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STUDYING FATTY ACID CONTENT IN RED BLOOD CELL MEMBRANES IN DIABETIC RETINOPATHY OF TYPE 2 DIABETES PATIENTS

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Abstract

Introduction. Studying pathogenetic pathways that are formed under conditions of hyperglycaemia and trigger further metabolic changes resulting in diabetes complications over time are of special interest both in theoretical and practical medicine. Membrane of the red blood cell that stays in blood constantly and relatively durable is an obvious participant of all metabolic changes and cannot but reflect changes in metabolism. **Objective**: to study content of fatty acids (FAs) in red blood cell membranes of patients with diabetic retinopathy (DR) with type 2 diabetes (T2D). **Materials and methods.** The study enrolled 73 subjects maximally matched by age and gender: 50 patients with established long-lasting T2D complicated with DR and 23 patients in the control group. All the biochemical tests were performed at the certified laboratory under the standard methods. Gas chromatography was applied to study FA spectrum. Statistical analysis was performed in IBM SPSS Statistics 23. **Results and discussion.** The key difference between the lipid metabolism of patients with complicated long-lasting T2D and healthy subjects was a significant difference in FA content redistribution in the red blood cell membranes represented by increased “saturation”. The content of saturated FAs (SAFAs)in patients with DR was higher than in the CG: 1.5-fold for palmitic (C:16), 2-fold for myristic(C:14), pentadecanoic (C:15) and margaric (C:17) ($P < 0.05$). The content of saturated stearic (C:18) FA was not changed significantly.The content of unsaturated FAs(USFAs) have changed multidirectionally: the content of linoleic

(C18:2) and arachidonic (C20:4) decreased 1.5-1.7-fold, respectively. The content of linolenic (C18:3) increased 2-fold, and content of oleic FA (C18:1) was not changed significantly. **Conclusion.** Study of the fluctuations of FA levels in red blood cell membranesplays animportant role in the development of insulin resistance, T2D and its microvascular complicationsthat can be applied in creating treatment and prevention strategies of DRin type 2 diabetes patients.

Key words: glycosilated haemoglobin; red blood cell membranes; type 2 diabetes; diabetic retinopathy.

Introduction

Diabetic retinopathy (DR), the most common complication of long-lasting and uncontrolled diabetes, is the reason of poor vision and blindness all over the world [1, 2]. Type 2 diabetes (T2D) results in about 90% of all cases of diabetes and ranks among 10 leading causes of death. A significant increase in the number of diabetes cases is predicted. It is anticipated that 629 million of adults in total will have diabetes till 2045 [3].

Endothelial dysfunction is well known in patients with hypercholesterolemia, and lipid peroxidation is the vascular wall results in local production of reactive radical forms that mediate recruiting of macrophages, cellular activation and proliferation, as well as chemical modification of vascular proteins due to the improvement of lipoxidation end-products. It is known that hyperlipidaemia and endothelial dysfunction may result in the development of DR, macular oedema which lead to exudation of blood serum lipids and lipoproteins [4, 5]. The studies [6] have found a significant correlation between HbA1c and total cholesterol, however, there were no association between blood serum lipids and DR. No association was also found between DR and blood serum lipids, course of diabetes, obesity and lifestyle[5].However, authors of the other studies have shown that mean levels of cholesterol, triglycerides and LDL were higher in patients with DR compared with those who were free from DR. Although the significant correlation was found with triglycerides only [7].

Koehrer et al. have studied the aspects of fatty acid spectrum of red blood cell membrane in patients with different degree of retinal damage. The authors showed that red blood cells of the diabetes patients with or without DR had a significant reduction in docosahexaenoic and arachidonic acids [8].

Currently, it is proved that increased blood plasma content of free fatty acids plays a key role in the development of T2D resulting in insulin resistance, therefore it is reasonable to establish the most informative biochemical markers that reflect lipid dysmetabolism, highlight

priority orientations for pharmacological correction and parameters to monitor efficacy of treatment regimens.

