10 Ascorbic Acid (Vitamin C)

10.1 Introduction

L-Ascorbic acid, also known as L-xyloascorbic acid, 3-oxo-L-gulofuranolactone (enol form), L-3-ketothreohexuronic acid lactone, antisorbutic vitamin and vitamin C, has the chemical formula $C_6H_8O_6$ and a molecular weight of 176.12. This water-soluble vitamin is important in forming collagen, a protein that gives structure to bones, cartilage, muscle, and blood vessels. It also helps maintain capillaries, bones, and teeth and aids in the absorption of iron. Ascorbic acid, a reducing agent, is necessary to maintain the enzyme prolyl hydroxylase in an active form, most likely by keeping its iron atom in a reduced state. The precursor molecule to the protein collagen, procollagen, contains an unusual amino acid sequence in that every third amino acid is a glycine and contains a high frequency of two amino acids not found in any other proteins - hydroxyproline and hydroxylysine. These latter two amino acids are converted from proline and lysine, respectively, after the procollagen molecule has been synthesized. The hydroxylation of proline and lysine in procollagen is carried out by the enzyme prolyl hydroxylase using ascorbic acid as a cofactor. The natural form of the vitamin is the L-isomer. Ascorbic acid plays an important role as a component of enzymes involved in the synthesis of collagen and carnitine; however, its most vital role is as a water-soluble vitamin in the human body (Sies & Stahl, 1995; Levine *et al.,* 1995).

Ascorbic acid is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical. As a scavenger of reactive oxygen and nitrogen oxide species, ascorbic acid has been shown to be effective against the superoxide radical ion, hydrogen peroxide, the hydroxyl radical and singlet oxygen (Weber, Bendich & Schalch, 1996).

Ascorbic acid protects folic acid reductase, which converts folic acid to folinic acid, and may help release free folic acid from its conjugates in food. Ascorbic acid facilitates the absorption of iron.

10.2 Deficiencies

Severe deficiency of ascorbic acid causes scurvy. Symptoms appear when the serum level falls below 0.2 mg/dl. A total body pool of less than 300 mg is associated with symptoms of scurvy, while maximum body pools are limited to about 2 g (IOM, 2000).

Several symptoms of ascorbic acid deficiency have been recognized including follicular hyperkeratosis, swollen and inflamed gums, loosening of teeth, dryness of the mouth and eyes, loss of hair and dry itchy skin. These symptoms reflect the role of ascorbic acid in the maintenance of collagen and blood vessel integrity. It is an acute or chronic disease characterised by hemorrhagic manifestations and abnormal osteoid and dentin formation. The psychological manifestations of scurvy include depression and hysteria. This potentially fatal disease can be prevented with as little as 10 mg ascorbic

acid per day, an amount easily obtained through consumption of fresh fruits and vegetables.

10.3 Food sources

Ascorbic acid is widely distributed in nature, mostly rich in fresh fruits and leafy vegetables such as guava, mango, papaya, cabbage, mustard leaves and spinach (Tee *et al.,* 1997). Animal sources of this vitamin such as meat, fish, poultry, eggs and dairy products contain smaller amounts and are not significant sources. Most food–based dietary guidelines are similar in that all recommend consumption of 5 servings of fruits and vegetables daily. If this recommendation is followed, daily intake of ascorbic acid will be 210 to 280 mg, depending on food content factors (Levine *et al.,* 1999). Ascorbic acid is the least stable of all vitamins and is easily destroyed during processing and storage. Juices are good foods to be fortified with ascorbic acid because their acidity reduces ascorbic acid destruction. Exposure to oxygen, prolonged heating in the presence of oxygen, contact with minerals (iron and copper) and exposure to light are destructive to the ascorbic acid content of foods.

