dietary Reference Values for vitamin C for children

| | D-A-CH (2013) | NNR (2012) | WHO/FAO (2004) | Afssa (2001) | IOM (2000) | SCF (1993) | NL (1992) | DH (1991) |
|---|---|---|---|---|---|---|---|---|
| **Age** (months) | 4-< 12 | 6-11 | 7-< 12 | Infants | 7-12 | 6-11 | 6-12 | 6-12 |
| **PRI** (mg/day) | 55 | 20 | 30 | 50[(a)] | 50[(a)] | 20 | 35[(a)] | 25[(a)] |
| **Age** (years) | 1-< 4 | 1-< 2 | 1-3 | 1-3 | 1-3 | 1-3 | 1-4 | 1-3 |
| **PRI** (mg/day) | 60 | 25 | 30 | 60 | 15 | 25 | 40 | 30 |
| **Age** (years) | 4-<7 | 2-5 | 4-6 | 4-6 | 4-8 | 4-6 | 4-7 | 4-6 |
| **PRI** (mg/day) | 70 | 30 | 30 | 75 | 25 | 25 | 45 | 30 |
| **Age** (years) | 7-< 10 | 6-9 | 7-9 | 7-9 | 9-13 | 7-10 | 7-10 | 7-10 |
| **PRI** (mg/day) | 80 | 40 | 35 | 90 | 45 | 30 | 50 | 30 |
| **Age** (years) | 10-< 13 | 10-13 | 10-18 | 10-12 | 14-18 | 11-14 | 10-13 | 11-14 |
| **PRI** (mg/day) | 90 | 50 | 40 | 100 | 75 (m)<br>65 (f) | 35 | 55 | 35 |
| **Age** (years) | 13-< 19 | 14-17 | | 13-19 | | 15-17 | 13-16 | 15-18 |
| **PRI** (mg/day) | 100 | 75 | | 110 | | 40 | 65 | 40 |
| **Age** (years) | | | | | | | 16-18 | |
| **PRI** (mg/day) | | | | | | | 70 (m)<br>65 (f) | |

(a): Adequate Intake
m, males; f, females; NL: The Netherlands

### 4.3. Pregnancy and lactation

From the fourth month of pregnancy onwards, D-A-CH (2013) added an intake of 10 mg/day to the RI for non-pregnant women (hence an RI of 110 mg/day), to help maintain body pools. For lactation,

D-A-CH considered a milk production of 0.75 L/day and an average vitamin C content of 65 mg/L, and added 50 mg/day to the RI for non-lactating women (i.e. an RI of 150 mg/day).

The Nordic countries (NNR, 2012) maintained their previous RI of 85 mg/day for pregnancy, i.e. higher by 10 mg/day compared to the RI for non-pregnant women, to cover the increased need due to the growth of the fetus and the increased catabolism of vitamin C (Olson and Hodges, 1987). During lactation, the RI was increased by 25 mg/day to 100 mg/day based on an average vitamin C content of 30 mg/L in human milk (Olson and Hodges, 1987) and an average milk secretion of 0.75 L/day.

WHO/FAO (2004) added throughout pregnancy an intake of 10 mg/day to the RNI for non-pregnant women (hence an RNI of 55 mg/day) to meet the needs of the growing fetus in the last trimester, as 8 mg/day is sufficient to prevent signs of scurvy in infants aged four to seven months (Irwin and Hutchins, 1976). During lactation, a vitamin C secretion via milk of 20 mg/day and an absorption efficiency of 85 % were the basis for recommending an extra 25 mg/day (hence an RNI of 70 mg/day).

For pregnant women, Afssa (2001) added 10 mg/day to the PRI for non-pregnant women (hence a PRI of 120 mg/day) to take into account haemodilution and the active transport of vitamin C through the cord and placenta (Morse et al., 1975). For lactating women, Afssa (2001) recommended an additional intake of 20-30 mg/day taking into account a vitamin C concentration in human milk of 40-60 mg/L (Salmenpera, 1984), and hence a PRI of 130 mg/day.

IOM (2000) noted that the maternal plasma ascorbate concentration decreases during pregnancy because of haemodilution and active transfer to the fetus (Morse et al., 1975; Choi and Rose, 1989), but that the amount of vitamin C required for transfer to the fetus was unknown and was estimated taking into account that intakes of 7 mg/day prevent scurvy in young infants (Van Eekelen, 1953; Goldsmith, 1961; Rajalakshmi et al., 1965). The EAR for near-maximal neutrophil concentration in non-pregnant women was therefore increased by 10 mg/day and, after adding twice an assumed CV of 10 % (because the SD of the requirement for vitamin C was unknown) and rounding, RDAs for pregnancy of 80 mg/day (14-18 years) and of 85 mg/day (adult women) were derived. For lactating women, the average amount of vitamin C secreted into milk during the first six months of lactation, i.e. 40 mg/day, was added to the EAR for non-lactating women. After adding twice an assumed CV of 10 %, RDAs for lactation of 115 mg/day (14 to 18 years) and 120 mg/day (adult women) were derived.

For pregnant women, the SCF (1993) proposed an additional intake of 10 mg/day to be added to the PRI for non-pregnant women to meet the needs of the fetus. For lactating women, an amount of 20 mg/day of vitamin C was considered to be secreted via milk and, assuming 85 % bioavailability, an intake of 25 mg/day was added to the PRI for non-lactating women.

For pregnant women, the Netherlands Food and Nutrition Council (1992) added 20 mg/day to the reference value for non-pregnant women, in agreement with the US National Research Council (NRC, 1980). For lactating women, the reference value was increased by 40 mg/day compared to non-lactating women, assuming a bioavailability of 80-90 % and a daily loss of 35 mg of vitamin C via milk.

The UK COMA (DH, 1991) considered that there is a moderate extra drain on tissue stores during pregnancy and thus increased the Reference Nutrient Intake by 10 mg/day during the third trimester. For lactating women, 30 mg/day were added to the Reference Nutrient Intake for non-lactating women to ensure the maintenance of maternal stores and to keep the concentrations in human milk in the upper half of the physiological range.

An overview of Dietary Reference Values for vitamin C for pregnant and lactating women is presented in Table 3.

**Table 3:** Overview of Dietary Reference Values for vitamin C for pregnant and lactating women

| | D-A-CH (2013) | NNR (2012) | WHO/ FAO (2004) | Afssa (2001) | IOM (2000) | SCF (1993) | NL (1992) | DH (1991) |
|---|---|---|---|---|---|---|---|---|
| **Pregnancy: additional intake** (mg/day) | (from 4th month onwards) 10 | 10 | 10 | 10 | 10 | 10 | 20 | (3rd trimester) 10 |
| **PRI** (mg/day) | 110 | 85 | 55 | 120 | 80 (14-18 y)<br>85 (≥ 19 y) | 55 | 90 | 50 |
| **Lactation: additional intake** (mg/day) | 50 | 25 | 25 | 20-30 | 40 | 25 | 40 | 30 |
| **PRI** (mg/day) | 150 | 100 | 70 | 130 | 115 (14-18 y)<br>120 (≥ 19 y) | 70 | 110 | 70 |

NL: The Netherlands; y: years.

## 5. Criteria (endpoints) on which to base Dietary Reference Values

The Panel considers that biomarkers of function for vitamin C, i.e. markers of collagen metabolism, of carnitine status, of oxidative damage, or of function of the immune system, cannot be used as a criterion for deriving the requirement for vitamin C. Other markers or outcomes may be considered for deriving this requirement.

### 5.1. Scurvy

Scurvy can be prevented by a vitamin C intake above 10 mg/day (see Section 2.2.3.1.). The Panel concludes that this low amount represents the intake associated with the appearance of severe deficiency but cannot be used as a criterion for deriving the requirement for vitamin C.

### 5.2. Biomarkers of body stores

The maintenance of the body pool and plasma and cellular concentrations of vitamin C can be considered as a criterion for establishing the requirement for vitamin C, assuming that body pools and plasma concentrations near saturation are associated with fulfilling vitamin C's coenzymatic and antioxidant functions.

Plasma ascorbate concentration is considered in this Opinion as the primary indicator of body stores (see Section 2). Plasma ascorbate concentration < 10 µmol/L is regarded as indicative of severe deficiency, and in the range from 10 to < 50 µmol/L as indicative of suboptimal status with a risk of insufficiency. A plasma concentration of 45-50 µmol/L was shown to correspond to the saturation of the pathways involved in the metabolic losses of ascorbic acid, and may represent the amount of ascorbic acid required to exert its physiological role (Kallner et al., 1979). A plasma ascorbate concentration of 50 µmol/L is indicative of adequate status that corresponds to a body pool of about 1.5 g with metabolic losses of about 3.0 % per day (or 40-50 mg/day). No additional increase in muscle ascorbate concentration was observed in non-smoking men at a plasma ascorbate concentration above around 50 µmol/L (Carr et al., 2013).

Ascorbate is excreted in urine even for low plasma ascorbate concentrations. For plasma concentrations above around 50 µmol/L, urinary ascorbate excretion increases sharply, and this is assumed to reflect near-saturation of body pools.

In healthy male adults, a plasma ascorbate concentration of 50 µmol/L is reached with a vitamin C intake between 60 and 100 mg/day, with intestinal losses of about 20 % and urinary excretion up to 25 % of the intake. Vitamin C intakes above 200 mg/day progressively elevate the plasma concentration to a plateau of 70-80 µmol/L or even above, at the expense of a dramatic increase in urinary excretion. Women reach a plasma ascorbate concentration of 50 µmol/L with a slightly lower intake of vitamin C compared to men.

The pharmacokinetics of vitamin C were compared in young and elderly men and women following a 500 mg oral dose administered to each subject either in a depleted state achieved by four to five weeks on a diet containing less than 10 mg vitamin C/day, or in a repleted state achieved following daily vitamin C doses of 500 mg/day for the three following weeks (Blanchard, 1991a). Men were taller and heavier than women and consequently had a greater body surface area. In the repleted state, compared to men, women exhibited a significantly greater peak plasma ascorbate concentration (Cmax), a shorter absorption lag time (tlag), as well as greater apparent volume of distribution (AVd), clearance (CL) and renal clearance (CLr) when these were expressed relative to body weight. However, when AVd, CL and CLr were expressed in absolute terms (i.e. L or mL/min), no sex-related differences were observed. In addition, Cmax was inversely linearly related to body weight, tlag was directly related to body surface area and dose, and AVd (L/kg) was inversely linearly related to body surface area, while CL (mL/h per kg) and CLr (mL/h per kg) were not significantly related to any of the body composition parameters examined (e.g. body weight, body surface area and body composition). The Panel notes that this study shows few sex-related differences in the pharmacokinetics of vitamin C, and that they may be related to differences in body weight and body surface area between the sexes (Blanchard, 1991a).

The Panel also notes that women reach the plateau of plasma ascorbate concentration at a lower vitamin C intake than men, but that the value of the plasma concentration at the plateau is similar in women and men (Levine et al., 1996; Levine et al., 2001; Levine et al., 2011) (see Section 2.3.2.).

In smokers, the plasma ascorbate concentration is usually lower than in non-smokers because of higher metabolic losses of vitamin C compared to non-smokers at similar vitamin C intakes.

IOM (2000) concluded from the available evidence that there were no consistent differences in the measurements of intestinal absorption, urinary excretion or plasma and leukocyte concentrations of ascorbate in older compared to younger adults. The Panel notes that no new data on the influence of ageing on these parameters have become available since then.

The Panel considers that in healthy adults, an intake of vitamin C that balances daily losses and maintains a plasma concentration of 50 µmol/L, corresponding to near-saturation of body pools with minimal urinary excretion, allows the fulfillment of the functions of vitamin C, and is a suitable criterion for deriving the requirement for vitamin C.

## 5.3. Markers of disease risk

### 5.3.1. Blood lipids

Randomised controlled trials (RCTs) on the effects of vitamin C at doses of 1 000 mg/day for eight months in healthy subjects showed no effect on blood lipids (Jacques et al., 1995; Jenner et al., 2000), whereas the same dose administered for four weeks decreased plasma LDL-cholesterol concentration by 16 % in healthy women (Gatto et al., 1996). No studies are available at lower doses.

