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# EFFECT OF DIETARY FATS ON ENDOGENOUS OLEIC ACID BIOSYNTHESIS IN RAT LIVER

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## Abstract

Aim: Determine the effect of dietary fats with different fatty acid composition on the biosynthesis of oleic acid and its metabolic precursors in the liver .

Methods: High linoleic sunflower oil (HLSO), high oleic sunflower oil (HOSO) and palm oil (PO) were used. Rats were fed a semi-synthetic fat-free diet (FFD) and fat diets containing 5 % of the above oils (instead of starch) for 30 days. Liver lipids were divided into 3 fractions: neutral lipids (NL), phospholipids (PL) and free fatty acids (FFA). The fatty acid composition of the fractions was determined by gas chromatography. The “activity” of fatty acid synthase was determined from the total content of the products of this reaction ($C_{16:0}$ and $C_{16:1}$). The “activity” of palmitic acid elongase was determined by the ratio $C_{18:0}/C_{16:0}$, as well as by the formula $(C_{18:0}+C_{18:1})/(C_{16:0}–C_{16:1})$. The “activity” of stearic acid desaturase (SCD1) was determined by the ratio $C_{16:1}/C_{16:0}$ (SCD16) and by the ratio $C_{18:1}/C_{18:0}$ (SCD18).

Results: In rats treated with fat diets, the content of palmitic and oleic acids is reduced only in the NL fraction, and to the greatest extent when consuming the diet with HLSO. The “activity” of palmitic acid elongase increases significantly with the consumption of a diet

with HLSO. SCD16 desaturase “activity” decreases with fat diet, while SCD18 desaturase “activity” increases. The level of SCD18 is significantly higher than the level of SCD16. Consumption of HLSO reduces the content of ω-3 PUFA in rat liver lipids, while the intake of HOSO increases it.

Conclusions: HLSO diet reduces the endogenous biosynthesis of oleic and palmitic acids, as determined by the analysis of the rat liver NL fraction. A fat diet reduces SCD16 “activity” but increases SCD18 “activity”, especially when fed a diet with HOSO. The diet with HLSO reduces the content of ω-3 PUFA in liver lipids.

**Keywords: fat nutrition; liver; fatty acids; palmitic acid; elongase; stearic acid desaturase; ω-3 PUFA.**

## Introduction

Oleic acid ($C_{18:1}$ n-9) is the main energetic fatty acid in human and animal lipids [1, 2]. It is oxidized in mitochondria much more easily than all other fatty acids, exceeding linoleic acid by 17 times and saturated fatty acids by hundreds of times [3]. Unlike polyunsaturated fatty acids (PUFA), oleic acid is more resistant to peroxidation and does not form pro-inflammatory mediators [4].

The main source of oleic acid in the animal body is endogenous intracellular biosynthesis from non-fat sources (carbohydrates, proteins, alcohols and organic acids) by converting them into acetyl-CoA, which, under the action of the fatty acid synthase (enzyme complex), is converted into palmitic acid ($C_{16:0}$) [5]. The latter is converted into stearic acid ($C_{18:0}$) under the action of the elongase enzyme, which is converted into oleic acid under the action of the stearyl-CoA desaturase (SCD) enzyme (Fig. 1) [6-8].

