



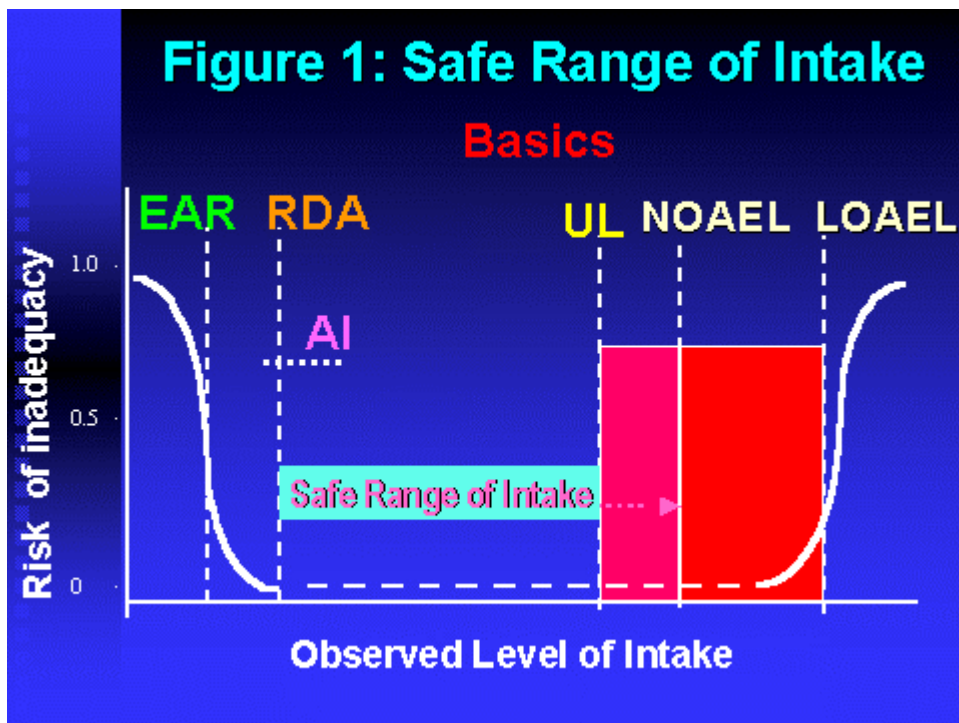
DIETARY REFERENCE INTAKES (DRIs): ANTIOXIDANT MICRONUTRIENTS

INTRODUCTION

Since their inception in 1943 by the National Research Council of the USA, the original objective of the Dietary Recommended Allowances (RDAs) has been “to provide standards to serve as a goal for good nutrition”. The attainment of this objective has always been hampered by the important limitation that the RDAs were developed primarily from minimalist criteria aimed at the prevention of clinical deficiencies, and, in the case of micronutrients, the respective deficiencies and their overt clinical signs. As a summary reminder of the previous VIC Update¹, the new approach by the Food and Nutrition Board of the Institute of Medicine of the United States to have defined DRIs represents a paradigm shift from **avoiding of deficiency states**, as determined by clinical manifestation and status, to **maximising health and improving quality of life**. The latter is determined by functional measures including the reduction of risk of chronic disease and by suggesting guidelines for groups and individuals. The DRI framework includes:

- the objective to determine recommendations to meet a variety of uses
- the contribution by nutrients in the risk reduction of chronic disease
- the inclusion and review of other food components
- the use and the rationale for functional end points, and
- the assessment of estimates of upper safe levels of nutrient intake

The term DRIs is a collective name and refers to a set of at least four nutrient-based reference values (Figure 1):



- **EAR** : **Estimated Average Requirement**; the nutrient intake value that is estimated to meet the nutrient requirements in 50% of the individuals in a given life-stage and gender group defined by a specified indicator of adequacy. The EAR is used as the basis in setting the RDA. If sufficient scientific evidence is NOT available to establish an EAR, no RDA can be set.
- **RDA** : **Recommended Dietary Allowance**; the RDA is the daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97 – 98 %) individuals in a given life-stage and gender group. If the standard deviation (SD) of the EAR is available, the $RDA = EAR + 2 SD_{EAR}$.
- **AI** : **Adequate Intake**; in a case where the scientific evidence is inadequate to set an EAR, the AI reference is used instead of an RDA. The AI is based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group of healthy people.
- **UL** : **Tolerable Upper Intake Level**; the UL is the highest level of nutrient intake that is considered unlikely to pose any risk of adverse health effects to almost all individuals in the general population. When possible, the UL is based on the no-observed-adverse-effect-level (NOAEL), which is the highest intake or experimental oral dose of a nutrient at which no adverse effects have been observed in the individuals investigated. If there is insufficient data to demonstrate a NOAEL, then the lowest-observed-adverse-effect-level (LOAEL), namely the lowest intake at which an adverse effect has been identified, may be used.