The Panel considers that the data provided by these studies on blood lipids cannot be used as a criterion for deriving the requirement for vitamin C.

### 5.3.2. Blood pressure

A prospective cohort study using the European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk cohort assessed the relationship between plasma concentrations of ascorbate at baseline and incident heart failure during a mean follow-up of about 12 years (20 299 participants). After adjusting for relevant confounders every 20 µmol/L difference in plasma, vitamin C was significantly associated with - 0.9 mmHg systolic blood pressure (SBP) and - 0.5 mmHg diastolic blood pressure (DBP). The risk of having high SBP in the fourth quartile of plasma ascorbate concentrations (≥ 66 µmol/L) was 22 % lower (odds ratio: 0.78, 95% CI 0.71-0.86) than in the first quartile (< 41 µmol/L) (Pfister et al., 2011).

The effect of vitamin C supplementation on blood pressure was evaluated in a meta-analysis of 29 RCTs (Juraschek et al., 2012). Doses of vitamin C ranged from 60 to 4 000 mg/day (median 500 mg/day), interventions lasted two to 26 weeks (median eight weeks) and trial size ranged from ten to 120 subjects. Fifteen trials permitted concurrent use of antihypertensive agents. Sixteen trials used vitamin C alone in their intervention arms, whereas 13 trials used a combination of vitamins and minerals that included vitamin C. Pre-treatment plasma ascorbate concentrations ranged from 38 to 83 µmol/L. There was a large heterogeneity across studies and greater reductions in SBP were observed in trials which administered vitamin C in combination with vitamins, minerals and blood pressure-lowering medications. Vitamin C supplementation when used alone significantly reduced SBP but not DBP. Sensitivity analyses showed that the effect on blood pressure did not depend on the dose of vitamin C administered or on plasma ascorbate concentrations at baseline, but rather on sample size and the accuracy of blood pressure measurements.

The Panel considers that the data provided by these studies on blood pressure cannot be used as a criterion for deriving the requirement for vitamin C.

### 5.4. Common cold

A number of human studies have addressed the relationship between the intake of high doses of vitamin C (generally > 500 mg/day) and the risk, severity and duration of common cold. Available evidence concerning doses up to 2 000 mg/day was not consistent or specific enough to estimate the vitamin C requirement based on this outcome, as previously discussed by IOM (2000).

A recent meta-analysis of randomised placebo-controlled trials (Hemila and Chalker, 2013) investigated the effect of vitamin C supplementation (> 200 mg/day) on common cold. Primary outcomes were the incidence and duration of common cold. Regular vitamin C supplementation did not reduce significantly the incidence of common cold in the general population (pooled risk ratio from 24 trials involving 10 708 participants: 0.97; 95 % CI 0.94-1.00). The pooled effect of vitamin C supplementation on the duration of common cold was a 14.2 % (95 % CI 7.3 %-21 %) reduction in children (14 trials), and a 7.7 % (95 % CI 3.7 %-12 %) reduction in adults (17 trials). The majority of trials used 1 g/day of vitamin C. Sensitivity analyses excluding non-randomised or not-double-blind trials did not change the estimates. The Panel notes that common colds were self-diagnosed in the majority of trials, and that no clear criteria were specified in the meta-analysis to assess either the severity or the duration of a common cold episode.

The Panel considers that the data available on common cold-related outcomes cannot be used as a criterion for deriving the requirement for vitamin C.

### 5.5. Chronic disease-related outcomes

Oxidative damage to cells and molecules has been implicated in the development of a number of chronic diseases. Because of its antioxidant role in the body, it has been hypothesised that vitamin C could affect the incidence, morbidity and/or mortality of such diseases.

The relationship between vitamin C intakes and/or body stores and chronic disease outcomes has been investigated mostly in observational (case-control, cross-sectional, prospective cohort) studies, where a positive, an inverse, or a lack of an association between vitamin C and disease outcomes might be counfounded by uncertainties inherent to the methodology used for the assessment of vitamin C intakes and/or vitamin C stores, and by the effect of other dietary, lifestyle, or undefined factors on the disease outcomes investigated.

IOM (2000) reviewed the evidence available in relation to vitamin C intakes and risk of cardiovascular diseases, cancers, cataracts, chronic obstructive pulmonary disease, infectious diseases, cognitive function and memory. Several, but not all, observational studies had reported an inverse correlation between vitamin C intakes and cardiovascular disease, some types of cancer and cataracts, whereas no evidence was found for an association between vitamin C intakes and the remaining disease outcomes.

Although useful in the generation of hypotheses about the role of vitamin C in chronic disease development, the results from these studies were not consistent or specific enough to derive a requirement for vitamin C.

A comprehensive search of the literature published between 1991 and 2011 was performed as preparatory work to this assessment, to identify relevant health outcomes upon which DRVs for vitamin C may potentially be based (Heinonen et al., 2012). Inconsistent associations between vitamin C intakes and/or plasma concentrations of ascorbate and risk of diabetes, bone mineral density, Parkinson's disease, Alzheimer's disease, dementia, preterm delivery, wheeze and eczema in the first two years of life, medulloblastoma during childhood, hearing loss, periodontal disease, and multiple sclerosis, among others, have been reported. The low number of studies available for each of these outcomes does not allow conclusions to be drawn on a putative role of vitamin C in the pathogenesis of these conditions.

More data have become available on the relationship between vitamin C intake and the risk of cardiovascular diseases, cancer and vision-related outcomes and mortality.

### 5.5.1. Cardiovascular disease-related outcomes

Results from epidemiological studies on the relationship between vitamin C intake or status and cardiovascular disease (CVD) risk are heterogeneous and difficult to compare because of the diversity of outcome measures assessed.

A prospective cohort study from the EPIC Norfolk cohort (Pfister et al., 2011) assessed the relationship between plasma concentrations of ascorbate at baseline and incident heart failure (either treatment for the disease or death from the disease) during a mean follow-up of 12.8 years (20 926 participants). After adjusting for relevant confounders, including myocardial infarction (MI) and ischemic heart disease, every 20 μmol/L difference in plasma ascorbate concentration was associated with an 8 % (95 % CI 2 %-13 %, P = 0.01) lower risk of incident heart failure. Excluding cases diagnosed in the first two years of follow-up did not change the results. The association was stronger in subjects who ever smoked (about 65 % in men and 42 % in women) and in subjects with hypertension.

Three prospective cohort studies and one case-control study investigated the association between vitamin C intakes and different CVD-related outcomes. The case (n = 108)-control (n = 142) study (Nam et al., 2003) reported significantly less cases of non-fatal ischaemic heart disease (MI or coronary stenosis ≥ 50 %) in the highest tertile of vitamin C intake (≥ 220.2 mg/day) compared with the lowest tertile (< 141.8 mg/day) in a population of which about 55 % were smokers, after adjustment for relevant confounders. The association remained significant after exclusion of subjects taking multivitamins or vitamin C supplements. In a prospective cohort study (Osganian et al., 2003) including 85 118 female nurses and with a follow-up of 16 years, an inverse association between vitamin C intakes from food and supplements combined (energy-adjusted mean about 300 mg/day, lowest to highest quintiles about 70-700 mg/day) and risk of incident CHD (non-fatal MI, fatal CHD and sudden death of probable coronary origin) was observed, whereas the association between dietary vitamin C intake and incident CHD was not significant in non-supplement users. The remaining prospective cohort studies did not find a significant association between vitamin C intakes and other CVD-related outcomes, such as CVD mortality (ischemic heart disease and stroke; 559 men followed-up for 15 years) (Buijsse et al., 2008) or death from stroke (34 492 postmenopausal women; mean duration of follow-up not reported) (Yochum et al., 2000).

The only large RCT available on vitamin C supplementation (500 mg/day) and cardiovascular morbidity and mortality was conducted in 14 641 male physicians, including 5.1 % with prevalent cardiovascular disease at randomisation. No effect of supplementation was observed during a mean follow-up of eight years (Sesso et al., 2008).

### 5.5.2. Cancer

The World Cancer Research Fund (WCRF) (2007) found insufficient evidence for an association between vitamin C and cancers of the mouth, pharynx, larynx, lung, stomach, pancreas, gallbladder, colon, rectum, breast, ovary, endometrium, prostate, kidney, bladder and skin. An inverse association was reported with oesophageal cancer, which was based on significant inverse associations in 10 out of 18 case-control studies but in none of the other studies considered (one cohort and three cross-sectional studies). Not all available studies addressed the relationship between this outcome and vitamin C intakes, but rather assessed the relationship with certain food groups.

Studies published after the WCRF report include two cohort studies showing no association between dietary and/or total vitamin C intake and breast cancer risk in premenopausal and postmenopausal women (Nagel et al., 2010; Hutchinson et al., 2012), a nested case-control study showing no association between vitamin C intake and gastric cancer risk (Jenab et al., 2006), a case-control study showing no association between vitamin C intake and risk of renal cell carcinoma (Hu et al., 2009), a nested case-control study showing no association between vitamin C intake or plasma ascorbate concentrations and urothelial cell carcinoma (Ros et al., 2012), a controlled trial showing no effect of vitamin C on colorectum, lung and other cancers (Gaziano et al., 2009), a meta-analysis of RCTs reporting no effect of vitamin C intakes on incidence and mortality from prostate cancer (Jiang et al., 2010), and a prospective cohort study showing no effect of total, dietary and supplemental intake of vitamin C on prostate cancer (Roswall et al., 2013).

### 5.5.3. Vision-related outcomes

In the IOM report, three case-control comparisons (Robertson et al., 1989; Jacques and Chylack, 1991; Leske et al., 1991) were described that showed an association between high vitamin C intakes or vitamin C supplement use and a decreased risk for cataract. An inverse association was also found in a prospective cohort study for long-term users of vitamin C supplements only (Hankinson et al., 1992), but not in another cohort study (Vitale et al., 1993). One cohort study published after the IOM report indicated that the use of vitamin C supplements may be associated with a higher risk of age-related cataract (hazard ratio 1.25; 95 % CI 1.05-1.50) in women followed up for 8.2 years and compared to non-users of vitamin C supplements (Rautiainen et al., 2010). A systematic search and meta-analysis of observational studies on the relationship between plasma ascorbate concentration and risk of age-related cataract showed that plasma ascorbate was inversely associated with age-related cataract in Asian (OR: 0.67; 95% CI: 0.57, 0.78, two studies) but not in Western (OR: 0.73; 95 % CI: 0.49, 1.08, five studies) populations (Cui et al., 2013).

Studies addressing other vision-related outcomes in predominantly middle-aged or older subjects have not found an association between vitamin C intake and open-angle glaucoma (one large cohort study, (Kang et al., 2003)), age-related maculopathy (two cross-sectional studies, (Smith et al., 1999; O'Connell et al., 2008)), and age-related macular degeneration (one case-control study, (Seddon et al., 1994)).

### 5.5.4. Mortality

No effect of vitamin C intake on total mortality was observed in a systematic review, with meta-analysis, of randomised trials investigating the relationship between supplements of beta-carotene, vitamins A, C and E, and selenium, either alone or in combination, on overall mortality (Bjelakovic et al., 2007), as well as in a randomised, double-blind, placebo-controlled, 2 x 2 x 2 x 2 factorial trial of male US physicians (receiving a multivitamin supplement or its placebo daily, vitamin E (400 IU) or its placebo on alternate days, vitamin C (500 mg) or its placebo daily, and beta-carotene (50 mg) or its placebo on alternate days) (Gaziano et al., 2009).

### 5.5.5. Conclusions on chronic disease-related outcomes

The Panel considers that the data available on vitamin C intake or status and cardiovascular disease-related outcomes and vision-related outcomes are not consistent or specific enough, and cannot be

used as a criterion for deriving the requirement for vitamin C. The Panel considers that there is no association between vitamin C intake and cancer-related outcomes or total mortality, and that available data on such outcomes cannot be used as a criterion for deriving the requirement for vitamin C.

