**Levitsky A. P., Gozhenko A. I., Velichko V. V., Selivanskaya I. A. The effect of dietary fat supplements on the activity of palmitic and stearic acid desaturases based on the results of a study of the fatty acid composition of neutral lipids in blood serum and liver of rats receiving a fat-free diet. Journal of Education, Health and Sport. 2022;12(1):197-206. eISSN 2391-8306. DOI <http://dx.doi.org/10.12775/JEHS.2022.12.01.016> <https://apcz.umk.pl/JEHS/article/view/JEHS.2022.12.01.016> <https://zenodo.org/record/5873836>**

The journal has had 40 points in Ministry of Education and Science of Poland parametric evaluation. Annex to the announcement of the Minister of Education and Science of December 1, 2021. No. 32343.<br>Has a Journal's Unique

Punkty Ministerialne 2 2019 - aktualny rok 40 punktów. Załącznik do komunikatu a mieszkie z 1201 r. p. 20143. Posiad Unikatowy Identyfikator Czasopisma: 201159.<br>Przypisane dyscypliny naukowe:Naukio kulturze fizycznej (Dzie

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**Received: 15.12.2021. Revised: 25.12.2021. Accepted: 18.01.2022.**

UDC 612.397+547.587+616.03

# **THE EFFECT OF DIETARY FAT SUPPLEMENTS ON THE ACTIVITY OF PALMITIC AND STEARIC ACID DESATURASES BASED ON THE RESULTS OF A STUDY OF THE FATTY ACID COMPOSITION OF NEUTRAL LIPIDS IN BLOOD SERUM AND LIVER OF RATS RECEIVING A FAT-FREE DIET**

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# **Abstract**

Background. Desaturase enzymes are involved in the formation of monoenoic acids from saturated fatty acids. One such enzyme is stearyl-CoA-desaturase (SCD1), which converts stearic acid to oleic acid. The aim of this work was to determine the effect of edible fats with different fatty acid compositions on SCD1 activity.

Methods. High linoleic sunflower oil (HLSO), high oleic sunflower oil (HOSO) and palm oil (PO) were used. The rats were fed for 30 days with a semi-synthetic diet that did not contain any fats (FFD) and fat diets containing 5 % of each of the above oils. In animals, lipids were extracted from serum and liver and divided into 3 fractions: neutral lipids (NL), phospholipids (PL), and free fatty acids (FFA).

The fatty acid composition of each fraction was determined by gas chromatography. The SCD18 activity was determined by the  $C_{18:1}$  n-9/ $C_{18:0}$  – ratio, and the SCD16 activity was determined by the  $C_{16:1}$  n-7/ $C_{16:0}$  ratio.

Results. A higher activity of SCD16 and SCD18 was found in the NL fraction, and the activity of SCD18 significantly exceeds that of SCD16. A decrease in the content of  $C_{16:0}$ ,  $C_{16:1}$  and  $C_{18:0}$  in the NL fraction of the liver and blood serum was shown. The activity of SCD16 in blood serum and liver decreases in rats fed fat diets, while the activity of SCD18 does not decrease, and even increases with the consumption of HOSO.

Conclusions. To determine the SCD1 activity, it is advisable to use the  $C_{18:1}/C_{18:0}$  ratio in terms of the level of fatty acids in the NL fraction. Fatty diet inhibits SCD16 activity, and consumption of HOSO increases SCD18 activity.

# **Keywords**: **fat food; fatty acid desaturase; oleic acid; palmitooleic acid.**

## **Introduction**

In humans and animals, the biosynthesis of fatty acids from various organic substances (carbohydrates, proteins, organic acids and alcohols) is carried out, preliminarily converting the latter into acetyl-CoA. From this compound, under the influence of a number of cofactors and under the action of enzymes, the original fatty acid molecule is formed – palmitic acid  $(C_{16:0})$  [1, 2]. Further transformations of palmitic acid under the influence of the elongase enzyme lead to the formation of stearic acid  $(C_{180})$ . Desaturase enzymes act on palmitic and stearic fatty acids, which split off two hydrogen atoms from the hydrocarbon chain and form a double bond [3, 4]. As a result, monounsaturated fatty acids palmitooleic ( $C_{16:1}$  n-7) and oleic  $(C_{18:1}$  n-9) are formed. It is these four acids  $(C_{16:0}$ ,  $C_{18:0}$ ,  $C_{16:1}$ ,  $C_{18:1}$ ) that form the basis of animal fats (triglycerides), which perform an important biological function in the body  $$ energy, by oxidation in mitochondria with the formation of ATP.

