BIOPHYSICS AND BIOCHEMISTRY

Effects of Supplementing the Rat Diet with Selenium on the Efficiency of Essential Linoleic Acid Metabolism

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Examination of the fatty-acid composition of lipids contained in the liver, spleen, blood plasma, aggregated lymphatic follicles of the small intestine, and mesenteric lymph nodes of rats fed diets supplemented with selenium revealed an appreciable effect of this element on the efficiency with which linoleic acid was metabolized to arachidonic acid, which was reflected in an increased 20:4/18:2 ratio. In contrast, Se was found to have little or no effect on levels of lipid peroxidation products in tissues and blood serum.

Key Words: *selenium in rat diet; lipid peroxidation; linoleic acid metabolism*

Arachidonic acid not only ensures the proper structural organization of cell membranes, in whose lipids it dominates among the polyunsaturated fatty acids (PUFA) with 20 or 22 carbon atoms [3], but also plays a no less important part in serving as a precursor for numerous products that are formed in the cyclooxygenase and lipoxygenase pathways and act as mediators of metabolic processes in the cell [10]. Only a small proportion of the arachidonic acid (20:4ω6) necessary for these purposes is supplied to the body through the diet, the rest being provided by biosynthesis from its dietary precursor linoleic acid (18:2ω6) in elongation and desaturation reactions. The efficiency of this biosynthesis depends on how much cis,cis-linoleic acid the diet contains [8], the activity of enzymes involved in the desaturation and elongation reactions [9], the rates of lipid peroxidation (LPO) in the body [12], and the availability of certain vitamins [2].

The relationship between the efficiency of 20:4 biosynthesis and the body's content of selenium (Se) is usually considered to be associated with the role of this element in the system of antioxidant defense [14]. The occurrence in various tissues of Se proteins distinct from those present in cellular and extracellular glutathione peroxidases does not exclude the possibility that under conditions of increased dietary Se intake the efficiency with which the dietary linoleic acid (18:2) is converted into arachidonic acid (20:4) may depend on other factors [6]. So far, this aspect has been studied inadequately. The objectives of the present investigation were to establish whether Se can influence the metabolism of PUFA, particularly arachidonic acid (20:4), and how this element affects LPO processes.

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MATERIALS AND METHODS

Four groups of male Wistar rats with an initial body weight of 110±10 g were used. For 6 weeks, they were fed an artificial food mixture consisting of casein as a source of protein (20%); maize starch that provided 56% of the required carbohydrate; minerals, and the essential water-soluble vi-

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tamins. The source of fiber was wheat bran (0.8 g/day per rat). The fat-soluble vitamins included vitamins A and D in the proportions generally used in feeding rats. For elucidating the possible synergistic actions of vitamin E and Se on LPO and on arachidonic acid (20:4) metabolism, the diets of both test groups contained Se in the same amount but one test group (Se 1) received vitamin E in the minimal amount sufficient to meet the physiological requirement while the other (Se 2) was fed a vitamin E-enriched diet. The two control groups were fed diets containing 0.192 mg/kg Se; the test groups (Se 1 and Se 2) received additional amounts of Se (2 mg/kg diet) in a yeast rich in this element. The levels of Se and vitamin E contained in the diets of the four groups are shown in Table 1.

The fat component of all diets was represented by sunflower oil and accounted for 23% of the calorie intake. The total caloric value of the diet was 94 kcal/day per rat. Essential linoleic acid (18:2) accounted for 0.95% of this value, which was sufficient to meet the requirement (not less than 0.7%) for this indispensable factor. All animals were fed without restriction and had free access to water.

PUFA were measured in the liver, spleen, blood plasma, aggregated lymphatic follicles of the small intestine, and mesenteric lymph nodes after their isolation and homogenation and extraction of the tissues as described by Folch *et al.* [7]. Methyl esters of fatty acids from the total lipid fraction of each of the tissues studied were obtained in a methylation reaction with BF_3 [11] and analyzed chromatographically using the conventional procedure [4]. LPO levels were estimated by determining the content of diene conjugates in the blood serum [5] and of thiobarbituric acid(TBA)-reactive products in the serum [1] and in the liver and aggregated lymphatic follicles [13].