Two panels of experts dealing with the Food and Nutrition Board's DRI evaluation process have successfully completed DRIs for the following nutrients^{2,3}

Calcium Panel : Calcium, Magnesium, vitamin D, **Fluoride and Phosphorus** : B-Vitamins and

Choline Panel : Vitamins B₁, B₂, B₆, B₁₂, niacin, folate, pantothenic acid, biotin, niacin, and choline.

More recently, the Food and Nutrition Board of the Institute of Medicine published a set of reference values for vitamin C, vitamin E, and selenium to replace the previously published RDAs for these nutrients⁴. *This Update highlights selected extracts from the report.* The Board has also examined the available evidence on β -carotene and other carotenoids (α -carotene, β -cryptoxanthin, lutein, lycopene, and zeaxanthin). The available evidence has been reviewed in relation to the minimum amounts in which these nutrients are needed to prevent deficiency disorders, as well as the amounts that might impact on chronic diseases

regardless of whether or not the protective mechanisms involve antioxidant properties. Importantly, the report provides a definition for a dietary antioxidant as:

- “a substance in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans”.

The criteria on which this definition is based include “the occurrence of the dietary antioxidant in human diets, the content of the dietary antioxidant has been measured in foods commonly consumed, and the substance decreases the adverse effects of reactive species *in vivo* in humans”. According to this definition, it is only vitamin C, vitamin E and selenium that meet the criteria of the definition. Although β -carotene and other carotenoids do not meet the criteria of the definition, they are known to “influence biochemical reactions that involve the oxidative process”.

Equally importantly, the report accepts the currently available scientific evidence regarding the damaging effects of high levels of reactive oxygen and nitrogen species, which may contribute to cellular dysfunction and disease. In relation to the latter, however, the report draws attention to the fact that although all humans are exposed to oxidative stress, it is only some who develop a chronic disease. As such, the report points out the urgent need for further research with a view to establishing a better understanding of this complex relationship. Similarly, while the report accepts the epidemiological evidence, which indicates that the liberal consumption of fruit and vegetables is associated with a lower risk of developing the so called chronic degenerative diseases, it also points out that the currently available evidence, although on occasions substantial, provides limited support for **individual** dietary antioxidants being protective on their own.

Vitamin C

The *in vivo* antioxidant function of vitamin C is defined as the scavenging of reactive oxidants in activated leukocytes, lung and gastric mucosa, and diminished lipid peroxidation as measured by urinary isoprostane excretion. The new RDA for vitamin C has been based on an intake that maintains near maximal neutrophil concentration with minimal urinary excretion of the vitamin.

As a water soluble antioxidant, vitamin C supplements (500 – 2000mg daily) have been shown to significantly decrease lipid oxidation products in plasma (low density lipoprotein; LDL) and in urine in seven of thirteen supplementation trials. Perhaps the most convincing evidence of the *in vivo* antioxidant function of vitamin C is derived from a study in smokers in whom supplementary vitamin C (2 g for 5 days) was associated with a significant reduction in urinary isoprostanes. The report points out, however, that the data on the effect of vitamin C on LDL oxidation are inconclusive and attributes this to the water soluble nature of the vitamin, which limits its partition into the LDL particle. Interestingly, the report also highlights a significant increase in the plasma concentration of thiobarbituric acid reactive substances, an indicator of oxidative stress, with a 500 mg oral dose of vitamin C. Furthermore, the beneficial effect of vitamin C on vasodilation is reported to be related to the antioxidant function of the vitamin and to be mediated by scavenging superoxide radicals, thus conserving intracellular glutathione and/or potentiating intracellular Nitric Oxide (NO) synthesis. In the leukocyte, vitamin C affords protection against the oxidant damage associated with the respiratory burst. Plasma vitamin C concentrations of 0.4 – 1.7 mg/dL (22 – 85 μ mol/L) have been shown to neutralize hypochlorous acid (HOCl), a powerful oxidant, generated by myeloperoxidase in activated neutrophils and monocytes. In this regard, increased vitamin C oxidation in the presence of inflammation in such conditions as rheumatoid arthritis or the adult respiratory distress syndrome indicates protection against activated neutrophil induced oxidant damage. Vitamin C scavenging of myeloperoxidase-derived oxidants from phagocytic white cells may also be protective against *in vivo* LDL oxidation since HOCl oxidized proteins have been identified in atherosclerotic lesions. With regard to the prevention of DNA and chromosomal damage by vitamin C as well as the reported enhancement of immune function by the vitamin, the report concludes that the current evidence is not compelling in apparently health individuals and cannot be used for the purpose of estimating vitamin C requirements. Similarly, although many studies suggest a protective effect of vitamin C against cardiovascular disease, site specific (breast, cervix,