### 5.6. Specific requirements during pregnancy and lactation

For pregnant women, haemodilution and active transfer of vitamin C to the fetus produce a decrease in maternal plasma ascorbate concentration inducing an additional need for vitamin C. The Panel notes that based on this, IOM (2000) added 10 mg/day to the EAR of non-pregnant women, while other authorities such as WHO/FAO (2004) recommended that the same value of 10 mg/day be added to the PRI or the value equivalent to the PRI (see Section 4.3.).

From the seven available studies (see Appendix 4 and Section 2.3.5.3.), mean milk vitamin C concentrations are in the range of 35-90 mg/L for mean maternal vitamin C intakes and mean maternal plasma concentrations, when reported, ranging from < 80 mg/day to 150 mg/day and from 30.1 to 84.1 µmol/L, respectively. However, two studies (Salmenpera, 1984; Daneel-Otterbech et al., 2005) made use of large samples (n = 142 and 200, respectively), providing data that had a relatively small SD (suggesting a normal distribution) and that were collected in Europe (Switzerland and Finland, respectively). Mean vitamin C concentration in breast milk in these two studies ranged from about 40 to 60 mg/L, with a midpoint at 50 mg/L. For women exclusively breastfeeding, the mean milk transfer over the first six months *post partum* is assumed to be 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009). From these values, the Panel notes that an average quantity of 40 mg/day of vitamin C is estimated to be secreted with milk.

## 6. Data on which to base Dietary Reference Values

Taking into account the available data on the relationship between vitamin C intake and its status, functions, and health effects, the Panel considers the maintenance of vitamin C status and total body pool as the most suitable criterion for deriving ARs for vitamin C. In the absence of knowledge about the variation in requirement, and taking into account the agreement between studies used for further calculations, the Panel considered a CV of 10 % to be justified. In addition, rounding to the closest 5 for ARs and PRIs is chosen as a general rule.

### 6.1. Adults

The Panel decided to determine the AR for vitamin C in healthy adults from the quantity of vitamin C that balances metabolic vitamin C losses and maintains fasting plasma ascorbate concentrations at about 50 µmol/L. The latter is considered by the Panel as indicative of an adequate vitamin C status (or vitamin C body pool) at which the different functions of vitamin C in the body can be fulfilled.

Taking a conservative approach, and based on the fact that a complete set of data was only available in men (see Sections 2.3. and 5.2.), the Panel selected metabolic losses of 50 mg/day, an absorption of 80 % and a urinary excretion of 25 % of the vitamin C intake. From these values, the mean daily vitamin C intake required to balance daily losses in men can be calculated using the following formula:

$$AR_{[mg/day]} = \text{metabolic losses}_{[mg/day]}/((\text{absorption}_{[\text{percentage of intake}]} - \text{urinary excretion}_{[\text{percentage of intake}]})/100)$$

According to this formula, a mean intake of 91 mg/day of vitamin C (rounded to 90 mg/day in Table 4) is required to balance daily losses. The Panel considers that this amount represents the AR, and assuming a CV of 10 %, a PRI of 110 mg/day of vitamin C is derived for men. This intake is associated with a plasma ascorbate concentration above 50 µmol/L in almost all subjects of the population of healthy men.

Women reach the plateau of plasma ascorbate concentration at a lower vitamin C intake than men, but the value of the plasma concentration at the plateau is similar in women and men (see Section 2.3.2.). As no values for metabolic losses are available in women, the AR for women is extrapolated from the

AR for men. This is done by isometric scaling (linear with body weight), since vitamin C is considered to be distributed throughout the whole body, since the multi-compartment models used to calculate the metabolic losses in men consider an exchange with only one whole body tissue pool, since few sex-related differences could be observed in the pharmacokinetics of vitamin C, and since a main part of the observed differences can be explained by body weight differences between sexes:

$AR_{women} = AR_{men}$ (body weight of women/body weight of men).

Thus, the Panel considers a value of 78 mg/day as the AR for vitamin C for women (rounded to 80 mg/day in Table 4). Assuming a CV of 10 %, and rounding to the closest 5, a PRI of 95 mg/day of vitamin C is derived for women.

Because of a scarcity of data on the influence of ageing, the Panel concludes that there are insufficient data to derive different DRVs for vitamin C for older adults compared to younger adults.

**Table 4:** Reference body weights, Average Requirements (ARs) and Population Reference Intakes (PRIs) of vitamin C for men and women

| Reference body weight[(a)] (kg) | | AR (mg/day) | | PRI (mg/day) | |
|---|---|---|---|---|---|
| **Men** | **Women** | **Men** | **Women** | **Men** | **Women** |
| 68.1 | 58.5 | 90 | 80 | 110 | 95 |

(a): Median body weight of 18 to 79-year-old men and women, respectively, based on measured body heights of 16 500 men and 19 969 women in 13 EU Member States and assuming a BMI of 22 kg/m$^2$ (see Appendix 11 in EFSA NDA Panel (2013)).

Values for ARs and PRIs were rounded to the closest 5, but PRIs were calculated based on the unrounded ARs.

### 6.2. Infants

The Panel considers that there are no new data to set an AR and thus a PRI for infants aged 7-11 months. The adequate intake (AI) resulting from observed intakes of breastfed infants is likely much higher than the requirement. Because foods consumed by infants in the second half year of life are often fortified with vitamin C, it was not considered suitable to use observed intakes as a basis for setting an adequate intake. The SCF (1993) set the PRI for infants aged 6-11 months at 20 mg/day. The Panel therefore retains this reference value for infants aged 7-11 months.

### 6.3. Children and adolescents

For children and adolescents, the Panel considers that there is no available data obtained in children that can be used to set the AR, thus the AR for vitamin C is extrapolated from the AR for adults taking into account differences in reference body weight (isometric scaling):

$AR_{child} = AR_{adult}$ (body weight of child/body weight of adult).

There are no data indicating that specific categories of age should be considered for vitamin C requirement for children and adolescents. As a consequence, the age categories as proposed in EFSA NDA Panel (2010) are applied for children and adolescents.

Assuming a CV of 10 %, and rounding to the nearest 5, the Panel derives the following PRIs (Table 5).

**Table 5:** Reference body weights, Average Requirements (ARs) and Population Reference Intakes (PRIs) of vitamin C for children and adolescents

| Age | Reference body weight (kg) | | AR (mg/day)[f] | | PRI (mg/day)[g] | |
|---|---|---|---|---|---|---|
| | Boys | Girls | Boys | Girls | Boys | Girls |
| 1-3 years | 12.2[a] | 11.5[a] | 15 | 15 | 20 | 20 |
| 4-6 years | 19.2[b] | 18.7[b] | 25 | 25 | 30 | 30 |
| 7-10 years | 29.0[c] | 28.4[c] | 40 | 40 | 45 | 45 |
| 11-14 years | 44.0[d] | 45.1[d] | 60 | 60 | 70 | 70 |
| 15-17 years | 64.1[e] | 56.4[e] | 85 | 75 | 100 | 90 |

(a): Median body weight-for-age of boys or girls, respectively, aged 24 months (WHO Multicentre Growth Reference Study Group, 2006).
(b): Median body weight of boys or girls, respectively, aged 5 years (van Buuren et al., 2012).
(c): Median body weight of boys or girls, respectively, aged 8.5 years (van Buuren et al., 2012)..
(d): Median body weight of boys or girls, respectively, aged 12.5 years (van Buuren et al., 2012)..
(e): Median body weight of boys or girls, respectively, aged 16 years (van Buuren et al., 2012)..
(f): ARs derived from the unrounded ARs for adults after adjustment on the basis of differences in reference weight, then rounded to the closest 5.
(g): PRIs derived from the unrounded ARs assuming a CV of 10 %, and then rounded to the closest 5.

### 6.4. Pregnancy and lactation

For pregnant women, in agreement with the value and approach of WHO/FAO (2004), which has also been endorsed by other expert Committees, and even though the scientific basis for this additional intake is limited, the Panel proposes an intake of 10 mg/day in addition to the PRI for non-pregnant women. This is compatible with the previous considerations that vitamin C is considered to be distributed in one whole body compartment.

For lactating women, an additional intake of vitamin C is necessary to balance vitamin C losses in human milk. An average quantity of 40 mg/day of vitamin C is estimated to be secreted with milk over the first six months *post partum* (see Section 5.6.). Assuming an absorption efficiency of vitamin C of 80 %, a mean vitamin C intake of 50 mg/day is required to balance the amount of vitamin C secreted in milk for exclusively breastfeeding women during the first six months of lactation, in addition to the AR of non-lactating women. Assuming a CV of 10 %, an additional intake of 60 mg/day is derived for exclusively breastfeeding women, in addition to the PRI for non-lactating women.

## CONCLUSIONS

The Panel concludes that an Average Requirement (AR) and a Population Reference Intake (PRI) for vitamin C can be derived for adults and children based on vitamin C status, and for pregnant and lactating women based on estimation of additional needs and factorial calculation of losses in breast milk, respectively.

For infants aged 7-11 months, the Panel retains the PRI set by the SCF (1993), i.e. 20 mg/day, as no suitable evidence has emerged since the previous assessment.

The Panel also considered several health outcomes that may be associated with vitamin C intake; however, the available data were considered insufficient for the setting of DRVs.

**Table 6:** Summary of Dietary Reference Values for vitamin C

| | PRI (mg/day) | |
|---|---|---|
| **Age** | **Males** | **Females** |
| 7-11 months | 20 | 20 |
| 1-3 years | 20 | 20 |
| 4-6 years | 30 | 30 |
| 7-10 years | 45 | 45 |
| 11-14 years | 70 | 70 |
| 15-17 years | 100 | 90 |
| ≥ 18 years | 110 | 95 |
| Pregnancy | - | + 10 |
| Lactation | - | + 60 |

## RECOMMENDATIONS FOR RESEARCH

Information related to the body pool of vitamin C is limited and the Panel suggests the development of methods for its precise measurement including isotope dilution methods in order to determine its size and variation. Relatively few data are available on the turnover of the body pool of vitamin C, and on metabolic and urinary losses, and the Panel proposes that methods for their estimation should be developed, taking into account different factors such as age (in children and adults), sex and smoking.

Future studies should also address the suitability of various biomarkers of status (such as plasma ascorbate vs leukocyte ascorbate) as best indicator of the requirement. The influence of genotype on plasma ascorbate concentration may also require future research.

The understanding of vitamin C's antioxidant activity, and of its potential protective effects on tissues and different health outcomes, may also require further research, as well as whether a vitamin C intake beyond the PRI affects relevant biomarkers of oxidative damage to lipids, DNA and proteins in intervention studies, including an assessment of dose-response relationship where relevant.

The Panel also suggests obtaining data in infants and children on vitamin C intakes in relation to plasma ascorbate concentrations, and collecting data on vitamin C intakes of European infants aged 7-11 months, distinguishing between fortified and non-fortified foods, and types of milk intake (breast milk, infant formula, cow's milk).

