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SOME INDICATORS IN RAT BLOOD SAMPLES TAKEN FROM THE PORTAL VEIN AND THE INFERIOR VENA CAVA AFTER CONSUMPTION OF DIFFERENT EDIBLE FATS

Anatoly Levitsky¹, Anna Maykova², Olga Makarenko²

¹State establishment “The institute of stomatology and maxillo-facial surgery
National Academy of Medical Sciences of Ukraine

e-mail: flavan@mail.ru

²Odessa National Mechnikov University

Abstract

Purpose: To determine the number of biochemical parameters in rats' serum derived from the portal vein and the inferior vena cava after consuming edible fats with different fatty acid composition.

Methods: White laboratory rats have been receiving in addition to standard vivarium diet 15% of the following edible fats for the 64 days: ordinary sunflower (high- linolenic), high oleic sunflower oil, butter, palm oil and coconut oil. At the end of this period, the rats' blood was taken under thiopental anesthesia from the portal vein and the inferior vena cava. Determination of elastase activity, alanine aminotransferase, urease, lysozyme, catalase, MDA content were conducted in the obtained serum and the degree of dysbiosis was calculated.

Results. The study showed that the activity of urease in serum of blood from the portal vein and the inferior vena cava was increased after consumption of all fats, except high-oleic sunflower oil. The activity of lysozyme, on the contrast, was decreased after feeding rats with butter, palm oil and coconut oil. The decrease of the activity of urease and elastase and an increase in the level of MDA and activity of alanine aminotransferase found in the serum of blood sample from the inferior vena cava, which indicated the involvement of the liver in that processes.

The conclusion. High-fat diets, except diet reach in high oleic sunflower oil, increased the degree of dysbiosis in rat's blood. The greatest increase was observed after the consumption of palm oil. The performed work allowed us to recommend the introduction of high oleic sunflower oil into a diet and reduction of a consumption of products containing palm oil to a minimum amount in order to prevent dysbiosis.

Key words: fat nutrition, portal vein, vena cava

Edible fats differ significantly from each other by their fatty acid composition. For example, ordinary sunflower oil mostly contains (50-60 %) of linoleic acid ($C_{18:2}$), high oleic sunflower oil contains 80-90 % of oleic acid ($C_{18:1}$), butter – 26-28 % of palmitic acid ($C_{16:0}$) too oleic acid, palm oil – 42-50 % of palmitic acid and 35-40 % of oleic acid, coconut oil – 45-48 % of lauric acid ($C_{12:0}$).

A number of studies presented a negative influence from saturated fatty acids such as palmitic acid to a human body. The glycerides of palmitic acid are hard to hydrolyze for a lipoprotein lipase enzyme and it can lead to hyperlipidemia and then to atherosclerosis [1-3] However, oleic acid can be better absorbed by the human body than any other fatty acids and that process has a positive influence on lipids metabolism [4].

It's also known that the liver is the main organ which is responsible for a metabolism of lipids. The products of lipids hydrolysis are transported to a portal vein into liver, where they are transported to a very-low-density lipoproteins (VLDL) [6].

The purpose of this work is the to determine some biochemical markers in a serum of rat's blood samples obtained from a portal vein and an inferior vena cava after digestion of edible fats with different fatty acid composition, specifically sunflower oil (high-linolenic), high-oleic sunflower oil, butter, palm and coconut oil.

Materials and methods of research

The experiments were performed on 36 white rats (males, 8-9 months, weight 255 ± 12 g). They were divided into 6 equal groups. The 1st group (control) received the standard

