МЕДИЦИНСКИЕ НАУКИ

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INFORMATION VALUE OF BIOCHEMICAL MARKERS FOR EVALUATION OF LIPID DYSMETABOLISM SECONDARY TO HYPERGLYCAEMIA IN PATIENTS WITH DIABETIC RETINOPATHY AND TYPE 2 DIABETES MELLITUS

Introduction. Diabetic retinopathy (DR) – the most common complication of long-term and uncontrolled type 2 diabetes mellitus (T2DM) – causes visual impairment and blindness all over the world. Hyperlipidaemia secondary to hyperglycaemia due to the endothelial dysfunction may contribute to the development of DR, macular oedema and impairment of blood-retinal barrier resulting in exudation of serum lipids and lipoproteins. Evaluation of the information value of biochemical markers determines priority trends of pharmacological correction and parameters for monitoring of the efficacy of therapeutic regimens.

Objective of this article was to study the information value of biochemical markers for evaluation of lipid dysmetabolism secondary to hyperglycaemia in patients with diabetic retinopathy.

Materials and methods. The study included 119 subjects maximally matched by the age and gender, allocated to three groups: 76 patients with the diagnosed long-term T2DM complicated with DR, 23 subjects without T2DM but with established metabolic changes by the lipid and carbohydrate metabolism parameters, 20 subjects without dysmetabolism – control group (CG). All biochemical tests were performed by the certified laboratory using the standard procedures. Fatty acids (FA) profile in red blood cell membranes was measured by the liquid chromatography method. Statistical analysis of data was performed using IBM SPSS Statistics 23 software package and MedStat software application.

Results. In patients with DR and T2DM, total cholesterol, Low-density lipoproteins (LDL), High-density lipoproteins (HDL) and triglycerides did not statistically differ compared with the healthy subjects. The main difference in the lipid metabolism of patients with complicated long-term T2DM 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 (SAFAs) in patients with DR was higher compared with 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 (USFAs) 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. Inversely, presence of the metabolic shifts in the form of increased total cholesterol and LDL secondary to hyperglycaemia, but without T2DM and its complications, is characterized by a slight increase in USFAs and polyunsaturated FA level in the composition of cellular membranes. This probably is a protective mechanism against worsening and complication of metabolic disorders, since FAs are strong natural inducers of unaffected β-cells of the pancreas preventing development of insulin resistance.

Conclusion. Study of the fluctuations of FA levels in the body of patients play a key role in the development of insulin resistance, T2DM and its microvascular complications, such as DR, making them an advantageous therapeutic approach.

Keywords: diabetic retinopathy, type 2 diabetes mellitus, endogenous regulation of homeostasis, red blood cell membranes.

According to the World Health Organization, type 2 diabetes mellitus (T2DM) is a reason of about 90 % of all cases of diabetes mellitus, and it is amongst 10 leading causes of death all over the world. The report of the International Diabetes Federation (IDF) predicts pronounced increase in the cases of diabetes, and it is expected that 629 million of adults in total will suffer from diabetes until 2045 [1]. Diabetic retinopathy (DR) – the most common complication of long-term and uncontrolled type 2 diabetes mellitus (T2DM) – causes visual impairment and blindness all over the world. [2, 3].

Dysfunction of vascular endothelium is considered as an important damaging factor in pathogenesis of DR and other vascular complications developed secondary to hyperglycaemia. Endothelial dysfunction is well-known in patient with hypercholesterolemia, and lipid peroxidation in the vascular wall results in a local production of reactive radical species that mediate recruiting of macrophages, cellular activation and proliferation, as well as chemical modification of vascular proteins due to improved end products of lipoxidation. Therefore, hyperlipidaemia due to endothelial dysfunction may contribute to the development of DR, macular oedema and impaired blood-retinal barrier resulting in exudation of serum lipids and lipoproteins [4, 5]. At the same time, contradictory reports on the effect of lipid profile on

retinopathy and maculopathy are available in the literature.

The study [6] has found a significant correlation between HbA1c and total cholesterol level, however, no association was reported between serum lipids and DR. The lack of association between the lipid profile and DR severity in this study complies with previous findings of multi-ethnic study in atherosclerosis that do not find relationship between DR and serum lipids, course of diabetes, obesity, and lifestyle [5]. Alternately, Rema et al. in Chennai Urban Rural Epidemiology Study, have shown that mean cholesterol, triglyceride and LDL levels were higher in patients with DR versus those who did not suffer from DR. However, significant correlation was found only with triglyceride content [7].

