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FEATURES OF LIPID METABOLISM IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE COMBINED WITH TYPE 2 DIABETES MELLITUS AND OBESITY

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ABSTRACT

Nonalcoholic fatty liver disease represents a widespread health impairment associated with development of a variety of complications, particularly: cardiovascular diseases. Thus, the current investigation of common pathogenetic mechanisms underlying the formation of nonalcoholic fatty liver disease, type 2 diabetes mellitus, obesity, and associated cardiovascular risk seems to be actual. Therefore, the aim of present research was to investigate cardiovascular risk markers such as apolipoprotein A1, apolipoprotein B and their ratio B/A1 in patients with nonalcoholic fatty liver disease associated with type 2 diabetes mellitus and obesity.

The study included 70 patients with nonalcoholic fatty liver disease: 48 patients with concomitant type 2 diabetes mellitus and obesity and 22 patients with concomitant type 2 diabetes mellitus and normal body weight; the control group involved 20 healthy volunteers.

The results showed that in patients with nonalcoholic fatty liver disease and type 2 diabetes mellitus, regardless the obesity, apolipoprotein A1 concentration was significantly decreased and inversely correlated with antropometric indices. Levels of apolipoprotein A1 and index HOMA-IR were also found to correlate inversely. Examined patients with nonalcoholic fatty liver disease combined with type 2 diabetes mellitus showed a significant increase in the apolipoprotein B concentration and the apolipoproteins B/A1 ratio. The maximum rates were observed in patients with nonalcoholic fatty liver disease associated with type 2 diabetes mellitus and obesity, the levels were significantly higher than those in patients with nonalcoholic fatty liver disease, type 2 diabetes mellitus and normal body weight, thus indicating to the earlier atherogenesis in patients with nonalcoholic fatty liver disease associated with type 2 diabetes mellitus and obesity.

Thus, patients with nonalcoholic fatty liver disease associated with type 2 diabetes mellitus and obesity might have significant coronary artery disease predictors, and they are at cardiovascular diseases risk group. Probably, the pathogenic mechanisms leading to such disturbances of apolipoproteins metabolism include adipose tissue dysfunction and adipocytokines synthesis abnormalities, as well as liver functional disorders per se affecting apolipoprotein metabolism enzymes.

Keywords: nonalcoholic fatty liver disease, cardiovascular risk, apolipoprotein A1, apolipoprotein B, apolipoproteins B/A1 ratio.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is widespread throughout the world and associated with the development of a variety of complications. In 2000, only in the USA simple steatosis of the liver was diagnosed in approximately 71 million people over the age of 18 [*Browning J. et al., 2004*]; NAFLD was responsible for 20% of all the requests for medical aid with newly diagnosed chronic liver disease [*Weston S. et al., 2005*]. Later, as a result of Dallas Heart Study conducted in the early 2000s, it was found that the prevalence of NAFLD was 31% in the general population [*Browning J. et al., 2004*]. However, in 2011 the study suggested a higher value of this figure: the prevalence of NAFLD was 46%, and nonalcoholic steatohepatitis made 12.2% [*Williams C. et al., 2011*].

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Cardiovascular diseases (CVDs) are considered to be the main risk factor for mortality in patients with NAFLD [*Targher G. et al., 2008*], but the pathogenic mechanisms underlying this relationship are not entirely clear. Data suggest an increased risk of CVDs in patients with NAFLD [*Edens M. et al., 2009*].

Currently, NAFLD is considered a hepatic manifestation of metabolic syndrome associated with the risk of CVDs development [*Kotronen A., Yki-Järvinen H., 2008; Fon Tacer K., Rozman D., 2011*]. Recently data were obtained on the genetic factors influencing the formation of cardiovascular risk (CVR) in patients with NAFLD: comparison between ADIPOR2 rs1044471 genotype and metabolic markers reliably confirmed the presence of genetic determination of CVR in NAFLD patients and showed an important role of ADIPOR2 in formation of CVR and associated metabolic phenotype in NAFLD patients [*Kolesnikova O., 2013*]. Possibly, there are common pathogenic mechanisms underlying this relationship – the formation of insulin resistance syndrome (IRS) and the subsequent development of lipid metabolism disorders. One of the pathogenetic mechanisms of NAFLD development and progression is known to include dyslipidemia: the increase of proatherogenic lipoprotein fractions: very low density lipoproteins (VLDL), low density lipoproteins (LDL) and triglycerides (TG) against the decrease of concentrations of high density lipoproteins (HDL) with antiatherogenic properties.

