**Batig V. M. The effect of omega-3 PUFA Liposan-3 on the content of essential fatty acids in lipid serum of rats with avitaminosis F. Journal of Education, Health and Sport. 2019;9(8):998-1006. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.3418924**
**http://ojs.ukw.edu.pl/index.php/johs/article/view/7472**

**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 OMEGA-3 PUFA LIPOSAN-3 ON THE CONTENT OF ESSENTIAL FATTY ACIDS IN LIPID SERUM OF RATS WITH AVITAMINOSIS F

**V. M. Batig**

**Bukovina State Medical University, Chernovtsy**
flavan.ua@gmail.com

**Abstract**

**Background.** Determine the effect of the drug "Liposan-3" on the content of PUFA in the serum lipids of rats receiving a fat-free diet.

**Methods.** Used the drug "Liposan-3α" containing 14% PUFA with a ratio of ω-6/ω-3 of 0.49. Rats were fed a semi-synthetic fat-free diet for 21 days (group 1), supplemented with 0.5 % liposan (group 2) or 1.0 % liposan (group 3). Controlled changes in live weight and feed intake. After euthanasia, the content of triglycerides, total cholesterol was determined in the blood serum, and the content of PUFA was determined in the fractions of neutral lipids (triglycerides + cholesterol esters) and free fatty acids. In addition, the content of malondialdehyde (MDA), the activity of catalase, and the antioxidant-prooxidant API index were determined in their blood serum.

**Results.** The actual liposan intake was 86.8 mg/kg per day, in terms of the amount of PUFA 11.9 mg/kg, including 8.1 mg/kg ω-3 PUFA. The animal weight gain increased by 30 %, the content of TG and cholesterol remained within the normal range, there was no increase in the

content of MDA, the activity of catalase did not decrease, and the API index increased. The content of ω-6 PUFA decreased in both fractions of serum lipids by 19-22 %, however, the containment of ω-3 PUFA increased by 2.5-3 times, while the ratio of ω-6/ω-3 PUFA decreased by 2.3- 3.3 times.

**Conclusion.** The drug "Liposan-3" in doses of 80-130 mg/kg increases the content in blood serum lipids of ω-3 PUFA and normalizes the ratio of ω-6/ω-3 of PUFA. Liposan-3 does not increase lipid peroxidation and has a stimulating effect on the growth of animals.

**Key words: fatty nutrition, essential fatty acids, ω-6 and ω-3 PUFAs, lipid peroxidation, PUFA preparation.**

## INTRODUCTION

Avitaminosis F occurs under nutritional deficiency in essential fatty acids, which include linoleic ($C_{18:2}$, ω-6), linolenic ($C_{18:3}$, ω-3), eicosapentaenoic ($C_{20:5}$, ω-3) and docosahexaenoic ($C_{22:6}$, ω-3) fatty acids [1-3].

A characteristic feature of avitaminosis F is impaired functional status of the nervous system, since essential fatty acids are a significant part of the lipids of nerve cell membranes and provide their functional activity [3-6].

It is known that disorders of the nervous system adversely affect the condition of the tissues of the oral cavity and, in particular, the condition of the periodontium [7-14].

In the pathogenesis of neurostomatological lesions, the processes of free radical lipid oxidation (LPO) play an important role [16].

Recently, PUFA have been shown to reduce the level of LPO in mitochondria [17]. In this work [17], large doses of PUFA preparation (120 mg/kg) were used for 42 days of full-feed compound feed containing up to 10 % fat.

The aim of our work was to investigate the effect of a new drug Liposan-3 [18] on the content of PUFAs in the serum of rats treated with a fat-free diet, that is, they had an alimentary deficiency of essential fatty acids.

## MATERIAL AND RESEARCH METHODS

The work used the preparation "Liposan-3α" (oil form) produced by SPA "Odessa Biotechnology" [18] containing almost 14 % PUFA, the composition of which is presented in

table 1. The content of PUFA was determined by gas chromatographic method on a mass spectrometer "Shimadzu» [19].

Table 1. The content of PUFA's into "Liposan-3α"

| PUFA | Abbreviated formula | Content, % |
|---|---|---|
| 1. Linoleum | $C_{18:2}$ ω-6 | 3,80 |
| 2. Linolenic | $C_{18:3}$ ω-3 | 0,25 |
| 3. Arachidonic | $C_{20:4}$ ω-6 | 0,50 |
| 4. Eicosapentaenoic | $C_{20:5}$ ω-3 | 5,12 |
| 5. Docosapentaenoic | $C_{22:5}$ ω-6 | 0,22 |
| 6. Docosahexaenic | $C_{22:6}$ ω-3 | 3,93 |
| Total PUFA<br>The sum of ω-6 PUFA<br>The sum of ω-3 PUFA<br>ω-6/ω-3 | | 13,82<br>4,52<br>9,30<br>0,49 |

The experiments were performed on 15 white Wistar rats (males, 3 months old) who received a fat-free diet (FFD), the composition of which is shown in Table 2 [20, 21]. All rats were divided into 3 equal groups: 1st received FFD, 2nd - FFD containing 0.5 % liposan (instead of the corresponding amount of starch) and 3rd received FFD containing 1.0 % liposan (instead of the appropriate amount of starch).