Objective of the work is aimed at studying content of fatty acids in red blood cell membranes of patients with diabetic retinopathy secondary to type 2 diabetes.

Materials and methods. The study enrolled 73 subjects maximally matched by age and gender. Out of them, 50 patients with established long-lasting T2D (Me = 16.0years, Min-Max 6-37years), who presented different degree of retinal damage in the form of DR by the results of ophthalmologic tests. A group of DR+T2D was formed. The control group (CG) enrolled23subjects who seek medical attention for preventive check-up at the Laboratory of Clinical Laboratory Diagnostics at O. O. Bogomolets National Medical University; they have no diagnosed dysmetabolism and complaints. Patients' age was Me 63 [QI÷QIII, 56-67] (Min-Max 45-80),in the control group: Me 56[QI÷QIII, 52-62], (Min-Max 22-79). Among patients, 58% were females, in the CG– 62%.

All biochemical tests were performed by the certified Clinical Diagnostics Laboratory at O. O. Bogomolets National Medical University using the standard procedures. Measurements were performed on semi-automated biochemical analyser BS-3000M (Sinnowa, China), using biochemical test kits Diagnosicum Zrt, (Hungary). The concentration of glycosilated haemoglobin (HbA1) was measured by ion exchange temperature-independent chromatography-spectrophotometry using a set of reagents and microcolumns Bio Systems (Spain). Investigations of FA composition were performed by gas chromatography method at the Experimental Study Laboratory of O. O. Bogomolets National Medical University Scientific and Research Institute of Experimental and Clinical Medicine. Nine the most informative FAs were identified in FA profile of the lipids of blood cells: where myristic C14:0, pentadecanoic C15:0, palmitic C16:0 margaric C17:0, stearic C18:0 form total of saturated fatty acids (SAFAs), and oleic C18:1, linoleic C18:2, linolenic C18:3 and arachidonic C20:4 form a group of unsaturated fatty acids (USFAs). Linoleic C18:2, linolenic C18:3, arachidonic C20:4 FAs form a total amount of polyunsaturated fatty acids (PUFAs), and they are regarded as essential. Statistical analysis of data was performed using IBM SPSS Statistics 23 software package. Check of the distribution of quantitative parameters throughout sample data for compliance with Gauss law was performed using Shapiro-Wilk one-sample test. The majority of parameters did not reflect normal distribution, therefore, non-parametric tests were used. Data in the groups were compared using one-way ANOVA on ranks by Kruskal-Wallis test, Dunn and Mann-Whitney test considering Bonferonni adjustment was used for pairwise comparison. For data description in the groups, median

(Me) and 25th (P_{25}) and 75th (P_{75}) percentile values were provided as [QI÷QIII]. For median interval estimation, a 95 % confidence interval was calculated. Graphs were provided as columns with specification of (95 % CI). Differences in the groups were specified as P-value with a statement of the level of significance. Data were considered to be different at $P < 0.05$.

Results and discussion. Analysis of comparison groups by the age, BMI and gender showed homogeneity in the groups with slight predominance of females. Gender differences in body weight and BMI were also non-significant: this parameter is the patients was 28.7 [25-30] kg/m^2, and in the CG– 29.41 [27-33] kg/m^2. Comparison of the biochemical parameters that are most applicable in clinical settings is provided on the diagrams (Fig. 1).