The main protein molecule of HDL, particularly their HDL2 subfraction that has the highest anti-atherogenic potential, is apolipoprotein A-1 (apo-A1) [*Schwartz R., 1987*]. In contrast, it is the concentration of apolipoprotein B (apo-B) that reflects the serum content of lipoproteins not belonging to the HDL class, i.e. non-HDL in the most accurate way.

The indices of apo-A1 and the concentration ratio of apo-B to apo-A1 (apo-B/A1) according to the INTERHEART study are the most reliable and stable predictors of cardiovascular events [*Liem A. et al., 2008; McQueen M. et al., 2008*]. According to the Penn Diabetes Heart Study (PDHS) and the study of inherited risk of coronary atherosclerosis (SIRCA) measuring of the apo-B concentration represents significant predictor of coronary artery disease in patients with type 2 diabetes mellitus (DM) [*Reilly M. et al., 2004; Martin S. et al., 2009; 2011*].

It is known that HDL2 concentrations and apo-A1 are reduced in patients with obesity. In the Framingham Offspring Study plasma concentrations of apo-A1 were shown to be significantly lower in patients with obesity [*Garrison R. et al., 1980*].

The results of the investigation for diabetes influence on the apo-A1 and apo-B concentrations are controversial [*Patel M. et al., 2012*].

Given the community of the basic pathogenetic mechanisms of NAFLD, type 2 DM, obesity and CVR, the aim of this study was to investigate the apo-A1 and apo-B concentrations in patients with NAFLD combined with type 2 DM, normal weight and obesity.

The study was conducted as part of the scientific research "Estimate ADIPOR2 gene polymorphism and features of nonalcoholic fatty liver disease course in patients with cardiovascular risk" (governmental registration number 0113U001139) at the Department of liver and gastrointestinal diseases of the GI "L.T. Malaya Therapy Institute of the National Academy of Medical Sciences of Ukraine".

Material and Methods

The study included 70 patients with NAFLD: 48 patients with concomitant type 2 DM and obesity (36 men and 12 women) and 22 patients with concomitant type 2 DM and normal body weight (14 men and 8 women). The patients' age varied from 37 to 67 averaging 42.1. All the patients were treated at the GI "L.T. Malaya Therapy Institute of the National Academy of Medical Science of Ukraine". The control group included 20 healthy volunteers matched for age and gender.

The study was approved by the local Ethics Committee and conformed to the principles outlined in the Declaration of Helsinki.

In all the patients we excluded other causes of fatty liver disease, such as: alcohol abuse (the use of >50 *g* ethanol per week for men, <30 *g* ethanol per week for women during the last year), infection with hepatitis viruses B, C, D), autoimmune or drug-induced hepatitis, Konovalov-Wilson disease, idiopathic hemochromatosis and congenital deficiency of α1-antitrypsin. The study did not include patients with severe stages of liver fibrosis and cirrhosis, decompensated diabetes, as well as those, who needed insulinotherapy. The verification of

NAFLD was carried out on the basis of ultrasound examination of the abdomen, as well as clinical and instrumental data. Diabetes was diagnosed according to the recommendations of the American Society of Diabetes and the Standardized clinical protocol of care at type 2 diabetes mellitus in Ukraine. The average duration of diabetes was 4.6 years, and in 7 cases it was newly diagnosed. All the patients complied with dietary recommendations, received oral hypoglycemic agents and had not previously undergone lipid-lowering therapy. Obesity was diagnosed according to the classification of the WHO International Group on Obesity (1997) by measuring body mass index (BMI). The type of anatomical fat distribution was assessed by anthropometric indices: waist circumference (WC), hip circumference (HC) and their ratio (WC/HC). Prior to involvement in the study, the written informed consent of all patients was obtained.

To assess long-term compensation of carbohydrate metabolism in all patients the concentration of glycated hemoglobin (HbA1c) was measured using the appropriate kit ("Reagent", Ukraine) by reaction with thiobarbituric acid, and total hemoglobin was defined using "Specol-11" spectrophotometer ("Carl Zeiss", Germany). The concentration of immunoreactive insulin and C-peptide were determined by ELISA using a standard kit ("DRG", Germany) at ELISA analyzer "HUMAREADER" ("Human", Germany), which were used to calculate the insulin resistance (IR) index HOMA-IR.

