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Plasma and hepatic antioxidant control and vitamin A nutritional status

I. Subirade[a], Y. Fernandez[a,*], B. Périquet[b], S. Mitjavila[a], F. Anglade[a] and A. Périquet[a]

[a]*Laboratoire des Xénobiotiques, I.N.R.A., 180 chemin de Tournefeuille, B.P. 3, 31931 Toulouse Cédex (France), Fax +33 61 28 52 44*

[b]*Service du Professeur Thouvenot, Biochimie, Secteur Chromatographie, Hôpital Purpan, 31059 Toulouse Cédex (France)*

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Abstract. Twenty-seven rats were divided into three groups and fed on diets containing 0.3, 6 or 60 RE (retinol equivalent) retinyl palmitate/g food. After 7 weeks, hepatic vitamin A uptake was found to be more efficient in vitamin A-deficient rats than in rats given adequate vitamin A. We showed that during the metabolic adaptation of the animals to the level of vitamin A in the diet, extensive modifications occur in the antioxidant defences of the organism. In parallel with the increase in the level of vitamin A, the decrease in the level of α-tocopherol in the plasma can bring about a greater susceptibility of the lipoproteins to oxidative stress. Similarly, the decrease in the hepatic α-tocopherol level and in glutathione peroxidase activity leads to the weakening of the liver's antioxidant defences.

Key words. Vitamin A; diet; rats; plasma; liver; scavengers; antioxidant enzymes.

Introduction

It has been reported that, like carotenoids[1], retinoids are in vitro antioxidants[2–4]; more exactly, they scavenge peroxyl radicals[5,6]. In a manner analogous to that proposed for β-carotene[7], they should provide better protection than α-tocopherol against peroxidation processes occurring at low pO_2[7,3]. However, direct demonstrations of the in vivo antioxidant activity of carotenoids and retinoids have been rather isolated[8,9] and contradictory evidence obtained. Thus, vitamin A excess can protect against[9] or potentiate[10] lipid peroxidation initiated by xenobiotics such as doxorubicin and carbon tetrachloride (CCl_4). Moreover, the toxic effect of CCl_4 can be potentiated or reduced depending on the species of animal considered[11].

It is known that the defences of the organism against oxidative damage involve enzymes [catalase, superoxide dismutase (SOD) and glutathione peroxidase] and molecules that trap radicals directly (α-tocopherol, uric acid, ascorbic acid)[12]; in the organism some of these molecules work cooperatively[13]. However, the protection against lipid peroxidation provided by the combinations of antioxidants was either additive, less than additive or synergistic[14]. Moreover, it has also been shown that modification of the level of an antioxidant such as α-tocopherol in the diet can alter the vitamin A levels in the organism[15] and the activity of certain enzymes involved in the defence against free radicals[16].

We propose that, in animals, metabolic adaptation to the level of vitamin A in the diet could bring about modifications of the antioxidant capacity of the organism and could explain some of the controversies described concerning the effect of vitamin A in vivo. For this purpose, we analyzed various plasma and hepatic parameters reflecting antioxidant defence in rats receiving a diet normal for vitamin A [6 RE (retinol equivalent)/g food]. The results were compared with those obtained with other animals receiving a diet deficient in (0.3 RE/g) or with an excess of (60 RE/g) vitamin A.

Materials and methods

Weanling male Sprague-Dawley rats (n = 27) were divided into three groups. For 7 weeks, they received a basal diet as described previously[17] but without vitamin A, containing 22% vitamin-free purified casein, and 21.8% sucrose and 43.6% corn starch as carbohydrate components. The diet was supplemented with 0.3, 6 or 60 RE retinyl palmitate/g food. The concentrations of other nutrients were the same for the three groups. At the end of the period, blood samples were obtained by cardiac puncture, and plasma was immediately separated after centrifugation (15 min at 4 °C, 1800*g*). The livers were rapidly removed, and a 10% homogenate was then prepared in ice-cold 3 mM EDTA, 154 mM KCl at pH 7.4. Cytosol was obtained after two successive centrifugations (9000*g* and 105,000*g*).