vivarium diet (fat content 6 %). The 2nd group received the diet with 15 % of the usual high-linoleic sunflower oil. The 3rd group received the diet with 15 % high oleic sunflower oil. The 4th group received the diet with 15 % butter. The 5th group received the diet with 15 % coconut oil. The composition of consumed fatty acid was determined by the chromatography [7]. The obtained results are presented in Table 1. The duration of the experiment was 64 days after that the blood samples were taken under thiopental anesthesia from rats' portal vein (PV) and rats' inferior vena cava (IVC). The following biochemical parameters from obtained serum were analyzed: inflammatory markers of elastase activity and content of malondialdehyde (MDA) [8], urease activity (marker of microbial contamination) [9], activity of lysozyme (non-specific immunity marker) [9], the activity of the antioxidant enzyme catalase [8], the activity of the alanine aminotransferase (ALT is a liver marker) [10]. The antioxidant-prooxidant index API was calculated from the ratio of catalase activity and the content of the MDA [8], the degree of dysbiosis was calculated from the ratio of the activity of urease and lysozyme by the Levitsky method. [9]. The final results of the experiments were analyzed by using the standard Student's t-test [11].

Table 1

Fatty acid composition of fats (% of the amount of fatty acids)

| Acid | Sunflower oil | Oil «Olivka» | Butter | Palm oil | Coconut oil |
|------------------|---------------|--------------|--------|----------|-------------|
| 8:0 caprilic | - | - | 1,25 | - | 2,00 |
| 10:0 caprinic | - | - | 2,67 | - | 3,02 |
| 12:0 laurinic | - | - | 2,97 | 0,19 | 46,57 |
| 14:0 myristic | 0,15 | 0,03 | 10,43 | 1,16 | 22,70 |
| 16:0 palmitic | 9,74 | 4,44 | 27,88 | 42,02 | 11,67 |
| 18:0 stearic | 3,90 | 3,07 | 12,73 | 4,87 | 13,60 |
| 18:1 oleic | 30,60 | 88,66* | 26,61 | 40,93* | 0,30 |
| 18:2 linoleic | 53,46 | 1,21 | 3,08 | 9,49 | 0,02 |
| 18:3 linolenic | 0,03 | 0,11 | 0,53 | 0,17 | - |
| 20:0 arachidic | 0,20 | 0,27 | 0,28 | 0,47 | 0,12 |
| 20:1 eicosenoic | 0,22 | 0,16 | 0,12 | 0,16 | - |
| 20:4 arachidonic | - | - | 0,05 | - | - |
| 22:0 behenic | 0,72 | 1,07 | - | 0,13 | - |
| 24:0 lignoceric | 0,25 | 0,81 | - | 0,10 | - |

* Sum of oleic acid isomers

Results and discussion

Table 2 presents the results of the determination of elastase activity, which is a proteolytic enzyme and a biochemical marker of inflammatory and dystrophic processes since it is produced by neutrophils [8]. From the presented data, it was obvious that both sunflower and palm oil can reduce to some degree the level of elastase activity in the serum of blood sample from the PV. This is probably due to the inhibition of these lipids by the inflammation process.

Table 2

Influence of different fats on the elastase activity in blood serum of rats

| №№ | Fat | The elastase activity, $\mu\text{kat} / \text{l}$ | |
|----|--------------------------------------|---|--|
| | | <i>v. porta</i> | <i>v. cava inf</i> |
| 1 | Control | $254,1 \pm 9,4$ | $214,5 \pm 8,6$ $p_1 < 0,05$ |
| 2 | Sunflower oil | $210,0 \pm 12,6$ $p < 0,05$ | $171,3 \pm 8,3$ $p < 0,05; p_1 < 0,05$ |
| 3 | High Oleic sunflower oil «Olivka» | $215,4 \pm 4,6$ $p < 0,05$ | $190,5 \pm 3,2$ $p < 0,05; p_1 < 0,01$ |
| 4 | Butter | $227,6 \pm 0,4$ $p > 0,05$ | $220,5 \pm 16,1$ $p > 0,3; p_1 > 0,5$ |
| 5 | Palm oil | $209,0 \pm 7,6$ $p < 0,05$ | $206,6 \pm 17,8$ $p > 0,3; p_1 > 0,6$ |
| 6 | Coconut oil | $241,9 \pm 8,5$ $p > 0,3$ | $288,7 \pm 12,9$ $p < 0,01; p_1 < 0,05$ |

Notes: p – in comparison with group 1; p_1 – compared to data for *v. porta*.

