the system of DNA methylation allow us to assume that regulation of gene activity by thyroid hormones may proceed via the blocking of DNA methylation. DNA demethylation may be among the structural changes necessary for the binding of thyroid hormones with DNA elements recognized by the thyroid hormone receptors and for further induction of the synthesis of specific mRNAs.

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Effect of the ω6/ω3 Ratio of Polyunsaturated Fatty Acids in the Rat Diet on Eicosanoid Content in the Blood Plasma and in the Liver

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The inclusion of fatty products rich in ω3 polyunsaturated fatty acids (PUFA), mainly eicosapentaenoic and docosahexaenoic, in the diet has recently been established to be therapeutic in the treatment of a number of diseases, in particular, cardiovascular diseases, diabetes mellitus, hypercholesterolemia, and allergic and skin disorders [3,6, 11]. The positive effects associated with an increased intake of highly unsaturated ω3 fatty acids are realized at the level of structural and functional alterations in the biomembranes, as well as at the level of biosynthesis of various eicosanoids, which direct the cell metabolism. The ω3 family of fatty acids has been shown to be just as essential as the acids of the linoleic group (ω6), which are regarded as vital in nutrition. The ω3 and w6 fatty acids supplied with food are precursors of diverse groups of eicosanoids modulating oppositely directed reactions. In a number of studies, a direct relationship has been shown to be absent between the content of PUFA of one of the groups in the diet and the synthesis of the corresponding group of prostaglandins (PG) in the organism [5,9]. One of the reasons for this lies in the competitive metabolic relationships between the two groups of fatty acids [12]. Therefore, it is of prime importance to choose the optimal ratio between the es-

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sential fatty acids ω6 and ω3 in the diet. The present study examines the effect of a diet containing various ω6/ω3 ratios on eicosanoid biosynthesis in the animal organism.

MATERIALS AND METHODS

The effect of various combinations of ω6 and ω3 PUFA was studied in experiments on growing male Wistar rats. The experiment lasted 3 months. All the types of rat diet were artificial food mixtures in which casein (20% of calories), corn starch (56% of calories), and lard, sunflower oil, and fish oil in diverse ratios (56% of calories) were the sources of protein, starch, and fat, respectively. The source of nutritional fiber was wheat bran added in the amount of 0.8 g per rat daily. All the diets contained the essential vitamins and minerals. Eikonol (Trinita, Moscow), a fish oil product, was the source of ω3 PUFA, and sunflower oil was the source of ω6 PUFA. During preparation of the fat mixtures, the ratio between the two groups of PUFA was varied in a wide range: from the routine (in nutritional practice) combinations of fatty products to the predominant or exclusive use of one fat as the source of either ω6 or ω3 fatty acids. The general biological follow-up of the animals involved daily examinations, recording of food intake, and determination of weight gain. After the completion of the experiments, the ether-anesthetized animals were decapitated and subjected to pathoanatomical examination. The blood collected was stabilized with anticoagulant (3.8% sodium citrate) and centrifuged at 4000 g for 15 min; the plasma was decanted and used in the subsequent investigations. Liver homogenates (10%) were prepared using 0.9% NaCl solution. Lipids were extracted from the plasma and tissues using a chloroform-methanol mixture (2:1) after Folch *et al.* [7]. Methyl esters of fatty acids were prepared after Kates [1]. The fatty acid composition was determined by gas-liquid chromatography under conditions described previously [2]. The content of thiobarbituric acid (TBA)-reactive products in the liver homogenates was measured after Ohkawa [14]. PG were extracted from the tissues and separated into classes by column chromatography after Auletta [4]. Quantitation of PG was performed by radioimmunoassay with ready-made kits (Institute of Isotopes, Hungary). Radioactivity was counted on a Rack-beta 1215 LKB-Wallac liquid-scintillation counter (Finland). The experimental results were processed by the methods of parametric statistics using Student's *t* test.