Antioxidant defences were evaluated in the biological samples. The following methods were used: urate and SH groups (or glutathione) using respectively a Boehringer test[18] and the method of Ellman[19]; total vitamin C after oxidation of ascorbic acid present[20]; vitamin A and vitamin E in the plasma and in the liver

* Corresponding author.

homogenate according to De Leenher et al.[21] and Périquet et al.[22] respectively; SOD and catalase activities, respectively according to Marklund[23] and Del Rio et al.[24]; total and selenium (Se)-dependent glutathione peroxidase activities[25] using *tert*-butyl hydroperoxide and H_2O_2 respectively as substrates.

Results are expressed as means ± SE by volume or in relation to proteins determined according to Lowry et al.[26] The significance of the differences was assessed using Student's *t* test.

Results

The mean body and liver weights of each group of nine animals at the end of the experimental period were respectively 435 ± 10 g and 17.86 ± 1.31 g for the animals on a diet containing 0.3 RE vitamin A/g food, 430 ± 8 and 17.83 ± 0.72 for 6 RE, and 435 ± 8 and 18.10 ± 1.12 for 60 RE. These values did not differ significantly among the three groups.

The results in figure 1 show the concentrations of retinol in the plasma and of total vitamin A in the liver for the various levels administered in the diet. For the plasma retinol, only slight variations were noted between the groups, but the concentration was significantly lower for the 0.3 RE animals. Moreover, the presence of retinyl palmitate was noted in the plasma of the 60 RE animals but at very low concentrations (0.09 ± 0.005 μM). On the other hand, the hepatic levels of total vitamin A rose very significantly with the level in the diet. However, when the dietary vitamin A increased from 0.3 RE to 6 RE (20-fold) or from 6 RE to 60 RE (10-fold), the corresponding increments in hepatic vitamin A were 200-fold and 12-fold respectively. Retinyl palmitate was the major form of vitamin A in the liver and it occurred in lower proportions for the 0.3 RE (58%) than for the 6 RE (78.7%) and the 60 RE (75.7%). Retinyl stearate, on the other hand, was present in higher proportions for the 0.3 RE (29%) than for the 6 RE (17%) and the 60 RE (18%). Other forms (retinyl esters: laurate, myristate, linoleate, pentadecanoate and heptadecanoate) were undetectable in the 0.3 RE group but represented 4% for 6 RE and 6% for 60 RE. Retinol represented 13% for 0.3 RE and only 0.6% and 0.3% respectively for 6 RE and 60 RE.

Table 1. Effect of different vitamin A intakes on plasma chain-breaking antioxidants.

	0.3 RE*	6 RE*	60 RE*
α-tocopherol (μM)	21.3 ± 2.45[a]	15.3 ± 1.75[a]	8.78 ± 0.64[b]
n	6	8	9
Total vitamin C (μM)	78.6 ± 6.12[a]	92.5 ± 8.58[a]	84.2 ± 6.28[a]
n	6	9	9
SH groups (μM)	225.5 ± 12.7[a]	222.4 ± 10.3[a]	225.8 ± 9.4[a]
n	6	9	9
Urate (μM)	25.0 ± 2.14[b]	31.7 ± 2.56[ab]	35.2 ± 1.37[a]
n	6	8	9

*0.3 RE, 6 RE and 60 RE are as described in figure 1.
Results are means ± SE (n = number of rats per group). Values in the same line followed by the same letter were not significantly different ($p < 0.05$).

For the plasma chain-breaking antioxidants (table 1), the concentrations of vitamin C and of SH groups were not significantly different among the three groups. However, the concentrations of α-tocopherol fell, and those of urate rose with increasing levels of vitamin A in the diet. The differences observed were significant for α-tocopherol between the control group (6 RE) and the 60 RE group and for urate between the 0.3 RE and the 60 RE groups.

In the liver (table 2) no variation was observed for vitamin C, SOD or catalase. However, the level of α-tocopherol and the activities of total and Se-dependent glutathione peroxidases decreased steadily with increasing intake of vitamin A, differences between the control group and the 60 RE group always being significant. Glutathione levels were not very different between the groups, although there was a significant difference between the 60 RE and the 6 RE groups.

