**Dvulit I. P. The effect of "Liposan-3C" preparation on contaits of PUFA in the liver of rats, receiving a fat-free diet. Journal of Education, Health and Sport. 2019;9(8):1025-1032. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.3462707**
**http://ojs.ukw.edu.pl/index.php/johs/article/view/7544**

**The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).**
**1223 Journal of Education, Health and Sport eISSN 2391-8306 7**

**Received: 16.08.2019. Revised: 22.08.2019. Accepted: 30.08.2019.**

UDK 616.31:615.015:577.16:612.397

# THE EFFECT OF "LIPOSAN-3C" PREPARATION ON CONTAITS OF PUFA IN THE LIVER OF RATS, RECEIVING A FAT-FREE DIET

**I. P. Dvulit**

**Lviv National Medical University named after Danylo Galytskij (Ukraine)**

**flavan.ua@gmail.com**

## Abstract

**Background.** To determine the effect of the PUFA preparation on the content of essential fatty acids in the liver lipids of rats receiving a fat-free diet (FFD).

**Methods.** The preparation "Liposan-3C" was used, containing a composition of fish oil, high oleic sunflower oil, β-carotene and tocopherol adsorbed on silica gel. Rats received with food (FFD) 0.5% or 1.0% of the drug for 22 days. The changes in rat live weight, feed intake and PUFA content in neutral lipids (triglycerides + cholesterol esters) and in the fraction of free fatty acids were evaluated.

**Results.** A 2-fold increase in weight gain was established in rats treated with Liposan-3C. In liver lipids, a decrease in the content of ω-6 PUFA and an increase in the content of ω-3 PUFA were found. The ration with the introduction of 0.5% Liposan turned out to be more effective, and when converted to PUFA with the introduction of 5.38 mg / kg. The ratio of ω-6 / ω-3 PUFA in liver lipids decreased by 2-3 times.

**Conclusion.** The new strew form of essential fatty acids, the drug "Liposan-3C" is an effective means to eliminate the deficiency of ω-3 fatty acids.

**Key words: essential fatty acids, ω-3 PUFAs, ω-6 PUFAs, liver lipids, avitaminosis F, fatty nutrition.**

## INTRODUCTION

Polyunsaturated fatty acids (PUFAs) belong to the group of essential nutrition factors [1, 2]. These include long-chain fatty acids having from two to six double bonds (Table 1). PUFAs, in turn, are divided into two groups depending on the location of the double bond with respect to the final (methyl) group of the acid radical, namely: ω-6 PUFAs, in which the double bond is located in the sixth carbon atom, counting from the methyl group, and on ω-3 PUFAs, in which the double bond is located at the third carbon atom, counting from the methyl group.

Table 1. Polyunsaturated fatty acids (PUFA)

| PUFA | Abbreviated formula | Number of double bonds | ω-6 або ω-3 |
|---|---|---|---|
| Linoleum | $C_{18:2}$ | two | ω-6 |
| Linolenic | $C_{18:3}$ | three | ω-3 |
| Arachidonic | $C_{20:4}$ | four | ω-6 |
| Eicosapentaenoic | $C_{20:5}$ | five | ω-3 |
| Docosapentaenoic | $C_{22:5}$ | five | ω-6 (ω-3 ?) |
| Docosahexaenic | $C_{22:6}$ | six | ω-3 |

All PUFAs perform two major functions in the body: the first is structural, related to their participation in the construction of biomembranes in the composition of phospholipids and cholesterol esters, and the second - regulatory, due to the physiological effects of products of their peroxidation, which are called eicosanoids and docosanoids [3].

Biologically active compounds that are formed by PUFA oxidation have different properties depending on the length of the radical and, most importantly, the location of the double bond at the ω-6 or ω-3 position [4]. Thus, ω-6 PUFA derivatives have pro-inflammatory, thrombogenic and immunosuppressive functions, whereas ω-3 PUFA derivatives, by contrast, have anti-inflammatory, thrombolytic and immunostimulatory functions [5].

