

## Micronutrients and ageing: intakes and requirements

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Ageing (and related diseases) may be described as a process which results from impaired immunological, genetic, neurological or endocrinological functions. Oxidative mechanisms may play an important role in the ageing process. It is important, therefore, to emphasize the relationship between health and nutrition in the elderly, particularly with regard to antioxidant micronutrient requirements. Indeed, accelerated ageing may be related to a deficit in the intakes of antioxidant vitamins (tocopherols, carotenoids and vitamin C) and trace elements (Zn and Se), as well as to an impaired adaptative mechanism against oxidative stress. Physiological modifications occurring during the lifetime and environmental influences are significant factors contributing to the impairment of micronutrient status, and these factors have to be considered when defining the specific requirements of the elderly. For Fe there is no evidence of benefit of supplementation in healthy subjects, but in the present state of knowledge combined supplementation, including Zn, Se, vitamins C and E and carotenoids, could be the best way to prevent accelerated ageing and reduce the risk of several common age-related diseases.

### Résumé

De nombreuses théories (immunologique, génétique et neuroendocrinologique) ont été proposées pour expliquer les processus de vieillissement et les pathologies qui s'y rapportent. Les mécanismes oxydatifs pourraient contribuer à ces processus et plus particulièrement à leur accélération. La théorie radicalaire repose sur une modification avec l'âge du rapport prooxydants ou antioxydants dû à une majoration des processus oxydatifs accompagnés d'une diminution des systèmes de défense subséquentes à une carence en un ou plusieurs micronutriments antioxydants. Les modifications physiologiques qui accompagnent le vieillissement et une alimentation sélective contribuent aux modifications d'apport et d'absorption des micronutriments. Ces altérations sont accentuées lors de pathologies chroniques. Il n'existe pas de réel consensus sur les recommandations d'apports en oligoéléments adaptés au sujet âgé. Néanmoins les études récentes montrent qu'une association zinc, sélénium, vitamines antioxydantes (vitamines A et E et caroténoïdes) pourrait être le moyen le plus efficace de lutte contre les pathologies du grand âge et également de prévention du vieillissement accéléré.

### Ageing: Antioxidants: Micronutrients

The elderly are a rapidly growing segment of the population, in both developed and developing countries. Thus, the maintenance of a healthy mental and physical state in the elderly appears to be one of the crucial public health challenges for the future. Since numerous clinical and epidemiological studies have confirmed the close relationship between health and nutrition, many reports have aimed to define specific nutrient requirements for ageing individuals. It is important, therefore, to emphasize the relationship between health and nutrition in the elderly,

particularly regarding the possible link between nutrition and chronic diseases.

For micronutrients (vitamins and trace elements), requirements and recommended dietary allowances are far from being well established (Mertz, 1990; Harman, 1995). In the present paper we will discuss the influence of antioxidant micronutrients with respect to recent data involving vitamins (tocopherols, carotenoids and vitamin C) and trace elements (Zn, Se, Mn and Cu) in cellular antioxidant defences and protection against accelerated ageing processes.

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**Abbreviation:** RDA, recommended dietary allowance.

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### Micronutrients and the ageing process

Ageing (and related diseases) may be described as a process which results from impaired immunological, genetic, neurological, endocrinological or antioxidant functions. The free radical theory of ageing (Sohal, 1993; Knight, 1995) suggests an age-related imbalance between pro-oxidant and antioxidant equilibrium, associated with a potential decrease in antioxidant defences. In animals, several workers have shown an inverse relationship between lifespan and the increased production of reactive oxygen species (see Cutler, 1985). Mitochondrial and nuclear DNA modifications were described in old animals. It has been shown *in vitro* that oxidative stress, administered at sublethal levels, led to genomic alterations and accelerated cellular ageing (Toussaint *et al.* 1998). In elderly subjects, especially in institutionalized patients, lipoperoxidation (monitored using indirect variables) is enhanced (Tolonen *et al.* 1988; Monget *et al.* 1996a; Coudray *et al.* 1997). Data concerning the relationship between age and DNA oxidation are not yet available from large-scale epidemiological studies, but in limited groups of elderly subjects we have observed a higher level of oxidized bases than in younger adults (Piperakis *et al.* 1998).