RESULTS

No differences were observed between the groups in body weight gains or in the weight of individual organs. Nor were any pathological changes found either on inspection of living rats or at autopsy.

Measurements of LPO products in the tissues and serum (Table 2) did not reveal significant intergroup differences in serum levels of diene conjugates. The increased dietary intake of Se or of Se in combination with the vitamin E supplement did not lead to a drop in TBA-reactive products either in the serum or in the aggregated lymphatic follicles of the small intestine.

TABLE 1. Se and Vitamin E Levels in Rat Diets

Diet	Se, mg/kg food	Vitamin E, mg per rat per day
Control group 1	0.192	0.64
Control group 2	0.192	0.14
Se 1 group	2.192	0.14
Se 2 group	2.192	0.64

The impact of Se on the metabolic fate of dietary fatty acids was evaluated from data on the fatty-acid composition of lipids contained in the liver, spleen, plasma, aggregated lymphatic follicles, and mesenteric lymph nodes. The fatty-acid composition of hepatic lipids is shown in Table 3. Supplementing the diet with Se appreciably influenced the efficiency with which linoleic acid was metabolized to arachidonic acid, and this influence was enhanced by the addition of vitamin E to the diet.

Analysis of data on the fatty-acid composition of lipids from other organs showed that the 20:4/18:2 ratio, which reflects the efficiency of linoleic to arachidonic acid transformation at a given level of dietary intake of essential 18:2, was markedly increased in the spleen, plasma, and aggregated lymphatic follicles of rats whose diet had been supplemented with Se; the increases in the liver and mesenteric lymph nodes were much smaller. The addition of vitamin E to the diet supplemented with Se raised the 20:4/18:2 ratio in all the tissues tested with the exception of mesenteric

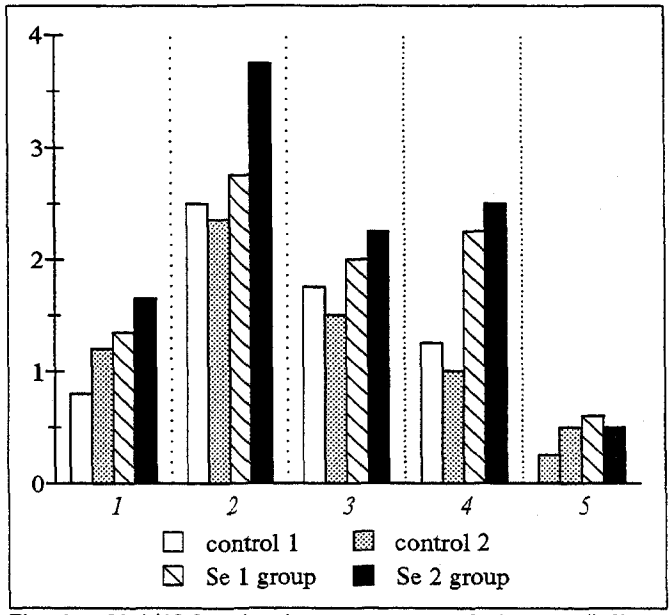

Fig. 1. 20:4/18:2 ratios in rat organs and tissues. *1*) liver; *2*) spleen; *3*) plasma; *4*) aggregated lymphatic follicles of small intestine; *5*) mesenteric lymph nodes.

TABLE 2. Levels of LPO Products in Rat Tissues and Blood

Group	TBA-reactive products			DC/OD 232/ml serum
	liver, nmol MDA/g	aggregated lymphatic follicles, nmol MDA/g	blood serum, nmol MDA/ml	
Control group 1	399.2±25.1	93.4±7.1	10.3±0.89	1.79±0.15
Control group 2	366.7±31.2	87.2±8.1	10.2±0.75	1.74±0.16
Se 1 group	380.2±29.6	101.3±9.4	9.7±0.90	1.61±0.14
Se 2 group	379.9±16.5	102.5±10.0	10.2±1.00	1.74±0.18

Note. MDA: malonic dialdehyde; DC: diene conjugates; OD: optical density.

lymph nodes. It should be noted that this ratio was also increased in all tissues, except the liver, of rats in control group 1, but the increase had mainly occurred under the influence of Se, which indicates that Se and vitamin E enhance each other's effect on the conversion of linoleic to arachidonic acid.