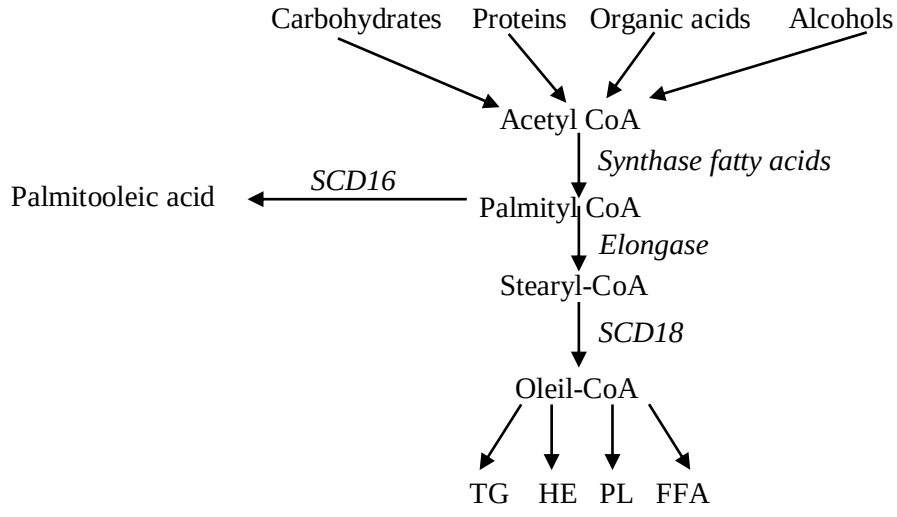


**Fig. 1.** Scheme of endogenous intracellular biosynthesis (EICB) of energetic fatty acids

The active form of oleic acid (oleyl-CoA) acylates various alcohol acceptors: monoglycerides, diglycerides, glycerophosphate, cholesterol [9], forming triglycerides in the greatest amount. From triglycerides in the liver, very low density lipoproteins (VLDL) are formed, which are increted by the liver into the blood and carried throughout the body, providing energy material to many organs and tissues, especially muscle tissue. Excess VLDL is deposited in fat depots and serves as a reserve for the reproduction of ATP in the working organs [5, 7].

It is believed that the source of these acids is food or they are formed by endogenous microbiota, which in its mass exceeds 1.5 times the mass of the liver [10].

In our previous works [11, 12], it was shown that in the composition of lipids of an animal organism in the conditions of keeping animals on a fat-free diet, such fatty acids (for example, PUFA) are also determined, which, as a rule, are not formed in the cells of the body according to the above scheme formation of energy fatty acids [13, 20-22].

The aim of this study was to determine the effect of dietary fats with different fatty acid composition on the endogenous biosynthesis of energy fatty acids (mainly oleic) in the liver of rats, since the liver is the biochemical center of fat metabolism.

**Methods**

We used ordinary (high-linoleic) sunflower oil, high-oleic sunflower oil "Olivka" produced by LLC "Biohimtekh" (Odessa) and palm oil produced in Malaysia. The fatty acid composition of these oils is presented in Table 1.

**Table 1**. Fatty acid composition of used fats (%)

| Fatty acid | Short formula | Sunflower oil | High oleic sunflower oil Olivka | Palm oil |
|---|---|---|---|---|
| Lauric | $C_{12:0}$ | 0 | 0 | 0,19 |
| Myristic | $C_{14:0}$ | 0,12 | 0,06 | 1,16 |
| Palmitic | $C_{16:0}$ | 6,53 | 4,15 | 42,02 |
| Palmitooleic | $C_{16:1}$ | 0,12 | 0,13 | 0,11 |
| Stearic | $C_{18:0}$ | 2,86 | 2,75 | 4,87 |
| Oleic | $C_{18:1}$ | 30,29 | 84,57 | 40,93 |
| Linoleic | $C_{18:2}$ ω-6 | 57,12 | 6,16 | 9,49 |
| α-linolenic | $C_{18:3}$ ω-3 | 0,08 | 0,21 | 0,17 |
| Arachidonic | $C_{20:4}$ ω-6 | 0 | 0 | 0 |
| Eicosapentaenoic | $C_{20:5}$ ω-3 | 0 | 0 | 0 |
| Docosapentaenoic | $C_{22:5}$ ω-3 | 0 | 0 | 0 |
| Docosahexaenoic | $C_{22:6}$ ω-3 | 0 | 0 | 0 |

Biological experiments were carried out on 24 white Wistar rats (males, 5 months old, body weight 225-235 g), divided into 4 equal groups. The 1st, control, received a fat-free diet (FFD), the composition of which is presented in Table 2. Rats of the 2nd, 3rd and 4th groups received 5 % oil with food, respectively: high-linoleic (HLSO), high-oleic (HOSO) sunflower oils and palm oil (PO). Oil was introduced into the diet instead of 5 % starch. The rats were fed for 30 days, and after euthanasia of the animals under thiopental anesthesia, the liver was isolated.