Findings relating to modifications in antioxidant defences in the elderly remain controversial. The discrepancy in the data could be due to the difficulties in defining appropriate population samples that are representative of the various strata of the elderly. How should 'elderly' be defined? A combination of lifestyle, nutritional habits, incidence of diseases and the ageing process itself should be considered. Nevertheless many authors have reported that elderly individuals have lowered Cu-Zn superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6) and GSH peroxidase (EC 1.11.1.9) activities in erythrocytes (see Ceballos-Picot *et al.* 1992). In France, we recently observed that modifications in antioxidant defences were significantly more important in institutionalized elderly individuals than in healthy free-living elderly individuals (Monget *et al.* 1996b; Schmuck *et al.* 1996). In fact, institutionalized individuals should receive particular care with respect to their antioxidant status.

*In ageing the changes in antioxidant defences might be due to a deficit of micronutrient intakes and/or might be related also to an impaired adaptive mechanism against oxidative stress*

**Selenium.** Several lines of evidence have shown that Se plays a key role in protecting cells against free radical attack. This powerful property is mainly due to its antioxidant effect as the constitutive trace element of the active site of GSH peroxidase (Richard *et al.* 1997) and as a limiting factor for the synthesis of this enzyme. Se can also modulate the immune system (MacKenzie *et al.* 1998), since in combination with vitamin E Se acts in protecting immunocompetent cells. Se also has a role in the detoxification of heavy metals (Hg, Cd and Pb) and xenobiotics. This function offers an interesting field of action in elderly patients on multiple medication. Recently reported beneficial effects of antioxidant supplementation on clinical

or biological variables suggested a deficient functional status in the elderly (Monget *et al.* 1996a,b; Girodon *et al.* 1997a,b).

**Zinc.** Zn forms part of numerous proteins and plays a key role in many metabolic reactions (Favier, 1994). The role of Zn as a biological antioxidant is complex (Bray & Bettger, 1990) and its influence on the ageing process is not completely understood. Zn is a component of antioxidant metalloenzymes (Cu-Zn superoxide dismutase) and metallothioneins. Cu-Zn superoxide dismutase activity is regulated by Cu intake (Hassan, 1988), but Zn plays a structural role in the maintenance of enzyme integrity (Coudray *et al.* 1992). Metallothioneins are low-molecular-weight proteins rich in cysteine whose main function is to transport metals (Bremner & Davies, 1975), but which can also detoxify reactive oxygen species (Rossman & Goncharova, 1998). Zn, by competition with other transition metals, protects SH groups from oxidation, and *in vitro* might slow down oxidative damage to SH groups. By preventing proteins from oxidation, Zn could contribute to SH group stabilization, and thus might influence transcription factors such as p53 (Verhaegh *et al.* 1998). Zn also functions as a cofactor for enzymes of nucleic acid catabolism (endonuclease and caspase; Perry *et al.* 1997). As described recently, Zn depletion results in cellular death by apoptosis (Parat *et al.* 1997). In isolated activated neutrophils (Nakamura *et al.* 1987) Zn would also decrease free radical production, leading to a potential negative effect on phagocytosis when inadequate Zn treatment is administered.

**Copper.** The role of Cu in oxidative stress remains highly controversial, since Cu acts both as an antioxidant and as a pro-oxidant. Cu ions are indeed pro-oxidants *in vitro* or when body Cu stores are very high. In contrast, Cu as an essential constituent of superoxide dismutase or as ferroxidase (EC 1.16.3.1) in caeruloplasmin acts as an antioxidant.

Is the importance of Cu deficiency, as for Zn or Se, related to the antioxidant properties of this trace element? What is the incidence of deficient status in the elderly? Further large-scale epidemiological studies and complementary intervention studies are needed to demonstrate functional deficiencies in the elderly (Schmuck *et al.* 1996). Nevertheless, a revised standpoint should be considered with respect to the possible cardiovascular risk associated with Cu deficiency.