10.4 Factors affecting requirements

Bioavailability, nutrient-nutrient interactions, gender and antioxidant protection are important factors affecting ascorbic acid requirement. The type of food consumed has not been shown to have a significant effect on the absorption of ascorbic acid. Although absorption of ascorbic acid decreases to about 50% and less with the single doses above 1g, some 70-90% of usual dietary intake of ascorbic acid (30 – 180 mg/day) is absorbed. Bioavailability was completed for 200 mg of ascorbic acid as a single dose. No ascorbic acid was excreted in the urine of six of seven volunteers until the 100 mg dose. At single dose of 500 mg and higher, bioavailability declined and the absorbed amount was excreted (Levine *et al.,* 1996).

Ascorbic acid is very labile, and the loss of ascorbic acid upon boiling milk provides one dramatic example of a cause of infantile scurvy. The ascorbic acid content of food is strongly influenced by season, transportation to market, shelf life, time of storage, cooking practices and chlorination of water.

Ascorbic acid is the most potent enhancer of non-heme iron absorption. A study by Hallberg (1987) showed that iron absorption from non-heme food sources can be increased significantly with a daily ascorbic acid intake of at least 25 mg for each meal (estimated for 3 meals/day). Higher ascorbic acid intakes should be considered if meals contain higher contents of nutrient inhibitors such as phytates and tannins.

10.5 Setting requirements and recommended intakes of ascorbic acid

There are no known local studies on ascorbic acid requirements of communities that the Technical Sub-Committee (TSC) on Vitamins could use as a reference when considering RNI for the vitamin. There are also very few reports of the biochemical status of the vitamin amongst the population groups. No reports of ascorbic acid deficiency have been made in this country for the past 70 years. The TSC therefore referred to the FAO/WHO (2002) consultation report and the IOM (2000) DRI recommendations. The rationale and steps taken in setting requirements and the levels recommended by these organisations as well as available reports of ascorbic acid status of communities in the country were considered.

The TSC on Vitamins decided to adapt the FAO/WHO (2002) values as the revised RNI for Malaysia, with appropriate modifications, given in bold in the following paragraphs according to age groups and summarised in Appendix 10.1.

Infants

Human milk is recognised as the optimal milk source for infants at least throughout the first year of life. It is recommended as the sole nutritional milk source for infants during the first 4 to 6 months of life. IOM (2000) estimated the AI for infants based on the average volume of milk intake of 780 ml and an average concentration of ascorbic acid of 50 mg/l in human milk. For infants 0-6 months, 40 mg per day was the estimated AI and for the 7-12 months infants, the AI was 50 mg per day, taking into consideration the amount of ascorbic acid from solid foods consumed at this stage.

The FAO/WHO expert consultation (FAO/WHO, 2002) estimated the mean ascorbic acid concentration in human mature milk as 40 mg/l. However, it was felt that the amount of ascorbic acid in human milk appears to reflect maternal dietary intake rather than the infants needs. Moreover, it was noted that 8 mg/day of ascorbic acid is sufficient to prevent scorbutic signs in infants. The Consultation therefore arbitrarily set the recommended intake for infants aged 0-6 months at 25 mg/day. The recommended intake for older infants was gradually increased to 30 mg per day.

RNI for infants

0 – 5 months	**25 mg/day**
6 – 11 months	**30 mg/day**

Children and adolescents

No data were available on which to base an estimated average requirement (EAR) for children 1 through 18 years of age. Thus, the IOM (2000) estimated the EARs and RDAs for children on the basis of relative body weight.

The FAO/WHO (2002) recommended intakes for ascorbic acid for children and adolescents were gradually increased from the recommended intake for infants. In deciding on recommended intake for older children, eg adolescents, the TSC considered the possible role that ascorbic acid can play in reducing the high prevalence of iron deficiency anemia in the country (Tee *et al.,* 1998). Hallberg (1987) had observed that the additional intake of at least 25 mg ascorbic acid promotes absorption of soluble non-haem iron. In addition, recent studies have pointed towards a possible antioxidant role for ascorbic acid, ie ability to scavenge reactive oxidants in activated leucocytes, lung, gastric mucosa and to protect against lipid peroxidation. The TSC therefore decided to increase the amount recommended by FAO/WHO (2002) by 25 mg ascorbic acid per day to all age groups from children 10 years and above.