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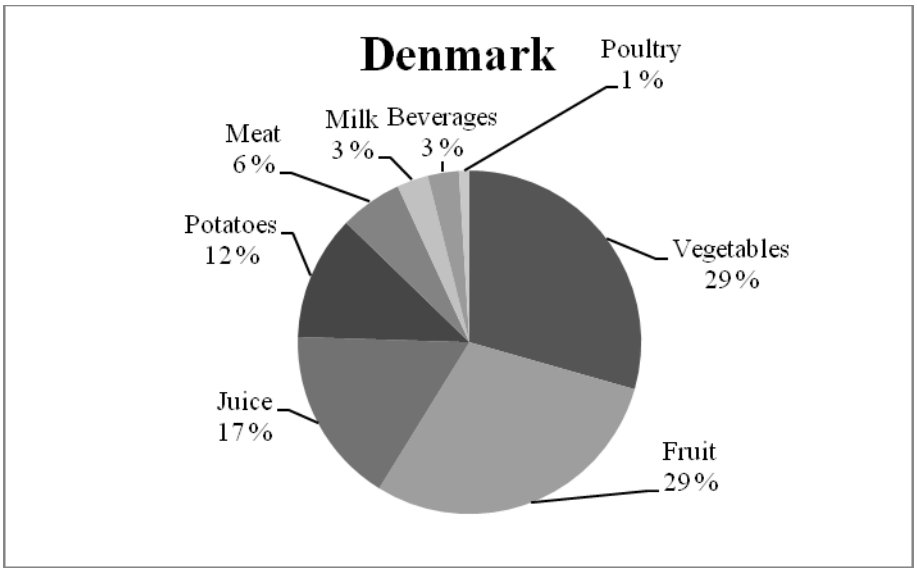
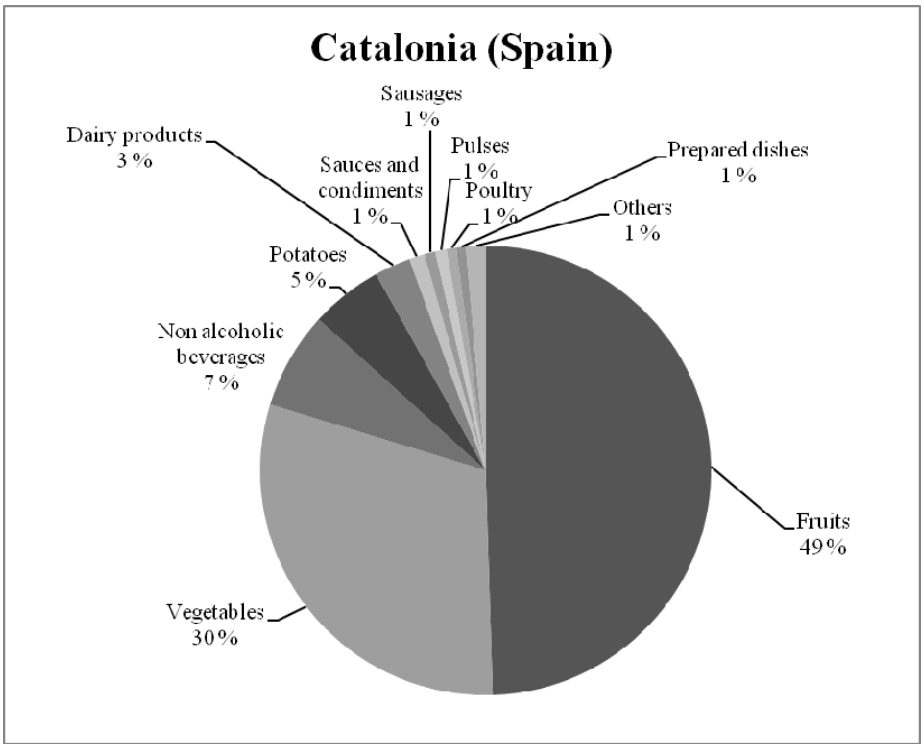
## APPENDICES

### Appendix 1. Foods contributing to vitamin C intake in Denmark, Spain and Germany




Adapted from Pedersen et al. (2010) (children and adults)

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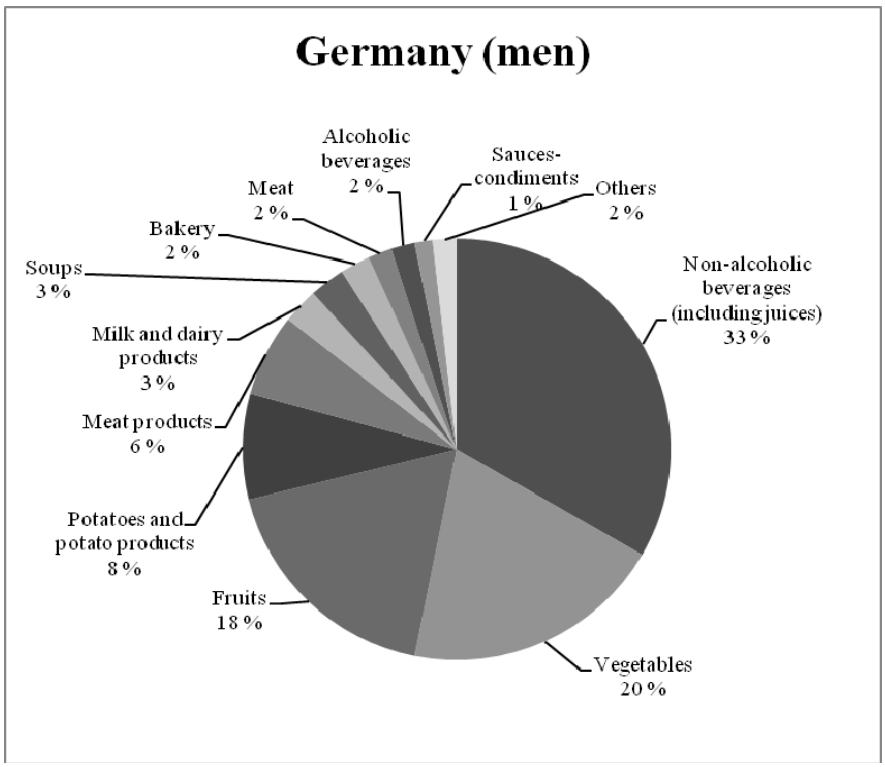


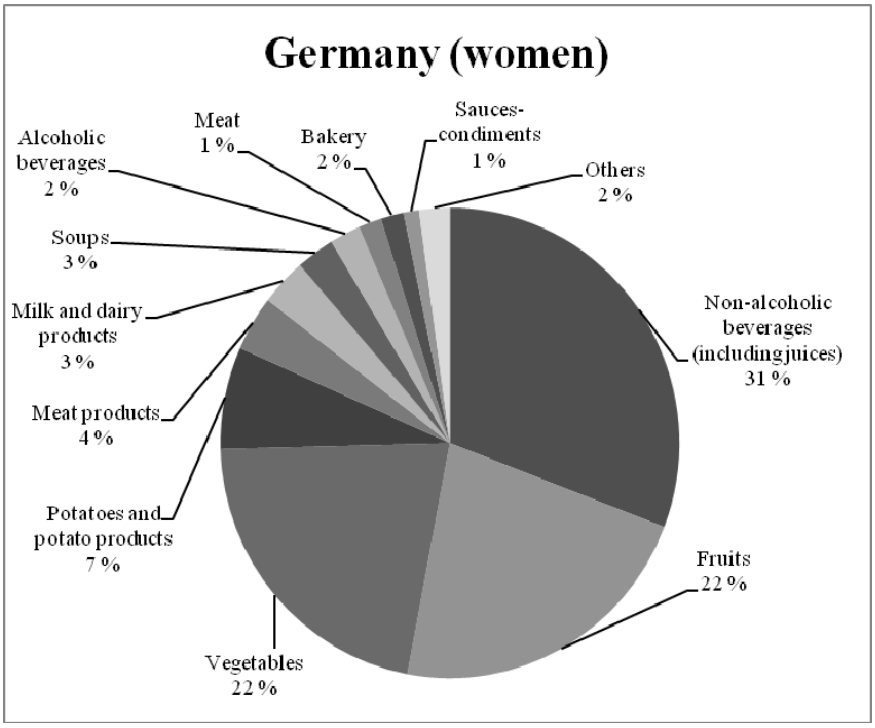


Adapted from DGE (2012) (adolescents and adults)

## Appendix 2A. Population, methods and period of dietary assessment in children and adolescents in European countries

| Country | Population | Dietary assessment method | Year of survey | Reference |
|---|---|---|---|---|
| **Austria** | Boys and girls aged 7-9 years | Three-day record | 2007-2008 | (Elmadfa et al., 2009a; Elmadfa et al., 2009b) |
| | Boys and girls aged 10-14 years | Three-day record | 2007-2008 | (Elmadfa et al., 2009a; Elmadfa et al., 2009b) |
| | Boys and girls aged 14-19 years | 24-hour recall | 2003-2004 | (Elmadfa et al., 2009a; Elmadfa et al., 2009b). *Mainly from a large Viennese sample.* |
| **Belgium** | Boys and girls aged 2.5-3 years | Three -day record | 2002-2003 | (Huybrechts and De Henauw, 2007). *Data collected in Flanders.* |
| | Boys and girls aged 4-6.5 years | Three -day record | 2002-2003 | (Huybrechts and De Henauw, 2007). *Data collected in Flanders.* |
| | Boys and girls aged 15-18 years | Two non consecutive 24-hour recall | 2004 | (De Vriese et al., 2006) |
| **Bulgaria** | Boys and girls aged 1-3 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| | Boys and girls aged 3-6 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| | Boys and girls aged 6-10 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| | Boys and girls aged 10-14 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| | Boys and girls aged 14-18 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| **Czech Republic** | Boys and girls aged 4-6 years | 48-hour recall | 2007 | (Elmadfa et al., 2009b) |
| | Boys and girls aged 7-9 years | 48-hour recall | 2007 | (Elmadfa et al., 2009b) |
| **Denmark** | Boys and girls aged 1-3 years | Seven-day record | 1995 | (Andersen et al., 1996) |
| | Boys and girls aged 4-5 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Boys and girls aged 6-9 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Boys and girls aged 10-13 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Boys and girls aged 14-17 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| **Finland** | Children aged 1 year | Three-day record | 2003-2005 | (Kyttälä et al., 2008; Kyttälä et al., 2010) |
| | Children aged 2 years | Three-day record | 2003-2005 | (Kyttälä et al., 2008; Kyttälä et al., 2010) |
| | Children aged 3 years | Three-day record | 2003-2005 | (Kyttälä et al., 2008; Kyttälä et al., 2010) |
| | Children aged 4 years | Three-day record | 2003-2005 | (Kyttälä et al., 2008; Kyttälä et al., 2010) |
| | Children aged 6 years | Three-day record | 2003-2005 | (Kyttälä et al., 2008; Kyttälä et al., 2010) |
| **France** | Boys and girls aged 1-3 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 4 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 5 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 6 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 7 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 8-9 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 10-12 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 13-18 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 19-24 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 25-30 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 31-36 mo | Consecutive 3-day record | 2005 | (Fantino and Gourmet, 2008) |
| | Boys and girls aged 3-10 years | Seven-day record | 2006-2007 | (Afssa, 2009) |
| | Boys and girls aged 11-14 years | Seven-day record | 2006-2007 | (Afssa, 2009) |
| | Boys and girls aged 15-17 years | Seven-day record | 2006-2007 | (Afssa, 2009) |