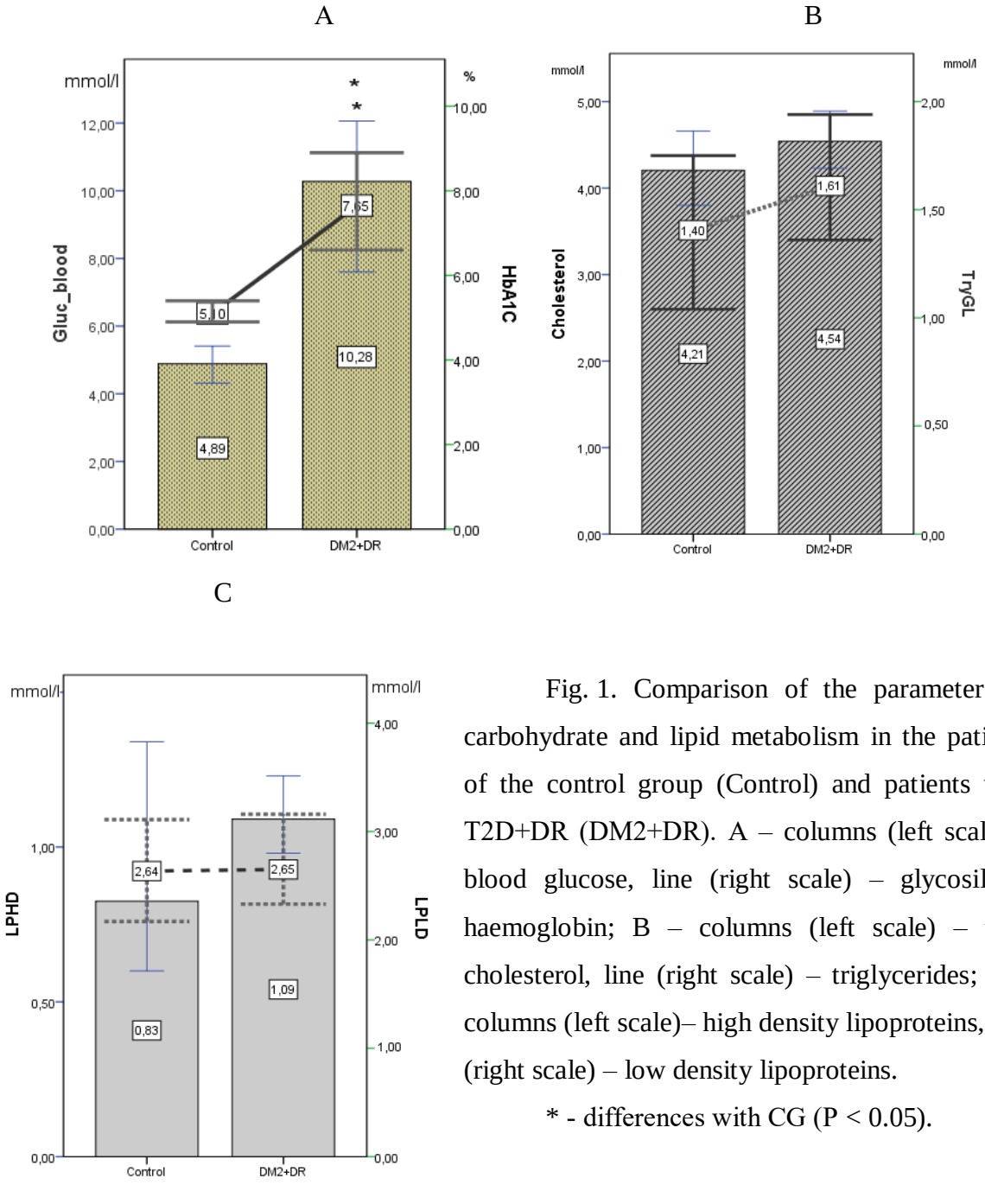


Fig. 1. Comparison of the parameters of carbohydrate and lipid metabolism in the patients of the control group (Control) and patients with T2D+DR (DM2+DR). A – columns (left scale) – blood glucose, line (right scale) – glycosilated haemoglobin; B – columns (left scale) – total cholesterol, line (right scale) – triglycerides; C – columns (left scale)– high density lipoproteins, line (right scale) – low density lipoproteins.

* - differences with CG ($P < 0.05$).

T2D+DR group included patients on dispensary follow-up due to diabetes, mean disease duration is 16 years, they obligatory take medications (tablet formulations or injections) and part of them take statins. All patients with T2D have vascular complication in the form of DR, which stage was determined by ETDRS. Thus, 12 patients suffered from initial moderate to severe non-proliferative DR, 24 – initial moderate and high risk proliferative DR and 14 patients – progressive proliferative DR. Therefore, T2D group was characterized with a long-term carbohydrate and lipid dysmetabolism.