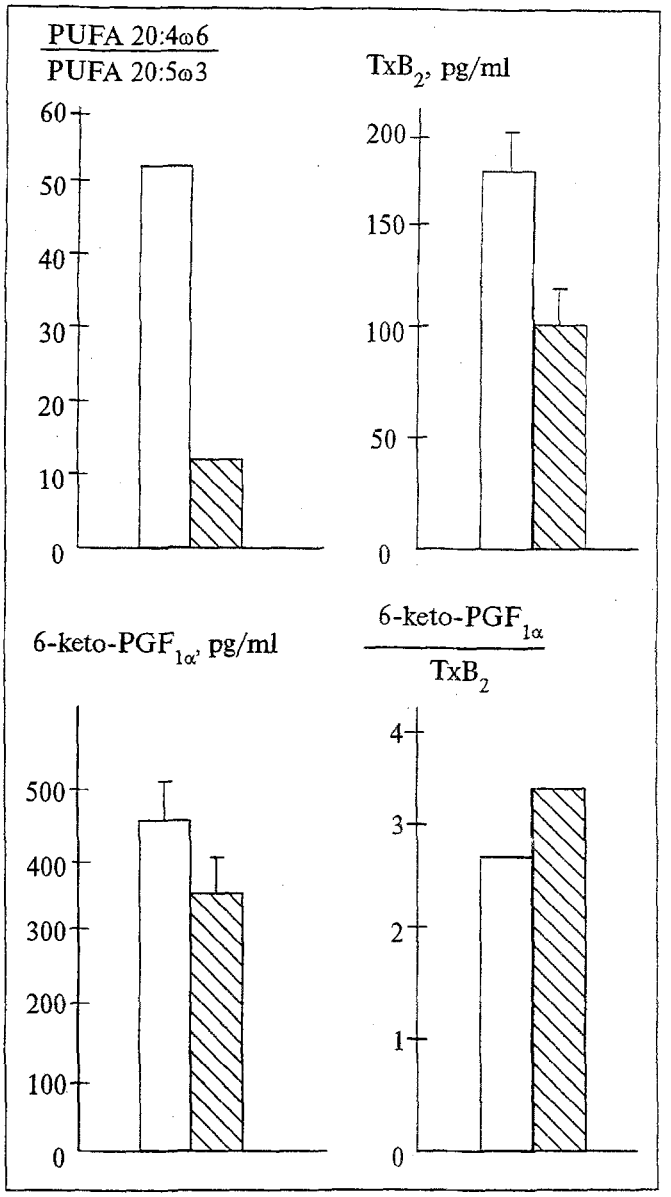

Fig. 1. PUFA ratio and eicosanoid content in blood plasma of rats. ω6/ω3 ratio in the diet. Light bars: 49; hatched bars: 5.2.

RESULTS

Table 1 shows the content of the main ω6 and ω3 fatty acids in the liver and plasma of experimental animals fed diets with diverse ratios of these acids. As is seen from the table, the addition of 20% of eikonol to the diet resulted in a marked increase of the percentage of ω3 acids in the plasma: the content of eicosapentaenoic acid (20:5ω3) and of docosahexaenoic acid (22:6ω3) increased 1.27- and 2.53-fold, respectively; on the other hand, the share of arachidonic acid (20:4ω6) somewhat decreased. This resulted in a sharp drop of the ω6/ω3 ratio in the plasma (more than 3-fold)).

TABLE 1. ω6/ω3 Ratio in the Diet and in the Organism of Rats

Fat composition of diet, %	ω6/ω3 ratio in diet	Content of main PUFA in plasma and liver					
		18:2 ω6	20:4 ω6	20:5 ω3	22:5 ω6	22:6 ω3	ω6/ω3
		Liver					
Lard/sunflower oil (50:50)	99.0	19.80	18.50	0.08	0.26	1.88	19.0
		Plasma					
Lard/sunflower oil (50:50)	49.0	17.26	21.57	0.91	0.18	1.24	24.05
		Plasma					
Sunflower oil/eikonol/lard (30:20:50)	5.2	16.23	18.62	1.16	0.22	3.14	7.71
		Liver					
Sunflower oil/eikonol (50:50)	1.9	18.22	10.23	4.33	2.40	8.42	1.90
		Liver					
Eikonol	0.12	10.19	6.47	6.52	3.24	6.03	0.54

In the liver of animals receiving equal shares of sunflower oil and eikonol as fatty components of the diet, the content of linoleic acid (18:2ω6) did not noticeably change in comparison with that in the group which received lard and sunflower oil (1:1). At the same time, the content of 18:4ω6 metabolites - arachidonic acid (20:4ω6) - markedly dropped: from 18.5 to 10.20%. The noted change in the content of ω6 acids in the tissue was associated with the suppression of arachidonic acid synthesis in the presence of a sufficient amount of its precursor, this being due to competitive relationships between the ω6 and ω3 fatty acids during the process of desaturation and elongation [10,13]. The share of ω3 acids sharply increased, this being manifested as a drop of the ω6/ω3 coefficient from 19 to 1.9. When the rats were fed a diet containing eikonol as the only source of fat, the above-mentioned trends were more pronounced. In this case a sharp drop of the linoleic acid content (almost 2-fold) was also observed. The data in Table 1 provide evidence that as the dietary ω6/ω3 ratio is changed, the 20:4ω6/20:5ω4 ratios of eicosenoic acids typically alter, this providing a basis for assessment of the biosynthesis of various types of eicosanoids, and, accordingly, of the amounts of transmitters for oppositely directed reactions. The data on the content of eicosanoids PGI_2 and TxA_2 in the plasma are presented in Fig. 1. The content of these PG was judged by the formation of their stable metabolites, 6-keto-$PGF_{2\alpha}$ and TxB_2, respectively. The addition of eikonol as a source of ω3 PUFA, i.e., a reduction of the ω6/ω3 ratio in the diet from 49 to 5.2, did not affect the content of $PGF_{2\alpha}$ and PGE in the plasma of animals (data not presented); on the