| Country | Population | Dietary assessment method | Year of survey | Reference |
|---|---|---|---|---|
| **Germany** | Boys and girls aged 6 years | Three-day record | 2006 | (Mensink et al., 2007; Elmadfa et al., 2009b) |
| | Boys and girls aged 7-9 years | Three-day record | 2006 | (Mensink et al., 2007; Elmadfa et al., 2009b) |
| | Boys and girls aged 10-11 years | Three-day record | 2006 | (Mensink et al., 2007; Elmadfa et al., 2009b) |
| | Boys and girls aged 12 years | Dietary history (over the last four weeks) | 2006 | (Mensink et al., 2007; Elmadfa et al., 2009b) |
| | Boys and girls aged 13-14 years | Dietary history (over the last four weeks) | 2006 | (Mensink et al., 2007; Elmadfa et al., 2009b) |
| | Boys and girls aged 14-18 years | Two non-consecutive 24-hour dietary recalls | 2005-2006 | (DGE, 2012) |
| **Greece** | Boys and girls aged 1-5 years | Three-day record (weighed food records and 24-hour recall or food diaries) | 2003-2004 | (Manios et al., 2008) |
| **Hungary** | Boys and girls aged 11-14 years | Three-day record | 2005-2006 | (Biro et al., 2007). *Data collected in Budapest.* |
| **Ireland** | Boys and girls aged 1 year | Four-day record | 2010-2011 | (IUNA, online-c) |
| | Boys and girls aged 2 years | Four-day record | 2010-2011 | (IUNA, online-c) |
| | Boys and girls aged 3 years | Four-day record | 2010-2011 | (IUNA, online-c) |
| | Boys and girls aged 4 years | Four-day record | 2010-2011 | (IUNA, online-c) |
| | Boys and girls aged 5-8 years | Seven-day record | 2003-2004 | (IUNA, online-b) |
| | Boys and girls aged 9-12 years | Seven-day record | 2003-2004 | (IUNA, online-b) |
| | Boys and girls aged 13-14 years | Seven-day record | 2005-2006 | (IUNA, online-a) |
| | Boys and girls aged 15-17 years | Seven-day record | 2005-2006 | (IUNA, online-a) |
| **Italy** | Boys and girls aged 0-< 3 years | Three-day food record | 2005-2006 | (Sette et al., 2010) |
| | Boys and girls aged 3-< 10 years | Three-day food record | 2005-2006 | (Sette et al., 2010) |
| | Boys and girls aged 10-< 18 years | Three-day food record | 2005-2006 | (Sette et al., 2010) |
| **The Netherlands** | Boys and girls aged 2-3 years | Two-day record (independent days) | 2005-2006 | (Ocké et al., 2008) |
| | Boys and girls aged 4-6 years | Two-day record (independent days) | 2005-2006 | (Ocké et al., 2008) |
| | Boys and girls aged 7-8 years | Two non-consecutive 24-hour recalls | 2007-2010 | (van Rossum et al., 2011) |
| | Boys and girls aged 9-13 years | Two non-consecutive 24-hour recalls | 2007-2010 | (van Rossum et al., 2011) |
| | Boys and girls aged 14-18 years | Two non-consecutive 24-hour recalls | 2007-2010 | (van Rossum et al., 2011) |
| **Norway** | Children aged 2 years | Food Frequency Questionnaire | 2007 | (Kristiansen and Andersen, 2009) |
| | Boys and girls aged 4 years | Four-day record | 2000 | (Elmadfa et al., 2009b) |
| | Boys and girls aged 9 years | Four-day record | 2000 | (Øverby and Andersen, 2002; Elmadfa et al., 2009b) |
| | Boys and girls aged 13 years | Four-day record | 2000 | (Øverby and Andersen, 2002; Elmadfa et al., 2009b) |
| | Boys and girls aged 16-19 years | Food Frequency Questionnaire | 1997 | (Johansson and Sovoll, 1999) |
| **Poland** | Boys and girls aged 1-3 years | 24-hour recall | 2000 | (Szponar et al., 2003) |
| | Boys and girls aged 4-6 years | 24-hour recall | 2000 | (Szponar et al., 2003) |
| | Boys and girls aged 7-9 years | 24-hour recall | 2000 | (Szponar et al., 2003) |
| | Boys and girls aged 10-12 years | 24-hour recall | 2000 | (Szponar et al., 2003) |
| | Boys and girls aged 13-15 years | 24-hour recall | 2000 | (Szponar et al., 2003) |
| | Boys and girls aged 16-18 years | 24-hour recall | 2000 | (Szponar et al., 2003) |

| Country | Population | Dietary assessment method | Year of survey | Reference |
|---|---|---|---|---|
| **Portugal** | Boys and girls aged 13 years | Food Frequency Questionnaire | 2003-2004 | (Elmadfa et al., 2009b) |
| **Slovenia** | Boys and girls aged 14-16 years | Food Frequency Questionnaire | 2003-2005 | (Elmadfa et al., 2009b) |
| **Spain** | Boys and girls aged 10-14 years | Two non-consecutive 24-hour recalls | 2002-2003 | (Elmadfa et al., 2009b). *Data collected in Catalonia.* |
| | Boys and girls aged 15-18 years | Two non-consecutive 24-hour recalls | 2002-2003 | (Elmadfa et al., 2009b). *Data collected in Catalonia.* |
| **Sweden** | Boys and girls aged 4 years | Four-day record | 2003 | (Enghardt-Barbieri et al., 2006) |
| | Boys and girls aged 8-9 years | Four-day record | 2003 | (Enghardt-Barbieri et al., 2006) |
| | Boys and girls aged 11-12 years | Four-day record | 2003 | (Enghardt-Barbieri et al., 2006) |
| **United Kingdom** | Boys and girls aged 6-9 mo | Seven-day record | 1986 | (Mills and Tyler, 1992) reported in (Noble et al., 2001). |
| | Boys and girls aged 1.5-3 years | Four-day food diary | 2008-2010 | (Bates et al., 2011) |
| | Boys and girls aged 4-10 years | Four-day food diary | 2008-2010 | (Bates et al., 2011) |
| | Boys and girls aged 11-18 years | Four-day food diary | 2008-2010 | (Bates et al., 2011) |

mo: months

## Appendix 2B. Vitamin C intake among children aged ~0-3 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Infants and/or young children** | | | | | |
| Boys | | | | | |
| Belgium | 2.5-3 | 102 | 91.02 | 46.06 | |
| Denmark | 1-3 | 129 | 55 | 0.4[a] | 25-104 |
| Finland | 1[b] | 257 | 80 | 34 | |
| | 2 | 112 | 54 | 33 | |
| | 3 | 236 | 67 | 44 | |
| The Netherlands | 2-3 | 327 | 68 | | 33–115 |
| Norway | 2 | 829 | 68 | 40 | |
| Poland | 1-3 | 70 | 55 | 48 | |
| United Kingdom | 6-9 mo | 130 | 114 | | |
| **Girls** | | | | | |
| Belgium | 2.5-3 | 95 | 90.16 | 39.68 | |
| Denmark | 1-3 | 149 | 64 | 0.3[a] | 23-81 |
| Finland | 1[b] | 198 | 75 | 31 | |
| | 2 | 118 | 53 | 30 | |
| | 3 | 235 | 60 | 38 | |
| The Netherlands | 2-3 | 313 | 62 | | 32–102 |
| Norway | 2 | 826 | 64 | 36 | |
| Poland | 1-3 | 48 | 44 | 35 | |
| United Kingdom | 6-9 mo | 128 | 119 | | |
| **Infants and/or young children (both sexes)** | | | | | |
| Bulgaria | 1-3 | 154 | 52.5 | 47.9 | |
| France | 1-3 mo | 64 | 60 | 13 | |
| | 4 mo | 60 | 79 | 21 | |
| | 5 mo | 69 | 83 | 29 | |
| | 6 mo | 58 | 80 | 27 | |
| | 7 mo | 66 | 86 | 29 | |
| | 8-9 mo | 67 | 92 | 40 | |
| | 10-12 mo | 63 | 76 | 34 | |
| | 13-18 mo | 66 | 74 | 35 | |
| | 19-24 mo | 66 | 59 | 33 | |
| | 25-30 mo | 65 | 54 | 32 | |
| | 31-36 mo | 62 | 55 | 33 | |
| Greece | 1-5 | 2 317[c] | 70 | n.a. | 54–88[d] |
| Ireland | 1 | 126 | 75[e] | 44[e] | |
| | 2 | 124 | 85[e] | 64[e] | |
| | 3 | 126 | 85[e] | 45[e] | |
| Italy[f] | 0-<3 | 52 | 90 | 58 | 28–205 |
| United Kingdom | 1.5-3 | 219 | 67.3 | 41.1 | 19.1-159.6[g] |
| | | | 73.1[e] | 55.0[e] | 20.2–192.6[e] |

(a): SE.
(b): Breast-fed children not included.
(c): Under-reporters excluded.
(d): P10–P90.
(e): Including supplements.
(f): Including fortified foods but excluding supplements.
(g): P2.5–P97.5.
n.a.: not available

## Appendix 2C. Vitamin C intake among children aged ~4-6 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Boys** | | | | | |
| Belgium | 4-6.5 | 236 | 97.05 | 48.83 | |
| Czech Republic | 4-6 | 641 | 157 | 95 | |
| Denmark | 4-5 | 81 | 88 | 41 | 37–151 |
| Finland | 4 | 307 | 60 | 41 | |
| | 6 | 364 | 72 | 49 | |
| Germany[(a)] | 6 | 102 | 91 | 45 | 27–176 |
| The Netherlands | 4-6 | 327 | 71 | | 35–118 |
| Norway[(a)] | 4 | 206 | 81 | 44 | |
| Poland | 4-6 | 82 | 66 | 46 | |
| Sweden | 4 | 302 | 90 | 71 | 23–182 |
| United Kingdom | 4-10 | 210 | 86.9 | 49.4 | 22.3–199.1[(b)] |
| | | | 95.6[(a)] | 58.5[(a)] | 22.3–239.5[(a,b)] |
| **Girls** | | | | | |
| Belgium | 4-6.5 | 228 | 90.54 | 36.30 | |
| Czech Republic | 4-6 | 446 | 157 | 95 | |
| Denmark | 4-5 | 78 | 78 | 32 | 31–138 |
| Finland | 4 | 247 | 61 | 41 | |
| | 6 | 349 | 61 | 36 | |
| Germany[(a)] | 6 | 102 | 91 | 51 | 32.6–190 |
| The Netherlands | 4-6 | 312 | 65 | | 29–112 |
| Norway[(a)] | 4 | 185 | 88 | 41 | |
| Poland | 4-6 | 84 | 61 | 50 | |
| Sweden | 4 | 288 | 88 | 74 | 29–155 |
| United Kingdom | 4-10 | 213 | 86.5 | 49.7 | 17.5–193.0[(b)] |
| | | | 92.2[(a)] | 54.5[(a)] | 17.5–206.3[(a,b)] |
| **Both sexes** | | | | | |
| Bulgaria | 3-6 | 199 | 70.8 | 62.6 | |
| Ireland | 4 | 124 | 94[(a)] | 53[(a)] | |

(a): Supplements included.
(b): P2.5–P97.5.

## Appendix 2D. Vitamin C intake among children aged ~7-9 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Boys** | | | | | |
| Austria | 7-9 | 146 | 125 | 92 | |
| Czech Republic | 7-9 | 940 | 172 | 113 | |
| Denmark | 6-9 | 172 | 101 | 53 | 37–210 |
| France | 3-10 | n.a. | 69.7 | 45.5 | |
| Germany[(a)] | 7-9 | 321 | 110 | 60 | 32–221 |
| Ireland | 5-8 | 145 | 70 | 39 | 19–135 |
| | | | 81[(a)] | 48[(a)] | 21–168[(a)] |
| The Netherlands | 7-8 | 153 | 73[(b)] | | 32–144 |
| Norway[(a)] | 9 | 402 | 102 | 56 | |
| Poland | 7-9 | 101 | 77 | 71 | |
| Sweden | 8-9 | 444 | 88 | 68 | 23–184 |
| **Girls** | | | | | |
| Austria | 7-9 | 146 | 110 | 73 | |
| Czech Republic | 7-9 | 765 | 172 | 113 | |
| Denmark | 6-9 | 151 | 91 | 38 | 41–161 |
| France | 3-10 | n.a. | 72.8 | 42.4 | |
| Germany[(a)] | 7-9 | 321 | 108 | 69 | 24.6–261 |
| Ireland | 5-8 | 151 | 75 | 47 | 23–175 |
| | | | 85[(a)] | 56[(a)] | 24–196[(a)] |
| The Netherlands | 7-8 | 151 | 82[(b)] | | 37–158 |
| Norway[(a)] | 9 | 408 | 95 | 55 | |
| Poland | 7-9 | 103 | 71 | 55 | |
| Sweden | 8-9 | 445 | 86 | 83 | 24–174 |
| **Both sexes** | | | | | |
| Bulgaria | 6-10 | 235 | 96.9 | 75.1 | |
| Italy[(c)] | 3-< 10 | 193 | 107 | 64 | 26–236 |

(a): Supplements included.
(b): Median (mean not available).
(c): Including fortified foods but excluding supplements.