**Iron.** In free-living healthy elderly individuals, Fe deficiency does not appear to be a major problem (Johnson *et al.* 1994). Fe requirements are lower in older subjects than in younger adults, especially in post-menopausal women. In individuals over 70 years old, although haemoglobin levels can be lowered, body weight : blood mass remains stable or slightly lowered. The prevalence of Fe deficiency and anaemia (following WHO criteria) is not high in healthy elderly subjects (Kikerby *et al.* 1991). Thus, Fe, a deficiency of which poses crucial problems in young women, should be considered in a totally different way in older populations.

It has been reported recently that excess Fe has a potential pro-oxidant effect. Free Fe is indeed implicated in oxidative damage. An inverse relationship between high Fe stores and the risk of coronary disease has been demonstrated

(Ascherio *et al.* 1994). The risk of cardiovascular diseases has been related to high dietary intakes of Fe, and excess Fe has been reported to have a role in carcinogenesis (Nelson *et al.* 1994). More recently, it was suggested that excess Fe could have a potential role in Alzheimer's disease (Smith *et al.* 1997). Thus, these findings suggest that excess Fe could result in a number of deleterious effects and accelerated ageing.

**Vitamins.** Some vitamins, complementary with anti-oxidant enzymes and thiol groups, play an antioxidant role in lipid (carotenoids, tocopherols) or aqueous mediums (vitamin C). They are particularly efficient against organic peroxides and singlet oxygen (Chow, 1991; Taylor Maine, 1996).

However, as observed for trace elements, an excess of these vitamins can lead to adverse effects. *In vitro*, vitamin C is a pro-oxidant when free metals are present (Podmore *et al.* 1998). Supplementation with high doses of  $\beta$ -carotene (five times the nutritional requirement) had an unexpected effect in smokers, and led to an increase incidence of lung cancers (The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group, 1994).

#### Antioxidant micronutrient status in the elderly

Data on the relationship between age and micronutrient status are still controversial (Favier, 1994). In studies focusing on Zn, the findings varied depending on the variables investigated. However, there appeared to be an inverse correlation between plasma Zn and age (Herberg *et al.* 1991). Our study, which measured the Zn pool exchange in elderly hospitalized patients, confirmed decreased storage of Zn in elderly patients. The deficits of Zn seemed to be more related to hospitalization and associated diseases than to age itself (Schmuck *et al.* 1996).

Cu status does not appear to be modified or increased with ageing. In the elderly, Cu:Zn tends to increase. This increase could lead to a decreased availability of Zn by stimulating the synthesis of metallothioneins.

Se status does not seem to be modified in healthy subjects until the age of 75 years (Neve, 1989; Olivieri, 1994), when the Se status is lowered. Using stable isotopes, a recent study showed a relationship between the decrease in the size of the Se pool and the incidence of associated diseases (Ducros *et al.* 1997).

For vitamins, most studies have failed to show a significant relationship between age and plasma retinol levels (Succari & Cals, 1998). Data focusing on vitamin E are difficult to discuss if they do not consider lipid or cholesterol levels. Indeed, when vitamin E:cholesterol was measured, no effect of age on vitamin E status was observed (Heseker & Schneider, 1994). For vitamin C, the main observation appears to be an inverse correlation between age and blood levels. Recently, Heseker & Schneider (1994) reported a lower level of plasma vitamin C in older men compared with younger controls with the same dietary intakes of vitamin C. Data on carotenoids are scarce, but a tendency to an increased level of plasma carotenoids with age has been reported in free-living subjects (Hallfrish *et al.* 1994), but we were unable to confirm these findings (Berr *et al.* 1998).