Koehrer et al. have studied features of fatty acid profile of red blood cell membrane in patients with different degree of retinal lesion. The authors have shown that red blood cells of patients suffering from diabetes mellitus with or without DR demonstrate pronounced reduction of docosahexaenoic acid and arachidonic acid levels [8].

Currently, it was proved that increased plasma content of free fatty acids plays a key role in the development of T2DM inducing insulin resistance, therefore, it is reasonable to establish the most informative biochemical markers that reflect lipid dysmetabolism and highlight priority trends of pharmacological correction and parameters for monitoring of the efficacy of therapeutic regimens.

Objective of this article was to study the information value of biochemical markers for evaluation of lipid dysmetabolism secondary to hyperglycaemia in patients with diabetic retinopathy.

Materials and methods. The study included 119 subjects maximally matched by the age and gender (Table 1). Out of them, 76 patients were allocated to the group with the diagnosed long-term T2DM (Me=16.4 years, QI÷QIII 9-20 years, Min-Max 5-36 years) who had different degree of retinal lesion in the form of DR according to ophthalmological examinations. We have included them as "DR+T2DM". Another 43 subjects came for preventive check-up to the Clinical Diagnostics Laboratory at O. O. Bogomolets National Medical University, they have no established dysmetabolism and complaints. Out of them, 23 subjects were diagnosed with metabolic changes by the parameters of lipid and carbohydrate metabolism, and this group was defined as "MC". Subjects, who had no increased glucose, glycosylated haemoglobin and cholesterol level were included to the control group (CG) (n = 20). Characteristics of the comparison groups is provided in Table 1.

Table 1

CHARACTERISTICS OF THE COMPARISON GROUPS BY AGE AND GENDER

Parameters	Control group n= 23	Metabolic changes group n= 20	DR+ T2DM n= 76	Significance of differences
Age, years Me; [QI÷QIII] (Maximum-Minimum)	57 [51-63] (22-79)	57 [50-65] (22-78)	63 [57.5-68.5] (44-84)	$P_{c1\text{-}c2}>0.05$ $P_{c1\text{-}DM}=0.03$* $P_{c2\text{-}DM}=0.03$*
Gender, females, % Proportion in the group	65% 15(23)	70% 14(20)	55% 42(76)	χ^2 P=0.34

Note: Determination of the statistical significance of differences was performed by Kruskal-Wallis test, and the parameter "gender" was compared in the contingency table "k*m" using χ^2 test.

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). Glycosilated haemoglobin (HbA1) concentration 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 forms 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 and MedStat software application. 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, and to compare data in a contingency table "k*m", χ^2 test was 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 that have been specified in tables 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 (Fig. , Table 2). First of all, we've performed analysis of the most wide-spread "screening" biochemical parameters used in clinical settings (Table 2). A special interest was called upon comparison of CG and MC groups that we have defined as patients without T2DM. CG included subjects who despite of the age and high body weight (BMI = 29.41) have no deviations from the reference values by the main parameters of carbohydrate and lipid metabolism. MC group included subjects who were not followed-up in dispensary settings, and did not consider themselves ill. However, interview of each such subject has found that they have some increased values of biochemical parameters during regular preventive measurements. As it shown in the Table, glycosilated haemoglobin levels in MC group were 2.4-fold higher ($P<0.05$) compared with CG, however blood glucose level was only slightly higher. In general, this is explained by a transient reduction in glucose level during blood sampling due to the fact that a subject familiar with fluctuations of the parameter may ensure sugar deprivation in advance. Such a situation only underlines the information value of HbA1C that currently is a golden standard for determination of carbohydrate dysmetabolism. At the same time, while MC group have no complaints typical for pronounced metabolic disorders in T2DM, we have analysed them as a "conditionally healthy".