## Appendix 2E. Vitamin C intake among children aged ~10-14 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Boys** | | | | | |
| Austria | 10-14 | 248 | 113 | 96 | |
| Bulgaria | 10-14 | 167 | 114 | 85.5 | |
| Denmark | 10-13 | 164 | 103 | 61 | 37–230 |
| France | 11-14 | n.a. | 90.1 | 44.0 | |
| Germany[(a)] | 10-11 | 199 | 119 | 108 | 27.6–257 |
| | 12 | 114 | 172 | 102 | 37.9–163 |
| | 13-14 | 214 | 197 | 135 | 61.2–407 |
| Hungary | 11-14 | 124 | 99.3 | 78.9 | |
| Ireland | 9-12 | 148 | 74 | 45 | 21–168 |
| | | | 91[(a)] | 90[(a)] | 21–220[(a)] |
| | 13-14 | 95 | 80 | 46 | 19–159 |
| | | | 95[(a)] | 74[(a)] | 25–224[(a)] |
| The Netherlands | 9-13 | 351 | 85[(b)] | | 39–165 |
| Norway[(a)] | 13 | 490 | 106 | 74 | |
| Poland | 10-12 | 128 | 71 | 63 | |
| | 13-15 | 118 | 106 | 89 | |
| Portugal | 13 | 987 | 161 | 94 | |
| Spain | 10-14 | 66 | 73 | 34 | |
| Sweden | 11-12 | 517 | 82 | 120 | 16–182 |
| United Kingdom | 11-18 | 238 | 89.7 | 65.1 | 15.9–256.3[(c)] |
| | | | 94.4[(a)] | 79.3[(a)] | 15.9-312.0[(a,c)] |
| **Girls** | | | | | |
| Austria | 10-14 | 248 | 113 | 67 | |
| Bulgaria | 10-14 | 180 | 102 | 77.1 | |
| Denmark | 10-13 | 196 | 101 | 59 | 35–204 |
| France | 11-14 | n.a. | 78.5 | 37.3 | |
| Germany[(a)] | 10-11 | 199 | 110 | 61 | 30.8–213 |
| | 12 | 114 | 222 | 243 | 57.5–400 |
| | 13-14 | 214 | 201 | 12 | 57.9–502 |
| Hungary | 11-14 | 111 | 94.3 | 69.9 | |
| Ireland | 9-12 | 150 | 79 | 50 | 23–173 |
| | | | 92[(a)] | 68[(a)] | 25–216[(a)] |
| | 13-14 | 93 | 69 | 44 | 22–164 |
| | | | 89[(a)] | 80[(a)] | 22–210[(a)] |
| The Netherlands | 9-13 | 352 | 82[(b)] | | 37–160 |
| Norway[(a)] | 13 | 515 | 103 | 81 | |
| Poland | 10-12 | 121 | 85 | 81 | |
| | 13-15 | 134 | 95 | 92 | |
| Portugal | 13 | 987 | 170 | 96 | |
| Spain | 10-14 | 53 | 77 | 37 | |
| Sweden | 11-12 | 499 | 78 | 79 | 18–180 |
| United Kingdom | 11-18 | | 79.0 | 52.2 | 17.7–209.2[(c)] |
| | | | 84.4[(a)] | 74.9[(a)] | 17.7–239.6[(a,c)] |

(a): Supplements included.
(b): Median (mean not available).
(c): P2.5–P97.5.

## Appendix 2F. Vitamin C intake among adolescents aged ~15-18 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Boys** | | | | | |
| Austria | > 13 | 1 527 | 91 | 96 | |
| Belgium | 15-18 | 405 | 89.7 | 42.1 | 59.0–113.0[(a)] |
| Bulgaria | 14-18 | 178 | 108 | 79.4 | |
| Denmark | 14-17 | 101 | 100 | 54 | 26–195 |
| France | 15-17 | n.a. | 77.2 | 34.8 | |
| Germany[(b)] | 15-19 | 506 | 87[(c)] | | |
| Ireland | 15-17 | 129 | 85 | 60 | 17–211 |
| | | | 101[(d)] | 96[(d)] | 18–245[(d)] |
| Italy[(e)] | 10-< 18 | 108 | 136 | 93 | 36–312 |
| The Netherlands | 14-18 | 352 | 92[(f)] | | 43–176 |
| Norway | 16-19 | 86 | 129 | | |
| Poland | 16-18 | 130 | 121 | 83 | |
| Slovenia | 14-17 | 1 010 | 146 | 105 | |
| Spain | 15-18 | 61 | 73 | 28 | |
| **Girls** | | | | | |
| Austria | > 13 | 1 422 | 90 | 93 | |
| Belgium | 15-18 | 401 | 87.7 | 38.4 | 60.0–109.0[(a)] |
| Bulgaria | 14-18 | 190 | 92.6 | 78.2 | |
| Denmark | 14-17 | 134 | 95 | 50 | 26–183 |
| France | 15-17 | n.a. | 91.0 | 48.0 | |
| Germany[(b)] | 15-19 | 536 | 87[(c)] | | |
| Ireland | 15-17 | 124 | 75 | 50 | 20–181 |
| | | | 94[(d)] | 113[(d)] | 20–208[(d)] |
| Italy[(e)] | 10-< 18 | 139 | 128 | 92 | 30–286 |
| The Netherlands | 14-18 | 354 | 84[(f)] | | 38–161 |
| Norway | 16-19 | 92 | 119 | | |
| Poland | 16-18 | 122 | 91 | 67 | |
| Slovenia | 14-17 | 1 214 | 149 | 85 | |
| Spain | 15-18 | 57 | 75 | 28 | |

(a): P25-P75.
(b): Including fortified foods but excluding supplements.
(c): Median.
(d): Supplements included.
(e): Including fortified foods but excluding supplements.
(f): Median (mean not available).

## Appendix 3A. Population, methods and period of dietary assessment in adults in European countries

| Country | Population | Dietary assessment method | Year of survey | Reference |
|---|---|---|---|---|
| **Austria** | Males and females aged 19-64 years | 24-hour recall | 2005-2006 | (Elmadfa et al., 2009a; Elmadfa et al., 2009b) |
| | Males and females aged 65 years and over | Three-day record | 2007-2008 | (Elmadfa et al., 2009a; Elmadfa et al., 2009b) |
| **Belgium** | Males and females aged 19-59 years | Two non consecutive 24-hour recalls | 2004-2005 | (De Vriese et al., 2006) |
| | Males and females aged 60-74 years | Two non consecutive 24-hour recalls | 2004-2005 | (De Vriese et al., 2006) |
| | Males and females aged 75 years and over | Two non consecutive 24-hour recalls | 2004-2005 | (De Vriese et al., 2006) |
| **Bulgaria** | Males and females aged 18-30 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| | Males and females aged 30-60 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| | Males and females aged 60-75 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| | Males and females aged >75 years | 24-hour recall | 1998 | (Abrasheva et al., 1998) |
| **Czech Republic** | Males and females aged 19-64 years | 24-hour recall | 2000-2001 | (Cifková and Škodová, 2004; Elmadfa et al., 2009b) |
| **Denmark** | Males and females aged 18-75 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Males and females aged 18-24 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Males and females aged 25-34 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Males and females aged 35-44 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Males and females aged 45-54 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Males and females aged 55-64 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| | Males and females aged 65-75 years | Seven-day record | 2003-2008 | (Pedersen et al., 2010) |
| **Estonia** | Males and females aged 19-64 years | 24-hour recall | 1997 | (ECOHOST/WHO, 1999; Elmadfa et al., 2009b) |
| | Males and females aged 19-34 years | 24-hour recall | 1997 | (ECOHOST/WHO, 1999) |
| | Males and females aged 35-49 years | 24-hour recall | 1997 | (ECOHOST/WHO, 1999) |
| | Males and females aged 50-64 years | 24-hour recall | 1997 | (ECOHOST/WHO, 1999) |
| **Finland** | Males and females aged 25-64 years | 48-hour recall | 2007 | (Paturi et al., 2008; Pietinen et al., 2010) |
| | Males and females aged 25-34 years | 48-hour recall | 2007 | (Paturi et al., 2008) |
| | Males and females aged 35-44 years | 48-hour recall | 2007 | (Paturi et al., 2008) |
| | Males and females aged 45-54 years | 48-hour recall | 2007 | (Paturi et al., 2008) |
| | Males and females aged 55-64 years | 48-hour recall | 2007 | (Paturi et al., 2008) |
| | Males and females aged 65-74 years | 48-hour recall | 2007 | (Paturi et al., 2008) |
| **France** | Males and females aged 18-79 years | Seven-day record | 2006-2007 | (Afssa, 2009) |
| | Males and females aged 18-34 years | Seven-day record | 2006-2007 | (Afssa, 2009) |
| | Males and females aged 35-54 years | Seven-day record | 2006-2007 | (Afssa, 2009) |
| | Males and females aged 55-79 years | Seven-day record | 2006-2007 | (Afssa, 2009) |

| Country | Population | Dietary assessment method | Year of survey | Reference |
|---|---|---|---|---|
| **Germany** | Males and females aged 15-80 years | Two non-consecutive 24-hour recalls | 2005-2006 | (DGE, 2012) |
| | Males and females aged 19-24 years | Two non-consecutive 24-hour recalls | 2005-2006 | (DGE, 2012) |
| | Males and females aged 25-34 years | Two non-consecutive 24-hour recalls | 2005-2006 | (DGE, 2012) |
| | Males and females aged 35-50 years | Two non-consecutive 24-hour recalls | 2005-2006 | (DGE, 2012) |
| | Males and females aged 51-64 years | Two non-consecutive 24-hour recalls | 2005-2006 | (DGE, 2012) |
| | Males and females aged 65-80 years | Two non-consecutive 24-hour recalls | 2005-2006 | (DGE, 2012) |
| **Greece** | Males and females aged 22 ± 2 years | 24-hour recall | 1989-2001 | (Elmadfa et al., 2009b). *Data from the EPIC Greek cohort* |
| **Hungary** | Males and females aged 18-59 years | Three-day record | 2003-2004 | (Zajkas et al., 2007; Elmadfa et al., 2009b) |
| | Males and females aged 18-34 years | Three-day record | 2003-2004 | (Zajkas et al., 2007; Elmadfa et al., 2009b) |
| | Males and females aged 35-59 years | Three-day record | 2003-2004 | (Zajkas et al., 2007; Elmadfa et al., 2009b) |
| | Males and females aged 60 years and over | Three-day record | 2003-2004 | (Zajkas et al., 2007; Elmadfa et al., 2009b) |
| **Ireland** | Males and females aged 18-64 years | Four-day record | 2008-2010 | (IUNA, 2011) |
| | Males and females aged 18-35 years | Four-day record | 2008-2010 | (IUNA, 2011) |
| | Males and females aged 36-50 years | Four-day record | 2008-2010 | (IUNA, 2011) |
| | Males and females aged 51-64 years | Four-day record | 2008-2010 | (IUNA, 2011) |
| | Males and females aged 65-90 years | Four-day record | 2008-2010 | (IUNA, 2011) |
| **Italy** | Males and females aged 18-< 65years | Three-day food record | 2005-2006 | (Sette et al., 2010) |
| | Males and females aged 65 years and over | Three-day food record | 2005-2006 | (Sette et al., 2010) |
| | Males and females aged 0-99 years | Three-day food record | 2005-2006 | (Sette et al., 2010). *Supplementary information on dietary supplements.* |
| **Latvia** | Males and females aged 17-26 years | Two non-consecutive 24-hour recalls + food frequency questionnaire | 2008 | (Joffe et al., 2009) |
| | Males and females aged 27-36 years | Two non-consecutive 24-hour recalls + food frequency questionnaire | 2008 | (Joffe et al., 2009) |
| | Males and females aged 37-46 years | Two non-consecutive 24-hour recalls + food frequency questionnaire | 2008 | (Joffe et al., 2009) |
| | Males and females aged 47-56 years | Two non-consecutive 24-hour recalls + food frequency questionnaire | 2008 | (Joffe et al., 2009) |
| | Males and females aged 57-64 years | Two non-consecutive 24-hour recalls + food frequency questionnaire | 2008 | (Joffe et al., 2009) |
| **Lithuania** | Males and females aged 19-64 years | 24-hour recall | 2007 | (Elmadfa et al., 2009b) |
| **The Netherlands** | Males and females aged 19-30 years | Two non-consecutive 24-hour recalls | 2007-2010 | (van Rossum et al., 2011) |
| | Males and females aged 31-50 years | Two non-consecutive 24-hour recalls | 2007-2010 | (van Rossum et al., 2011) |
| | Males and females aged 51-69 years | Two non-consecutive 24-hour recalls | 2007-2010 | (van Rossum et al., 2011) |