**Table 2.** The composition of diets for rats (%)

| Components | Free fat diet FFD | Fats diet |
|---|---|---|
| Corn starch | 64 | 59 |
| Soybean meal defatted | 20 | 20 |
| Ovalbumin | 6 | 6 |
| Sucrose | 5 | 5 |
| Mineral mixture | 4 | 4 |
| Vitamin mixture | 1 | 1 |
| Vegetable oil | 0 | 5 |

Lipids were extracted from the liver according to the Dole method [15] and divided into three fractions: neutral lipids (NL), phospholipids (PL), and free fatty acids (FFA) [16, 17]. The combined lipid fractions of each group of rats were used to determine the fatty acid composition by gas chromatography [18].

The “activity” of the fatty acid synthase enzyme was determined from the products of this reaction ($C_{16:0}$+$C_{16:1}$).

The “activity” of the elongase enzyme was determined by the ratio of the content of stearic ($C_{18:0}$) and palmitic (C16:0) acids [6], as well as by our proposed ratio ($C_{18:0}$+$C_{18:1\ n-9}$)/($C_{16:0}$–$C_{16:1\ n-7}$).

The “activity” of the stearyl-CoA desaturase (SCD) enzyme was determined by the ratio $C_{18:1\ n-9}/C_{18:0}$ (SCD18) and by the ratio $C_{16:1\ n-7}/C_{16:0}$ (SCD16) [8].

**Results**

Table 3 presents the results of determining the content of fatty acids in the lipid fractions of the liver of rats treated with FFD. Almost 75-80 % of lipids are in the neutral lipid fraction (triglycerides + cholesterol esters), 18-20 % in the phospholipid fraction and only 3-5% in the FFA fraction.

Fatty acids, which are the product of endogenous intracellular biosynthesis, account for 62 % to 80 % of all fatty acids in lipid fractions. The remaining 15-38 % are fatty acids, among which the majority (60-80 %) are PUFA. The question of the sources of these fatty

acids in an animal organism that does not receive lipids remains unclear. We assume that these fatty acids are synthesized by the endogenous microbiota, since the administration of the antibiotic lincomycin reduces the content of PUFA, while the administration of the antidysbiotic agent restores it [20].

**Table 3.** The content (%) of fatty acids, products of endogenous intracellular biosynthesis (EICB) in fractions of lipids in the liver of rats fed a fat-free diet (FFD)

| Indicators | Neitral lipids (NL) | Phospholipids (PL ) | FFA |
|---|---|---|---|
| A. EICB Products<br>Palmitic acid ($C_{16:0}$) | 31,43 | 27,08 | 22,20 |
| Palmitooleic acid ($C_{16:1}$, n-7) | 11,19 | 4,59 | 5,97 |
| Stearic acid ($C_{18:0}$) | 2,65 | 24,79 | 12,16 |
| Oleic acid ($C_{18:1}$, n-9) | 39,85 | 16,25 | 22,35 |
| Total | 85,11 | 72,71 | 62,68 |
| B. Fatty acids of other origin<br>including PUFA | 14,89<br>9,14 (61,4 %) | 27,29<br>21,53 (78,9 %) | 37,32<br>30,11 (80,7 %) |
| ω-3 PUFA | 0,64 | 2,42 | 4,01 |

Note: PUFA =$C_{18:2}$+$C_{18:3}$+$C_{20:4}$+$C_{20:5}$+$C_{22:5}$+$C_{22:6}$

Table 4 presents the results of determining the content of fatty acids in the neutral fraction of lipids in the liver of rats receiving fatty diets. It can be seen that the consumption of dietary fats reduces the content of fatty acids, the products of endogenous intracellular biosynthesis (EICB), and most of all with the consumption of high-linoleic sunflower oil (by 26 %), while high-oleic sunflower oil reduces the content of EICB products by only 5 %.