Discussion

In our experimental conditions, the dietary intake of vitamin A determines its liver storage: in the vitamin A-deficient state, hepatic vitamin A uptake is more efficient than in the replete state. Note that only deficiency causes a notable alteration in the various forms stored. The drop in blood retinol after 7 weeks of deficient diet shows that the homeostatic control mechanism no longer maintains the level of circulating retinol, in agreement with preliminary studies[27].

During the metabolic adaptation of the animals to the level of vitamin A in the diet, extensive modifications of the parameters involved in antioxidant defences were observed. The decrease in the level of α-tocopherol (in the plasma and in the liver), in parallel with the increase in vitamin A in the diet, could be related to results observed in the plasma with β-carotene-supplemented diets[28]. The results from the liver complete those of other authors[29,30] obtained simply with vitamin A-deficient diets.

In the plasma, considering the increase in the levels of urate with the level of dietary vitamin A, the chain-breaking antioxidant status is apparently little modified, since α-tocopherol and urate are able to trap 2 and 1.3 peroxyl radicals respectively per molecule[12]. However, α-tocopherol is the most important lipid-soluble chain-breaking antioxidant in the plasma, and its content in circulating low density lipoprotein (LDL) is a major

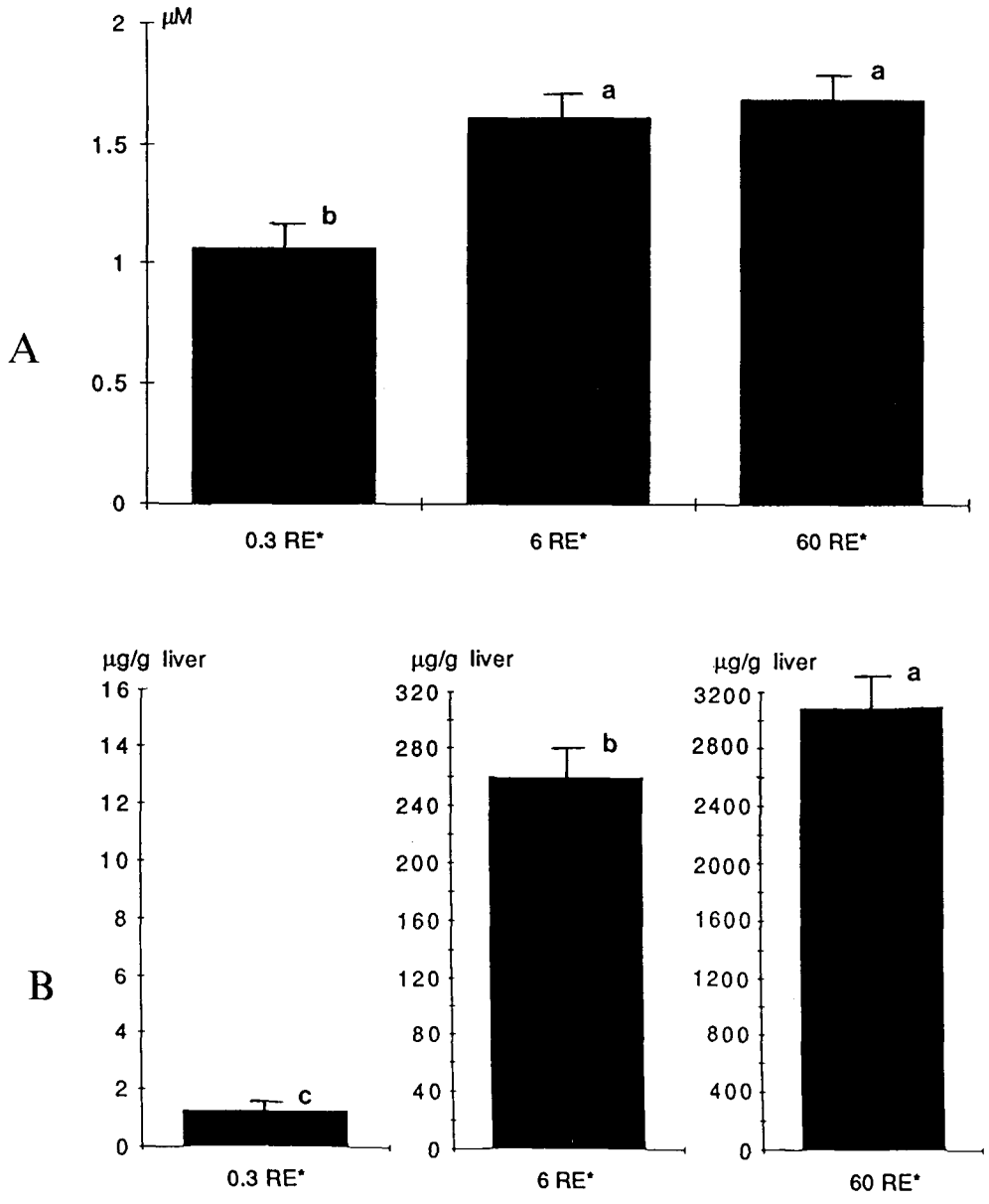

Figure 1. Effect of different vitamin A intakes on plasma and hepatic vitamin A content.
*0.3 RE, 6 RE and 60 RE are samples from groups of rats receiving these amounts of retinyl palmitate/g food. Results are means ± SE of nine rats for plasma retinol (*A*) and hepatic total vitamin A (*B*). Values of a parameter in the three groups followed by the same letter were not significantly different ($p < 0.05$).

factor in determining resistance to lipid peroxidation[31]. Thus, the increase in the level of vitamin A in the diet can indirectly induce a greater susceptibility of the lipoproteins to oxidative stress.