Elastase activity in rats' serum obtained from blood samples from the inferior vena cava (IVC) was significantly lower in correlation to both, control group and relevant indicators from VP after consuming ordinary sunflower oil and high oleic sunflower oil, which may indicate the ability of liver to inactivate a certain amount of elastase under the influence of sunflower oil due to its proteinase inhibitors [12]. The consumption of butter and palm oil did not reduce the activity of elastase in the blood from IVC and the consumption of coconut oil even significantly increased the activity of this enzyme (table 2).

Table 3 shows the results of the determination of MDA content which is the final product of peroxide oxidation of unsaturated fatty acids. It is clear that a sunflower oil consumption of different fats did not significantly affect that indicator in serum from VP. However MDA level is twice higher in serum from IVC and it is three times higher than the corresponding indicator from VP after consumption of palm oil. Taking into account that such a significant increase in MDA content is also observed in the intact group, it could be an assumption that the liver excreted MDA into the blood.

Table 3

Influence of different fats on the content of MDA in blood serum of rats

| №№ | Fat | MDA content , mmol / l | |
|----|--------------------------------------|-------------------------|---|
| | | <i>v. porta</i> | <i>v. cava inf</i> |
| 1 | Control | 0,75±0,08 | 1,84 ± 0,05 p ₁ < 0,01 |
| 2 | Sunflower oil | 0,78 ± 0,03 p > 0,5 | – |
| 3 | High Oleic sunflower oil «Olivka» | 0,97 ± 0,08 p > 0,05 | 1,82 ± 0,05 p > 0,1; p ₁ < 0,01 |
| 4 | Butter | 0,81 ± 0,08 p > 0,5 | 1,97 ± 0,25 p > 0,1; p ₁ < 0,002 |
| 5 | Palm oil | 0,88 ± 0,04 p > 0,05 | 2,51 ± 0,32 p < 0,05; p ₁ < 0,001 |
| 6 | Coconut oil | 0,78 ± 0,06 p > 0,5 | 1,55 ± 0,07 p < 0,01; p ₁ < 0,01 |

Notes: p – in comparison with group 1; p₁ – compared to data for *v. porta*.

At the same time, it could be seen from the results in the table. 4 that the catalase activity in serum from VP was not changed after a consumption of fats and the level of catalase activity was slightly different from its level in serum from IVC, with the exception of rats which received high oleic oil diet. In that case, there was a reliable decrease in its level.

Table 4

Influence of different fats on the catalase activity in blood serum of rats

| №№ | Fat | the catalase activity, μkat / l | |
|----|--------------------------------------|---------------------------------|---|
| | | <i>v. porta</i> | <i>v. cava inf</i> |
| 1 | Control | 0,28 ± 0,01 | 0,30 ± 0,06 p ₁ >0,3 |
| 2 | Sunflower oil | 0,25 ± 0,02 p>0,05 | 0,19 ± _____ p > 0,1; p ₁ <0,05 |
| 3 | High Oleic sunflower oil «Olivka» | 0,28 ± 0,01 p=1 | 0,31 ± 0,15 p > 0,1; p ₁ > 0,1 |
| 4 | Butter | 0,27 ± 0,01 p > 0,3 | 0,18 ± 0,02 p > 0,1; p ₁ < 0,01 |
| 5 | Palm oil | 0,26 ± 0,01 p > 0,2 | 0,25 ± 0,04 p > 0,3; p ₁ > 0,1 |
| 6 | Coconut oil | 0,27 ± 0,02 p > 0,3 | 0,25 ± 0,14 p > 0,5; p ₁ > 0,1 |

Notes: p – in comparison with group 1; p₁ – compared to data for *v. porta*.

Table 5 presents the results of the determination of the activity of alanine aminotransferase (ALT), which is a liver marker. Indeed, the liver excreted this enzyme into the blood, which was exemplified by a significant increase in its activity in serum samples from IVC. It was not found any significant difference of ALT activity in rat blood, even with a different fat consumption.

