Invited commentary

The effects of iron and copper status and of dietary carbohydrates on the activity of rat intestinal β-carotene 15,15′-dioxygenase

In much of the world, the primary dietary sources of vitamin A (an essential nutrient for vision, the immune system, reproduction and growth) are coloured fruits and vegetables rich in carotenoids (Underwood & Arthur, 1996; Olson, 1999). A sobering fact in this regard is that millions of young children, pregnant women and lactating mothers do not ingest enough carotenoids and vitamin A to fulfil their physiological needs (Sommer & West, 1996; Underwood & Arthur, 1996; Olson, 1999). As a consequence, a variety of public health strategies are being used to improve the vitamin A statuses of these at risk groups (Sommer & West, 1996; Underwood & Arthur, 1996).

Two factors primarily determine the amount of vitamin A that is derived from dietary carotenoids: (1) the bioavailability of the ingested food carotenoids (Castenmiller & West, 1998; Olson *et al*. 1999) and (2) the mechanism of conversion of provitamin A carotenoids into vitamin A. In the former instance, the widely accepted view that, on average, 6 μg all-*trans* β-carotene in food is equivalent to 1 μg all-*trans* retinol is being re-evaluated; (2) the question of whether a molecule of β-carotene is cleaved to one or to two molecules of vitamin A has been debated since 1960 (Olson, 1999). Central cleavage of β-carotene to two molecules of vitamin A clearly seemed to be the major pathway in the mid-1960s (Goodman & Huang, 1965; Olson & Hayaishi, 1965), but interest in the stepwise cleavage of β-carotene to one molecule of vitamin A has recently resurged (Wang & Krinsky, 1998). The current view is that central cleavage is clearly the predominant pathway in healthy animals and human subjects (Nagao *et al*. 1996), although stepwise cleavage is enhanced in intestinal tissue by oxidative stress (Wang & Krinsky, 1998; Olson, 1999).

The central cleavage of β-carotene into retinal, the immediate product, requires molecular O. The postulated mechanism is that a molecule of O adds across the central (15,15′) double bond of β-carotene to give an unstable dioxetane ring, which then rearranges with cleavage to yield two molecules of retinal (Olson, 1999). Most dioxygenases require metal ions, usually Fe or Cu, to bind O initially at the active site of the enzyme. In support of this view, O-phenanthroline and α,α′-dipyridyl, which bind strongly to Fe, inhibit the enzyme (Olson & Hayaishi, 1965; Olson, 1999). In contrast, the storage of vitamin A formed from β-carotene seems to be normal in Fe-deficient rats (Swanson & Parker, 1993) and normal or enhanced in Cu-deficient rats (Rachman *et al*. 1987; Dulin *et al*. 1995). Finally, because the cleavage enzyme has never been highly purified, uncertainty has continued to exist about the possible role of metals in its activity.

Fe and Cu are closely linked both physiologically and nutritionally (Fairbanks, 1999; Turnlund, 1999). In the transport of Fe from the intestinal mucosa into the plasma, the oxidation of Fe from the ferrous to the ferric state, which then is bound to transferrin, is catalysed by caeruloplasmin (ferroxidase I) and ferroxidase II, both Cu-containing proteins. Furthermore, Cu and Fe are necessary components of cytochrome oxidase, the terminal enzyme of electron transport in the reduction of O to H_2O. Finally, Cu also seems to be involved in the formation of erythrocytes. Thus, at least at three key points in the utilization and function of Fe, Cu is required. The intestinal absorption of Fe, however, does not seem to require Cu (Fairbanks, 1999).

Thus, in Cu deficiency, anaemia would be expected, probably associated with a decline in energy production and an accumulation of Fe in internal organs. In rats, but not necessarily in other species, fructose or sucrose, when substituted for maize starch in the diet, seems to enhance the onset of Cu deficiency (Turnlund, 1999).

That preamble brings us to the interesting paper by During *et al*. (2000) published in this issue of the *British Journal of Nutrition*. Groups of rats were fed low, medium or high amounts of ferric citrate in combination with a Cu-deficient or Cu-adequate diet. Either starch or fructose was supplied as the carbohydrate source. The question was: ‘What effect do these different dietary regimens have on the carotene cleavage enzyme in the intestine?’ Let us consider first the starch-fed rats.

In Cu-deficient rats, the concentration of Fe in the liver, but rather oddly, not in the intestinal mucosa, expectedly doubled at the higher two levels of Fe intake. The activity of the intestinal cleavage enzyme was also consistently higher in Cu-deficient than in Cu-sufficient rats and was directly proportional to the Fe content of the intestinal mucosa.

When fructose was fed, the effects of Cu-deficient diets on tissue concentrations of Cu and Fe were similar to those seen with starch, except that the Cu concentration in the intestinal mucosa, oddly enough, was higher than with starch. On the other hand, the activity of the intestinal cleavage enzyme in fructose-fed, Cu-deficient rats was higher than in starch-fed, deficient rats. Thus, although none of the rats was highly deficient in Cu, as indicated by significant amounts in the tissues, dietary fructose did reduce the growth rate of the rats relative to the starch-fed groups and did enhance carotene cleavage activity.

So what does this study tell us? First, the cleavage enzyme clearly does not require Cu for activity. Second, the enzyme activity is proportional to the Fe content of the tissue, which reflects that in the diet. This latter finding is

a bit surprising, in that one might expect the enzyme to sequester Fe, such that its activity would reach an optimum at medium intake levels of Fe. As the level of dietary Fe also enhanced cleavage activity in Cu-sufficient rats fed on fructose, but not in those fed on starch, however, other factors are clearly at work here. This study confirms and extends recent studies of the same group (During *et al.* 1999) that Fe is essential for carotenoid cleavage.

The number of factors, therefore, that influence the activity of the intestinal carotenoid cleavage enzyme keeps growing. The intestinal enzyme activity is enhanced by vitamin A deficiency, polyunsaturated fats in the diet, Cu depletion, fructose feeding, and glutathione, and is inhibited by protein deficiency, heavy metals that bind to its essential sulfhydryl groups, and some aromatic phytochemicals. Whether carotenoid cleavage enzymes in the liver and other organs are similarly affected merits attention.

In closing, it makes sense that an enzyme responsible for vision and for the integrity of several key physiological processes for the vast majority of humans worldwide would be influenced both by nutritional status and by diet. Further developments must certainly be in the offing.

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