**Table 4.** Effect of dietary fats on the fatty acid composition of the fraction of neutral lipids in rat liver

| Indicators | Dietary fats 5 % | | |
|---|---|---|---|
| | High linoleic sunflower oil | High oleic sunflower oil | Palm oil |
| A. EICB Products<br>Palmitic acid ($C_{16:0}$) | 21,62 | 20,66 | 25,27 |
| Palmitooleic acid ($C_{16:1}$, n-7) | 6,08 | 4,61 | 5,69 |
| Stearic acid ($C_{18:0}$) | 1,69 | 1,31 | 2,23 |
| Oleic acid ($C_{18:1}$, n-9) | 33,37 | 54,10 | 44,86 |
| Total | 62,76 | 80,68 | 78,05 |
| B. Fatty acids of other origin<br>including PUFA | 37,24<br>30,44 | 19,32<br>13,31 | 21,95<br>16,31 |
| ω-3 PUFA | 0,46 | 0,72 | 0,66 |

Note: see table 3.

Table 5 presents the results of determining the content of fatty acids in the phospholipid fraction of the liver of rats fed fat diets. It can be seen that the consumption of fats reduces the content of EICB products, however, significantly less than in the NL fraction. Thus, the consumption of HOSO reduces the content of these acids by 13.7 %, the consumption of HLSO by 9.4 % and the consumption of palm oil by only 2.3 %.

**Table 5.** The effect of dietary fats on the fatty acid composition of the phospholipid fraction rat liver

| Indicators | Dietary fats ,5 % | | |
|---|---|---|---|
| | High linoleic sunflower oil | High oleic sunflower oil | Palm oil |
| A. EICB Products<br>Palmitic acid ($C_{16:0}$) | 24,70 | 21,35 | 25,26 |
| Palmitooleic acid ($C_{16:1}$, n-7) | 2,98 | 1,98 | 3,43 |
| Stearic acid ($C_{18:0}$) | 22,14 | 19,70 | 20,61 |
| Oleic acid ($C_{18:1}$, n-9) | 16,07 | 19,75 | 21,76 |
| Total | 65,90 | 62,78 | 71,06 |
| B. Fatty acids of other origin<br>including PUFA | 34,10<br>26,20 | 37,22<br>30,08 | 28,99<br>23,49 |
| ω-3 PUFA | 0,24 | 3,81 | 1,13 |

Note: see table 3.

Table 6 presents the results of determining the content of EICB products in the FFA fraction of liver lipids in rats fed fat diets. It can be seen that the consumption of HLSO reduces the content of fatty acids in EICB products by 23.5 %, while the consumption of HOSO and palm oil has practically no effect on the content of these acids.

**Table 6.** Effect of dietary fats on the fatty acid composition of the FFA fraction of rat liver lipids

| Indicators | Dietary fats, 5 % | | |
|---|---|---|---|
| | High linoleic sunflower oil | High oleic sunflower oil | Palm oil |
| A. EICB Products<br>Palmitic acid ($C_{16:0}$) | 17,89 | 17,10 | 20,42 |
| Palmitooleic acid ($C_{16:1}$, n-7) | 4,92 | 3,25 | 5,48 |
| Stearic acid ($C_{18:0}$) | 7,49 | 9,31 | 9,68 |
| Oleic acid ($C_{18:1}$, n-9) | 24,40 | 31,885 | 28,14 |
| Total | 47,95 | 61,51 | 63,72 |
| B. Fatty acids of other origin<br>including PUFA | 52,05<br>37,60 | 38,49<br>30,94 | 36,28<br>29,07 |
| ω-3 PUFA | 1,64 | 3,35 | 2,86 |

Note: see table 3.

On fig. 2 shows the results of determining the "activity" of fatty acid synthase by the total content of the products of this reaction ($C_{16:0}$+$C_{16:1}$). It can be seen that the “activity” of this enzyme in the liver of fat-fed rats decreases in all lipid fractions, however, to the greatest extent in the NL fraction and when HOSO is consumed.