Table 5

Influence of different fats on the activity of ALT in blood serum of rats

| №№ | Fat | ALT, μ kat / l | |
|----|--------------------------------------|-------------------------------|---|
| | | <i>v. porta</i> | <i>v. cava inf</i> |
| 1 | Control | 0,38 \pm 0,04 | 0,64 \pm 0,02 $p_1 < 0,01$ |
| 2 | Sunflower oil | 0,35 \pm 0,02 $p > 0,3$ | 0,59 \pm 0,08 $p > 0,3; p_1 < 0,01$ |
| 3 | High Oleic sunflower oil «Olivka» | 0,32 \pm 0,01 $p < 0,05$ | 0,47 \pm 0,03 $p < 0,05; p_1 < 0,01$ |
| 4 | Butter | 0,24 \pm 0,02 $p < 0,05$ | 0,57 \pm 0,03 $p > 0,05; p_1 < 0,01$ |
| 5 | Palm oil | 0,34 \pm 0,01 $p > 0,05$ | 0,52 \pm 0,02 $p < 0,05; p_1 < 0,01$ |
| 6 | Coconut oil | 0,36 \pm 0,04 $p > 0,3$ | 0,53 \pm 0,02 $p < 0,05; p_1 < 0,01$ |

Notes: p – in comparison with group 1; p_1 – compared to data for *v. porta*.

Table 6 presents the results of the determination of an activity of lysozyme, which was an indicator of the level of nonspecific immunity. It could be seen that consumption of butter, palm and coconut oil significantly decreased the level of activity of that enzyme in the serum of blood sample from both VP and IVC, which may be a result of an inhibition of its absorption from the intestine. However, when rats consumed palm oil, there was a significant decrease in the activity of lysozyme in the serum of blood from IVC, possibly due to a decrease in the lysozyme synthesizing function of the liver [13].

Table 6

Influence of different fats on the lysozyme activity in blood serum of rats

| №№ | Fat | the lysozyme activity, u / l | |
|----|--------------------------------------|------------------------------|--|
| | | <i>v. porta</i> | <i>v. cava inf</i> |
| 1 | Control | 71 \pm 3 | 66 \pm 3 $p_1 > 0,1$ |
| 2 | Sunflower oil | 73 \pm 6 $p > 0,3$ | 76 \pm 2 $p < 0,05; p_1 > 0,3$ |
| 3 | High Oleic sunflower oil «Olivka» | 78,5 \pm 5 $p > 0,05$ | 68 \pm 3 $p > 0,3; p_1 > 0,05$ |
| 4 | Butter | 59 \pm 4 $p < 0,05$ | 55 \pm 3 $p < 0,05; p_1 > 0,3$ |
| 5 | Palm oil | 45 \pm 4 $p < 0,01$ | 15 \pm 1 $p < 0,001; p_1 < 0,001$ |
| 6 | Coconut oil | 38 \pm 2 $p < 0,001$ | 44 \pm 3 $p < 0,01; p_1 > 0,05$ |

Notes: p – in comparison with group 1; p_1 – compared to data for *v. porta*.

Fig. 1 shows the effect from different edible fats on the activity of urease, which is an indicator of bacterial contamination. It could be seen that the activity of urease in the

bloodstream VP exceeded the corresponding indicator in blood from IVC, in all groups, including intact, which may indicate the ability of the liver to inactivate urease, which is a very aggressive microbial toxin [14].