Interesting fact was found that CG and T2D+DR group did not significantly differed by total cholesterol and LDL level. HDL and triglyceride values have no significant difference in the groups at all. We believe that the lack of difference in biochemical parameters reflects low information value of the specified parameters for characterization of long-term dysmetabolism.

Thus, to study features of FA metabolism in the body, we have used analysis of fatty acid composition in red blood cell membrane that was performed on a gas-liquid chromatograph. Blood is a transportation medium for lipoproteins, therefore measurement of plasma FAs reflects total amount of chylomicrons circulating in different directions – to the cell and in the opposite direction, thus, measurement of plasma FA level does not provide complete information on the use of fatty acids by the cells. Red blood cell membrane is regarded as the most informative model for analysis of FA profile in the patient's body [9,10], and it reflects general features of FA consumption in the cells, their building into the cellular membrane and how it takes place in all the cells of the body.

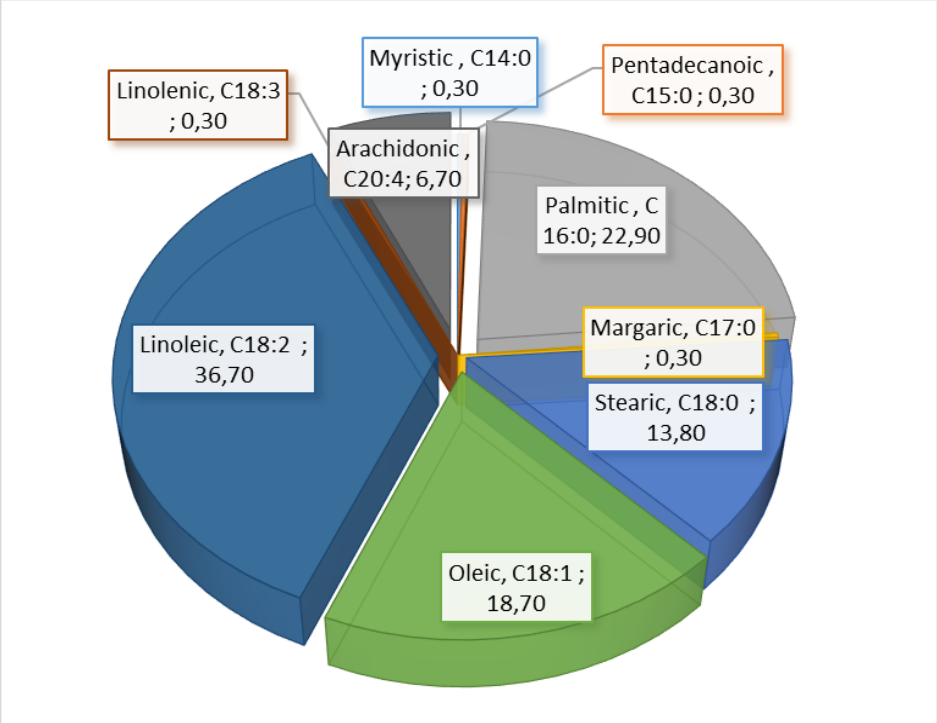
Analysis of FA content showed a significant difference between groups of healthy subjects and patients with T2D (Fig. 2).

Only relative content of two FAs – saturated stearic C18:0, and unsaturated oleic C18:1 did not differ in the groups. Other measurements had a clear tendency as follows: saturated FAs in patients with T2D+DR were virtually 1.5-2-fold higher, and unsaturated linoleic C18:2 and arachidonic C20:4 1.5-1.7-fold lower. Unsaturated linolenic C18:3 FA, which content in patients with T2D+DR also increased, was an exception.