#### Micronutrient requirements in the elderly

Physiological modifications occurring during the lifetime and environmental influences are significant factors contributing to the impairment of trace element status in the elderly. Physiological modifications of the gastrointestinal tract, decreased pancreatic function, frequent lesions caused by gastritis, intestinal pH modifications resulting in decreased absorption, loss of appetite and selection of foods may be responsible for the deficiency. However, the Euronut Seneca Study (1992) reported a relatively good nutritional status in free-living European elderly subjects. In contrast, in hospitalized subjects an important incidence of deficiencies and malnutrition has been described by many authors (Bunker & Clayton, 1989; Schmuck *et al.* 1996; Coudray *et al.* 1997; Table 1). In the healthy elderly an efficient adaptive mechanism to these modifications occurs for some elements. However, the presence of chronic diseases significantly affects absorption and bioavailability of trace elements, mainly as a consequence of drug treatment (anti-acids, diuretics, antibiotics and steroids). In elderly individuals, as in younger adults, the concept of risk groups must be considered with respect to environmental factors (loneliness and low socio-economic level) or depression, functional impotencies and hypertension. The risk increases during long-term hospitalization or when physical or mental stress suddenly affects the potential mechanism of adaptation. Taking into consideration the physiological modifications associated with ageing, the incidence of chronic disease and the decreased intakes, the Standing Committee on Dietary Reference Intakes (World Health Organization/Food and Agriculture Organization/International Atomic Energy Authority, 1996) suggested the adoption of new age-ranges (51–70, 71–80 and over 81 years) in defining the specific requirements of the elderly.

#### Recommended dietary allowances

Few studies focus on recommended dietary allowances (RDA) for micronutrients in the elderly (Guigoz, 1994; Table 2). Where they exist, they are extrapolated from RDA for younger adults. In France, the recommended nutrient intakes (apports nutritionnels conseillés; Dupin *et al.* 1992) relate to subjects aged 65 years and more with a mean

**Table 1.** Trace element intakes in the healthy elderly (From Bunker & Clayton, 1989)

	Healthy free-living elderly	Long-term institutionalized elderly	RDA	ANC
Zn (mg/d)	9.0	5.9	12–15	12
Cu (mg/d)	1.2	0.7–1.0	1.5–3*	2.5*
Fe (mg/d)	9.2	8.9	10	10
Mn (mg/d)	5.3	4.0	3.5*	4
Cr ( $\mu$ g/d)	22.9	26.8	50–200*	–
Se ( $\mu$ g/d)	64.7	37.5	55–75	70

RDA, recommended dietary allowances (National Research Council, 1989); ANC, apports nutritionnels conseillés (recommended nutrient intakes; Dupin *et al.* 1992).

\* Estimated safe and adequate daily intake (National Research Council, 1989).

**Table 2.** National recommended nutrient intakes for the elderly (From Guigoz, 1994)

	USA RDA	UK DRV	Germany ENZ	Canada RNI (ANR)	France ANC	
Age (years)	> 51	> 50	> 65	50–74	> 65	
Energy (MJ)	8.0–9.6	8.0–9.7	7.1–8.0	7.5–9.6	7.1–8.3	6.3–8.8
Fe (mg/d)	10	8.7	10	8.9	8.9	10
Zn (mg/d)	12–15	7.0–9.5	12–15	9–12	9–12	12
Se (µg/d)	55–75	60–75	20–100	50	50	70
Cu (mg/d)	1.5–3.0	1–2	1.5–3.0	–	–	2–5
Mn* (mg/d)	2.0–5.0	–	2.0–5.0	3–5	3–5	4
Cr (µg/d)	50–200	–	50–200	–	125	–
Vitamin E (mg/d)	8–10	–	–	–	–	12
Vitamin C (mg/d)	60	–	–	–	–	80
Carotenoids (mg/d)	–	–	–	–	–	4–5

RDA, recommended dietary allowances; DRV, dietary reference value; ENZ, empfehlungen für die nährstoffzufuhr; ANR, apports nutritionnels recommandés; RNI, recommended nutrient intake; ANC, apports nutritionnels conseillés.

\* Estimated safe and adequate daily intakes.

body weight of 60 kg. In the USA, the RDA (National Research Council, 1989) relate to adults over 51 years old. For Cu, Mn and Cr only estimated safe and adequate dietary intakes are proposed. Recommendations give information only on the range of intakes estimated to fulfil the needs of a group or a general population. Thus, recommended values are above the minimal requirements and aim to fulfil the needs of 95 % of individuals; so, these recommendations are typically to be considered with caution for the elderly, and requirements largely depend on individual factors. For trace elements, new proposals from World Health Organization/Food and Agriculture Organization/International Atomic Energy Authority (1996) or the US Committee on Dietary Intakes (RDA workshop, 1996) suggested revising the RDA. What should RDA represent? Should they aim only to prevent deficiency, and should they be defined just in terms of safety? Or should they aim to maintain the nutrient status (even if minimal) in order to prevent a decrease in biological variables? Or should they aim to cause optimal and protective effects against age-related diseases?