RNI for children

1 – 3 years	**30 mg/day**
4 – 6 years	**30 mg/day**
7 – 9 years	**35 mg/day**

RNI for adolescents

Boys 10 - 18 years	**65 mg/day**
Girls 10 - 18 years	**65 mg/day**

Adults

The classic disease of severe ascorbic acid deficiency, scurvy, is now rare in most countries. Other human experimental data that can be utilised to set a ascorbic acid requirement, based on a biomarker other than scurvy, are limited. The IOM (2000) recommended intakes of ascorbic acid are based on an amount of the vitamin that is thought to provide antioxidant protection as derived from the correlation of such protection with neutrophil ascorbate concentrations. It is however recognised that there are no human data to directly quantify the dose-response relationship between ascorbic acid intake and in vivo antioxidant protection.

Based on ascorbic acid intakes sufficient to maintain near-maximal neutrophil concentrations with minimal urinary loss, IOM (2000) set an EAR of 75 mg/day of ascorbic acid for men. Based on this, and assuming a coefficient of variation of 10%, RDA for ascorbic acid for men was computed to be 120% of estimated requirement or 90 mg/day. Since no similar data were available for women, it is assumed that women will have lower requirement due to their smaller lean body mass, total body water, and body size. The RDA for women was thus set at 75 mg/day.

The IOM noted that at a ascorbic acid intake of 90 mg/day, the plasma ascorbate concentration reaches 50 µmol/l which has been shown to inhibit LDL oxidation *in vitro* systems. Although it is not known whether ascorbic acid prevents LDL oxidation *in vivo,* if it does this might be relevant in the prevention of heart disease. Also, since neutrophils

are at 80 percent saturation at an EAR of 75 mg/day, this should potentially protect intracellular proteins from oxidative injury when these cells are activated during infectious and inflammatory processes.

FAO/WHO (2002) calculated the dietary intake from physiologic requirements. At saturation the whole body content of ascorbate in adult males is approximately 20 mg/kg, or 1500 mg. Clinical signs of scurvy appear when the whole body content falls below 300–400 mg, and the last signs disappear when the body content reaches about 1000 mg. In these experiments, ascorbate in the whole body was catabolised at an approximate rate of 2.9 percent/day.

There is a sigmoidal relationship between intake and plasma concentrations of ascorbic acid. At low doses, dietary ascorbic acid is almost completely absorbed, but over the range of usual dietary intakes (30–180 mg/day), absorption may decrease to 75 percent because of competing factors in the food.

A body content of 900 mg falls halfway between tissue saturation and the point at which clinical signs of scurvy appear. Assuming an absorption efficiency of 85 percent, and a catabolic rate of 2.9, the average intake of ascorbic acid can be calculated as:

900 x 2.9/100 x 100/85 = 30.7 mg/day, which can be rounded off to 30 mg/day.

The recommended nutrient intake (RNI) would therefore be:

900 x (2.9 + 1.2)/100 x 100/85 = 43.4 mg/day, which can be rounded off to 45 mg/day.

No turnover studies have been done in women, but from the smaller body size and whole body content of women, requirements might be expected to be lower. However, in depletion studies plasma concentrations fell more rapidly in women than in men. FAO/WHO (2002) therefore made the same recommendation for non-pregnant, non-lactating women as for men.

An intake of 45 mg/day will ensure that measurable amounts of ascorbate will be present in the plasma of most people and will be available to supply tissue requirements for metabolism or repair at sites of depletion or damage. A whole body content of around 900 mg of ascorbic acid would provide at least 1 month's safety interval, even for a zero intake, before the body content falls to 300 mg.

It has been reported that elderly people generally have lower plasma and tissue ascorbate levels than young people, often because of poor dentition or mobility problems. However, FAO/WHO (2002) felt that the requirements of elderly people do not differ substantially from those of younger people in the absence of pathology, which may influence absorption or renal functioning. The recommended intake for the elderly are therefore the same as those for adults (45 mg/day).