Conventionally, total SUFAs, PUFAs and USFAs in the observational groups is compared (Fig. 3). By the parameters in the CG, content of USFAs (63 %) prevails in the membranes under normal conditions, and this is 1.7-fold higher that SUFAs content (37 %). PUFAs constitute virtually half (43.1 %) of membrane FAs. In case of long-term complicated T2D, redistribution of FAs completely differs: the main content of membrane FAs is presented with SUFAs (53.2 %) that is 1.4-fold higher compared with the normal value

(P < 0.05). There is a significant (P<0.05) 1.3-fold decrease to 46.8 % in the proportion of USFAs compared to the CG. Proportion of PUFAs decreases to 1/3 of membrane FAs (29.3 %) that is 1.5-fold (P<0.05) lower than in the CG.

A



B

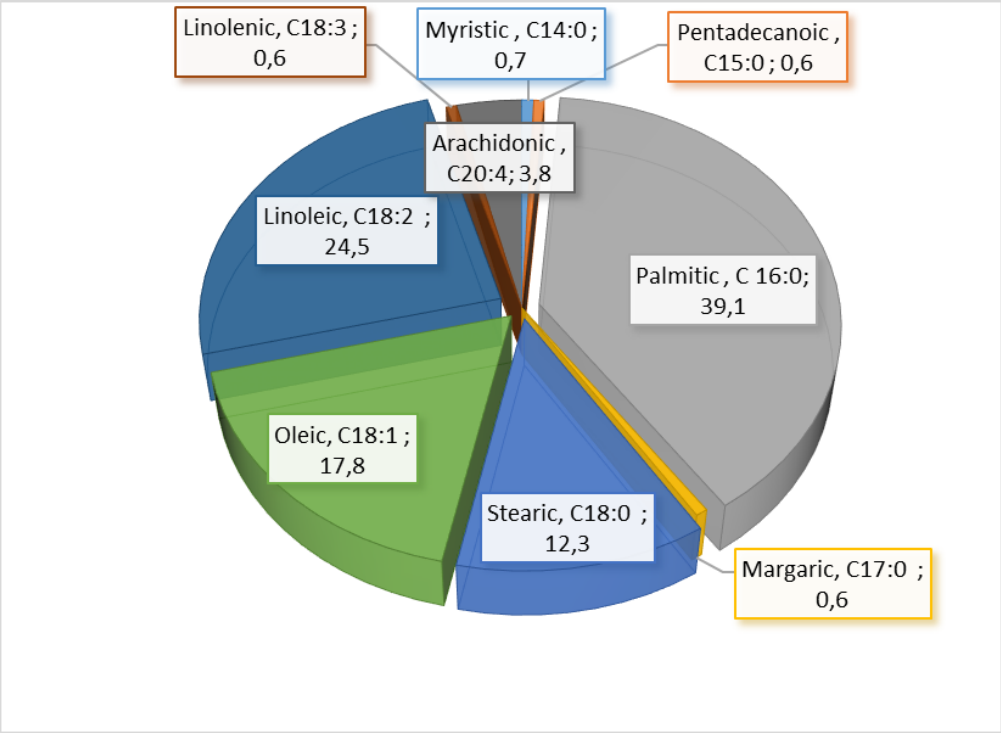


Fig. 2. Content of the main FAs (%) identified in red blood cell membranes of the control group (A) and patients with T2D+DR (B)

