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Effect of Pollack Liver Oil on Lipid Metabolism in Rat Testes

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Rat testes were examined by biochemical and histochemical methods after 1, 3, 6, and 12-month pollack liver fat-supplemented diet (0.1 g/kg). Phospholipid concentration in the testicular tissue increased by 16% and cholesterol concentrations decreased by almost 20% after feeding fish oil for 1-3 months, while after 6-12-month diet further increase in cholesterol level in the testes and degeneration of some gonadal cells were noted. The most pronounced changes were seen in more differentiated elements of the spermatogenic epithelium in convoluted tubules.

Key Words: *testis; lipid metabolism; pollack liver oil*

Pollack liver oil (PLO) containing polyunsaturated fatty acids and vitamin A in very high concentrations is now widely used in medical practice [2,5,8]. However, long-term treatment with PLO improving the efficiency of therapy of chronic diseases leads to structural and functional changes in the testes and impairs reproductive function [6,7]. These disorders can be due to disturbances in lipid metabolism caused by excessive fat consumption [1,9].

We examined some parameters of lipid metabolism in the testes of rats after long-term treatment with PLO.

MATERIALS AND METHODS

The study was carried out on adult albino rats weighing 200 g. Group 1 animals were fed normal fodder (control 1), group 2 rats (control 2) received sunflower oil (SO, 0.1 g/kg). In group 3, PLO containing 45% polyunsaturated fatty acids (21% eicosapentaenoic and 22% docosahexaenoic acids) and 250 U vitamin A (retinol) was added to the standard fodder (0.1 g/kg). The quality of fish oil was controlled by the content of polyunsaturated fatty acids by the acid, peroxide, and iodine numbers. Residual pesticides did not surpass the maximum permissible dose, and the content of toxicants did not surpass the standards approved by the Russian Ministry of Health. The rats were sacrificed 1, 3, 6, and 12 months after the start of the experiment.

The lipids were detected and identified by thin-layer chromatography on 6×6 cm plates coated with CSC silica gel (5-7-μ particles). Phospholipids were measured using the universal molybdenum reagent after treatment with 72% perchloric acid. Total cholesterol in lipid extracts was evaluated using ferric chloride staining in orthophosphoric and sulfuric acid with subsequent colorimetry at 550 nm. Cholesterol concentration was evaluated using a calibration curve.

For histological studies, the testes were fixed in Stieve fluid and 10% formalin and embedded in paraffin. Sections (6 μ) were stained with Nile blue A (phospholipids) and Sudan black B (total lipids).

RESULTS

The concentrations of cholesterol (CH) and phospholipids (PL) in lipid extract from rat testes depended on the duration of feeding SO- or PLO-containing diet.

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The changes were more pronounced in animals fed PLO (Table 1).

One-month treatment with SO caused no significant changes in lipid metabolism, though CH concentration tended to decrease and PL content slightly increased in this group compared to control 1. More pronounced changes in lipid metabolism were observed in animals receiving PLO: the content of CH in testes decreased by 19.8% and that of PL increased by 16.1% ($p<0.05$) in comparison with control 1. The differences from control-2 were less pronounced and were significant only for CH. The CH/PL index slightly decreased in animals receiving SO and dropped by more than one-third in the PLO group.

Histological analysis of the testicular tissue from rats receiving SO or PLO for 1 months showed no considerable changes in the distribution of PL and total lipids. Like in intact animals, the cytoplasm of spermatogenic epithelial cells and sustenocytes contained light phospholipid inclusions (mainly in the perinuclear zone and at the cell periphery). Finely dispersed PL particles were seen in the nuclei. More intensive reaction was seen in differentiated cells (spermatides and spermatocytes). In the interstitial tissue, precipitate in the form of small lumps was seen in glandulocytes. Sudanophilic granules (total lipids) were more or less evenly distributed in the cytoplasm of convoluted tubule cells without forming compact accumulations. In glandulocytes large lipid granules grouped around the nucleus or, sometimes, at the cell periphery. These reactions and distribution of PL and total lipids were seen in practically all cells of convoluted tubules and interstitium. However in 1-3% tubules and 4-5% glandulocytes the intensity of staining for PL and total lipids was increased or decreased probably due to functional peculiarities of spermatogenesis [4].