The concentration of total cholesterol and its fractions – HDL and TG – was determined by enzymatic method on the biochemical analyzer "Humalayzer" № 2106-1709 using the appropriate kit ("Human", Germany). LDL value was calculated with the standard formula of W.T. Friedewald. The concentration of cholesterol in the VLDL was determined by the ratio of TG/2.22.

The concentrations of apo-A1 and apo-B were measured using immunoturbodimetric method and the appropriate kit ("Dialab", Austria).

The statistical analysis was performed using the software package SPSS V 11.0. All the numerical data were presented as descriptive characteristics: mean, standard deviation. The association between the dependent and independent variables was determined using linear regression analysis and Pearson's correlation coefficient. Statistical significance between the group means were evaluated by Student's *t*-test for unpaired samples, and the differences were considered statistically significant at $p<0.05$.

Results

In the analysis of the nutritional status of all patients with concomitant obesity the average BMI was 32.10±0.83 *kg/m²*, that was significantly higher than in patients with NAFLD combined with type 2 DM and normal body weight and in control group patients (21.70±0.92 *kg/m²* and 22.70±1.14 *kg/m²*, respectively) (Table 1). In all the patients with NAFLD combined with type 2 DM and obesity there was observed the abdominal type of obesity: the main pathogenetic link for IRS formation. That is, in NAFLD patients with type 2 DM and obesity the WC and WC/HC indices were significantly higher than those in the group with normal body weight and in the control group (113.7±5.6 *cm* vs 84.9±4.2 *cm* and 83.9±6.9 *cm*, respectively, $p<0.05$; 1.09±0.06 vs 0.78±0.03 and

Table 1.

Clinical and instrumental parameters in nonalcoholic fatty liver disease patients and the control group

Parameters	Patients with nonalcoholic fatty liver disease		Control group (N = 20)
	type 2 diabetes mellitus and obesity (N = 48)	type 2 diabetes mellitus (N = 22)	
Body mass index, *kg/m²*	31.6 ± 1.32*,**	21.7 ± 0.92*,***	22.7 ± 1.14
Waist circumference, *cm*	113.7 ± 5.6*,**	84.9 ± 4.2*,***	83.9 ± 6.9
Ratio of waist circumference to hip circumference	1.09 ± 0.06*,**	0.78 ± 0.03*,***	0.78 ± 0.03

Notes: ** - p<0.05 compared to the control group;*
*** - p<0.05 compared to patients with normal body weight;*
**** - p <0.05 compared to patients with concomitant obesity.*

0.78±0.03, $p<0.05$, respectively). The analysis on the association of anthropometric parameters found a strong correlation between BMI and WC ($\rho=+0.83$, $p<0.05$), as well as BMI and WC/HC ($\rho=+0.81$, $p<0.05$).

The parameters of carbohydrate metabolism in patients with NAFLD and in the control group are presented in Figure. All NAFLD patients were detected as having hyperglycemia. The fasting glucose serum concentration in patients with concomitant obesity was 8.37±0.12 *mmol/L* compared to 7.29±0.22 *mmol/L* in patients with NAFLD and normal body weight; glycated hemoglobin levels in patients with NAFLD combined with type 2 DM and obesity was also significantly higher than those in the group of patients with NAFLD, type 2 DM and normal body weight (7.47±0.33 HbA1c, µmol of fructose/*g* Hb vs. 6.89±0.42 HbA1c, µmol of fructose/*g* Hb, $p<0.05$) and in healthy subjects (7.47±0.33 HbA1c, µmol of fructose/*g* Hb and 5.35±0.42 HbA1c, µmol of fructose/*g* Hb, $p<0.05$).

The analysis of insulin sensitivity showed its significant decrease in patients with NAFLD, and the lowest sensitivity was observed in patients with concomitant type 2 DM and obesity: HOMA-IR index measured in conventional units (conv. units) was significantly higher than those observed in the group of patients with NAFLD in combination with type 2 diabetes and normal weight (5.11±0.22 vs. 2.87±0.07, $p<0.05$) and in the control group (5.11±0.22 vs 1.16±0.07, $p<0.05$). There was a direct strong correlation between HOMA-IR index and the steatosis degree ($\rho=+0.79$, $p<0.05$).