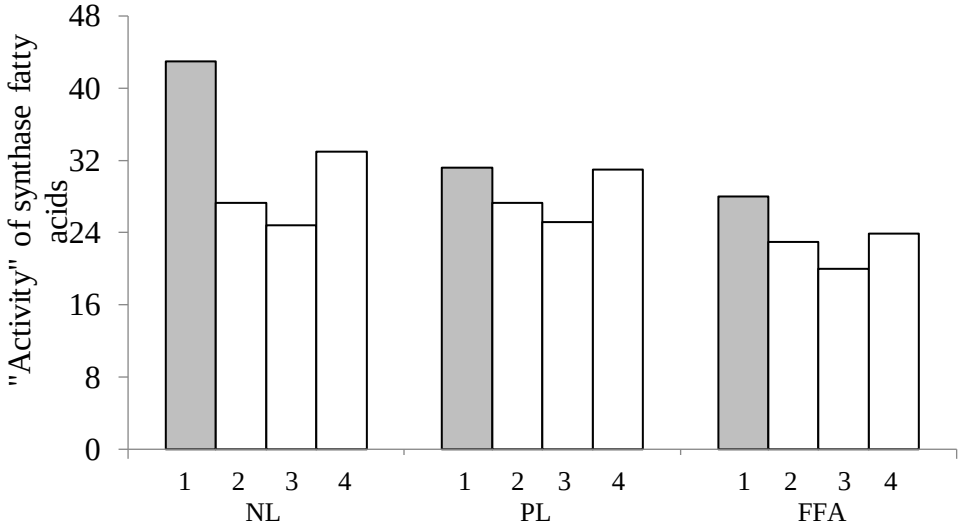


**Fig. 2.** "Activity" of fatty acid synthase in the liver of rats fed with fat
(1 – FFD, 2 – +5 % HLSO, 3 – +5 % HOSO , 4 – +5 % PM)
NL – neutral lipids; PL – phospholipids; FFA – free fatty acids

On fig. 3 shows the results of determining the “activity” of the palmitic acid elongase enzyme by two methods: usually recommended by the ratio $C_{18:0}/C_{16:0}$ [8] and proposed by us according to the ratio ($C_{18:0}$+$C_{18:1\ n-9}$)/($C_{16:0}$–$C_{16:1\ n-7}$).