### Supplementation

Several Se supplementation trials conducted in elderly subjects (Bortoli *et al.* 1991; Peretz *et al.* 1991; Simonoff *et al.* 1992; Monget *et al.* 1996a), using 66–125 µg/d in an organic or inorganic form, combined (or not) with other antioxidant micronutrients, had beneficial effects on status, immunity, lipoperoxidation and erythrocyte sensitivity to

haemolysis (Table 3). Moreover, epidemiological data highlighted the key role of Se in protecting against age-associated diseases (cardiovascular disease and cancers). Thus it seems that an intake of 50–100 µg Se/d should be achieved in elderly subjects with subclinical Se deficiency.

The effects of Zn supplementation on immunity in the elderly gave interesting results (Table 4); with doses from 15 to 100 mg/d, beneficial effects were observed. The supplement was often a combined mixture of trace elements and vitamins, thus the specific effect of Zn was difficult to estimate. However, the efficiency of combined supplementation underlines the complementary effect of such a mixture. Recently, using combined supplementation for 6 months in very old subjects (> 80 years old), we observed a decrease in lipoperoxidation (Monget *et al.* 1996b) and erythrocyte-induced haemolysis (Girodon *et al.* 1997a).

The MinVitAox Study (Monget *et al.* 1996a,b; Girodon *et al.* 1997a,b) clearly demonstrated the incidence of multiple deficiencies in the hospitalized elderly, and suggested that combined supplementation would be beneficial in this population. In this study, the incidence of infections was significantly lowered in the supplemented group.

### Conclusions

Micronutrient intakes and status are frequently deficient in elderly subjects, resulting in age-related oxidative diseases. In view of the relationship between nutrition and health in the elderly, more attention should be devoted to fulfilling the requirements of the elderly; revised dietary allowances

**Table 3.** Selenium supplementation in the elderly

Reference	Supplementation	Duration (months)	Plasma Se	Erythrocyte GPx	TBARS	Immunity
Peretz <i>et al.</i> (1991)	100 µg	6	↗	↗	NS	Restoration
Bortoli <i>et al.</i> (1991)	66 µg selenite + 20 mg vitamin E	1	↗	↗	↘	
Simonoff <i>et al.</i> (1992)	120 µg selenite	1	↗	ND	↘	
Monget <i>et al.</i> (1996a)	100 µg selenite + anti-oxidant micronutrients	12	↗	↗	NS	↗IL-1

GPx, GSH peroxidase (EC 1.11.1.9); TBARS, thiobarbituric acid-reactive substances; IL-1, interleukin 1; ↗, significant increase; ↘, significant decrease; ND, not determined.

**Table 4.** Zinc supplementation and immune functions in the elderly

Reference	Supplement	Response
Boukaiba <i>et al.</i> (1993)	20 mg	Restoration of thymulin activity
Bogden <i>et al.</i> (1994)	15 mg + other trace elements	Increased response to cutaneous tests (DHST)
Galan <i>et al.</i> (1994)	20 mg + antioxidants	IL-1 increase

DHST, delayed-type hypersensitivity test; IL-1, interleukin 1.

**Table 5.** Revised dietary allowances and requirements for trace elements in the elderly

Group ...	>75 years, hospitalized, dependant, chronic pathologies	Healthy elderly people
Zn (mg/d)	20–30	10
Se (µg/d)	70–100	50–70
Cr (µg/d)	100–200	50–100
Fe	Dependent on degree of anaemia	None

are shown in Table 5. There is now good evidence that appropriate supplementation can reduce the risk of several common age-related diseases. Combined supplementation with antioxidant trace elements and vitamins could be the best way to prevent accelerated ageing and age-related diseases. For Fe, there is no evidence of the benefit of supplementation in healthy elderly subjects, but according to our present state of knowledge, protection should be optimal using supplements which contain Zn, Se, vitamin C, E and carotenoids.

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