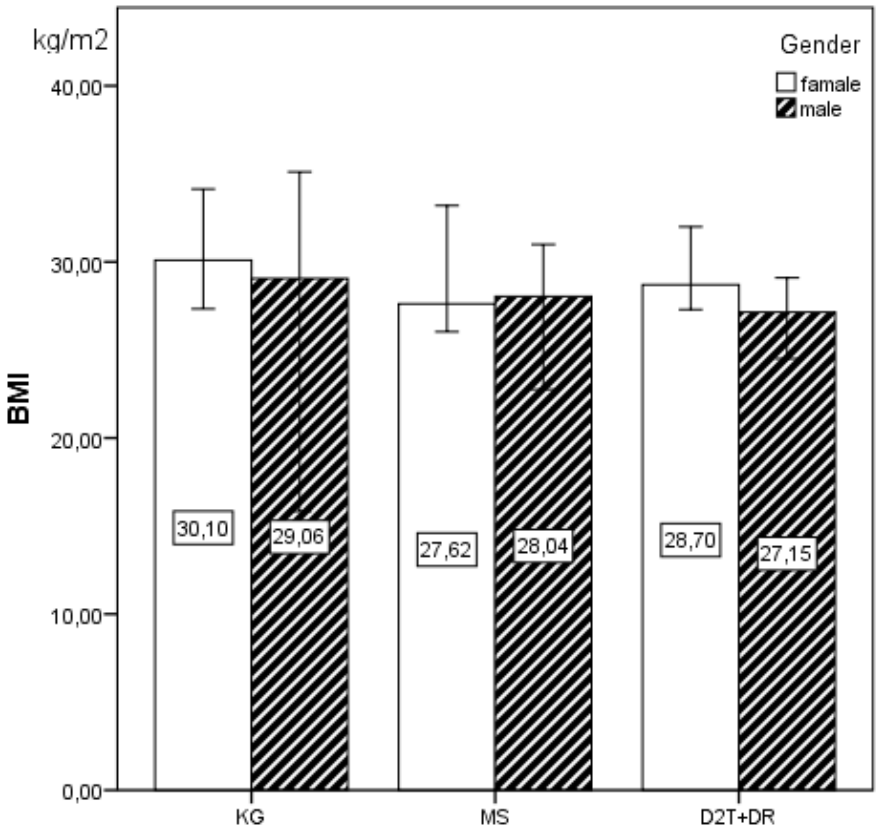

Fig. 1. Distribution of BMI in CG, MC group and patients with T2DM+DR depending on the gender. Light columns stand for females, dark – for males.

Table 2

PARAMETERS OF THE GROUPS OF INTEREST AND PATIENTS WITH T2DM (N, ME; [QI÷QIII])

Parameters	Control group n= 23	Metabolic changes group n= 20	DR+ T2DM n= 76	Significance of differences
BMI, kg/m2	29.41 [27.5-33.8]	27.69 [26.4-30.72]	28.05 [25.45-32]	$P_{CG-MC}>0.05$ $P_{MC-DM}>0.05$ $P_{CG-DM}>0.05$
HbA1C (%)	5.1 [4.9-5.5]	12.3 [7.0-13.8]	7.7 [6.6-9.5]	$P_{CG-MC}<0.05$* $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
Blood glucose, mmol/L	5.0 [4.4-5.4]	5.9 [5.0-7.6]	10.0 [7.5-12.0]	$P_{CG-MC}<0.05$* $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
Cholesterol, mmol/L	4.2 [3.82-4.66]	5.4 [4.68-5.96]	4.5 [3.72-5.30]	$P_{CG-MC}<0.05$* $P_{MC-DM}<0.05$* $P_{CG-DM}>0.05$
HDL, mmol/L	0.82 [0.60-1.34]	1.16 [0.79-1.26]	1.15 [0.87-1.40]	$P_{CG-MC}>0.05$ $P_{MC-DM}>0.05$ $P_{CG-DM}>0.05$
LDL, mmol/L	2.63 [2.17-3.11]	3.38 [2.96-14.05]	2.6 [1.77-3.41]	$P_{CG-MC}<0.05$* $P_{MC-DM}<0.05$* $P_{CG-DM}>0.05$
Triglycerides, mmol/L	1.4 [1.04-1.75]	1.35 [1.12-1.85]	1.55 [1.18-2.01]	$P_{CG-MC}>0.05$ $P_{MC-DM}>0.05$ $P_{CG-DM}>0.05$

Note: Determination of the statistical significance of differences was performed using Kruskal-Wallis test.