In the liver, the decrease in the activity of the glutathione peroxidases with the level of dietary vitamin A could be attributed to a decrease in the level of Se[14]. Since it has been demonstrated that excessive Se intake decreases the concentrations of retinol and retinyl palmitate in the liver[32], we can put forward the hypothesis that there is an inverse relationship between the level of vitamin A and the level of hepatic Se. Thus the decrease in the glutathione peroxidase activities contributes, together with the decrease in α-tocopherol, to lowering antioxidant defences in the hydrophilic and hydrophobic phases of the hepatic cells of animals having received an excess of vitamin A.

In view of the present vogue for the wide use of antioxidants and megadoses of vitamins in preventive medicine, these results, showing the delicate metabolic

Table 2. Effect of different vitamin A intakes on hepatic vitamin A, E, C, glutathione and antioxidant enzymes.

	0.3 RE*	6 RE*	60 RE*
α-tocopherol (μg/g liver)	43.5 ± 3.92^a	27.9 ± 0.81^b	21.1 ± 1.21^c
n	6	6	6
Total vitamin C (μg/g liver)	347.7 ± 12.2^a	383.7 ± 24.0^a	360.1 ± 40.1^a
n	6	9	9
Glutathione (μg/g liver)	1206.0 ± 27^{ab}	1129.0 ± 33^b	1254.0 ± 37^a
n	6	9	9
Total glutathione peroxidase (nmol NADPH oxidized/min/mg protein)	660.4 ± 44.6^a	586.5 ± 28.6^a	480.5 ± 30.3^b
n	6	9	9
Se-dependent glutathione peroxidase (nmol NADPH oxidized/min/mg protein)	543.8 ± 38.7^a	505.4 ± 30.5^a	394.3 ± 25.1^b
n	6	9	9
SOD (units/mg protein)**	7.74 ± 0.37^a	9.26 ± 0.77^a	8.33 ± 0.87^a
n	6	9	9
Catalase (μmol H_2O_2 decomposed/min/mg protein)	336.8 ± 21.2^a	348.4 ± 10.9^a	300.2 ± 32.4^a
n	6	6	6

*0.3 RE, 6 RE and 60 RE are as described in figure 1.
Results are means ± SE (n = number of rats per group). Values in the same line followed by the same letter were not significantly different ($p < 0.05$).
**One unit of SOD was defined as the amount of protein that inhibited pyrogallol autoxidation by 50%. Glutathione peroxidase and SOD activities were measured in the cytosol; catalase activity was measured in the homogenate.

balance between several factors involved in the protection against oxidative damage and vitamin A, should be a warning and an incentive to investigate this type of interaction in humans.

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