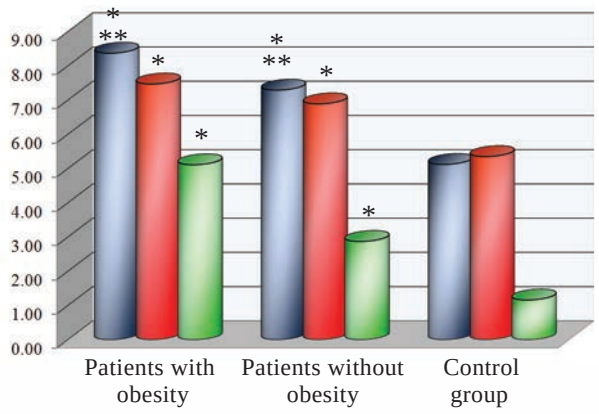

FIGURE *Carbohydrate metabolism parameters in NAFLD patients and in the control group.*
Fasting glycemia, *mmol/L;* HbA1c, µmol of fructose/*g* Hb; HOMA-IR, conv. units * *p<0.05 compared to the control group; ** p<0.05 compared to patients with normal body weig*

Table 2 presents the parameters of fat metabolism in the target groups. All NAFLD patients showed signs of lipid metabolism disorders. The levels of total cholesterol were 7.20±0.52 *mmol/L* in NAFLD patients with type 2 DM and obesity, and 5.80±0.98 *mmol/L* in NAFLD patients with type 2 DM and normal weight. It should be noted that the increase in total cholesterol levels in patients with the combined course of NAFLD, type 2 DM and obesity was significant when compared to the control group and the group of patients with normal body weight ($p<0.05$). The concentrations of VLDL and TG in groups of patients with NAFLD regardless of obesity were also significantly higher than those of the control group. The maximum concentration of VLDL was observed in the group of NAFLD patients with type 2 DM and obesity; the concentration amounted to 3.38±0.27 *mmol/L*, while the levels of VLDLs in the group with normal body weight and in the control group were 2.10±0.11 *mmol/L* and 0.68±0.08 *mmol/L*, respectively. Patients with NAFLD were characterized by increased levels of TG; in the obese group TG concentration was 5.10±0.13 *mmol/L*, while in patients with normal body weight it made 3.20±0.23 *mmol/L* that was significantly higher than concentration in the control group. There was found a significant decrease in the concentration of HDL cholesterol in patients of NAFLD groups: in the group of patients with concomitant type 2 DM and obesity cholesterol concentration was 0.62±0.09 *mmol/L*, whereas in patients with NAFLD combined with type 2 DM and normal weight it made 0.78±0.06 *mmol/L*. Comparison of the groups revealed significantly lower concentrations of HDL cholesterol in patients with NAFLD combined with type 2 DM and obesity compared to patients with normal body weight (0.62±0.09 *mmol/L* vs. 0.78±0.06 *mmol/L*, $p<0.05$) and in patients of the control group (0.62±0.09 *mmol/L* vs. 1.21±0.11 *mmol/L*, $p<0.05$). When analyzing the types of dyslipidemia observed in the groups of patients with NAFLD, we found prevalence of type IIA and IV according to D. Fredrickson: respectively, 16.7% and 58.3%, in the group of patients with NAFLD combined with type 2 DM and obesity, and 36.3% and 13.6% in the group of patients with NAFLD combined with type 2 DM and normal body weight, respectively.

Table 2.

Biochemical parameters of the examined subjects

Parameters	Patients with nonalcoholic fatty liver disease		Control group (N=20)
	type 2 diabetes mellitus and obesity (N=48)	type 2 diabetes mellitus (N=22)	
Total cholesterol, *mmol/L*	7.2±0.52*,**	5.8 ± 0.98*,***	3.2 ± 0.35
Tryglycerides, *mmol/L*	5.1±0.13*,**	3.2±0.23*,***	1.12±0.08
LIPOPROTEINS:			
High density *mmol/L*	0.62±0.09*,**	0.78±0.06*,***	1.21±0.11
Very low density. *mmol/L*	3.38±0.27*,**	2.1±0.11*,***	0.68±0.08
Low density, *mmol/L*	3.32±0.08*	3.2±0.08*	0.44±0.08
Apolipoprotein A-1, *mg/dl*	121.0±12.3*,**	138.1±10.9*,***	198.0±20.1
Apolipoprotein-B. *mg/dl*	112.9±5.6*	97.6±7.5*	61.9±3.8
Ratio of apo-B to apo-A1	0.93±0.016*,**	0.85±0.09*,***	0.31±0.02