Table 7

Effect of different fats on the urease activity in blood serum of rats

| №№ | Fat | the urease activity, $\mu\text{kat} / \text{l}$ | |
|----|--------------------------------------|---|---|
| | | <i>v. porta</i> | <i>v. cava inf</i> |
| 1 | Control | $1,04 \pm 0,23$ | $0,62 \pm 0,35$ $p_1 > 0,3$ |
| 2 | Sunflower oil | $1,97 \pm 0,16$ $p < 0,05$ | $1,50 \pm 0,29$ $p > 0,05; p_1 > 0,05$ |
| 3 | High Oleic sunflower oil «Olivka» | $1,44 \pm 0,30$ $p > 0,2$ | $0,62 \pm 0,26$ $p=1; p_1 < 0,05$ |
| 4 | Butter | $2,06 \pm 0,20$ $p < 0,05$ | $1,23 \pm 0,30$ $p > 0,05; p_1 < 0,05$ |
| 5 | Palm oil | $2,61 \pm 0,24$ $p < 0,01$ | $1,38 \pm 0,35$ $p > 0,05; p_1 < 0,05$ |
| 6 | Coconut oil | $1,84 \pm 0,36$ $p > 0,05$ | $1,25 \pm 0,32$ $p > 0,05; p_1 > 0,05$ |

Notes: p – in comparison with group 1; p_1 – compared to data for *v. porta*.

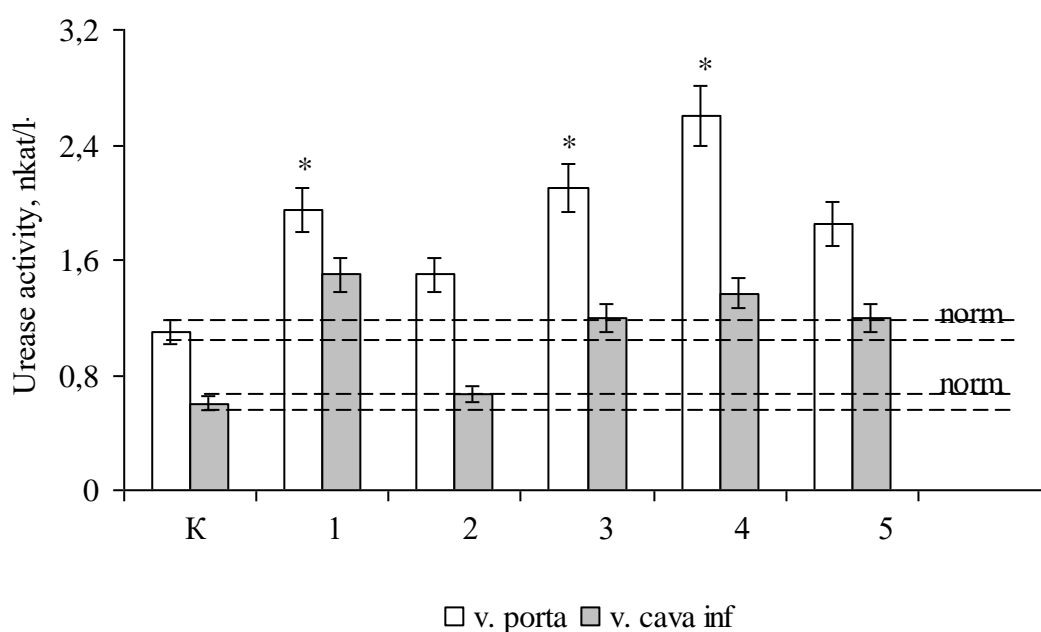


Fig. 1. Urease activity in blood serum of rats receiving different edible fats

The use of edible fats increased the level of urease activity two times or more in the serum of blood from VP, especially after consumption of sunflower oil, palm oil and butter. The level of urease activity in serum of the rats' blood from IVC after obtaining a fatty diet, also significantly exceeded of urease activity in intact animals. This fact could be explained

by the inability of the liver to neutralize very high amounts of urease that came through VP from the intestines of rats which consumed all fats with the exception of high oleic sunflower oil. Only consumption of that oil contributed to a preservation of the activity of urease in the blood from IVC at a normal level (Fig. 1).

The degree of dysbiosis was calculated by the ratio of relative activity of urease and lysozyme by the Levitsky method is given in Fig. 2, which showed that the consumption of edible fats with the exception of high oleic sunflower oil, caused the development of dysbiosis in the serum of blood sample from VP and from IVC and to the greatest extent after consumption of palm oil. The latter one has such a negative effect on that indicator that the degree of dysbiosis increased almost ten times in the serum blood sample from VC.