| Country | Population | Dietary assessment method | Year of survey | Reference |
|---|---|---|---|---|
| **Norway** | Males and females aged 18-70 years | Two randomly distributed 24-hour recalls + Food Propensity Questionnaire | 2010-2011 | (Holm Totland et al., 2012) |
| | Males and females aged 18-29 years | Two randomly distributed 24-hour recalls + Food Propensity Questionnaire | 2010-2011 | (Holm Totland et al., 2012) |
| | Males and females aged 30-39 years | Two randomly distributed 24-hour recalls + Food Propensity Questionnaire | 2010-2011 | (Holm Totland et al., 2012) |
| | Males and females aged 40-49 years | Two randomly distributed 24-hour recalls + Food Propensity Questionnaire | 2010-2011 | (Holm Totland et al., 2012) |
| | Males and females aged 50-59 years | Two randomly distributed 24-hour recalls + Food Propensity Questionnaire | 2010-2011 | (Holm Totland et al., 2012) |
| | Males and females aged 60-70 years | Two randomly distributed 24-hour recalls + Food Propensity Questionnaire | 2010-2011 | (Holm Totland et al., 2012) |
| **Poland** | Males and females aged 19-25 years | 24-hour recall | 2000 | (Szponar et al., 2003) |
| | Males and females aged 26-60 years | 24-hour recall | 2000 | (Szponar et al., 2003) |
| | Males and females aged 61 years and over | 24-hour recall | 2000 | (Szponar et al., 2003) |
| **Portugal** | Males and females aged 18-≥ 65 years | Food frequency questionnaire | 1999-2003 | (Lopes et al., 2006; Elmadfa et al., 2009b). *Data collected in Porto.* |
| | Males and females aged 18-39 years | Food frequency questionnaire | 1999-2003 | (Lopes et al., 2006). *Data collected in Porto* |
| | Males and females aged 40-49 years | Food frequency questionnaire | 1999-2003 | (Lopes et al., 2006). *Data collected in Porto* |
| | Males and females aged 50-64 years | Food frequency questionnaire | 1999-2003 | (Lopes et al., 2006). *Data collected in Porto* |
| | Males and females aged 65 years and over | Food frequency questionnaire | 1999-2003 | (Lopes et al., 2006; Elmadfa et al., 2009b). *Data collected in Porto.* |
| **Romania** | Males and females aged 19-64 years | Personal interview | 2006 | (Elmadfa et al., 2009b) |
| | Males and females aged 65 years and over | Personal interview | 2006 | (Elmadfa et al., 2009b) |
| **Spain** | Males and females aged 18-24 years | Two non-consecutive 24-hour recalls | 2002-2003 | (Serra-Majem et al., 2007). *Data collected in Catalonia*. |
| | Males and females aged 24-44 years | Two non-consecutive 24-hour recalls | 2002-2003 | (Serra-Majem et al., 2007). *Data collected in Catalonia* |
| | Males and females aged 45-64 years | Two non-consecutive 24-hour recalls | 2002-2003 | (Serra-Majem et al., 2007). *Data collected in Catalonia* |
| | Males and females aged 65-75 years | Two non-consecutive 24-hour recalls | 2002-2003 | (Serra-Majem et al., 2007). *Data collected in Catalonia* |
| **Sweden** | Males and females aged 18-80 years | Four-day record | 2010-2011 | (Amcoff et al., 2012) |
| | Males and females aged 18-30 years | Four-day record | 2010-2011 | (Amcoff et al., 2012) |
| | Males and females aged 31-44 years | Four-day record | 2010-2011 | (Amcoff et al., 2012) |
| | Males and females aged 45-64 years | Four-day record | 2010-2011 | (Amcoff et al., 2012) |
| | Males and females aged 65-80 years | Four-day record | 2010-2011 | (Amcoff et al., 2012) |
| **United Kingdom** | Males and females aged 19-64 years | Four-day food diary | 2008-2010 | (Bates et al., 2011) |
| | Males and females aged 65 years and over | Four-day food diary | 2008-2010 | (Bates et al., 2011) |

## Appendix 3B. Vitamin C intake among adults aged ~19-65 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | | Vitamin C intake including supplements (mg/day) | | |
|---|---|---|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 | mean | SD | P5–P95 |
| **Men** | | | | | | | | |
| Austria | 19-64 | 778 | 130 | 120 | | | | |
| Belgium | 19-59 | 413 | 87.5 | 32.4 | 64.0–106.0[(a)] | | | |
| Czech Republic | 19-64 | 1 064 | 111 | 119 | | | | |
| Denmark | 18-75 | 1 569 | 102 | 54 | 47–163[(b)] | | | |
| Estonia | 19-64 | 899 | 82 | 96 | | | | |
| Finland | 25-64 | 730 | 98 | 88 | | | | |
| France | 18-79 | 776 | 91.3 | 55.5 | | | | |
| Germany | 15-80 | 6 160 | 96[(c)] | | | | | |
| Hungary | 18-> 60 | 473 | 79 | 50 | | | | |
| Ireland | 18-64 | 634 | 81 | 55 | 19–192 | 114 | 152 | 19–310 |
| Italy | 18-64.9 | 1 068 | 126 | 79 | 38–275 | | | |
| Lithuania | 19-64 | 849 | 69 | 53 | | | | |
| Norway | 18-70 | 862 | 105 | 77 | n.a.-258 | | | |
| Portugal | 18-≥ 65 | 917 | 116 | 53.9 | 51.5–219 | | | |
| Romania | 19-64 | 177 | 82 | 48 | | | | |
| Sweden | 18-80 | 792 | 93 | 57 | 23–193 | | | |
| United Kingdom | 19-64 | 346 | 91.4 | 71.5 | 12.7–259.3[(d)] | 101.3 | 89.6 | 12.7–288.5[(d)] |
| **Women** | | | | | | | | |
| Austria | 19-64 | 1 345 | 133 | 105 | | | | |
| Belgium | 19-59 | 460 | 91.2 | 39.1 | 63.0–114.0[(a)] | | | |
| Czech Republic | 19-64 | 1 094 | 138 | 142 | | | | |
| Denmark | 18-75 | 1 785 | 114 | 63 | 50–190[(b)] | | | |
| Estonia | 19-64 | 1 113 | 82 | 81 | | | | |
| Finland | 25-64 | 846 | 118 | 82 | | | | |
| France | 18-79 | 1 142 | 94.3 | 48.7 | | | | |
| Germany | 15-80 | 7 593 | 97[(c)] | | | | | |
| Hungary | 18-> 60 | 706 | 80 | 52 | | | | |
| Ireland | 18-64 | 640 | 79 | 51 | 18–184 | 141 | 304 | 20–474 |
| Italy | 18-64.9 | 1 245 | 123 | 74 | 35–259 | | | |
| Lithuania | 19-64 | 1 087 | 66 | 62 | | | | |
| Norway | 18-70 | 925 | 111 | 71 | n.a.-243 | | | |
| Portugal | 18-≥ 65 | 1 472 | 131 | 62.6 | 51.6–235 | | | |
| Romania | 19-64 | 341 | 76 | 41 | | | | |
| Sweden | 18-80 | 1 005 | 96 | 53 | 29–194 | | | |
| United Kingdom | 19-64 | 461 | 87.6 | 66.7 | 15.6–237.2[(d)] | 122.1 | 149.6 | 15.6–545.8[(d)] |

(a): P25-P75.
(b): P10-P90.
(c): Median, including fortified foods but excluding supplements.
(d): P2.5–P97.5.
n.a.: not available

## Appendix 3C. Vitamin C intake among adults aged ~19-34 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Men** | | | | | |
| Bulgaria | 18-30 | 208 | 99.8 | 82.2 | |
| Denmark | 18-24 | 105 | 96 | 47 | 31–194 |
| | 25-34 | 234 | 111 | 63 | 48–212 |
| Estonia | 19-34 | 396 | 85 | 105 | |
| Finland | 25-34 | 137 | 103 | 97 | |
| France | 18-34 | n.a. | 90.3 | 66.7 | |
| Germany[(a)] | 19-24 | 469 | 90[(b)] | | |
| | 25-34 | 614 | 91[(b)] | | |
| Greece | 22 ± 2 | 500 | 146[(c)] | 130[(c)] | |
| Hungary | 18-34 | 136 | 77 | 49.9 | |
| Ireland | 18-35 | 276 | 84 | 59 | 17–213 |
| | | | 129[(c)] | 168[(c)] | 17–506[(c)] |
| The Netherlands | 19-30 | 356 | 96[(d)] | | 45–180 |
| Norway | 18-29 | 138 | 102 | 77 | |
| | 30-39 | 136 | 107 | 88 | |
| Poland | 19-25 | 191 | 117 | 97.1 | |
| Portugal | 18-39 | 179 | 127 | 63.8 | 51.5–230 |
| Spain | 18-24 | 127 | 73.9 | | |
| | 25-44 | 326 | 93.1 | | |
| Sweden | 18-30 | 132 | 78 | 59 | 11-187 |
| **Women** | | | | | |
| Bulgaria | 18-30 | 204 | 94.2 | 78.9 | |
| Denmark | 18-24 | 150 | 109 | 56 | 41–235 |
| | 25-34 | 340 | 118 | 67 | 42–248 |
| Estonia | 19-34 | 459 | 89 | 76 | |
| Finland | 25-34 | 180 | 101 | 70 | |
| France | 18-34 | n.a. | 82.1 | 45.7 | |
| Germany[(a)] | 19-24 | 486 | 87[(b)] | | |
| | 25-34 | 852 | 92[(b)] | | |
| Greece | 22 ± 2 | 451 | 145[(c)] | 120[(c)] | |
| Hungary | 18-34 | 176 | 77.3 | 59.3 | |
| Ireland | 18-35 | 255 | 74 | 51 | 16–178 |
| | | | 146[(c)] | 384[(c)] | 17–536[(c)] |
| The Netherlands | 19-30 | 347 | 86[(d)] | | 39–165 |
| Norway | 18-29 | 143 | 97 | 67 | |
| | 30-39 | 169 | 116 | 76 | |
| Poland | 19-25 | 211 | 82.1 | 60.7 | |
| Portugal | 18-39 | 299 | 136 | 70.4 | 55.1–237 |
| Spain | 18-24 | 182 | 78.6 | | |
| | 25-44 | 376 | 96.7 | | |
| Sweden | 18-30 | 202 | 83 | 48 | 18-168 |
| **Both sexes** | | | | | |
| Latvia[(c)] | 17-26 | 378 | 98.72 | | |
| | 27-36 | 206 | 93.79 | | |

(a): Including fortified foods but excluding supplements.
(b): Median.
(c): Supplements included.
(d): Median (mean not available).
n.a.: not available

