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Can dietary micronutrients influence tissue antioxidant capacity?

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Numerous recent studies have indicated possible roles for free-radical-mediated processes in human pathology. This has led to a great interest in the substances thought to prevent the deleterious effects of these substances *in vivo*, the antioxidants. The biochemistry of free-radical reactions, the manner in which they damage cells and the antioxidants inhibiting their reaction with cellular constituents, have been the subject of a number of recent reviews (see Halliwell & Gutteridge, 1989). Some of the antioxidants are micronutrients derived directly from the diet, while others require an adequate supply of specific micronutrients to ensure they remain at appropriate concentrations within the body. Examples of the former are vitamin E and the carotenoids, while the latter are exemplified by the absolute requirement of glutathione peroxidase (*EC* 1.11.1.9) for Se or cytosolic superoxide dismutase (*EC* 1.15.1.1) for Zn and Cu.

The aim of the present short review is to examine aspects of some of these antioxidant micronutrients with particular reference to the impact which dietary manipulation can have on the 'antioxidant capacity' of tissues. Antioxidant capacity is a concept which has arisen to describe the overall ability of tissues to inhibit free-radical-mediated processes; it is dependent on the concentrations of individual antioxidants and activity of protective enzymes, but also reflects interactive effects of the different antioxidants. A question of particular current interest is whether supplementation of adequately nourished subjects with antioxidants will lead to an enhanced ability to reduce possible deleterious effects of free-radical activity and the incidence of certain common pathologies. The possibility that reported protective effects of some antioxidant micronutrients may be due to properties other than their ability to inhibit or scavenge free-radical reactions will also be briefly examined.

The commonest and probably most important of the antioxidant micronutrients are shown in Table 1. Vitamin E, the major lipid-soluble antioxidant, is capable of breaking the chain of free-radical-mediated lipid peroxidation in cell membranes, thus preventing formation of lipid hydroperoxides. Carotenoids appear to have antioxidant properties as do some quinones, such as ubiquinone. These also can react directly with free radicals. Vitamin C can directly scavenge radicals in the cytosolic fraction of cells, and may also play a role in regeneration of vitamin E. The trace elements Zn, Cu, Se and Mn are essential components of the antioxidant enzymes superoxide dismutase (Cu, Zn or Mn)

Table 1. *Antioxidant micronutrients*

Substances with endogenous antioxidant properties	Substances essential for synthesis of antioxidant enzymes
Vitamin E	Se
Carotenoids	Zn
Vitamin C	Cu
Ubiquinone	Mn
	(Fe)*

* The pro-oxidant effects of dietary Fe are probably more important in free-radical biology than fluctuations in catalase (*EC* 1.11.1.6) activity due to changes in Fe nutrition.

or glutathione peroxidase (Se). Fe is essential for the activity of catalase (*EC* 1.11.1.6), but the pro-oxidant effects of Fe are probably more important than any antioxidant effects in this context.

EFFECTS OF MICRONUTRIENT DEFICIENCIES

Depletion of body stores by feeding diets low in individual micronutrients has been used, to examine the role of those micronutrients in the protective antioxidant systems. There is ample evidence that feeding of animals on diets depleted in vitamin E leads to an increased susceptibility of tissues to free-radical-mediated lipid peroxidation (for example, see Jackson *et al.* 1983; Quintanilha & Packer, 1983), but it is considerably more difficult to find clear evidence that free-radical-mediated processes lead to tissue degeneration in vitamin E-deficient animals. Vitamin E deficiency in man arises infrequently as a secondary effect of defects in fat absorption or metabolism, such as in patients with abetalipoproteinaemia (Muller *et al.* 1983). Tissues from these patients also have increased peroxidizability *in vitro*. It is much less clear whether deficiency of the other antioxidant micronutrients leads to an overall change in the ability of tissues to withstand free-radical-mediated stress. Insufficient studies of carotenoids, vitamin A and ubiquinone have been undertaken, while vitamin C depletion does not occur in commonly utilized laboratory animals and relatively little information is available from appropriate species such as the guinea-pig.

It is widely accepted that depletion of the dietary Se content will lead to a depression in the activity of the antioxidant enzyme glutathione peroxidase; this has been described in both human populations and experimental animals (Burk, 1983; Hill *et al.* 1987). In animals, concurrent Se and vitamin E deficiency frequently occurs and the lack of glutathione peroxidase activity due to Se deficiency contributes to an increased susceptibility to free-radical-mediated damage. The situation concerning the effects of Zn, Cu or Mn deficiency on the susceptibility of tissues to free-radical-induced damage is more complicated. In particular, in the case of Zn, the clinical manifestations of Zn deficiency appear in the absence of changes in the activity of most Zn-dependent enzymes, including cytosolic Cu, Zn-dependent superoxide dismutase (Golden, 1989; Jackson & Lowe, 1992). However, several workers have suggested that some of the manifestations of Zn deficiency may be attributable to increased free-radical activity, perhaps by removing an antagonistic effect against Fe-catalysed systems (Wilson, 1989).

