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Dietary Modulation of the Immunocompetent System and Nonspecific Resistance in Rats by Different Ratios of Essential Fatty Acids

M. N. Volgarev, M. M. Levachev, E. N. Trushchina, K. V. Sergeeva, S. N. Kulakova, L. E. Aseeva, O. K. Mustafina, and T. V. Tokareva

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The immunomodulatory effect of dietary ω6 and ω3 polyunsaturated fatty acids (PUFA) is accomplished through their competitive incorporation into lipid structures of cell and subcellular membranes, thereby affecting their functional characteristics [6,10,12]. Various proportions between these PUFA both alter the substrate provision for eicosanoid synthesis [11,14] and indirectly affect immune functions by modulating the level of peroxidative processes and the antioxidant state of the organism [13]. Since both ω6 and ω3 PUFA occur in the diet in varying combinations, their quantitative ratios have to be taken into account in order to achieve a particular biological effect.

The purpose of the present study was to elucidate the effect of fixed ratios of ω6 and ω3 PUFA on the state of the immunocompetent system and nonspecific resistance in rats.

MATERIALS AND METHODS

The effect of different combinations of ω6 and ω3 PUFA was studied on male Wistar rats weighing initially 135±3.2 g. During 3 months the animals were fed isocaloric mixed rations, containing casein (20% of total calorie content), corn starch (56%), test fatty mixtures of lard, sunflower oil, and eikonol (24%, Table 1), and wheat bran (0.8 g/day per rat). All diets were supplemented with vitamins and minerals in accordance with the recommendations of the Institute of Nutrition; the α-tocopherol supplement was 0.5 mg/day per rat.

The functional activity of the immunocompetent system was studied 5 days after the animals has been immunized intraperitoneally with a 20% sheep erythrocyte suspension. For assessment of the B immunity the dynamics of antibody (hemagglutinin) titer in the blood serum [8] and the number of antibody-producing spleen cells (plaque-forming cells, PFC) [5] were determined. The functional activity of T lymphocytes was assessed in the reaction of lymphocyte blast transformation (RLBT) induced by phytohemagglutinin (PHA) [1], and the number of T cells in the blood was determined using the method of spontaneous rosette formation with sheep erythrocytes [9]. For evaluation of the functional and metabolic activity of the mononuclear phagocyting system the

Institute of Nutrition, Russian Academy of Medical Sciences, Moscow

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TABLE 1. Fat Composition of Experimental Diets

Group	Fat constituent of diet	ω6/ω3 PUFA ratio	Number of animals
1	Lard:sunflower oil:eikonol (47:28:15%)	2.1	30
2	Lard:sunflower oil:eikonol (36:19:45%)	1.1	30
3	Lard:sunflower oil (70:30%)	49.0	40
4	Lard:sunflower oil:eikonol (28:47:15%)	6.1	30

Note. Eikonol is a type of fish oil produced at the Trinita industrial plant.

nitroblue tetrazolium reduction test (NBT test) was used in a monolayer culture of peritoneal macrophages [15]. The structure of the spleen, mesenteric lymph nodes, and Peyer's glands was studied using histological (staining with hematoxylin and eosin) and histochemical (PAS reaction, RNA-specific staining after Brachet) methods. The areas of lymph folicles and germinative centers (GC) in the spleen were determined morphometrically with a MOP-Videoplan device. The fatty acid composition of lipids from the spleen, lymph nodes, Peyer's glands, and lymphocytes was determined by gas-liquid chromatography [4] after chloroform:methanol (2:1) extraction and methylation. Antibacterial activity [2] and serum content of β-lysine and lysozyme [3] were measured nephelometrically using 24-hour cultures of *E.coli* strain M-17, *B.subtilis* strain № 83, and *M.lysodekticus* strain ATCC 4698, respectively. Phagocyting activity was determined using a 24-hour *E.coli* culture.

RESULTS

The study of the parameters of the humoral and cell immunity revealed a slight immunostimulating effect of all eikonol-containing diets in comparison to that of a diet (group 3), whose fatty component consisted of lard and sunflower oil only (ω6/ω3=49). The antibody titer and the number of PFC and T cells (RFC), as well as their functional activity were maximal in group 2, which was maintained on a diet with a minimal ω6/ω3 ratio (ω6/ω3=1.1) (Table 2); in animals, maintained on diets with ω6/ω3 ratios equal to 2.1 and 6.1 these parameters were somewhat lower, and in animals of group 3 they dropped to minimal values. The functional and metabolic activity of peritoneal macrophages was reliably elevated in animals maintained on diets with low ω6/ω3 ratios (Table 2).