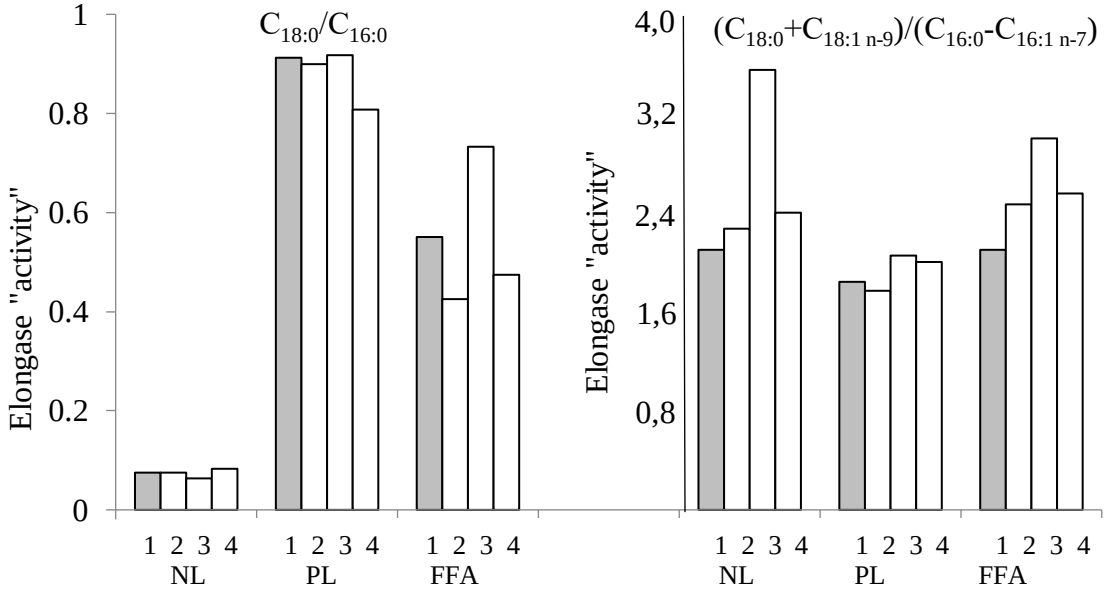


**Fig. 3.** Effect of dietary fats on the “activity” of palmitic acid elongase in rat liver lipids
(1-4 – see Fig. 2)

It can be seen that the first method gives very poor results when using the fraction of neutral lipids. Phospholipid fractions give higher results, but do not reveal differences between groups. Differentiation is manifested only when using indicators of the FFA fraction.

The "activity" of elongase, when determined by the method proposed by us, is several times higher, and a clearer differentiation by groups is observed when using the indicators of the NL and FFA fractions. It follows from these data that elongase activity increases the consumption of HOSO the most.

On fig. 4 shows the results of determining the "activity" of stearic acid desaturase (SAD) by two methods: by the ratio of $C_{16:1\ n-7}/C_{16:0}$ (SCD16) and by the ratio of $C_{18:1\ n-9}/C_{18:0}$ (SCD18). It can be seen that the indicators of SCD18 are an order of magnitude greater than those of SCD16. Moreover, they differ significantly in the nature of the change in enzyme "activity": SCD16 levels decrease with fat nutrition, while SCD18 levels increase significantly, especially after the consumption of HOSO.

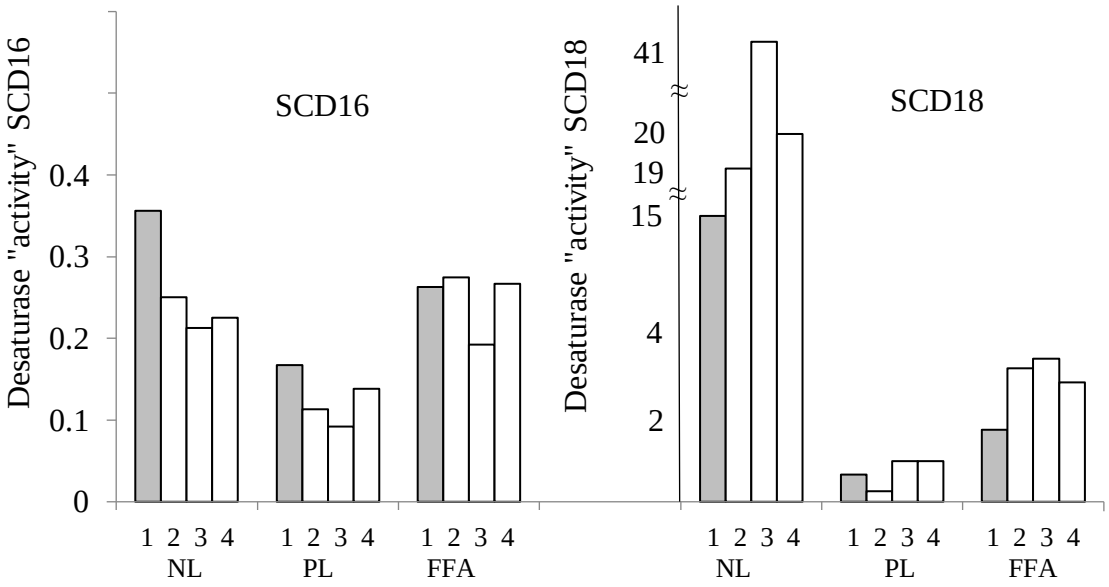


**Fig. 4.** Effect of dietary fats on the "activity" of SCD16 and SCD18 desaturases in rat liver lipids
(1-4 – see Fig. 2)

Based on the results of our studies, presented in Table 3, it follows that 15-34 % of liver fatty acids are formed outside of EICB. In the composition of these fatty acids, PUFA prevail (60-80 %), including ω-3 PUFA.