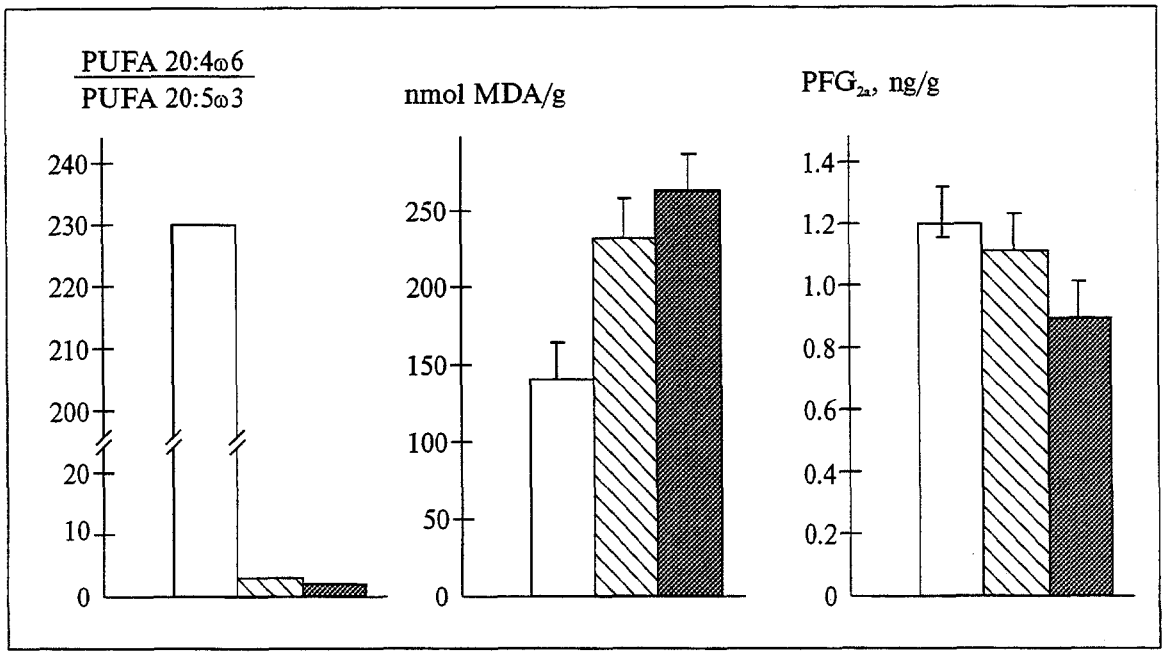

Fig. 2. PUFA ratio and MDA and $PGF_{2\alpha}$ content in liver of rats. ω6/ω3 ratio in the diet. Light bars: 99; hatched bars: 1.9; dark bars: 0.12.

other hand, the presence of ω3 in the diet exerted an effect upon PGI_2 and, especially, TxA_2 generation, leading to inhibition of the biosynthesis of these PG.

The use of eikonol is based on the ability of long-chain ω3 PUFA to act as specific inhibitors of TxA_2 formation, possibly, at the level of TxA_2-synthetase [8,15]. The 6-keto-$PGF_{1\alpha}$/Txb_2 ratio in the plasma of rats fed a diet with a 5.2 ω6/ω3 ratio was higher (3.36) than the same parameter in the group of animals not receiving eikonol (2.66). In view of the depressor and antiaggregating functions of PGI_2, and, on the contrary, the pressor and proaggregating properties of TxA_2, the above changes concerning these PG must be regarded as a positive effect of eikonol inclusion in the animal diet. It should be mentioned that the balance between PGI_2 and TxA_2, regulated by means of the diet, may also be physiologically important due to TxA_2 being a major factor in myocardial ischemia and subsequent changes in the myocardium.

An increase in the ω3 content of the diet affects the induction of lipid peroxidation (LPO) in the organism. Under experimental conditions all the animal diets contained α-tocopherol in an amount compatible with the rat requirements of this vitamin. As is seen from Fig. 2, the addition of large amounts of PUFA results in LPO induction, as is indicated by the data on the content of TBA-active products in the liver. At the same time, ω3 PUFA cause a more pronounced increase of this parameter: for instance, the content of malonic dialdehyde (MDA) in the rat liver increased from 145.9±13.1 to 258.5±18.4 nmol MDA/g liver for a change of the ω6/ω3 ratio in the diet from 99 to 0.12.

Along with the marked changes of eicosenoic acid content in the liver caused by dietary PUFA (ω6/ω3=0.12), inhibition of $PGF_{2\alpha}$ formation was noted in the organ. Replacement of endogenous arachidonic acid, a substrate for diene eicosanoids, with ω3 PUFA may partially account for the decrease of $PGF_{2\alpha}$ content in the liver of the animals. Thus, we obtained data attesting to the possible substrate-mediated regulation of eicosanoid synthesis by the PUFA ratio in the diet.

The results of this study provide evidence that the ω6/ω3 PUFA ratio in the diet may be a useful index reflecting the nature of the biological effect of two types of essential fatty acids. Structural changes of membrane lipids, changes of LPO intensity, and shifts of the balance between the synthesis of diverse types of eicosanoids contribute to the metabolic alterations caused by changes of the dietary ω6/ω3 ratio of PUFA.

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