Notes: ** p<0.05 compared to the control group;*
*** p<0.05 compared to patients with normal body weight;*
**** p<0.05 compared to patients with concomitant obesity.*

The analysis of the relation in the group of patients with NAFLD combined with type 2 DM and obesity showed the presence of a strong direct correlation between the concentrations of TG, VLDL and the degree of hepatic steatosis (ρ=+0.76, $p<0.05$; and ρ=+0.81, $p<0.05$, respectively).

In all NAFLD patient groups regardless of obesity some changes in the level of apo-A1 in serum were observed (Table 2). However, the concentration of apo-A1 was significantly lower in the group of patients with NAFLD combined with type 2 diabetes and obesity compared to patients with NAFLD combined with type 2 diabetes and normal body weight (121.0±12.3 *mg/dl* vs. 128.0±10.9 *mg/dl*, $p<0.05$) and the control group (121.0±12.3 *mg/dl* vs. 198.0±20.1 *mg/dl*, $p<0.05$). Differences in apo-A1 concentrations between NAFLD patients combined with type 2 DM and without obesity was also significantly different from the control group (138.1±10.9 *mg/dl* vs. 198.0±20.1 *mg/dl*, $p<0.05$). The observed changes depended on the severity of the IRS, which was reflected by a moderate correlation observed between the concentrations of apo-A1 and the HOMA-IR index in patients with NAFLD combined with type 2 DM and obesity (ρ=+0.51, $p<0.05$) and patients with NAFLD combined with type 2 DM and normal body weight (ρ=+0.45, $p<0.05$). The same relationship was observed between apo-A1 and BMI (ρ=+0.38, $p<0.05$), WC (ρ=+0.76, $p<0.05$) and WC/HC (ρ=+0.81, $p<0.05$).

The analysis of apo-B concentrations showed the inverse relation. In patients with NAFLD combined with type 2 DM regardless of the obesity there was an increase in the concentration of apo-B compared with the control group. In patients with NAFLD combined with type 2 DM and obesity compared to NAFLD patients with type 2 DM and normal weight the levels of apo-B were significantly higher (112.9±5.6 *mg/dl* vs. 97.6±7.5 *mg/dl*, respectively; $p<0.05$). In the group of NAFLD patients with type 2 DM and without obesity the apo-B concentrations were also higher than those in the control group (97.6±7.5 *mg/dl* vs. 61.9±3.8 *mg/dl*, $p<0$ .05). There was a negative correlation between apo-B and apo-A1 (ρ=-0.61, $p<0.05$). Apo-B value also positively correlated with IR degree – HOMA-IR (ρ=+0.76, $p<0.05$) and WC/HC (ρ=+0.69, $p<0.05$). The ratio of apo-B and apo-A1 was also significantly increased in the examined groups of NAFLD and type 2 DM. In NAFLD patients with type 2 DM and obesity apo-B/A1 was 0.93±0.016; in NAFLD patients with type 2 diabetes and normal body weight it made 0.85±0.09, and in the control group = 0.31±0.02. We revealed significant differences when comparing apo-B/A1 in

NAFLD patients with type 2 DM and obesity versus NAFLD patients with with type 2 DM and normal body weight ($p<0.05$). In this case, there was a positive correlation of this index with BMI ($\rho=+0.79$, $p<0.05$), WC/HC ($\rho=+0.67$, $p<0.05$) and HOMA-IR ($\rho=+0.72$, $p<0.05$).

Discussion

The investigation on apo-A1 and apo-B concentrations revealed significant changes in patients with NAFLD combined with type 2 DM either at obesity, or at normal body weight. In NAFLD patients with type 2 DM and obesity there was a significant decrease in the concentration of apo-A1.