It is believed that food sources of PUFA are linoleic ($C_{18:2\ n-6}$) and α-linolenic ($C_{18:3\ n-3}$) [4, 6]. However, these acids are absent in our FFD. Therefore, they are formed in the animal

body. We believe that the biosynthesis of these fatty acids occurs due to the endogenous microbiota [19]. It is not clear only what types of bacteria carry out their biosynthesis.

Fatty nutrition reduces the EICB of fatty acids: high linoleic sunflower oil reduces by 26.2 % (NL fraction), by 22.6 % (PL fraction) and by 43.7 % (FFA fraction). Palm oil reduces by 8.3 % (NL fraction), by 13.7 % (FL fraction), but slightly increases (by 1.7 %) in the FFA fraction.

High oleic sunflower oil reduces the content of fatty acids in EICB by 5.2 % (NL fraction), by 13.7 % (PL fraction) and only by 1.9 % (FFA fraction).

Fat diet not only reduces the EICB of energy fatty acids, but also greatly reduces the endogenous biosynthesis of ω-3 PUFA. Thus, the consumption of high-linoleic sunflower oil reduces the content of ω-3 PUFA in the neutral lipid fraction by 72 %, in the FFA fraction by 59 %, and by 10 times in the phospholipid fraction. In contrast to HLSO, high oleic sunflower oil increases the content of ω-3 PUFA by 12.5 % (NL fraction), by 57.4 % (FL fraction) and only decreases in the FFA fraction (by 16.5 %).

The consumption of palm oil reduces the endogenous biosynthesis of ω-3 PUFA significantly less than HLSO: in the PL fraction by 53.3 % and in the FFA fraction by 28.7 %, and even slightly increases in the NL fraction (by 3.1 %).

The ability of high-linoleic sunflower oil to reduce endogenous ω-3 PUFA biosynthesis can be explained by the negative effect of linoleic acid on those bacteria that produce endogenous ω-3 PUFA. However, this assumption still needs to be proven.

We found a decrease in the "activity" of the fatty acid synthase enzyme, which forms palmitic acid, under the influence of dietary fats, and most of all with the consumption of HOSO . On this basis, it can be assumed that the inhibition of the "activity" of the synthase is carried out by oleic acid (retroinhibition).

We believed that the decrease in the intensity of EICB of fatty acids may depend on the “activity” of the elongase enzyme, however, it turned out that with fat nutrition, the “activity” of elongase does not decrease, and even increases when HOSO is consumed, which clearly registers our proposed method for determining its “activity”.

A similar situation occurred with the "activity" of stearyl-CoA desaturase (SCD18), the level of which increased significantly, especially after the consumption of HOSO.

In contrast to SCD18, SCD16 "activity" is reduced with a fat diet, and to the greatest extent with the consumption of HOSO. It can be assumed that oleic acid inhibits the formation of palmitooleic acid, which provides additional formation of stearic, and then oleic acid. But this assumption also requires proof.

## Conclusions

In the animal body, along with the classical endogenous intracellular biosynthesis of fatty acids (mainly energy ones), there are other ways of formation of fatty acids, including those considered essential (ω-3 PUFA).

Fatty nutrition reduces the intensity of EICB of fatty acids, and this is most pronounced in the NL fraction when consuming ordinary high-linoleic sunflower oil. Moreover, the consumption of this oil also inhibits the endogenous biosynthesis of essential fatty acids, especially in the PL fraction (10 times!).

In contrast to HLSO, high oleic oil stimulates the endogenous biosynthesis of essential fatty acids and activates enzymes (elongase and SCD18-desaturase), which are involved in the formation of energy fatty acids, mainly oleic, however, the consumption of HOSO most strongly inhibits the first phase of EFA biosynthesis – synthase activity fatty acids.

## Acknowledgments

The authors declare that there are no conflicts of interest.

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