In rats feeding SO- or PLO-supplemented diet for 3 months no marked changes in lipid metabolism were found compared to the previous term. In the majority of convoluted tubules and interstitium the intensity and distribution of total lipids and PL in rats receiving SO or PLO were practically the same. However in rats treated with PLO the content of PL was decreased in 8-12% tubules. This decrease was observed mainly in highly differentiated spermatogenic cells and sometimes in sustenocytes. These cells were characterized by more intensive staining for total lipids.

In rats treated with SO for 6 months, CH concentration in the testes considerably increased, while the content of PL decreased in comparison with animals receiving SO for 3 months ($p<0.05$), but the differences from controls 1 and 2 were negligible.

Treatment with PLO produced more pronounced changes: CH concentration increased by more than one-third in comparison with control 1 and PL concentration decreased by 18.5% ($p<0.05$). The differences from the corresponding values in control 2 were less pronounced, but still significant except the CH/PL ratio, which increased 1.5 times compared to the control (Table 1).

In control 2, the content and distribution of PL and total lipids in spermatogenic epithelium, sustenocytes, and glandulocytes at this term did not change in the absolute majority of testicular convoluted tubules. However the number of tubule profiles with PL-depleted spermatogenic cells increased to 5-6%. Accumulation of sudanophilic substances in these tubules was noted not only in highly differentiated cells, but also in some sustenocytes.

In experimental rats the number of tubule profiles with abnormal reaction to PL or total lipids increased to 20-25% by 6 months. In some tubules the content of PL in spermatogenic epithelial cells markedly decreased: PL were not detected in the spermatide nuclei and in the majority of spermatocyte nuclei; small PL granules were seen only at the cytoplasm periphery. The number, size, and location of PL granules remained practically unchanged in spermatogonia and majority of sustenocytes. Sudan staining showed accumulation and enlargement of lipid inclusions, presenting as 2-5-μ black droplets at the periphery of spermatide and spermatocyte cytoplasm. In some cells lipid inclusions occupied the whole cytoplasm except a narrow perinuclear zone. Tubules with marked accumulation of PL in the spermatogenic epithelium and reduced content of total lipids were also seen. These tubules (about 6-8% profiles) were often located near the tubules with opposite cell reaction.

The content of PL and total lipids in the majority of glandulocytes did not change at this term of PLO treatment. The number of cells with increased or decreased content of PL and total lipids increased only by 3-4% in comparison with control 1 and 2.

Treatment with SO for 12 months produced significant changes in lipid metabolism ($p<0.05$). The concentration of CH in the testicular tissue increased by 19.6% and the content of PL decreased by 13.7% in comparison with control 1. In rats receiving PLO changes in lipid metabolism were similar, but more pronounced than in control 2. The content of CH by 67 and 39.7% surpassed that in control groups 1 and 2, respectively, and the concentration of PL decreased by 29.8 and 18.6%, respectively. The CH/PL ratio moderately increased in control 2 control and 2.5-fold increased in the PLO group.