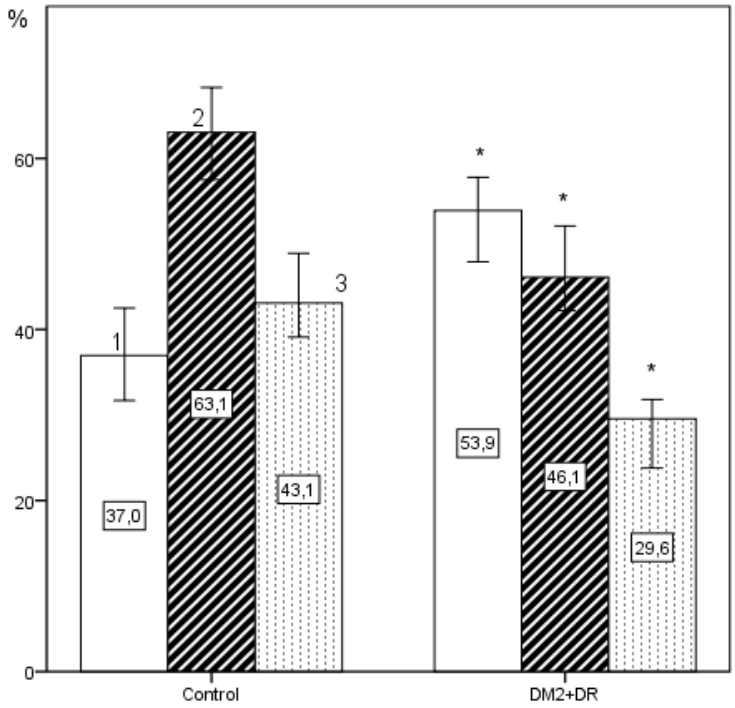


Fig. 3. Content of total amount of different FA types (%), measured in red blood cell membranes of the study groups. 1 – SUFAs, 2 – USFAs, 3 – PUFAs, where * – difference with the CG ($P < 0.05$).

Thus at the background of T2D, lipid dysmetabolism is characterized by the significant redistribution of FAs in cellular membranes. This may be a significant reason for changing functional ability of cellular membrane of both red blood cell, manifested as worsening of its flexibility and loss of deformation properties that is an obstacle for tissue oxygenation in the capillaries and worsens hypoxia, and for other cells, since it contributes to the reduction of intercellular interaction, possibility for exocytosis, ligand acceptance, receptor interaction, etc. [11, 12].

A historical belief exists that diabetes is a disorder, first of all associated with glucose dysmetabolism. And chronic hyperglycaemia induces diabetic condition, the main danger of which includes the development of multiple microvascular complications [13].

Investigating the origin of diabetes has shown that may people with obesity and insulin resistance will never have diabetes.Whenpancreatic β-cells function normally, FAs are strong inducers of insulin secretion, and particularly this is the compensatory mechanism that prevents development of insulin resistance [13]. It was experimentally shown that continuous (2-4 days) raises of plasma FAs levels initially reduced and then potentiated glucose-induced secretion of insulin in healthy volunteers. Furthermore, in patients with obesity with and

without diabetes, when chronically increased plasma FA levels were suddenly reduced, insulin secretion reduced by 30-50 % [14], highlighting that increased plasma FAs maintained 30-50 % of basal insulin secretion.

However, if FA plasma level increased for more than several hours, FA-induced insulin resistance develops that has a favourable action on accumulation of carbohydrates for use by vital tissues such as central nervous system. However, FA-induced insulin resistance becomes counterproductive [13].

Since FA may induce insulin resistance both in liver and muscles, it may be expected that in all people with excessive body weight or obesity that may have increased plasma FA levels, glucose levels may be also increased. However, only a half of people with excessive body weight have abnormal glucose levels. National Health and Nutrition Examination Survey (NHANES III) has shown that only one in four people with excessive body weight or obesity had impaired fasting glucose or impaired glucose tolerance, and 23 % suffered from diabetes [15].

Since long-term exposure to increased FA levels plays a key role in the development of insulin resistance and T2D, then the use of FA-lowering drugs is a consistent decision. However, their benefit is limited, since initial reduction in plasma FA levels after, for example, administration of nicotinic acid, is steadily accompanied by a dramatic FA peak [13] that increases insulin resistance, at least temporarily. Therefore, studies of the mechanism of effect of increased FA levels that result in insulin resistance, as well as their critical and maximally permissible levels that are protective and ensure compensatory mechanism of stimulation of pancreatic β-cells secondary to metabolic disorders are actively ongoing.

CONCLUSION:

Patients with T2D complicated with DR, total cholesterol, LDL, HDL and triglyceride levels have no statistically significant difference compared with healthy subjects.