## Appendix 3D. Vitamin C intake among adults aged ~35-64 years in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Men** | | | | | |
| Bulgaria | 30-60 | 224 | 106 | 84.9 | |
| Denmark | 35-44 | 318 | 98 | 50 | 37–188 |
| | 45-54 | 336 | 105 | 57 | 37–216 |
| | 55-64 | 336 | 104 | 51 | 36–202 |
| Estonia | 35-49 | 319 | 84 | 93 | |
| | 50-64 | 185 | 69 | 81 | |
| Finland | 35-44 | 177 | 96 | 86 | |
| | 45-54 | 190 | 99 | 85 | |
| | 55-64 | 226 | 96 | 87 | |
| France | 35-54 | n.a. | 86.2 | 44.4 | |
| Germany[a] | 35-50 | 1 946 | 97[b] | | |
| | 51-64 | 1 460 | 102[b] | | |
| Hungary | 35-59 | 199 | 79.4 | 47.6 | |
| Ireland | 36-50 | 205 | 77 | 53 | 19–182 |
| | | | 96[c] | 106[c] | 19–274[c] |
| | 51-64 | 153 | 78 | 49 | 20–178 |
| | | | 113[c] | 170[c] | 20–296[c] |
| The Netherlands | 31-50 | 348 | 95[d] | | 44–180 |
| | 51-69 | 351 | 90[d] | | 42–172 |
| Norway | 40-49 | 179 | 96 | 64 | |
| | 50-59 | 192 | 104 | 70 | |
| Poland | 26-60 | 865 | 94.4 | 81.1 | |
| Portugal | 40-49 | 197 | 117 | 60.5 | 49.7–229 |
| | 50-64 | 295 | 114 | 49.8 | 52.9–212 |
| Spain | 45-64 | 265 | 110 | | |
| Sweden | 31-44 | 183 | 87 | 49 | 25–179 |
| | 45-64 | 308 | 94 | 59 | 24–199 |
| **Women** | | | | | |
| Bulgaria | 30-60 | 224 | 95.1 | 75.5 | |
| Denmark | 35-44 | 412 | 114 | 67 | 40–222 |
| | 45-54 | 359 | 112 | 62 | 38–222 |
| | 55-64 | 326 | 117 | 62 | 41–228 |
| Estonia | 35-49 | 376 | 86 | 91 | |
| | 50-64 | 280 | 65 | 74 | |
| Finland | 35-44 | 211 | 110 | 76 | |
| | 45-54 | 232 | 128 | 93 | |
| | 55-64 | 223 | 130 | 82 | |
| France | 35-54 | n.a. | 82.1 | 45.7 | |
| Germany[a] | 35-50 | 2 648 | 96[b] | | |
| | 51-64 | 1 740 | 103[b] | | |
| Hungary | 35-59 | 295 | 86.4 | 55.6 | |
| Ireland | 36-50 | 232 | 80 | 50 | 20–182 |
| | | | 103[c] | 108[c] | 22–256[c] |
| | 51-64 | 153 | 87 | 53 | 22–202 |
| | | | 192[c] | 344[c] | 24–1,082[c] |
| The Netherlands | 31-50 | 351 | 89[d] | | 41–171 |
| | 51-69 | 353 | 94[d] | | 44–178 |
| Norway | 40-49 | 256 | 111 | 66 | |
| | 50-59 | 193 | 113 | 74 | |
| Poland | 26-60 | 1 035 | 80.4 | 70.8 | |
| Portugal | 40-49 | 340 | 138 | 62.4 | 60.6–248 |
| | 50-64 | 494 | 133 | 60.5 | 51.6–241 |
| Spain | 45-64 | 337 | 135 | | |
| Sweden | 31-44 | 247 | 90 | 53 | 33–183 |
| | 45-64 | 358 | 101 | 52 | 30–197 |
| **Both sexes** | | | | | |
| Latvia[c] | 37-46 | 272 | 93.69 | | |
| | 47-56 | 304 | 96.28 | | |
| | 57-64 | 217 | 87.28 | | |

(a): Including fortified foods but excluding supplements.
(b): Median.
(c): Supplements included.
(d): Median (mean not available).

## Appendix 3E. Vitamin C intake among adults aged ~65 years and over in European countries

| Country | Age (years) | N | Vitamin C intake excluding supplements (mg/day) | | |
|---|---|---|---|---|---|
| | | | mean | SD | P5–P95 |
| **Men** | | | | | |
| Austria | > 64 | 147 | 102 | 59 | |
| Belgium | 60-74 | 416 | 94.5 | 40.8 | 65.0–118.0[a] |
| | > 75 | 389 | 86.4 | 43.6 | 55.0–110.0[a] |
| Bulgaria | 60-75 | 186 | 89.1 | 69.8 | |
| | > 75 | 101 | 85.8 | 61.0 | |
| Denmark | 65-75 | 240 | 96 | 51 | 38–198 |
| Finland | 65-74 | 229 | 92 | 72 | |
| France | 55-79 | n.a. | 96.1 | 60.1 | |
| Germany[b] | 65-80 | 1 165 | 98[c] | | |
| Hungary | ≥ 60 | 138 | 79 | 52.8 | |
| Ireland | ≥ 65 | 106 | 72 | 52 | 14–174 |
| | | | 102[d] | 146[d] | 14–347[d] |
| Italy | ≥ 65 | 202 | 127 | 74 | 43–261 |
| Norway | 60-70 | 217 | 115 | 84 | |
| Poland | > 60 | 226 | 76.7 | 60.6 | |
| Portugal | ≥ 65 | 246 | 121 | 57.3 | 56.8–227 |
| Romania | > 64 | 177 | 77 | 39 | |
| Spain | 65-75 | 122 | 122 | | |
| Sweden | 65-80 | 169 | 110 | 57 | 37–211 |
| United Kingdom | ≥ 65 | 96 | 85.7 | 50.1 | 18.5–238.6[e] |
| | | | 109.7[d] | 104.9[d] | 18.5–314.8[d,e] |
| **Women** | | | | | |
| Austria | > 64 | 202 | 115 | 60 | |
| Belgium | 60-74 | 406 | 92.1 | 52.3 | 55.0–117.0[a] |
| | > 75 | 355 | 86.3 | 41.7 | 56.0–110.0[a] |
| Bulgaria | 60-75 | 194 | 83.2 | 69.8 | |
| | > 75 | 113 | 79.4 | 64.9 | |
| Denmark | 65-75 | 198 | 104 | 55 | 30–208 |
| Finland | 65-74 | 234 | 97 | 68 | |
| France | 55-79 | n.a. | 104 | 52 | |
| Germany[b] | 65-80 | 1 331 | 100[c] | | |
| Hungary | ≥ 60 | 235 | 72.4 | 39.9 | |
| Ireland | ≥ 65 | 120 | 81 | 46 | 23–189 |
| | | | 132[d] | 333[d] | 23–358[d] |
| Italy | ≥ 65 | 316 | 127 | 84 | 34–282 |
| Norway | 60-70 | 164 | 115 | 72 | |
| Poland | > 60 | 365 | 68.8 | 53.2 | |
| Portugal | ≥ 65 | 339 | 118 | 56.5 | 46.7–212 |
| Romania | > 64 | 341 | 76 | 40 | |
| Spain | 65-75 | 122 | 119 | | |
| Sweden | 65-80 | 198 | 108 | 57 | 38-216 |
| United Kingdom | ≥ 65 | 128 | 80.3 | 49.8 | 14.0–200.0[e] |
| | | | 96.7[d] | 87.2[d] | 14.0–338.0[d,e] |

(a): P25-P75.
(b): Including fortified foods but excluding supplements.
(c): Median.
(d): Supplements included.
(e): P2.5-P97.5.
n.a.: not available

## Appendix 4. Vitamin C concentration of human milk from healthy mothers not taking supplements

| Reference | N (number of samples) | Country | Maternal intake (mg/day); mean ± SD | Maternal plasma (µmol/L); mean ± SD | Stage of lactation | Vitamin C concentration (mg/L) mean ± SD | median | Range (mg/L) |
|---|---|---|---|---|---|---|---|---|
| Byerley and Kirksey (1985) | 5[a] (5) | USA | 156 ± 115 | Not reported | 7-20 weeks | ~83 ± 30.5[b] | | ~47-122[b] |
| Daneel-Otterbech et al. (2005) | 142[a] (142) | Switzerland | Not reported | Not reported | 1.0-21.8 months | 61.6 ± 13.6[c] | | |
| Ortega et al. (1998a) | 41 [d] | Spain | 149.7 ± 73.2 | 84.1 ± 168.7 [f] | 13-14 days<br>40 days | 76.0 ± 52.1[g]<br>87.4 ± 57.3[g] | | |
| | 16 [e] | | 152.4 ± 97.9 | 73.3 ± 84.2 [f] | 13-14 days<br>40 days | 41.2 ± 35.7[g]<br>42.5 ± 51.6[g] | | |
| Ortega et al. (1998b) | 12[h] (24) | Spain | < 80[i] | 30.1 ± 36.3 [b] | 13-14 days<br>40 days | 39.7 ± 38.8[c]<br>89.0 ± 67.8[c] | 40.0[c]<br>79.7[c] | |
| Salmenpera (1984) | 200[d] (565) | Finland | 138<br>range: 48-277 | 58.5 ± 19.3[g]<br>48.8 ± 22.1[g]<br>54.5 ± 26.1[g]<br>53.9 ± 24.4[g]<br>60.8 ± 26.7[g]<br>61.3 ± 26.7[g] | 0 months<br>2 months<br>4 months<br>6 months<br>9 months<br>12 months | 61.8 ± 9.9<br>59.1 ± 11.8<br>49.7 ± 10.6<br>46.8 ± 10.2<br>44.6 ± 5.6<br>41.4 ± 11.3 | | |
| Sneed et al. (1981) | 7[a] | USA | 83 ± 55<br>152 ± 115 | 43.7 ± 33.5[g]<br>49.4 ± 14.8[g] | 5-7 days<br>43-45 days | 53.1 ± 17.1<br>61.0 ± 10.2 | | |
| Thomas et al. (1980) | 6[a] | USA | 131 ± 88 | 31.0 ± 18.2[g] | 6 months | 35.2 ± 12.0 | | |

(a): smoking status not reported.
(b): estimated from figure.
(c): calculated using a correction for the density of milk of 1.032 g/mL according to Neville et al. (1988).
(d): non-smokers.
(e): smokers. Among them, three subjects took supplements (mean intake of vitamin C from supplements 30 ± 79.9 mg/day).
(f): serum.
(g): calculated using a conversion factor of 5.678 (Young and Huth, 1998).
(h): smokers (~24 %) and non-smokers.
(i): data for intakes ≥ 80 mg/day excluded as included women taking supplements.

## ABBREVIATIONS

| | |
|---|---|
| Afssa | Agence française de sécurité sanitaire des aliments |
| AI | Adequate Intake |
| AR | Average Requirement |
| BMD | Bone mineral density |
| BMI | Body mass index |
| CC | Case control |
| CHD | Coronary heart disease |
| CI | Confidence interval |
| COMA | Committee on Medical Aspects of Food Policy |
| CRP | C-reactive protein |
| CV | Coefficient of variation |
| CVD | Cardiovascular disease |
| d | day |
| D-A-CH | Deutschland- Austria- Confoederatio Helvetica |
| DBP | Diastolic blood pressure |
| DNA | Deoxyribonucleic acid |
| DH | Department of Health |
| DR | Dietary recall |
| DRV | Dietary Reference Value |
| EAR | Estimated Average Requirement |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EPIC | European Prospective Investigation into Cancer and Nutrition |
| EU | European Union |
| EURRECA | EURopean micronutrient RECcommendations Aligned |
| FAO | Food and Agriculture Organization |
| FFQ | Food Frequency Questionnaire |

| | |
|---|---|
| GLUT | Glucose transporter |
| GST | Glutathion-S-transferase |
| Hp | Haptoglobin |
| IOM | U.S. Institute of Medicine of the National Academy of Sciences |
| LDL | Low-density lipoprotein |
| LRNI | Lower Reference Nutrient Intake |
| LTI | Lowest Threshold Intake |
| m | male |
| MI | Myocardial infarction |
| NADH | reduced nicotinamide adenine dinucleotide |
| NADPH | reduced nicotinamide adenine dinucleotide phosphate |
| NNR | Nordic Nutrition Recommendations |
| NHANES | National Health and Nutrition Examination Survey |
| NRC | National Research Council |
| OJ | Official Journal |
| PRI | Population Reference Intake |
| RCT | Randomised controlled trial |
| RDA | Recommended Dietary Allowance |
| RI | Recommended Intake |
| RNI | Recommended Nutrient Intake |
| SBP | Systolic blood pressure |
| SCF | Scientific Committee for Food |
| SD | Standard deviation |
| SE | Standard error |
| SU.VI.MAX | Supplementation en vitamines et minéraux antioxydants [*French prospective study on supplementation with vitamins and minerals*] |
| SVCT | Sodium-dependent vitamin C transporter |
| UK | United Kingdom |

| | |
|---|---|
| UL | Tolerable Upper Intake Level |
| UNU | United Nations University |
| US | United States of America |
| WCRF | World Cancer Research Fund |
| WHO | World Health Organization |
| WR | Weight record method |
| y | year |