colon and rectum, pancreas, lung and stomach) malignancies, cataracts, asthma and obstructive pulmonary disease, the common cold as well as cognitive function and memory, the data “are not consistent or specific enough” for the purpose of estimating vitamin C requirements based on these specific biomarkers.

In terms of factors affecting vitamin C requirements, 70 – 90% of the vitamin is absorbed at usual dietary intake levels, whereas absorption decreases to 50% or less with single oral doses in excess of 1 g. The bioavailability of vitamin C naturally found in foods or in the form of a supplement has not been shown to be significantly different from that of pure synthetic ascorbic acid. Because vitamin C participates in redox reactions it interacts with many other dietary constituents or endogenous compounds including glutathione (sparing), tocopherol (regenerating), flavonoids (synergism), iron (enhanced absorption of non-haem iron) and copper (inhibition of absorption). Available data in cigarette smokers indicate that the turnover of the vitamin is increased by 35 mg/day and as such smokers need an additional 35 mg of the vitamin daily to achieve comparable nutriture to that of non-smokers; passive smokers are urged to ensure that they meet the daily new RDA for the vitamin. The available evidence regarding vitamin C status and physical activity or stress in humans does not allow a definitive conclusion to be made as to whether vitamin C requirements are increased.

The DRIs for vitamin C are overall higher than the 1989 RDAs (Table 1). It is be noted that the RDAs for children 1 – 13 years of age are lower than the AI for infants 0 – 12 months of age. This is because the AI has been estimated on the basis of milk consumption and volume of milk consumed whereas the RDAs have been estimated on the assumed differences in body weight from adults for whom there are some data respectively. The data that have been used to derive the two estimates are, therefore, totally different and, hence, cannot and should not be compared. In terms of safety, osmotic diarrhoea and related gastrointestinal disturbances were selected as the critical points on which to base a UL. The report indicates that no causal relationship could be shown between excess vitamin C intake and other adverse effects such as kidney stone formation, excess iron absorption, reduced vitamin B12 and copper absorption, pro-oxidant effects, dental enamel erosion or allergic response in adults and children. Nevertheless, individuals with haemochromatosis, glucose-6-phosphate dehydrogenase deficiency and renal disorders may be especially susceptible to adverse effects from excess vitamin C intake and should be cautious about ingesting more vitamin C than the new RDAs.

The report also highlights areas of research in urgent need of attention which include the establishment of functional measures of vitamin C status, large scale studies with children/adolescents 1 –18 years of age, population studies on the relationship between vitamin C status and chronic disease, the safety of large doses of the vitamin from the pro-oxidant concept point of view as well as the effect of high doses of vitamin C during pregnancy in relation to fetal or neonatal risk of withdrawal, haemolysis or oxidant damage.

Vitamin E

Vitamin E is thought to function primarily as a chain breaking antioxidant that prevents the propagation of lipid peroxidation. The RDAs in the report are based largely on induced vitamin E deficiency in humans and the correlation between hydrogen peroxide induced haemolysis and plasma α -tocopherol concentrations. For the purpose of defining the RDAs in the report, vitamin E activity of α -tocopherol is limited to that available from the naturally occurring forms of α -tocopherol. Other naturally occurring forms of the vitamin (β -, γ - and δ -tocopherols and the tocotrienols) do not contribute towards the vitamin E requirements because they, once absorbed, are not converted to α -tocopherol by humans and, further, are poorly recognized by the α -tocopherol transfer protein (α -TTP) in the liver.