The concentration of apo-B significantly exceeded this parameter in patients with NAFLD combined with type 2 DM and obesity, as compared to patients with NAFLD combined with type 2 DM and normal weight. The identified changes are likely to be regarded as "non-traditional" factors contributing to the cardiovascular risk development in NAFLD patients with type 2 DM and obesity, as it is known that "traditional" factors of cardiovascular risk include dyslipidemia, type 2 DM, abdominal type of obesity and IRS formation showing the early process of atherosclerosis.

The evidence of the obtained results is the fact that disorders in synthesis of atherogenic factors – the atherogenic lipid fractions and cytokines – are observed during the formation of NAFLD combined with obesity and type 2 DM. The increased levels of LDL and TG are meant against HDL lowering. It is known that reduction of cholesterol plasma concentration is associated with the increased risk of atherosclerosis. Anti-atherogenic properties of HDL are caused, perhaps, by its role in ensuring the reverse cholesterol transport to the liver, as well as endothelial function [*Lewis G., Rader D., 2005*]. Changes mediated by HDL cholesterol metabolism can contribute to the formation of so-called "dysfunctional HDL" observed at NAFLD combined with type 2 DM and obesity and may lead to the development of endothelial dysfunction [*Navab M. et al., 2005; Musunuru K., 2010*]. It is apo-A1 serum level that most reliably reflects HDL concentration.

Apo-A1 is a polypeptide consisting of 243 amino acids; its molecular weight is 28.1 *kDa* [*Zannis V. et al., 2004*]. Apo-A1 is mainly expressed in the intestine and the liver, but in minor amounts it can be determined in other tissues. Apo-A1 secreted in intestine then enters the blood flow in complex with chylomicrons and is integrated into the HDL by lipoprotein lipase [*Herbert P. et al., 1982; Brunham L. et al., 2006*]. In its turn, apo-A1 secreted in the liver is released into the bloodstream in the fat-free or minimally enriched forms and plays an important role in cholesterol biosynthesis *de novo* [*Zannis V. et al., 2004*]. In further transformation apo-A1 enriched with phospholipids and cholesterol takes the form of discoid and then spherical particles using enzyme lecithin-cholesterol acyltransferase. Both discoid and spherical particles interact with class B scavenger receptors type I that is important to realize anti-atherogenic functions of HDL.

Apo-B is a major protein of VLDL and LDL – 40% and 95%, respectively; this apolipoprotein is synthesized mainly in the liver. Apo-B is represented in two forms of apo-B-48 and apo-B-100. Apo-B-48 is synthesized in the intestine, where it conjugates with TG from food and free cholesterol from the intestinal lumen to form a chylomicron particles; then it is metabolized by the liver. Apo-B-100 is synthesized in hepatocytes and is presented in LDL, intermediate density lipoproteins (IDL) and VLDL. Apo-B is required for the binding of LDL particles to the corresponding receptors, providing capture of LDL cholesterol and thus its absorption. Excess particles containing apo-B are the major trigger of the atherogenesis process. The particles of small, dense LDL have a more pronounced atherogenic potential than large floating LDL molecules due to their increased ability to enter the subintimal space and provide their further adhesion to the matrix proteoglycans followed by oxidation, which increases the risk of atherothrombotic events [*Lamarche B. et al., 1997; Betteridge D., Morrell J., 1999*]. Probably, the effects of apo-A1 and apo-B in patients with NAFLD combined with type 2 DM and obesity are realized by carrier proteins in fatty liver that, in its turn, also modifies their metabolism and is involved in the regulation of lipid metabolism. In patients with NAFLD combined with type 2 DM and obesity the relationship between the development of IRS and impaired concentration of apo-A1 and apo-B was demonstrated. The pathophysiological changes that might underlie the relationship

between IRS, NAFLD, type 2 DM, obesity and increased cardiovascular risk can include both direct and indirect mechanisms.

First, at development of NAFLD combined with type 2 DM and obesity the metabolic abnormalities, which lead to decrease in the HDL concentration, are observed. Hypertriglyceridemia that is frequently observed in NAFLD combined with type 2 DM and obesity can result in decrease of HDL levels; it affects HDL exchange by enhancing their catabolism [*Despres J. et al., 1989*], which is realized due to cholesterol esters transport protein (CETP), which mediates the more intense transfer of TG from TG-enriched lipoproteins (VLDL and chylomicrons) to HDL compared to the normal one [*Patsch J. et al., 1983; Tall A., 1990*].