Unfortunately, an excessive amount of saturated fatty acids, especially palmitic acid, negatively affects the physiological functions of the body, causing the development of a number of diseases (atherosclerosis, myocardial dystrophy, type 2 diabetes mellitus, metabolic syndrome) [5-8].

At the same time, monoenoic fatty acids (palmitooleic and oleic) formed from saturated fatty acids are largely devoid of pathogenic effects, are easily transported from the

sites of formation (mainly from the liver and the mucous membrane of the small intestine) to organs and tissues that use these acids in as an easily oxidizable substrate [1, 2]. Therefore, the role of desaturase enzymes can be regarded as very positive [9].

However, quite recently (in 2020) a work by Norwegian researchers appeared which presents data on a negative relationship between the level of desaturases and the main indicators of metabolic syndrome [3]. In this work, the activity of stearyl-CoA-desaturase (SCD1) was determined by the ratio of the content of desaturase reaction products ( $C_{16:1}$  and  $C_{18:1}$ ) and substrates ( $C_{16:0}$  and  $C_{18:0}$ ), and the source of desaturases was the whole blood of people with an increased BMI divided into two groups: without metabolic disorders and with those. It turned out that in persons with metabolic disorders, the SCD activity in the ratio  $C_{16:1}/C_{16:0}$  (SCD16) is 33 % higher, and in the ratio  $C_{18:1}/C_{18:0}$  (SCD18) it is 22.4 % higher.

We believe that such results could result from the use of whole blood as a source of desaturases, which, in addition to triglycerides (the main MUFA transporters), contains a significant amount of structural (membrane) lipids (phospholipids, sphingomyelins, cholesterol esters), which contain a significant amount of non-monounsaturated fatty acids, and polyunsaturated, having two or more double bonds and formed by completely different desaturases [2].

Therefore, the purpose of our work was to determine the activity of desaturases of palmitic and stearic acids (SCD16 and SCD18) in blood serum and in the liver, which is the main organ producing energy fatty acid. In addition, we set the task to determine how fat nutrition affects the activity of these desaturases.

#### **Material and research methods**

The following edible fats were used in the work: regular, high-linoleic sunflower oil (HLSO), high-oleic sunflower oil (HOSO) and palm oil. The content of fatty acids in them was determined by the gas chromatographic method [10].

Biological experiments were carried out on 30 white Wistar rats (male, 5 months old, body weight 225-235 g), divided into 5 groups. The first two groups were kept on a fat-free diet (FFD), the composition of which is presented in Table 1. Group 3 received FFD, in which 5 % of starch was replaced with 5 % of HLSO, the 4th group received the same diet, but instead of HLSO, HOSO was used, and the 5th group received FFD with 5 % palm oil. The rats were kept on these diets for 30 days, after which they were sacrificed under thiopental anesthesia, blood serum was obtained, and the liver was isolated.

Components	<b>FFD</b>		Fat diets			
		$\overline{2}$	3	4	5	
Corn starch	64	19	59	59	59	
Soybean meal	20	20	20	20	20	
Ovalbumin	6	6	6	6	6	
Mineral mixture	$\overline{4}$	$\overline{4}$	$\overline{4}$	$\overline{4}$	$\overline{4}$	
Vitamin mixture						
Sucrose	5	50	5	5	5	
Sunflower oil	$\theta$	$\Omega$	5	$\Omega$	$\Omega$	
High oleic sunflower oil	$\theta$	$\Omega$	⋂	5	$\Omega$	
Palm oil	$\Omega$	0		$\Omega$	5	

Table 1. The composition of the diets (in%) for rats [11]

Lipids were extracted from these biological objects according to Dole's method [12] and divided into three fractions: neutral lipids (NL), which included triglycerides and cholesterol esters, phospholipids (PL) and free fatty acids (FFA) [13-16].

Lipid fractions from serum and liver of each group of rats were pooled and the content of fatty acids was determined in them by gas chromatography in triplicate. The activity of desaturase SCD16 was determined from the ratio of the content of  $C_{16:0}$  and  $C_{16:1}$ , and the activity of SCD18 was determined from the ratio of  $C_{18:0}$  and  $C_{18:1}$ . Only the isomers  $C_{16:1}$  n-7 and C18:1 n-9 were taken into account.

### **Results**

In fig. 1 shows the results of SCD16 determination in three fractions of serum and liver lipids from rats fed a fat-free diet. It can be seen that the highest activity of SCD16 is in the fraction of neutral lipids, and the lowest is in the fraction of phospholipids.