For reasons already mentioned above for the adolescents, the TSC on Vitamins has proposed that 25 mg per day ascorbic acid be added on to the FAO/WHO (2002) recommended intake of 45 mg per day for all groups above 10 years of age.

RNI for adults

Men	**19 – 65 years**	**70 mg/day**
Women	**19 – 65 years**	**70 mg/day**

RNI for elderly

Men	**> 65 years**	**70 mg/day**
Women	**> 65 years**	**70 mg/day**

Pregnancy and lactation

During pregnancy there is a moderate extra drain on ascorbic acid, particularly during the last trimester. It has been reported that 8 mg/day of ascorbic acid is sufficient to prevent scorbutic signs in infants aged 4–17 months. FAO/WHO (2002) therefore provided an extra 10 mg/day throughout pregnancy, to bring the recommended intake to 55 mg/day. This enables reserves to accumulate to meet the extra needs of the growing foetus in the last trimester.

During lactation, it has been estimated that 20 mg/day of ascorbic acid is secreted in milk. For an assumed absorption efficiency of 85 percent, an extra 25 mg will be needed by the mother. FAO/WHO (2002) therefore recommended that the RNI should be set at 70 mg to fulfill the needs of both the mother and infant during lactation.
For the same reasons mentioned for the adolescents, the TSC for Vitamins suggested to add an additional 25 mg per day of ascorbic acid to the FAO/WHO (2002) recommended intake for pregnant and lactating women.

RNI for

Pregnancy	**80 mg/day**
Lactation	**95 mg/day**

Discussions on revised RNI for Malaysia

The RNI values for ascorbic acid for Malaysia, adapted from FAO/WHO (2002), but with the addition of 25 mg per day for all age groups above 10 years of age, are also the same as those adopted by the Working Group for the Harmonisation of RDAs in SEAsia (2002). The SEA Group also decided to provide for an additional amount mentioned. Appendix 10.1 provides a summary of these revised RNI, compared with the previous Malaysian RDI (Teoh, 1975), the FAO/WHO (2002) recommendations and the values recommended by IOM (2000).

The revised RNI for Malaysia is higher than the 1975 Malaysian RDI for all age groups. For the infants and young children, the increase is about 50%. For all other groups, the increase is much more, because of the additional amount mentioned above. For the adolescents and adults, the increase ranged from 116-225%. The increase for the pregnant and lactating women is much less, being only about 60%. The recommended intakes are easily achieved if one follows the Malaysian dietary guidelines. The higher recommendations for the vitamin are deemed reasonable and justifiable. The higher recommended levels are well below the UL and hence pose no health hazard. The revised intakes are still lower than the IOM recommended intakes for most age groups by about 15-60%.

10.6 Toxicity and tolerable upper intake levels

The review by IOM (2000) reported no evidence suggesting that ascorbic acid is carcinogenic or teratogenic or that it causes adverse reproductive effects. High intakes of the vitamin have been reported to have low toxicity; adverse effects have been reported primarily after very large doses (greater than 3 g/day). Data obtained showed little increase in plasma steady-state concentrations at intakes above 200 mg/day. Saturable intestinal absorption and renal tubular reabsorption data suggest that overload of ascorbic acid is unlikely in humans. Possible adverse effects associated with very high intakes have been reviewed and include: diarrhea and other gastrointestinal disturbances, increased oxalate excretion and kidney stone formation, increased uric acid excretion, pro-oxidant effects, systemic conditioning ("rebound scurvy"), increased iron absorption leading to iron overload, reduced vitamin B12 and copper status, increased oxygen demand, and erosion of dental enamel. The tolerable upper intake levels (ULs) as proposed by IOM (2000) for various age groups are tabulated in Table 10.1.