The hypothesis that oxidized LDL is a causative compound in the development of cardiovascular disease is based on solid scientific evidence and is strongly supported by consistent finding in animal models. Vitamin E does inhibit LDL oxidation in vitro, and

additionally it could also affect atherogenesis by other mechanisms based on the following in vitro findings:

- Inhibition of smooth muscle proliferation through the inhibition of protein kinase C
- Inhibition of platelet adhesion, aggregation and platelet release reactions
- Inhibition of plasma generation of thrombin, which binds to platelet receptors and induces aggregation
- Decrease in monocyte adhesion to the endothelium and decrease in monocyte superoxide production
- Potentiation of the synthesis of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation
- Inhibition of the expression of intracellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1) induced by exposure to oxidized LDL.

In humans, three large prospective cohort studies have reported an inverse association between estimated dietary intake of vitamin E and coronary heart disease risk. However, of the four large scale, published, randomized, double blind clinical trials, which tested the ability of vitamin E to prevent myocardial infarction, only one, a secondary prevention trial supplementing with 400 or 800 IU α -tocopherol, has been strongly positive in terms of prevention. In interpreting these findings, it is interesting to note that the three trials, which did not show a preventive effect, all employed 400 IU or less of supplemental vitamin E. The available evidence the report concludes is insufficient for a recommendation to be made for supplemental vitamin E for the prevention of heart disease in the general population. A similar conclusion is reached for the role of supplemental vitamin E in diabetes mellitus, despite the available findings, which indicate that these patients show accelerated lipid and lipoprotein oxidation and that treatment with vitamin E can partially reverse this process.

With regard to cancer, the report concludes that the evidence for a protective effect of vitamin E is weaker than that for cardiovascular disease. Observational epidemiological studies provide only limited evidence for a protective association and only for some cancer sites, especially the prostate. Supplemental vitamin E has been shown in some individuals to reverse the age associated decline in immune function. It is uncertain whether a similar effect can be shown in younger populations. Further studies are needed in order to elucidate the role of vitamin E in the prevention of cataracts and the management of defined neurological disorders such as Parkinson's and Alzheimer's disease, Down's syndrome and tardive dyskinesia. Interestingly, the report speculates that even if additional evidence in terms of positive outcomes was to be obtained, it may not necessarily lead to changes in the current recommendations for the whole population but rather for those at higher risk for the defined conditions, at least initially.

Most dietary vitamin E is found in foods that contain fat and the absorption of the vitamin requires micelle formation and chylomicron secretion by the gastrointestinal tract. The amount of fat necessary for the absorption of vitamin E has, however, not been determined. In terms of nutrient-nutrient interactions, the tocopheroxyl radical can be reduced by vitamin C thereby oxidizing the latter and returning vitamin E to its reduced state. However, the extent to which vitamin E is recycled in humans and which antioxidant species are preferentially used for this recycling is not known. Vitamin E requirements have been reported to increase with an increased intake of polyunsaturated fatty acids (PUFAs) and the suggestion has been made that 0.4 mg of α -tocopherol per gram PUFAs should be consumed by adults. The report acknowledges the "simplicity" of this recommendation in view of the importance of the degree of fatty acid unsaturation in relation to requirements, but it nevertheless highlights the need for an increased vitamin E intake in people on high PUFA diets.

The DRIs for vitamin E are overall higher than the 1989 RDAs (Table 2), indeed in some age groups the RDAs have increased by as much as 50%. The report acknowledges that some physicians, on the basis of the results of the one trial that documented strongly positive preventive outcomes for myocardial infarction are prescribing vitamin E supplements in the range of 400 or 800 IU. It is not, however, known how vitamin E works at these high doses and the mechanism of action could include both antioxidant and non-antioxidant functions. The available evidence is also inconclusive in terms of increased requirement for the vitamin in relation to physical exercise, extreme body size and composition and cigarette smoking.

Safety-wise, there are no data of adverse effects from the consumption of vitamin E naturally occurring in foods. There is some evidence, however, that supplemental vitamin E may be associated with an increased risk of haemorrhagic effects in premature infants. In adults, the available data has not demonstrated any consistent causal adverse effects at the UL level in apparently healthy individuals. Nevertheless, caution is advised, since the database on the safety of large doses of vitamin E has been primarily derived from small groups of individuals with small doses (mostly less than 2 000 mg/day) for relatively short periods (weeks to a few months).