The liver is central organ at to the metabolism of fatty acids, including metabolism of PUFA [6]. In animals (in particular rodents), in the presence of only linoleic acid (C18: 2), biochemical reactions of other PUFAs occur in the liver [7]. Unfortunately, in humans, the level of such reactions is insufficient for the formation of long-chain PUFAs (eicosapentaenoic and docosahexaenoic), so deficiency of these acids in the diet requires mandatory consumption of products containing these PUFAs [2].

The main source of all PUFAs is the fish of the northern seas, especially fish oil [8]. The main disadvantage of the latter is its rapid oxidation with the formation of toxic peroxidation products [9].

In the laboratory of prof. A. P. Levitsky was proposed to use fish oil in a heap with a natural antioxidant complex, which includes β-carotene, α-tocopherol and oleic acid, which were called "Katomas" [10]. A mixture of fish oil and Katomas in certain proportions and on silica gel media is produced by the SPA Odessa Biotechnology called "Liposan-3C" [11]. Liposan-3C - is a loose form of PUFA, one gram of which contains 94.1 mg of essential fatty acids, of which 62.3 mg are ω-3 PUFA at a ratio of ω-6 / ω-3 0.51.

The purpose of this study was to investigate the effect of Liposan-3C consumption on the content of PUFAs in the liver of rats treated with a fat-free diet.

**MATERIAL AND RESEARCH METHODS**

The work used a strew form of Liposan-3α, namely Liposan-C, in which the liquid form of Liposan-3α was adsorbed on silica gel "Orisil-300" (manufacturer of Liposan-3C SPA Odessa Biotechnology) in accordance with TU U 10.8-37420386 -002: 2015).

The content of PUFA in the preparations of Liposan-3 is presented in table 2, which shows that one gram of the drug "Liposan-3C" contains 94.1 mg of PUFA, of which 62.3 mg are ω-3 PUFA.

Biological experiments were performed on 18 white Wistar rats (females, 3 months old, initial live weight 93-123 g, who received a semi-synthetic fat-free diet for 22 days (fat content <0.6%) (Table 3) [12]. All rats were divided into 3 equal groups: 1 - control, received fat-free diet (FFD), 2 - received FFD + 0,5% of the drug "Liposan-3C" and 3 – received FFD + 1,0 % Liposan-3C.

Animal euthanasia was performed on day 23 under thiopental anesthesia (20 mg / kg) by total bleeding from the heart. The liver was isolated and the lipids were extracted into the

homogenate, which was extracted with Dole extraction [13], and then separated into two neutral lipid fractions (triglycerides and cholesterol esters) and free fatty acids [14].

Table 2. The content of PUFA in the preparations "Liposan-3α" (liquid form) and "Liposan-3C" (Strew form)

| PUFA | Abbreviated formula | Content, % | |
|---|---|---|---|
| | | liquid form | strew form |
| Linoleum | $C_{18:2}$ | 3,90 | 2,60 |
| Linolenic | $C_{18:3}$ | 0,30 | 0,20 |
| Arachidonic | $C_{20:4}$ | 0,65 | 0,43 |
| Eicosapentaenoic | $C_{20:5}$ | 5,12 | 3,41 |
| Docosapentaenoic | $C_{22:5}$ | 0,22 | 0,15 |
| Docosahexaenic | $C_{22:6}$ | 3,93 | 2,62 |
| Total PUFA | | 14,12 | 9,41 |
| The sum of ω-6 PUFA | | 4,77 | 3,18 |
| The sum of ω-3 PUFA | | 9,35 | 6,23 |
| ω-6/ω-3 | | 0,51 | 0,51 |

Table 3. The composition of fat-free diet (FFD) for rats [12]

| Component | Content, % |
|---|---|
| Wheat starch | 70 |
| Soybean meal | 20 |
| Sugar | 5 |
| Mineral mixture [12] | 4 |
| Vitamin mix [12] | 1 |

The content of PUFA in both fractions was determined by gas chromatography on a Shimadzu chromatographic mass spectrometer [15].

Lipid extracts from 3 rats were pooled before fatty acid analysis and the final results are presented as mean values per group.