Nile blue staining was weakened in 10-15% tubules, mainly in mature cells (spermatides and spermatocytes), sometimes in glandulocytes and only occasionally in spermatogonias and sustenocytes. Sudan staining showed accumulation of total lipids in both

TABLE 1. Effect of SO and PLO on Lipid Metabolism in Rat Testes at Different Terms of Treatment

Duration of treatment, months	CH, mg/g		PL, mg/g		CH/PL	
	SO	PLO	SO	PLO	SO	PLO
1	191±8	166±14	855±26	959±56	0.22	0.18
3	182±12	178±16	860±29	896±55	0.21	0.19
6	221±10	279±13	798±19	674±42	0.28	0.41
12	249±16	348±18	714±42	581±34	0.35	0.60
Control	208±10		827±21		0.25	

spermatogenic epithelial cells and sustenocytes. In some cells sudanophilic granules occupied the whole cytoplasm except narrow perinuclear zone. It is known that the diet enriched with vegetable oil (*n*-6 fatty acids) can lead to fatty degeneration of cells and cause membrane destructive processes associated with lipid peroxidation. Degenerative changes in the blood, liver, and small intestine were observed in rats receiving 9-18% peanut oil with food [3,9].

The most pronounced changes in the testes were observed in animals receiving PLO for 1 year. The number of tubules with abnormal content of PL and total lipids increased to 50-53%. PL content decreased in the majority of cells. In some tubules PL completely disappeared from all spermatides and spermatocytes and most spermatogonias, while in sustenocytes PL were seen as fine powder-like granules located at the cytoplasm periphery. Sudanophilic granules often occupied the whole cytoplasm. Signs of fatty degeneration were also noted in glandulocytes, primarily in cells adjacent to convoluted tubules with pronounced changes in spermatogenic epithelium.

At the same time, accumulation of PL was observed in 7% tubules. Large lumps of precipitate were seen in the cytoplasm of spermatides and spermatocytes, some spermatogonias and sustenocytes. These tubule profiles were often adjacent to tubules with low PL activity in the cells.

Hence, changes in lipid metabolism in rat testes directly depend on the duration of PLO treatment. During the first 3 months of PLO-supplemented diet the content of PL in the rat testes increased and the level of CH markedly decreased. According to published data, the hypolipidemic (reduction of the content of cholesterol, β-lipoproteins, and triglycerides) and membrane-stabilizing (increase in membrane-bound PL) effects of PLO make it an effective agent for prevention and treatment of cardiovascular diseases [2,5,8]. However long-term treatment with PLO leads to a pronounced decrease of PL concentration and increase of CH concentration. Even a moderate decrease of PL level against the background of CH concentration reduces mobility of fatty acids, increases lipid microviscosity and membrane rigidity [3,9]. These shifts impair the structure of membrane enzymes and metabolic and trophic function of cell membranes. Long-term treatment with PLO induce degeneration of the spermatogenic epithelium, primarily differentiated cells in testicular convoluted tubules. Tubules with PL-depleted spermatides and spermatocytes and overloaded with lipid inclusions were often seen during this period. Spermatogonias, sustenocytes, and glandulocytes were rarely involved in fatty degeneration.

REFERENCES

1. G. O. Kaminskaya, *Probl. Tuber.,* No. 5, 71-74 (1982).
2. A. N. Klimov, *Vestn. Akad. Med. Nauk SSSR,* No. 3, 9-15 (1988).
3. E. M. Kreps, *Cell Membrane Lipids* [in Russian], Leningrad (1984).
4. E. Ruzin-Range, *Spermatogenesis in Animals* [in Russian], Moscow (1980).
5. M. A. Samsonov, M. M. Levachev, A. V. Pogozhaeva, *et al., Vopr. Pitaniya,* No. 5, 14-18 (1990).
6. V. M. Chertok and T. A. Botvich, *Byull. Eksp. Biol. Med.,* **123,** No. 3, 240-243 (1997).
7. V. M. Chertok and T. A. Botvich, *Ibid.,* **125,** No. 6, 699-701 (1998).
8. W. S. Harris, W. E. Connor, and S. M. Goodnight, *J. Golden Gubilu,* **15,** 125-126 (1982).
9. L. A. Pich, H. H. Drapper, and P. D. Cole, *Lipids,* **23,** No. 2, 370-372 (1988).
10. P. M. Verschuren, *Food Chem. Toxicol.,* No. 9, 35-44 (1989).