Research recommendation in the report include the development of biomarkers for use in the assessment of vitamin E status, large scale studies in the age group 1 - 18 years, further research on the relationship between oxidant stress and vitamin E status, especially in relation to disease prevention, vitamin E kinetics and metabolism and more research on the role of the other forms of the vitamin.

Selenium

Selenium functions through selenoproteins, which form part of the oxidant defense enzymes. The RDAs for selenium are based on the amount needed to maximize the synthesis of the selenoprotein glutathione peroxidase, as assessed by the plateau in the activity of the plasma isoform of this enzyme.

In humans some but not all observational studies have shown that people with self-selected diets that produce high plasma and nail selenium levels tend to have a lower incidence of cancer (skin, stomach and prostate). Some studies have shown that intakes of selenium above those needed to maximize selenoproteins have an anticancer effect in humans. The available evidence, however, is insufficient for it to serve as the basis for determining selenium requirements in humans.

Most dietary selenium is highly bioavailable with the bioavailability of selenomethionine being greater than 90%. Inorganic salts of selenium (selenate and selenite) used in dietary supplements have approximately equivalent bioavailability, which generally exceeds 50%. The new selenium RDAs are largely similar to the 1989 RDAs (Table 3) and any differences, especially in men, are due to the differences in the criteria and the more recent data used in setting the new RDAs. Hair and nail brittleness and loss were used as the critical end points on which the UL was decided upon as these are the most frequently reported signs and symptoms of chronic selenosis. The report calls for research on new biomarkers for the assessment of selenium status, large scale studies in the 1 – 18 years age group of the population, the better understanding of the function of selenoproteins, the health consequences of an inadequate selenium intake and controlled intervention trials in order to understand better the role of selenium in cancer prevention.

β -Carotene and Other Carotenoids

Blood concentrations of carotenoids are considered to be good biomarkers for the consumption of fruits and vegetables. The totality of the available evidence supports consistently an association between increased fruit and vegetable consumption and a lower risk of several chronic diseases. The very same evidence, however, does not exclude the possibility that the said observed association may be due to other food components in these foods or other behavioural correlates, which may also be responsible for the apparent protection afforded by carotenoid rich foods. The importance of the accepted in vitro antioxidant properties of β -carotene and other carotenoids to health is not known at present. In terms of health outcomes, there can be little doubt, however, regarding the function of carotenoids ((α -carotene, β -carotene, β -cryptoxanthin) as vitamin A precursors and the prevention of vitamin A deficiency. Requirements for carotenoids based on the basis of their provitamin A activity will be dealt with by the Panel in a future report in conjunction with the DRIs for vitamin A. Although the report makes no DRI recommendations for β -carotene and other carotenoids, it nevertheless highlights the importance of an increased consumption of fruit and vegetables in health. Regarding β -carotene supplements, the report concludes that,

at present, there is no evidence that such supplements confer any health benefits and they may indeed be harmful in certain sections (smokers) of the general population. In this regard, three large scale double blind, randomised trials using high dose β -carotene supplements have provided no evidence of a protective role of β -carotene in lung or total cancers, except perhaps for prostate cancer, or total mortality. Such supplements, therefore, when used, should be for the purpose of improving vitamin A status in populations at high risk for vitamin A deficiency. For this purpose and since it is known that dietary fat is important in ensuring optimal carotenoid absorption, especially in populations consuming low fat diets, the ingestion of such supplements with the concomitant addition of fat to the diets of such populations is thought to be beneficial. In one such study, the addition of 18 g/day of olive oil improved the absorption of β -carotene from 5 to 25%.

In conclusion, the new DRIs represent another important milestone in our efforts to better understand nutrient requirements in relation to health and disease. They also highlight the very serious gaps in our knowledge that still remain in great need of understanding and answers. This is perhaps hardly surprising in view of the complex and multifactorial nature of health and disease. Further research will undoubtedly equip us better to deal with the role of food in health promotion and disease prevention. In the meantime, however, and in view of the emerging trend of some RDAs that are set higher than previous recommendations against a background of continued poor dietary habits and stressful lifestyles, the concern as to whether we can obtain all our nutrients in the amounts recommended from food alone can be seen to be gathering momentum. Whether of course we will become increasingly reliant on supplements to meet our nutrient requirements remains to be seen.