The fatty-acid composition of dietary lipids is known to undergo the greatest changes in the liver, where it is modified substantially in accordance with the body's requirements. The liver is also the organ in which the major metabolic conversion of 18:2 to 20:4 takes place [15]. Our analysis of the fatty-acid composition in the livers of both the control and test rats (Table 3) revealed high levels of arachidonic acid, indicating that the animals had adequate amounts of the essential cis,cis-linoleic acid. This was confirmed by the values of the 20:3/20:4 ratio (Holman's ratio [8]). The physiological significance of this ratio stems from the fact that a value in excess of 0.4 is associated with a deficiency of essential fatty acids. The content of 18:2 was somewhat lower in the test groups than in the control groups, although its dietary content was the same in all groups; this difference may be attributed to an increased rate of 18:2 conversion to 20:4 under the influence of Se.

TABLE 3. Fatty-Acid Composition of Lipids in Rat Liver (Expressed as Percentages of Total Fatty Acids)

Fatty acids	Control group 1	Control group 2	Se 1 group	Se 2 group
12:0	0.06±0.01	0.07±0.01	0.07±0.01	0.11±0.02
14:0	0.37±0.05	0.27±0.04	0.45±0.05	0.40±0.03
15:0	0.16±0.02	0.18±0.01	0.17±0.03	0.17±0.02
16:0	19.29±1.87	19.63±1.15	21.34±1.93	20.52±2.72
16:1	3.04±0.27	3.14±0.26	3.37±0.35	2.78±0.24
17:0	0.30±0.05	0.21±0.04	0.18±0.02	0.12±0.02
18:0	12.68±1.22	12.88±1.29	12.20±1.17	14.69±1.37
18:1	21.78±2.56	23.14±2.00	24.38±1.96	21.68±2.78
18:2	19.98±1.10	15.17±1.62	14.48±1.51	12.39±1.40
20:0	0.06±0.01	0.09±0.01	0.09±0.02	0.20±0.03
18:3	0.09±0.02	0.11±0.04	0.05±0.01	0.05±0.01
20:1	0.12±0.02	0.18±0.03	0.16±0.01	0.13±0.02
20:2	0.05±0.01	0.07±0.02	0.22±0.03	0.23±0.03
20:3	0.26±0.02	0.45±0.05	0.43±0.05	0.56±0.05
20:4	17.68±1.80	18.84±1.92	17.58±1.52	21.02±1.96
20:5	0.04±0.01	0.05±0.01	0.04±0.01	0.04±0.01
24:0	0.39±0.05	0.61±0.05	0.52±0.04	0.53±0.06
22:4+24:1	0.54±0.03	0.77±0.06	0.65±0.05	0.75±0.06
22:5	0.41±0.03	0.39±0.05	0.37±0.04	0.38±0.04
22:6	2.70±0.27	3.75±0.22	3.25±0.30	3.25±0.24
20:4/18:2	0.88	1.24	1.21	1.70
20:3/20:4	0.015	0.024	0.024	0.027

It is of interest that the metabolic activity of 18:2 conversion to 20:4 in the Se 2 group, whose diet had been supplemented with Se, was twice that in control group 1 (the 20:4/18:2 ratio was 1.7 vs. 0.88), which had received the same amounts of dietary vitamin E. This may be an indication that this element itself affected arachidonic acid metabolism in the liver.

Thus, we see that Se has no appreciable effect on the levels of LPO products, indicating that its impact on antioxidant defense was very small. In contrast, examination of the fatty-acid composition of lipids contained in the liver, spleen, blood plasma, aggregated lymphatic follicles of the small intestine, and mesenteric lymph nodes revealed that Se had a marked effect on the metabolic conversion of 18:2 to 20:4, which was reflected in increased 20:4/18:2 ratios. These findings may be taken as evidence of a hitherto unreported impact of selenium on the metabolism of an essential acid in the rat organism.

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