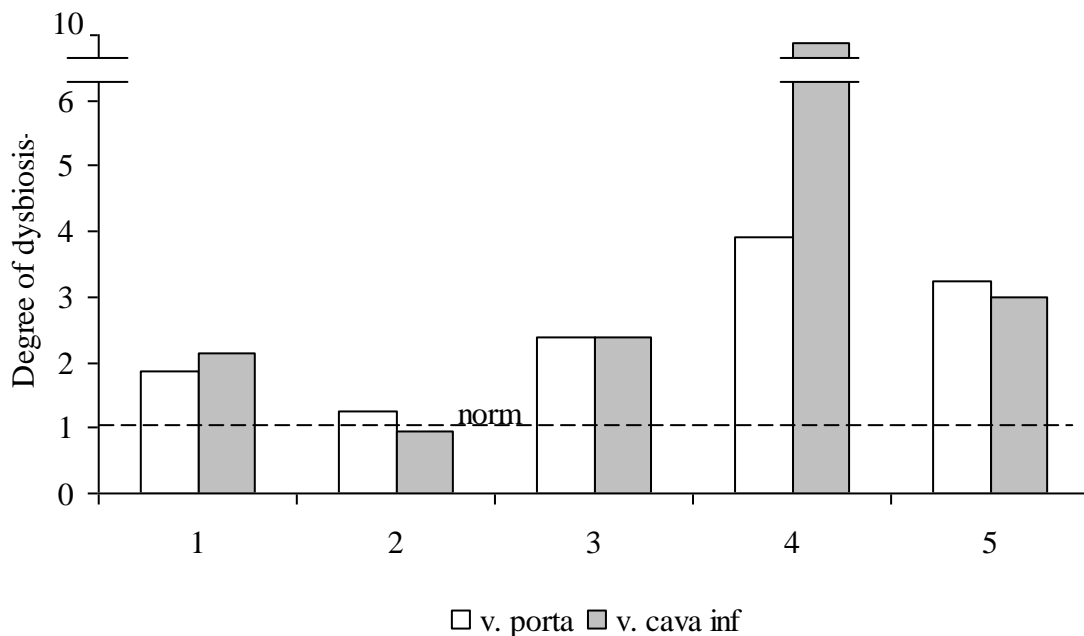


Fig. 2. Degree of dysbiosis in blood serum of rats receiving different edible fats

Thus, the research of the influence of different edible fats, which differ in their fatty acid composition, concluded that all fats, with the exception of high oleic sunflower oil, increased intestinal absorption of microbial urease toxin and reduced the lysozyme synthesizing function of the liver. As a result, there was a significant increase in the degree of dysbiosis, especially after consumption of palm oil. The exception was high oleic sunflower oil, which did not cause a development of dysbiosis.

Studies have shown that the liver was involved in the elimination of not only urease, but also a proteolytic enzyme elastase, which indicated a protective anti-inflammatory function of the liver. At the same time, the liver excreted a final product of peroxide oxidation of fatty acids – malonic dialdehyde and the enzyme alanine aminotransferase to the blood,

where the last one was a marker of an inflammatory process. The lowest ALT activity in the blood from IVC was observed after consumption of high oleic sunflower oil.

Conclusions

1. Edible fats, especially palm oil, increased the absorption of microbial factors (in particular urease) and reduced the lysozyme synthesizing function of the liver, with the exception of ordinary and high oleic sunflower oil, which caused the development of dysbiosis of blood as from VP, and in the systemic blood circulation.

2. The liver was able to neutralize the aggressive urease enzyme, but was excreted into the blood malodialdeald and ALT.

3. The obtained data indicate the harmful effect of most edible fats, especially high-palmitine, which use in quantities of 15 % or more. It gives grounds to recommend the use of high-oleic sunflower oil.

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