**RESULTS AND DISCUSSION**

The results of feeding animals are presented in table 4, which shows that the consumption of Liposan-3C increases more than twice the increase in live weight of animals not only by increasing feed intake (by 14-23%), but possibly due to improving metabolism. The amount of PUFA intake was 5.38 mg / kg per day in group 2 rats and 10.62 mg / kg per day in group 3 rats. These doses correspond to human consumption doses [16].

Table 4. Results of feeding rats for 22 days using Liposan-3C

| Indicators | 1 gr. FFD | 2 gr. FFD+0,5 % Liposan-3C | 3 gr. FFD+1,0 % Liposan-3C |
|---|---|---|---|
| Average live weight, g | 216,7 | 231,2 | 218,6 |
| Weight gain, % | 14,60 | 32,60 | 27,47 |
| Feed intake for 1 day, g | 21,53 | 26,45 | 24,67 |
| Dose of PUFA, mg / kg / day | 0 | 5,38 | 10,62 |

Table 5 presents the results of determining the content of PUFAs in neutral lipids (triglycerides + cholesterol esters) of rat liver. From these data it is seen that the consumption of feed with 0.5% liposan significantly reduces the content of ω-6 PUFA, namely linoleic, arachidonic and docosapentaenoic, but significantly increases the content of ω-3 PUFA: eicosapentaenoic 4-fold. Due to this, the ratio of ω-6 / ω-3 is doubled.

Table 6 presents the results of determining the content of PUFA in the fraction of free fatty acids of rat liver treated with liposan-3C. It is seen that liposan consumption decreases the content of ω-6 PUFAs, especially arachidonic acid, but significantly increases the content of ω-3 PUFAs: eicosapentaenoic twice and docosahexaenoic acid 2.3 times, and the ratio of ω-6 / ω-3 is almost 3 times.

Thus, our studies have shown that the new drug "Liposan-3C" increases the content of ω-3 PUFA in liver lipids and significantly improves the ratio of ω-6 / ω-3. The drug "Liposan-3C" in 2 times increases the gain of live weight of animals with its introduction in the amount of 0.5% in the fat-free diet, which is a daily reach of PUFA in the diet of 5.38 mg / kg.

Table 5. Effect of Liposan-3C on PUFA content in rat serum neutral lipid fraction with avitaminosis F (% of total fatty acids)

| PUFA | 1 гр. | 2 гр. | 3 гр. |
|---|---|---|---|
| | | Liposan-3 | |
| | FFD | + 0,5 % | + 1,0 % |
| Linoleum, $C_{18:2}$, ω-6 | 9,77 | 5,75 | 10,73 |
| Linolenic, $C_{18:3}$, ω-3 | 0,28 | 0,20 | 0,24 |
| Arachidonic, $C_{20:4}$, ω-6 | 2,47 | 0,99 | 1,16 |
| Eicosapentaenoic, $C_{20:5}$, ω-3 | 0,05 | 0,20 | 0,22 |
| Docosapentaenoic, $C_{22:5}$, ω-6 | 0,55 | 0,52 | 0,77 |
| Docosahexaenoic, $C_{22:6}$, ω-3 | 0,21 | 0,09 | 0,25 |
| Σ PUFA ω-6 | 12,45 | 6,77 | 12,14 |
| Σ PUFA ω-3 | 0,88 | 0,92 | 1,23 |
| ω-6/ω-3 | 14,15 | 7,36 | 9,87 |

Table 6. The effect of Liposan-3C on the content of PUFAs in the fraction of free fatty acids of rat serum with avitaminosis F (% of total fatty acids)

| PUFA | 1 gr. | 2 gr. | 3 gr. |
|---|---|---|---|
| | | Liposan-3 | |
| | FFD | + 0,5 % | + 1,0 % |
| Linoleum, $C_{18:2}$, ω-6 | 11,72 | 10,59 | 10,00 |
| Linolenic ,$C_{18:3}$, ω-3 | 0,25 | 0,08 | 0,19 |
| Arachidonic, $C_{20:4}$, ω-6 | 10,12 | 6,05 | 6,75 |
| Eicosapentaenoic, $C_{20:5}$, ω-3 | 0,41 | 0,86 | 0,75 |
| Docosapentaenoic, $C_{22:5}$, ω-6 | 2,30 | 5,20 | 3,94 |
| Docosahexaenoic, $C_{22:6}$, ω-3 | 0,73 | 0,45 | 0,48 |
| Σ PUFA ω-6 | 22,57 | 17,09 | 17,23 |
| Σ PUFA ω-3 | 2,96 | 6,14 | 4,88 |
| ω-6/ω-3 | 7,63 | 2,78 | 3,53 |

## CONCLUSIONS

1. Strew form of the preparation of PUFA "Liposan-3C" contains 9-10% of essential fatty acids at a ratio of ω-6 / ω-3 0,51.