Table 2. The composition of the fat-free diet (FFD) for rats [20, 21]

| Component | g/kg |
|---|---|
| Maize starch | 660 |
| Soybean meal | 150 |
| Ovalbumin | 50 |
| Sugar | 90 |
| Mineral mixture [21] | 40 |
| Mineral mixture [21] | 10 |
| Liposan-3 | 5 or 10 (starch replacement) |

The duration of the experiment was 21 days and throughout the period controlled live weight and dietary intake.

To determine the condition of LPO in the blood serum, the content of malondialdehyde (MDA) thiobarbituric was determined. After feeding was completed, the rats were euthanized under thiopental anesthesia (20 mg/kg) by total bleeding from the heart. A serum was obtained to determine the lipid content and fatty acid composition of two fractions: neutral lipids

(triglycerides + cholesterol esters) and free fatty acids (FFA) [19]. Also, serum triglycerides (TG) and total cholesterol (CH) were determined in serum by enzymatic micromethods [22].

To determine the condition of LPO in serum, the content of malondialdehyde (MDA) was determined by the thiobarbitur method [23] and the activity of the catalase antioxidant enzyme [24], and the antioxidant-prooxidant index API [25] was calculated by the ratio of catalase activity and MDA content.

The results of the experiments were subjected to a standard statistical processing [26].

**RESULTS AND DISCUSSION**

Table 3 presents the results of actual consumption of PUFA rats treated with FFD. It is seen that the introduction of 0.5 % liposan into the diet significantly increases the weight gain of rats (86 g compared with 66 g in the 1st group). The actual dose of ω-3 PUFA was 8.1 mg/kg in group 2 and 12.4 mg/kg in group 3, which is an order of magnitude lower than in the experiment [17].

Table 3. Actual doses of PUFA consumption by rats treated with FFD

| Indicators | 1 gr. | 2 gr. | 3 gr. |
|---|---|---|---|
| | L i p o s a n | | |
| | 0 | 0,5 % | 1,0 % |
| Initial weight (average weight), g | 94 (127) | 120 (163) | 123 (145) |
| Feed intake, g/d. | 19,4 | 28,2 | 19,3 |
| Daily intake of liposan (mg/rat) | 0 | 14,1 | 19,3 |
| Liposan dose (mg/kg) | 0 | 86,5 | 133,1 |
| Dose of PUFA (mg/kg) | 0 | 11,9 | 18,4 |
| Dose of ω-3 PUFA (mg/kg) | 0 | 8,1 | 12,4 |

Table 4 shows how liposan supplementation affects the levels of triglycerides (TG) and total cholesterol (CH) in the serum of rats treated with FFD. It is seen that in rats of group 1, the level of TG in serum is below normal, whereas the level of CH corresponds to the norm. The introduction of liposan increases the level of TG and CH to the norm.

Table 5 presents the results of determining the content of PUFA in neutral lipids (TG + cholesterol esters) of serum of rats with avitaminosis F. F. It is seen that the supplement to liposan feed reduces the content of ω-6 PUFA by 22 %, but increases the content of ω-3 PUFA in 2,5 times, which reduces the ratio of ω-6 PUFA/ω-3 PUFA by 3.3 times. The dose of liposan 0.5 % (in terms of PUFA 12 mg/kg) was more effective.

Table 4. Effect of "Liposan-3" on the content of lipids in the serum of rats with avitaminosis F

| Groups | Triglycerides, mmol/l | | Cholesterol, mmol/l | |
|---|---|---|---|---|
| | experiment | norm [27] | experiment | norm [27] |
| 1. FFD | 0,46±0,07 | 0,6 – 2,3 | 0,76±0,07 | 0,5 – 2,8 |
| 2. + 0,5 % liposan | 0,64±0,05 p<0,05 | | 0,92±0,09 p>0,05 | |
| 3. + 1,0 % liposan | 0,68±0,13 p>0,05 | | 1,02±0,09 p<0,05 | |