During encroaching Cu deficiency the activity of Cu-dependent enzymes has been shown to fall, but whether superoxide dismutase is particularly sensitive in this respect is not clear (Mills, 1991). However, in contrast to the pattern seen in Zn deficiency many of the characteristic features of Cu deficiency can be attributed to a reduced activity of certain Cu-dependent enzymes, such as lysyl oxidase. It is worthy of note that the predominant features are not those which are thought to be associated with an increase in oxidative stress. Mn deficiency and its effect on the mitochondrial Mn-dependent superoxide dismutase has not been sufficiently studied to permit any further conclusions to be drawn.

EFFECTS OF MICRONUTRIENT SUPPLEMENTATION

The possibility that supplementation with antioxidant micronutrients can reduce the susceptibilities of tissues to oxidative stress is the subject of current interest. Reported inverse relationships between habitual antioxidant intake and the incidences of cardiovascular diseases and certain cancers (Gey *et al.* 1987) have raised the possibility that antioxidant supplements may be beneficial to large population groups. It is pertinent, therefore, to consider the evidence for an influence of such supplements on tissue antioxidant capacity.

A number of studies have indicated that vitamin E supplementation of apparently replete subjects leads to an enhancement of circulating α-tocopherol levels and the same appears to be true for the other organic antioxidants listed in Table 1. However, the effect of some of these (particularly carotenoids and ubiquinone) on antioxidant capacity still requires clarification and potential toxic effects of high doses of substances such as vitamin A and β-carotene have been reported.

The effects of supplementation with the inorganic elements listed in Table 1 are not so straightforward. These elements appear to be under close homeostatic control and hence supplementation of replete subjects will not automatically lead to an increase in body burden. This is exemplified by the situation with Zn where an increase in dietary intake is associated with homeostatic adaptations to reduce the proportion of Zn absorbed from the diet and excreted into the gut in order to regulate body levels (Jackson *et al.* 1984). Similar mechanisms are thought to function for Cu (Turnlund, 1991). It is not logical, therefore, to assume that an increase in dietary Zn or Cu intake will lead to an enhancement of antioxidant capacity in subjects who have adequate Zn and Cu status.

Se is somewhat different. Se supplementation of normal subjects caused both an increase in circulating Se concentrations and an increase in urine Se content, demonstrating an increased absorption of the element, but tissue glutathione peroxidase activities showed little or no change in activity (Jackson *et al.* 1989; Table 2). In retrospect, such a finding is not surprising since any stimulation of glutathione peroxidase activity by Se supplementation would imply that either Se was limiting for the synthesis of the enzyme or that excess provision of an essential cofactor could provide a stimulus for the *de novo* synthesis of the enzyme. The latter possibility would imply some potential storage or detoxifying effect of glutathione peroxidase, roles which have not been ascribed to this protein.

It is also worth noting that antioxidant nutrients can play protective roles against cellular damage by processes not directly related to their ability to inhibit free-radical reactions. Thus, we have recently described protective effects of α-tocopherol on the

Table 2. *Effect of selenium supplementation on Se status**

(Mean values and standard deviations)

	Post-placebo therapy		Post-Se therapy		Statistical significance of difference between treatments
	Mean	SD	Mean	SD	
Plasma Se concentration (μg/l)	77	19	136	36	$P<0{\cdot}001$
Glutathione peroxidase (*EC* 1.11.1.9) activity:					
Plasma (units/l)	439	169	443	228	NS
Erythrocyte (units/g Hb)	32·5	14·8	35·9	15·4	NS

Hb, haemoglobin; NS, not significant.

* Data derived from Jackson *et al.* (1989). Subjects received 1 mg Na_2SeO_3/d or placebo for 2–6 months.

skeletal muscle plasma membrane which appear to be related to its ability to stabilize or change the fluidity of the membrane rather than to an antioxidant effect (Phoenix *et al.* 1991; Page *et al.* 1993), while Se is essential for normal thyroid function with a deficiency leading to secondary changes in cellular viability (Arthur *et al.* 1993).

In summary it is apparent, therefore, that manipulation of the dietary content of vitamins E, A, C, and the carotenoids may lead to important changes in tissue antioxidant potential but, with the exception of Se deficiency, little effect of the other trace elements is likely. Such elements are closely regulated because of their potential for toxic effects and/or importance in maintenance of tissue structure and function; hence, our ability to modifiy their content by dietary means is limited.

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