There are research works on kinetics of apo-A1 in patients with obesity. As a result of these studies a 30% reduction in the apo-A1 plasma retention time was shown in obese patients compared to those with normal weight. In this case, apo-A1 rate in obese patients did not differ from that in patients with normal body weight and good lipid profile. Thus, obesity enhances clearance of cholesterol in the plasma. These results are supported by research that showed a strong correlation between the level of intra-abdominal fat on magnetic resonance imaging device and clearance of apo-A1 (ρ=0.98, p<0.05) [*Brinton E., Terry J., 1996*]. Gain in apo-A1 clearance can serve as the main reason for the low level of apo-A1.

Such an increase in the catabolism of HDL in patients with NAFLD combined with type 2 DM and obesity might be also due to other indirect mechanisms – increased activity of hepatic lipase and CETP. First, it is known that the level of hepatic lipase activity increases during obesity development [*Dullaart R. et al., 1994; Deeb S. et al., 2003*]. High activity of the hepatic lipase is associated with a decrease in HDL2 concentration by hydrolysis of TG of their surface, thus reducing the stability of the particles and increasing HDL clearance [*Dullaart R. et al., 1994; Deeb S. et al., 2003*]. In its turn, CETP may play a role in enhancing the clearance of HDL: the activity of this enzyme is increased in obesity [*Dullaart R. et al., 1994*]. CETP activity, regardless of hepatic lipase and lipoprotein lipase, negatively correlates with the HDL levels [*Arai T. et al., 1994*]. CETP provides the transfer of TG from TG-enriched HDL and chylomicrons to HDL particles and also mediates the reverse transport of cholesterol esters from HDL to VLDL and chylomicrons [*Rashid S. et al., 2002*]. This results in the formation of cholesterol-enriched TG that directly leads to lower levels of HDL cholesterol [*Rashid S. et al., 2002*]. In turn, HDL particles rich in TG are good substrate for hepatic lipase, the activity of which, in its turn, also leads to an increase of HDL clearance [*Rashid S. et al., 2002*].

Similar results were obtained in the experimental work [*Karavia E. et al., 2012*: in mice with genetically deficient apo-A1 the researchers observed formation of hepatic steatosis, which was probably caused by the influence of apo-A1 to lipoprotein metabolism. That is, apo-A1 represents a structural component of the secreted chylomicrons in the intestine, it regulates the degree of their formation and secretion into the bloodstream and is an inhibitor of lipoprotein lipase [*Yamamoto M. et al., 2003*]; accordingly, apo-A1 deficiency enhances the lipolysis, thus increasing their catabolism.

The revealed changes of apo-A1 and apo-B metabolism in patients with NAFLD combined with type 2 DM and obesity can be caused by changes in the activity of hepatic lipase [*Miksztowicz V. et al., 2012*]. The activity of hepatic lipase in NAFLD is enhanced [*Miksztowicz V. et al., 2012*], it correlates with HOMA-IR and reduction in adiponectin, HDL, cholesterol and apo-A1 levels. In turn, such changes in the activity of hepatic lipase, might be conditioned by deficient insulin regulation of the enzyme due to IRS development and adipose tissue dysfunction observed at NAFLD combined with type 2 DM and obesity.

Conclusion

In patients with NAFLD combined with type 2 DM abse a significant reduction in apo-A1 concentration was found regardless of obesity. This index was significantly lower in patients with NAFLD combined with type 2 DM and obesity, which may indicate the formation of cardiovascular risk in this group of patients.

The concentration of apo-A1 was inversely dependent on anthropometric factors: BMI, WC, WC/HC. Furthermore, there was found an inverse correlation between the level of apo-A1 and HOMA-IR index which confirmed the role of IRS

in the metabolic regulation of apo-A1 and cardiovascular risk formation.

In examined patients with NAFLD combined with type 2 DM a significant increase in apo-B concentration and apo-B/A1 ratio was shown. The maximum values of these parameters were observed in patients with NAFLD combined with type 2 DM and obesity, these levels were significantly higher than those in patients with NAFLD combined with type 2 DM and normal body weight, indicating the earlier processes of atherogenesis in patients with NAFLD combined with type 2 DM and obesity.

Thus, given these results, it is conceivable that in patients with NAFLD combined with type 2 DM and obesity there are reliable predictors of coronary arteries disease, and, consequently, these patients are at risk of early cardiovascular events.

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