2. The stability of the drug "Liposan-3C" to oxidation is due to the use of hepatoprotector "Katomas", which contains antioxidants vitamin E, β-carotene and oleic acid.

3. Consumption of "Liposan-3C" in the composition of the fat-free diet in the amount of 0.5% 2 times increases the weight gain, increases the content of ω-3 PUFA and significantly improves the ratio ω-6 / ω-3.

4. The more effective daily dose of Liposan was 57.2 mg / kg, which in terms of ω-3 PUFA was 3.56 mg / kg.

## REFERENCES

1. Gavrisjuk VK. The use of omega-3 polyunsaturated fatty acids in medicine. Ukrainian pulmonological journal. 2001; 3: 5-10. (in Russian)

2. Shih EV, Mahova AA. Long-chain polyunsaturated fatty acids of the ω-3 family in the prevention of diseases in adults and children: a view of a clinical pharmacologist. Nutrition issues. 2019; 88(2): 91-100. (in Russian)

3. Caron JP, Gandy JC, Bronen JL [and others]. Omega-3 fatty acids and docosahexaenolic acid oxymetabolites modulate the inflammatory response of equine recombinant interleukin-1β-stimulated equine synoviocytes. Prostaglandins, Ofter Lipid Mediator. 2019; 142(1): 1-8.

4. Titov VN, Lisitsyn DM. Fat acids. Physical chemistry, biology and medicine. Tver, Triada, 2006: 672. (in Russian)

5. Levitsky AP. The pathophysiology of high-fat diet and ways to prevent its complications. Bulletin of the XVII readings to them. V.V. Podvysotsky. Odessa, 2018: 120-124. (in Russian)

6. Titov VN. Fatty acids, lipids (transport forms of fatty acids) and apolipoproteins (lipid-carrying macromolecules) are a single functional system. Clinical Laboratory Diagnostics. 2007; 1: 3-10. (in Russian)

7. Titov VN, Rozhkova TA, Amelyushkina VA. Fatty acids, triglycerides, hypertriglyceridemia, hyperglycemia and insulin. M., INFRA, 2016. (in Russian)

8. Lisitsyn AN. Scientific support of modern technologies for the production of new types of oil and fat products. Oilseeds. 2012; 2(151-152): 1-10. (in Russian)

9. Sarkisian VA, Kochetkova AA, Bessonov VV [and others]. Toxicological characteristics of the main products of lipid oxidation. Nutrition issues. 2016; 85(6): 80-85. (in Russian)

10. Levitsky AP. The ideal formula of fatty food. Odessa, KP OGT, 2002: 64. (in Russian)

11. TU U 10.8-37420386-002:2015 «The diet supplementary «Liposan» (vitamin F)». The diet supplementary «Liposan» (vitamin F).TU U 10.8-37420386-002:2015. Conclusion of the Ministry of Healthcare of the Ukraine № 05.03.02-06/22333 from 27.05.2015. (in Ukrainian)

12. Levitsky AP, Makarenko OA, Demyanenko SA. Methods of experimental dentistry (teaching aid). Simferopol, Tarpan, 2018: 78. (in Russian)

13. Keyts M. Metods of lipidology. Recciving, analise and identification of lipids. M., Mir, 1975: 334. (in Russian)

14. Rezvuhin AI, Shaladrova IJu, Potapova IA [and others]. Chromatography-mass spectrometric determination of free fatty acids in serum. Questions of medical chemistry. 1993; 39(5): 55-58. (in Russian)

15. Levitsky AP, Makarenko OA, Khodakov IV. Methods to investigate fats and oils. Odessa, KP OGT, 2015: 32. (in Russian)