The FAO/WHO (2002) report pointed out that the potential toxicity of excessive doses of supplemental ascorbic acid relates to intra-intestinal events and to the effects of metabolites in the urinary system. Intakes of 2–3 g/day of ascorbic acid produce unpleasant diarrhoea from the osmotic effects of the unabsorbed vitamin in the intestinal lumen in most people. Gastrointestinal disturbances can occur after ingestion of as little as 1 g because approximately half of the amount would not be absorbed at this dose. Oxalate is an end product of ascorbate catabolism and plays an important role in kidney stone formation. Excessive daily amounts of ascorbic acid produce hyperoxaluria. The risk of oxalate stones formation may become significant at high intakes of ascorbic acid (>1 g), particularly in subjects with high amounts of urinary calcium. The FAO/WHO Consultation felt that 1 g ascorbic acid appears to be the advisable upper limit of dietary intake.

Table 10.1 Tolerable Upper Intake Levels (UL) of ascorbic acid for various age groups

Age groups	mg/day of preformed ascorbic acid
Infants	Not possible to establish; source of intake should be formula and food only
Children	
1 – 3 years	400
4 – 8 years	650
9 – 13 years	1,200
Adolescents, 14 – 18 years	1,800
Women, ≥ 19 years	2,000
Men, ≥ 19 years	2,000
Pregnant women	
14 – 18 years	1,800
> 19 years	2,000
Lactating women	
14 – 18 years	1,800
> 19 years	2,000

Source: IOM (2000)

10.7 Research Recommendations

The following priority areas of research are recommended:

- Content of ascorbic acid in breast milk and complementary foods given to infants.
- Ascorbic acid content in a variety of foods especially cooked and processed fruits and vegetables.
- Studies on health benefits of ascorbic acid in the occurrence of chronic diseases and influence on ageing.

10.8 References

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Appendix 10.1 Comparison of recommended intake for ascorbic acid: RDI Malaysia (1975), RNI Malaysia (2005), FAO/WHO (2002), and RDA of IOM (1998)

Malaysia (1975)		Malaysia (2005)		FAO/WHO (2002)		IOM (1998)	
Age groups	**RDI (mg/day)**	**Age groups**	**RNI (mg/day)**	**Age groups**	**RNI (mg/day)**	**Age groups**	**AI (mg/day)**
Infants		Infants		Infants		Infants	
< 1 year	20	0 – 5 months	25	0 – 6 months	25	0 – 6 months	40
		6 – 11 months	30	7 – 11 months	30	7 – 12 months	50
							RDA (mg/day)
Children		Children		Children		Children	
1 – 3 years	20	1 – 3 years	30	1 – 3 years	30	1 – 3 years	15
4 – 6 years	20	4 – 6 years	30	4 – 6 years	30	4 – 8 years	25
7 – 9 years	20	7 – 9 years	35	7 – 9 years	35		
Boys		Boys		Boys		Boys	
10 – 12 years	20	10 – 18 years	65	10 – 18 years	40	9 – 13 years	45
13 – 15 years	30					14 – 18 years	75
16 – 19 years	30						
Girls		Girls		Girls		Girls	
10 – 12 years	20	10 – 18 years	65	10 – 18 years	40	9 – 13 years	45
13 – 15 years	30					14 – 18 years	65
16 – 19 years	30						
Men		Men		Men		Men	
20 – 39 years	30	19 – 65 years	70	19 – 65 years	45	19 – 30 years	90
40 – 49 years	30	> 65 years	70	> 65 years	45	31 – 50 years	90
50 – 59 years	30					51 – 70 years	90
≥60 years	30					> 70 years	90
Women		Women		Women		Women	
20 – 39 years	30	19 – 65 years	70	19 – 65 years	45	19 – 30 years	75
40 – 49 years	30	> 65 years	70	> 65 years	45	31 – 50 years	75
50 – 59 years	30					51 – 70 years	75
≥60 years	30					>70 years	75
Pregnancy		Pregnancy		Pregnancy		Pregnancy	
1st trimester	30		80		55	14 – 18 years	80
2nd trimester	50					19 – 30 years	85
3rd trimester	50					31 – 50 years	85
Lactation		Lactation		Lactation		Lactation	
1st 6 months	50		95		70	14 – 18 years	115
2nd 6 months	30					19 – 30 years	120
						31 – 50 years	120