The difference in the activity of SCD18 is even greater (Fig. 2): in neutral lipids, its level is ten times higher than the level of SCD16 in the PL and FFA fractions.



Fig. 1. SCD16 activity according to the results of a study of serum and liver lipids in rats treated with FFD ( $NL$  – neutral lipids,  $PL$  – phospholipids,  $FFA$  – free fatty acids)



Fig. 2. SCD18 activity according to the results of a study of serum and liver lipids in rats treated with FFD ( $NL$  – neutral lipids,  $PL$  – phospholipids,  $FFA$  – free fatty acids)

Table 2 shows the results of determining the content of  $C_{16:0}$ ,  $C_{16:1}$ ,  $C_{18:0}$  and  $C_{18:1}$  in the fraction of neutral lipids in the blood serum of rats receiving fat and fat diets.

Table 2. Effect of fat supplements on the content of palmitic, palmitooleic, stearic and oleic acids in neutral lipids of rat blood serum

		Content, %				
$N_2$ $N_2$	Group		Palmitooleic $n-7$ $C_{16:1}$ ,	Stearic C <sub>18:0</sub>	$n-9$ $C_{18:1}$ , Oleic	
1	<b>FFD</b>	26,13	10,81	1,97	37,93	
$\overline{2}$	$FFD + 50 %$ sucrose	25,39	10,88	1,94	45,20	
3	$FFD + 5$ % high linoleic sunflower oil (HLSO)	19,30	7,03	1,56	31,76	
$\overline{4}$	$FFD + 5$ % high oleic sunflower oil (HOSO)	21,54	5,89	1,78	50,08	
5	$FFD + 5 %$ palm oil (PO)	26,27	7,96	2,24	42,46	

Note: FFD – Fat free diet

It can be seen that the consumption of HLSO and HOSO reduces the content of palmitic and stearic acids. The content of palmitooleic acid in the blood serum of rats fed with fat diets is also significantly reduced.

Table 3 shows the results of determining the content of  $C_{16:0}$ ,  $C_{16:1}$ ,  $C_{18:0}$  and  $C_{18:1}$  in neutral lipids of rat liver. It can be seen that the content of  $C_{16:0}$ ,  $C_{18:0}$ , and especially  $C_{16:1}$  is significantly reduced in rats fed with fat diets.

Table 3. Influence of fat supplements on the content of palmitic, palmitooleic, stearic and oleic acids in neutral lipids of rat liver



Note: FFD – Fat free diet

In fig. 3 shows the results of determining the activity of desaturases in the fraction of neutral lipids in rat blood serum. It can be seen that SCD16 activity decreases in rats fed fatty diets. Especially in rats treated with HOSO. SCD18 activity in rats fed fat diets did not decrease.

In fig. 4 shows the results of determining the activity of desaturases in the fraction of rat liver neutral lipids. It can be seen that fatty diets are characterized by a decrease in SCD16 activity and an increase in SCD18 activity.



Fig 3. Influence of fat supplements on the activity of desaturases (SCD16 and SCD18) according to the results of a study of neutral lipids in rat blood serum



Fig. 4. Influence of fat supplements on the activity of desaturases (SCD16 and SCD18) according to the results of a study of neutral lipids of rat liver

# **Discussion**

Our data confirm the results of a study by Norwegian authors [3], indicating a significant predominance of SCD18 activity: 25-28 times more than SCD16 activity. According to our data, the SCD18 activity in the blood serum NL fraction was 46 times higher than the SCD16 activity. In the liver, in the NL fraction, the excess of SCD18 activity over SCD16 was 42.

In other lipid fractions of blood serum and liver, the activity of desaturases turned out to be significantly lower: the level of SCD18 in the PL fraction of blood serum and liver was 22-23 times lower than that for the NL fraction.

The excess of SCD18 activity over SCD16 determines the important role of oleic acid formed under the action of SCD18, which is the main energetic substance of the animal organism [4, 8, 9].

We found that the consumption of dietary fats in physiological doses (less than 20 %) of the energy consumed) does not decrease the activity of SCD18, and even increases with the consumption of HOSO.

In contrast, SCD16 activity decreases with fat intake, most of all with HOSO consumption.

It should be noted that palm oil, despite the high content of palmitic acid, differs little in its effect on the activity of desaturases from the action of ordinary sunflower oil, which contains only 6-7 % of this acid.

#### **Conclusions**

To determine the activity of desaturases SCD16 and SCD18, it is advisable to use a neutral lipid fraction containing a significant amount of triglycerides – the end products of the desaturase reaction.

Fatty diet inhibits SCD18 activity.

High oleic sunflower oil activates SCD18.

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