T2DM group included patients on dispensary follow-up due to diabetes mellitus, mean disease duration is 16 to 20 years, they obligatory take medications (tablet formulations or injections), and part of them administer statins. All patients with T2DM have vascular complication in the form of DR, which stage was determined by ETDRS. Thus, 30 patients suffered from initial moderate to severe non-proliferative DR, 34 – initial moderate and high risk proliferative DR, and 37 patients – progressive proliferative DR. Therefore, T2DM group was characterized with a long-term carbohydrate and lipid dysmetabolism.

Interesting fact was found that CG and MC group significantly differed by total cholesterol and LDL level, and patients with T2DM+DR did not differ from CG by these parameters. HDL and triglyceride values have no significant difference in the groups at all. We believe that the lack of difference in biochemical parameters of the CG and T2DM+DR 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 conditionally healthy subjects and patients with T2DM. At the same time, no significant differences in terms of any measurement were reported in CG and MC group (Table 3).

Only relative content of two FAs – saturate 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 T2DM+DR were virtually 1.5-2-fold higher, and unsaturated linoleic C18:2 and arachidonic C20:4 1.5-1.7-fold lower, respectively. Unsaturated linolenic C18:3 FA, which content in patients with T2DM+DR also increased, was an exception.

Table 3

CONTENT OF THE MAIN FAS (%), MEASURED IN RED BLOOD CELL MEMBRANES OF THE STUDY SUBJECTS AND PATIENTS WITH T2DM+DR (N, ME; [QI÷QIII])

Fatty acid	Control group n= 23	Metabolic changes group n= 20	DR+ T2DM n= 76	Significance of differences
C14:0 Myristic	0.35 [0.3-0.6]	0.4 [0.3-0.5]	0.7 [0.4-1.2]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
C15:0 Pentadecanoic	0.35 [0.3-0.6]	0.4 [0.3-0.5]	0.6 [0.4-1.0]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
C16:0 Palmitic	24.7 [16.4-30.5]	21.5 [13.2-23.4]	39.1 [26.9-44.1]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
C17:0 Margaric	0.35 [0.3-0.6]	0.4 [0.3-0.5]	0.6 [0.4-1.0]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
C18:0 Stearic	12.3 [10.8-12.7]	11.5 [10.3-12.4]	11.3 [9.7-13.9]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$ $P_{CG-DM}<0.05$
C18:1 Oleic	18.6 [17.1-20.4]	18.0 [16.4-19.3]	17.8 [16.2-20.3]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$ $P_{CG-DM}<0.05$
C18:2 Linoleic	37.1 [31.0-41.6]	40.1 [36.0-46.2]	23.3 [17.0-32.4]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
C18:3 Linolenic	0.35 [0.3-0.6]	0.4 [0.3-0.5]	0.6 [0.4-1.0]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*
C20:4 Arachidonic	6.6 [3.2-9.3]	6.1 [4.4-8.4]	3.8 [1.8-8.7]	$P_{CG-MC}>0.05$ $P_{MC-DM}<0.05$* $P_{CG-DM}<0.05$*

Note: Determination of the statistical significance of differences was performed using Kruskal-Wallis test.

Conventionally, total SUFAs, PUFAs and USFAs in the observational groups was compared (Fig. 2). By the parameters in the CG, content of USFAs 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. Presence of metabolic shifts by the biochemical parameters in the form of increased blood glucose and total cholesterol in MC group is accompanied by slight non-significant increase in USFAs and PUFAs and decrease in SUFAs in MC group compared to the CG. In case of long-term complicated T2DM, 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.