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

| PUFA | 1 гр. | 2 гр. | 3 гр. |
|---|---|---|---|
| | FFD | + 0,5 % liposan | + 1,0 % liposan |
| Linoleum, $C_{18:2}$, ω-6 | 12,30 | 10,03 | 10,50 |
| Linolenic, $C_{18:3}$, ω-3 | 0,38 | 0,50 | 0,39 |
| Arachidonic, $C_{20:4}$, ω-6 | 3,20 | 2,03 | 2,75 |
| Eicosapentaenoic, $C_{20:5}$, ω-3 | 0,16 | 1,09 | 0,47 |
| Docosahexaenoic, $C_{22:6}$, ω-3 | 0,41 | 0,85 | 0,68 |
| Docosapentaenoic, $C_{22:5}$, ω-6 | 0,2 | 0,16 | 0,15 |
| Σ PUFA ω-6 | 15,71 | 0,95 | 16,54 |
| Σ PUFA ω-3 | 12,22 | 2,44 | 5,01 |
| ω-6/ω-3 | 13,40 | 1,54 | 8,70 |

Table 6 presents the results of determining the content of PUFA in the fraction of free fatty acids of the serum of rats. The introduction of liposan reduces the content of ω-6 PUFA by 19 %, but increases the content of ω-3 PUFA almost 3 times and 2.3 times reduces the ratio of ω-6/ω-3 PUFA.

Table 6. The effect of Liposan-3 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. |
|---|---|---|---|
| | FFD | + 0,5 % liposan | + 1,0 % liposan |
| Linoleum, $C_{18:2}$, ω-6 | 8,89 | 10,41 | 9,96 |
| Linolenic ,$C_{18:3}$, ω-3 | 0,19 | 0,23 | 0,23 |
| Arachidonic, $C_{20:4}$, ω-6 | 6,42 | 6,75 | 7,56 |
| Eicosapentaenoic, $C_{20:5}$, ω-3 | 0,12 | 0,71 | 0,87 |
| Docosahexaenoic, $C_{22:5}$, ω-6 | 0,17 | 0,38 | 0,41 |
| Docosapentaenoic, $C_{22:6}$, ω-3 | 0,87 | 2,11 | 2,66 |
| Σ PUFA ω-6 | 15,48 | 12,54 | 17,93 |
| Σ PUFA ω-3 | 1,18 | 3,05 | 3,76 |
| ω-6/ω-3 | 13,12 | 5,75 | 4,77 |

As shown in table 7, liposan-3α not increases in the serum of rats the content of MDA-the final product of LPO [16], has little effect on the activity of catalase, but significantly increases the index of API when using feed with 0.5 % liposan.

Table 7. Effect of Liposan-3 on indicators LPO in rat serum with avitaminosis F

| Indicators | 1 gr. | 2 gr. | 3 gr. |
|---|---|---|---|
| | FFD | + 0,5 % liposan | + 1,0 % liposan |
| MDA, mmol/l | 0,78±0,02 | 0,73±0,03<br>p>0,3 | 0,78±0,06<br>p=1 |
| Catalase, mkat/l | 0,31±0,04 | 0,33±0,01<br>p>0,3 | 0,30±0,01<br>p>0,8 |
| API | 3,97±0,15 | 4,52±0,19<br>p<0,05 | 3,85±0,23<br>p>0,3 |

Thus, studies have shown that even small doses of PUFA (12 mg/kg) can significantly affect the fatty acid composition of serum lipids, increase the content of ω-3 PUFA and reduce the content of ω-6 PUFA, normalizing the ratio of ω-6/ω -3 PUFA. At the same time, it remains unknown, due to which rats treated with FFD contained linoleic acid and other essential fatty acids in the serum lipids. Probably, the source of these acids can be triglycerides of spare fats, for the complete utilization of which it is necessary to extend the life of the experiment by 2-3 months.

It is possible that endogenous microbes that populate the intestine may be a possible source of linoleic acid. However, more research is needed to answer these questions.

**CONCLUSIONS**

1. Introduction to the composition without fatty diet of the drug "Liposan-3α" containing 14 % PUFA (ratio ω-6/ω-3 equal to 0.49) causes a decrease in serum lipids ω-6 PUFA and a significant increase (in 2.5-3 times) the content of ω-3 PUFA.

2. The introduction of liposan-3α at doses of 86-133 mg / kg normalizes in the serum the content of triglycerides and cholesterol and does not increase the level of lipid peroxidation, and the dose of 86 mg / kg was more effective.

**REFERENCES**

1. Spector AA. Essentiality of fatty acids. Lipids. 1999; 34: 1-3.

2. La Guardia M, Giammanco S, Di Majo D [and others]. Omega 3 fatty acids: Biological activity and effects on human health. Panminerva med. 2005; 47(4): 245-257.