Morphological study of the peripheral lymphoid organs revealed no essential structural differences between the experimental groups. The area of spleen lymph follicles did not differ statistically in all experimental groups: group 1 - 61.5±22.7, group 2 - 65.8±28.1, group 3 - 64.5±23.4, group 4 - 63.7±30.3 $\omega\times10^3$. The specific area of germinative centers was found to be maximal in group 2 (63.3%) maintained on a diet with ω6/ω3=1.1 (group 1 - 42.9%, group 3 - 58.4%, group 4 - 47.6%). The lymphoid organs of animals of group 1 and, especially, of group 2 exhibited a pronounced macrophagal-plasmocytic reaction. Namely, aggregates of large lymphoid cells, young and mature plasma cells and macrophages (the latter often in cooperation with small lymphocytes) are observed in the red splenic pulp near the marginal zones of the lymph follicles, as well as in the perivascular and subcapsular zones. There are active GC in the mesenteric lymph nodes, and large lymphoid cells, macrophages, and plasma cells are seen in the paracortical and interfollicular zones and in the parenchymal cords. The interfollicular zones and cupolas of Peyer's glands are also predominantly populated by macrophages and plasma cells (in comparison with those in group 3). No marked differences between the experimental groups were observed in the state of the thymus-dependent zones of the lymphoid organs.

The effect of various ω6/ω3 PUFA ratios on the fatty acid composition of lipids in lymphocytes, spleen, mesenteric lymph nodes, and Peyer's

TABLE 2. Immunological Parameters and Functional Activity of Peritoneal Macrophages from Rats Maintained on Diets with Different ω6/ω3 PUFA Ratios

Group	Antibody titer, log	PFC, $\times10^6$ splenocytes	RFC, %	RLBT, %	NBT test, conventional units
1	4.5±0.2	41.0±3.9	69.3±1.7	37.0±0.5	0.093±0.01
2	4.7±0.2	42.0±2.7	71.8±1.3	43.5±1.8*	0.128±0.03*
3	3.9±0.3	35.0±2.9	67.0±1.5	35.0±2.4	0.076±0.01
4	4.7±0.2	39.0±0.7	70.5±6.3	39.0±2.2	0.084±0.02

Note. Here and in Table 3 an asterisk indicates reliable differences ($p<0.05$) of groups 1,2, and 4 in comparison with group 3.

TABLE 3. Parameters of Nonspecific Resistance of Rats Maintained on Diets with Different ω6/ω3 PUFA Ratios ($M \pm m$)

Group	Antibacterial activity of serum, %	β-Lysines, %	Lysozyme, μg/ml	Phagocytosis, %	
				activity of phagocytosis	intensity of phagocytosis
1	79.2±2.5	69.2±2.1	7.8±0.4	55.3±1.5	2.8±0.2
2	82.5±2.9	70.1±1.2	8.1±0.2	64.8±1.5*	3.0±0.2*
3	75.6±3.2	67.1±2.5	7.4±0.4	57.5±1.5	2.8±0.2
4	80.5±3.1	66.0±2.1	6.9±0.4	59.0±2.4	2.9±0.2

glands was also studied. An increased content of ω3 PUFA in the diet induced a similar alteration of tissue (cell) lipids in all studied cells and organs. It consisted in an increased partial content of eicosapentaenoic (20:5 ω3) and docosahexaenoic (22:6 ω3) fatty acids and, simultaneously, in a reduced content of ω6 PUFA, primarily arachidonic acid (20:4 ω6). In lymphocytes from animals maintained on a diet with a ω6/ω3 ratio of 1.1 these indexes were: 20:5 ω3 - 4.17%, 22:6 ω3 - 0.98%, 20:4 ω6 - 6.18% (vs. 0.24%, 0.18%, and 12.15% in the group maintained on a diet with a ω6/ω3 ratio of 49). The changed proportion between the eicosaenoic acids is considered as a substrate prerequisite for altered proportions in the biosynthesis of E_1, E_2, and E_3 eicosanoids.

The data on the nonspecific resistance in rats are presented in Table 3. These parameters were found to show a tendency to increase in animals fed a diet high in ω3 PUFA. The indexes of phagocytic activity of neutrophils were also reliably higher in these animals, which is consistent with the elevated functional and metabolic activity of peritoneal macrophages. The antibacterial activity of the blood serum, which represents an integral index of the combined activity of complement, lysozyme, β-lysines, and other factors of natural immunity, showed a slight tendency to increase in animals of groups 1, 2, and 4, in comparison to that in group 3.

Thus, directed shifts in the partial content of essential PUFA in the diet offer the possibility of modulating the state of immune reactivity and nonspecific resistance of the organism. An increased dietary consumption of ω3 PUFA leads to an increased content of eicosaenoic ω3 acids in tissue lipids and exerts a stimulating effect on the immune status and factors of nonspecific resistance. The obtained results suggest that the ω6/ω3 PUFA ratio is useful for characterization of the fatty component of a diet.

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