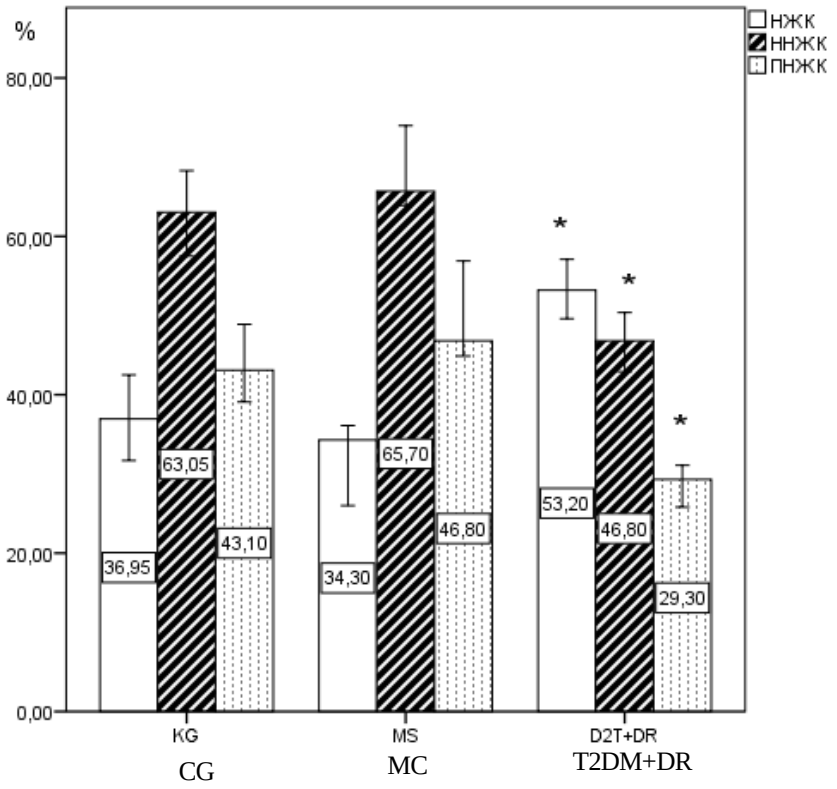

*Fig. 2. Content of total amount of different FA types (%), measured in red blood cell membranes of the study groups. Light columns stand for SUFAs, striated – USFAs, columns with dots – PUFAs, where * - difference with the CG (P<0.05).*

Therefore, metabolic disorders accompanied by shifts in the biochemical parameters of carbohydrate and lipid metabolism, but not transformed in the diabetes, are characterized with slight fluctuations in fatty acid profile of membrane in the form of increase in USFAs and PUFAs. In other words, tendency of changes has a reversal form than at the background of T2DM, where lipid dysmetabolism is characterized by the significant redistribution of FAs in cellular membranes. This may be a significant reason for changes in functional ability of cellular membrane of both red blood cell (in the form of its reduced flexibility and lose of deformation properties that prevents tissue oxygenation in capillaries and worsens hypoxia) and other cells, since it contributes to the reduction of intercellular interaction, possibility to exocytosis, ligand acceptance, receptor interaction, etc. [11,12].

For historical reasons, concepts of T2DM pathogenesis run on an axis of "glucose-insulin", and diabetes is first of all a disorder associated with impaired glucose metabolism. Certainly, this concept has been confirmed by a great deal of data that chronic hyperglycaemia induces diabetic condition, the main threaten of which involves many microvascular complications [13].

Boden et al. have studied causes of diabetes and made a conclusion why many people with obesity resistant to insulin will never suffer from diabetes. In people with normal pancreatic β-cells, 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 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. This is a very reasonable mechanism for sustainable survival and functioning of the brain during starvation or in the late pregnancy, when insulin resistance in mother accumulates glucose for growing foetus, however, FA-induced insulin resistance becomes counterproductive in other cases [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, that is not the case, since 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 23 % of people with excessive body weight or obesity (BMI ≥25 kg/m^2) 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 T2DM, 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:

1. Patients with DR and T2DM complicated with DR, total cholesterol, LDL, HDL and triglyceride levels have no statistically significant difference compared with healthy subjects.

2. The main difference in the lipid metabolism of patients with complicated long-term T2DM 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.

3. 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.

4. Inversely, presence of the metabolic shifts in the form of increased total cholesterol and LDL secondary to hyperglycaemia, but without T2DM and its complications, is characterized by a slight increase in USFAs and PUFAs level in the composition of cellular membranes. This probably is a protective mechanism against worsening and complication of metabolic disorders, since FAs are strong natural inducers of unaffected β-cells of the pancreas preventing development of insulin resistance.

5. Study of the fluctuations of FA levels in the body of patients play a key role in the development of insulin resistance, T2DM and its microvascular complications, making them an advantageous therapeutic approach

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