3. 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)

4. Sears B, Perry M. The role of fatty acids in insulin resistance. Lipids in Health a Disease. 2015; 14(121):1-9.

5. Wan X, Gao X, Bi J [and others]. Use of n-3 $PUFA_5$ can decrease the mortality in patients with systemic inflammatory response syndrome: a systematic review and meta-analysis. Lipids in Health a Disease. 2015; 14(23):1-13.

6. Allam-Nadoul B, Guévard F, Barbier O [and others]. Effect of n-3 fatty acids on the expression of inflammatory genes in THP-1 macrophases. Lipids in Health a Disease. 2016; 15(69): 1-7.

7. Grigor'ev IV, Nikolaeva LV, Artamonov ID. The protein composition of human saliva against a background of various psychoemotional condition. Biochemistry. 2003; 68(4): 501-503. (in Russian)

8. Modina TN, Mamaeva EV. Pathology of parodontical tissues and vegetative homeostasis in youth schoolboys. Pediatric dentistry and prevention. 2006; 3-4: 3-7. (in Russian)

9. Kovach IV, Dychko JeN, Hotims'ka JuV [and others]. The estimation of role of simpato-adrenaline system in glossalgi pathogenesis. The medical perspectives. 2012; XVII(3): 124-127. (in Ukrainian)

10. Mysula IR, Suhovolec' IO. The course of periodontitis at hypoergic and hyperergic types of inflammatory reaction simultaneous to adrenaline myocardiopathy. Medical chemistry. 2013; 15(3(56): 27-30. (in Ukrainian)

11. Nasonova TI. Nasonova TI. Correction of clinical and metabolic disorders in patients with cerebrovascular disease on the background of metabolic syndrome. Endocrinology.2015; 20(4): 677-687. (in Ukrainian)

12. Kononova OV, Borisenko AV, Levitsky AP. The influence of oral gels of quertulin and adrenergic blockers upon the state of periodontium in rats with adrenalin stress. Announcer of stomatology. 2016; 4(97): 8-11. (in Russian)

13. Sloboda MT. The results of investigation of vegetative nervous system at parodonte lesion in young persons with deformation dorsopathy. Odessa Medical Journal. 2016; 1 (153): 54-58. (in Ukrainian)

14. Borisenko AV, Kononova OV, Levitsky AP. The comparative effects of quertulin and adrenoblocators oral geles on the biochemical indices of rat serum after common action adrenaline and lincomycin. Journal of Education, Health and Sport. 2017; 7(8): 1062-1069.

15. Baraboi VA. The frey radical mechanisms of neurodegenerative pathology (review). Journal of the Academy of Medical Sciences of Ukraine. 2001; 7(2): 219-231. (in Russian)

16. Butiugin IA, Volchegorskii. The condition of the lipid peroxidation system in mixed saliva in patients with chronic generalized periodontitis. Clinical Laboratory Diagnostics. 2014; 2: 44-47. (in Russian)

17. Ketsa OVb Marchenko MM. The effect of essential lipophilic nutrients on free radical processes in the mitochondrial fraction of rat liver. Nutrition issues. 2019; 88(2): 32-39. (in Russian)

18. Levitsky AP, Khodakov IV, Levitsky YuA [and others]. Preparation of irreplaceable fat acids of "Liposan". Patent of Ukraine 108571. IPC A61P 9/00. Publ.: 25.07.2016. Bul. № 14. (in Ukrainian)

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

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

21. Eggum B. Methods to evaluate utilization of proteins by animal. Moskva, Kolos, 1977: 190. (in Russian)

22. Tets NU. The encyclopedia of clinical laboratory tests. Moskva, Labinform, 1997: 128, 459-460. (in Russian)

23. Stalnaya ID, Garishvili TG. The method of revelation of malonic dialdehyde with thiobarbituric acid. Moskva, Meditsina, 1977: 66-68. (in Russian)

24. Girin SV. The modification of the method of the determination of catalase activity in biological substrates. Laboratory diagnostics. 1999; 4: 45-46. (in Russian)

25. Levitsky AP, Denga OV, Makarenko OA. [and others]. Biochemical markers of inflammation of oral cavity tissue: method guidelines. Odessa, KP OGT, 2010: 16. (in Russian)

26. Truhacheva NV. Mathematical Statistics in biomedical research using application package Statistica. Moscow, GJeOTAR-Media, 2012: 379. (in Russian)

27. Evans GO. Animal Clinical Chemistry a Practical Handbook for Toxicologiks and Biomedical Researchers. 2 ed. UK: Taylor and Francis Group, 2009: 308.