The main difference in the lipid metabolism of patients with complicated long-term T2D and healthy subjects was a significant difference in redistribution of FA content in red blood cell membranes in the form of increased “saturation”. The content of saturated FAs in patients with DR was higher than in the CG: namely: palmitic (C:16) 1.5-fold, myristic (C:14), pentadecanoic (C:15) and margaric (C:17) 2-fold ($P < 0.05$). The content of saturated stearic (C:18) FA was not significantly changed.

Change in the content of unsaturated FAs was multidirectional: content of linoleic (C18:2) and arachidonic (C20:4) reduced 1.5-1.7-fold, respectively. The content of linolenic

(C18:3) increased 2-fold, and as for oleic FA (C18:1), its content was not changed significantly.

Study of the fluctuations of FA levels in red blood cell membranesplays animportant role in the development of insulin resistance, T2D and its microvascular complicationsthat can be applied in creating treatment and prevention strategies of DRin type 2 diabetes patients.

References

1. Balashevich L.I., Izmaylov A.S. Diabeticheskaya oftal'mopatiya. SPb: Chelovek; 2012. 396 s. [in Russia]
2. Pasechnikova N.V. Diabeticheskaya makulopatiya. Sovremennyye aspekty patogeneza, kliniki, diagnostiki, lecheniya. Kiyev: iz-vo OOO «Karbon LTD». 2010. 154 s [in Russia]
3. International diabetes federation annual report 2015. https://www.oedg.at/pdf/1606_IDF_ Atlas_2015_UK.pdf
4. Chernobryvtsev O.P. The endothelial dysfunction factors in diabetes mellitus 2 type. *Journal of Education, Health and Sport formely of Health Sciences*. 2019;9(1):525-533.
5. Benarous R, Sasongko MB, Qureshi S, et al. Differential association of serum lipids with diabetic retinopathy and diabetic macular edema. Invest Ophthalmol Vis Sci. 2011 Sep 27;52(10):7464-9. doi: 10.1167/iovs.11-7598.
6. Cetin E. N., Bulgu Y., Ozdemir S., et al. Association of serum lipid levels with diabetic retinopathy. Int J Ophthalmol. 2013; 6(3): 346–349.
7. Rema M, Srivastava BK, Anitha B, et al. Association of serum lipids with diabetic retinopathy in urban South Indians--the Chennai Urban Rural Epidemiology Study (CURES) Eye Study-2. Diabet Med. 2006 Sep;23(9):1029-36.
8. Koehrer P. Erythrocyte Phospholipid and Polyunsaturated Fatty Acid Composition in Diabetic Retinopathy/ P. Koehrer, S.Saab, O. Berdeaux //2014 journal.pone 0106912
9. Petrenko O. V. , Natrus L. V., Tavartkiladze K. Aspects of fatty acid content in blood cells of patients with diabetic retinopathy. Archive of Ukrainian Ophthalmology. – 2017. – Vol. 5, No. 2.
10. Weijers Rob N.M. Lipid Composition of Cell Membranes and Its Relevance in Type 2 Diabetes Mellitus. Curr Diabetes Rev. 2012 Sep; 8(5): 390–400.

11. Shin S, Ku Y, Babu N, Singh M. Erythrocyte deformability and its variation in diabetes mellitus. Indian J Exp Biol. 2007 Jan;45(1):121-8.

12. Hussein J. S. Cell membrane fatty acids and health / J. S. Hussein // Int. J. Pharm. Sci. – 2013. – Vol. 5, suppl. 3. – P. 38–46.

13. Boden G, Chen X and Iqbal N. Acute lowering of plasma fatty acids lowers basal insulin secretion in diabetic and nondiabetic subjects. Diabetes 1998 Oct; 47(10): 1609-1612.https://doi.org/10.2337/diabetes.47.10.1609

14. Boden G, Laakso M. Lipids and Glucose in Type 2 Diabetes. What is the cause and effect? Diabetes Care 2004 Sep; 27(9): 2253-2259. https://doi.org/10.2337/diacare. 27.9.2253

15. Benjamin SM, Valdez R, Geiss LS, Rolka DB, Narayan KM: Estimated number of adults with prediabetes in the US in 2000: opportunities for prevention. Diabetes Care 26:645–649, 2003