[For the application of the DRIs in daily practice, the reader is referred to the previous Update on the subject¹].

Table 1. Comparison of the 1989 and 2000 Recommended Dietary Intake for Vitamin C[&]

Population group	RDAs [#] 1989 ⁵ (mg/day)	DRIs 2000 ⁴ (mg/day)		
		AI*	RDAs [#]	UL [^]
Infants				
0 – 6 months	30	40	-	-
7 – 12 months	35	50	-	-
Children				
1 – 3 years	40	-	15	400
4 – 8 years	45	-	25	650
Boys				
9 – 13 years	50	-	45	1200
14 – 18 years	60	-	75	1800
Girls				
9 – 13 years	50	-	45	1200
14 – 18 years	60	-	65	1800
Men				
19 – 30 years	60	-	90	2000
31 – 50 years	60	-	90	2000
51 – 70 years	60	-	90	2000
> 70 years	-	-	90	2000
Women				
19 – 30 years	60	-	75	2000
31 – 50 years	60	-	75	2000
51 – 70 years	60	-	75	2000
> 70 years	-	-	75	2000
Pregnancy				
14 - 18 years	70	-	80	1800
19 – 50 years	70	-	85	2000
Lactation				
14 - 18 years	90 – 95	-	115	1800
19 – 50 years	90 - 95	-	120	2000

[&] The age groups range was approximated in the case of the 1989 RDAs for the purpose of the comparison * Adequate Intake; # Recommended Dietary Allowances; ^ Tolerable Upper Intake Level

Table 2. Comparison of the 1989 and 2000 Recommended Dietary Intake for Vitamin E (α -tocopherol)[&]

Population group	RDAs [#] 1989 ⁵ (mg/day)	DRIs 2000 ⁴ (mg/day)		
		AI [*]	RDAs [#]	UL [^]
Infants				
0 – 6 months	3	4	-	-
7 – 12 months	4	5	-	-
Children				
1 – 3 years	6	-	6	200
4 – 8 years	7	-	7	300
Boys				
9 – 13 years	10	-	11	600
14 – 18 years	10	-	15	800
Girls				
9 – 13 years	8	-	11	800
14 – 18 years	8	-	15	800
Men				
19 – 30 years	10	-	15	1000
31 – 50 years	10	-	15	1000
51 – 70 years	10	-	15	1000
> 70 years	-	-	15	1000
Women				
19 – 30 years	8	-	15	1000
31 – 50 years	8	-	15	1000
51 – 70 years	8	-	15	1000
> 70 years	-	-	15	1000
Pregnancy				
14 - 18 years	10	-	15	800
19 – 50 years	10	-	15	1000
Lactation				
14 - 18 years	11 – 12	-	19	800
19 – 50 years	11 - 12	-	19	1000

[&] The age groups range was approximated in the case of the 1989 RDAs for the purpose of the comparison

^{*} Adequate Intake; [#] Recommended Dietary Allowances; [^] Tolerable Upper Intake Level

Table 3. Comparison of the 1989 and 2000 Recommended Dietary Intake for Selenium[&]

Population group	RDAs [#] 1989 ⁵ (µg/day)	DRIs 2000 ⁴ (µg/day)		
		AI [*]	RDAs [#]	UL [^]
Infants				
0 – 6 months	10	15	-	45
7 – 12 months	15	20	-	60
Children				
1 – 3 years	20	-	20	90
4 – 8 years	20	-	30	150
Boys				
9 – 13 years	40	-	40	280
14 – 18 years	50	-	55	840
Girls				
9 – 13 years	45	-	40	280
14 – 18 years	50	-	55	400
Men				
19 – 30 years	70	-	55	400
31 – 50 years	70	-	55	400
51 – 70 years	70	-	55	400
> 70 years	-	-	55	400
Women				
19 – 30 years	55	-	55	400
31 – 50 years	55	-	55	400
51 – 70 years	55	-	55	400
> 70 years	-	-	55	400
Pregnancy				
14 - 18 years	65	-	60	400
19 – 50 years	65	-	60	400
Lactation				
14 - 18 years	75	-	70	400
19 – 50 years	75	-	70	400

[&] The age groups range was approximated in the case of the 1989 RDAs for the purpose of the comparison

^{*} Adequate Intake; [#] Recommended Dietary Allowances; [^] Tolerable Upper Intake Level