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# **Sex and Age Differences in Lipoprotein Metabolism Proatherogenic Changes under the Experimental Metabolic Syndrome in Hamsters**

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Additional information is available at the end of the chapter

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## **Abstract**

The unbalanced high-calorie diet can be the cause of a number of pathological states, including metabolic syndrome (MS). It is well known that the risk of MS increases with age, but gender differences in age-related lipid metabolism changes under this pathology are not fully understood.

In order to investigate the mechanisms of atherogenic dyslipidemia under the MS, we study the dynamics of some parameters of lipid and lipoprotein metabolism in hamsters of different sex and age. In our experiments, we found some age and gender differences in lipid and lipoprotein metabolism in healthy hamsters and hamsters with MS. In general, the obtained results demonstrate dyslipidemia development in males feeding high-calorie diet, irrespective of age. We suppose that hypertriglyceridemia in males under the high-calorie diet developed due to the accumulation of triacylglycerols (TAGs) in hepatocytes and as a result very low density lipoprotein 1 (VLDL1) over secretion by liver. However, in females feeding high-calorie diet atherogenic dyslipoproteinemia develops only with aging. It can be assumed that the reason why high-calorie diet in females leads to the pathological changes in VLDL morphology and hypertriglyceridemia development is reducing the hepatocytes sensitivity to insulin. Herewith, insulin resistance in females does not cause lipolysis activation in adipose tissue, which is probably associated with the ability of female

sex hormones to suppress lipolysis in adipose tissue regardless of sensitivity to insulin.

**Keywords:** lipoprotein metabolism, metabolic syndrome, hamsters, age differences

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## 1. Introduction

Metabolic syndrome (MS) is the complex of hormonal and metabolic disorders that increase the risk of type 2 diabetes mellitus and cardiovascular system (CVS) diseases [59, 78]. It was found the close pathogenic link between obesity, hypertension, insulin resistance (IR) and atherogenic dyslipidemia in the 60's of the last century [54].

According to clinical observations, MS was already registered in 20–25% of the adult population of the industrialized countries in 2004 [36, 61]. In epidemiological studies was found that among examined 8814 men and women older than 20 years the incidence of MS according to the US National Cholesterol Education Program (NCEP) criteria was about 24% among men and 25% among women in the USA [5, 6]. In another epidemiological study examining men of all ages, selected by random sampling, MS was diagnosed in 26.2% of the cases [37]. This prevalence of MS in the population increases with age and is highest among the elderly [24, 38]. In the results of other research groups related to the analysis of men and women, conducted in the USA, MS according to NCEP criterion was diagnosed in 6.7% people aged 20–29 years, in 43.5% people aged 60–69 years, and in 42% people aged 70 years [37,38]. It is also known that MS at a younger age is more common in men, but in women the incidence of MS increases gradually with age – especially during menopause [13, 78].

However, gender differences in age-related lipid metabolism changes under MS are not fully understood. In order to investigate the mechanisms of atherogenic dyslipidemia under MS, we study the dynamics of some parameters of lipid and lipoprotein metabolism in hamsters of different sex and age.

## 2. Material and methods

Experiments were planned to develop a diet-induced MS in Golden Syrian hamsters of different sex and age (4 weeks, 20 weeks, 1 year at the beginning of the experiment), which were kept in a standard vivarium condition. Animals were fed a standard normal diet (intact group), and for 5 weeks a high-calorie diet that contained 29% of fats (predominantly saturated) with fructose addition – 1 g daily per 100 g body weight (MS groups) [27, 63]. Blood and liver samples were taken after decapitation in necessary terms and prepared according to individual procedures.

Experiments were carried out according to the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Strasbourg, 1985).

Lipoprotein fractions (very low density lipoproteins (VLDL); low density lipoproteins (LDL), and high density lipoproteins (HDL)) were determined using electroforesis. Total LDL and apoB-containing lipoproteins (apoB-LP) in blood serum and hepatic cytosol were determined by gradient gel electrophoresis [1]. Using these data (apoB-LP concentration and data of LP fractions percentage), we calculated the content of every LP fraction. Total lipids (TL) were fractionated by thin layer chromatography on the plates with silica layers Silufol U.V.254" (Sklarny Kavalier, Czech Republic).

Triacylglycerol (TAG) content was determined by enzymatic assay ("KONE," Finland).

Free and esterified cholesterol (CE) was determined by enzymatic assays ("Boehringer Mannheim GmbH diagnostica," Germany). Total lipid concentration was determined by standard test using vanillin reagent (Eagle Diagnostics, USA).

Cholesterol esterification rate and cholesterol ester (CE) transfer was estimated in the HDL fraction received by centrifugation and then incubation of material and measuring of cholesterol and CE before and after incubation (for determination of cholesterol esterification rate – adding 5,5'-dithiobis(2-nitrobenzoic acid) [82].

Lipoprotein lipase (LPL, EC 3.1.1.34) activity and hepatic triglyceride lipase (HL, EC 3.1.1.3) were determined by the method of Lithell and Boberg [52].

Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) activity was measured using assay kit (Cayman Chemical, USA), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) using assay kit (Biocompare, USA), and malate dehydrogenase (EC 1.1.1.40) using assay kit (Biovision, USA).

Lysosomal acid lipase (LAL, EC 3.1.1.3) activity was measured in hepatic mitochondrial/lysosomal fraction by the substrate hydrolysis – 4-methylumbelliferone determined fluorometrically ( $E=449$  nm, 410 nm) [86]. Protein content was determined by Lowry in Miller modification.

Statistical analysis was performed using nonparametric van der Waerden criterion [21, 87] with packet Excel and Statistica, and the correlation coefficient was calculated by Spearman.

### 3. Results

Changes in blood hormone levels observed under the MS led to a shift in the lipolysis/lipogenesis balance and were accompanied by the excessive production of the free fatty acids (FFA).

According to our data, blood FFA levels in animals fed high-calorie diet were significantly increased in all experimental groups except the young females (Table 1).

Indeed, the FFA level was increased by approximately 40% in male experimental animals independently of age, and in young females this index was practically unchanged, but in the adult it increased also by 40% under experimental pathology. However, even such increased FFA level was 17% lower than in intact males of the same age.

Sex	Age	Group	Free fatty acids content, mmol/L
Males	4 weeks	Intact	1.02±0.07
		MS	1.44±0.29*
	20 weeks	Intact	1.64±0.16
		MS	2.29±0.25*
Females	4 weeks	Intact	0.91±0.42
		MS	0.85±0.03
	20 weeks	Intact	0.85±0.04
		MS	1.20±0.14*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* – p≤0.05 vs the same age intact group.

**Table 1.** The serum free fatty acids content in male Syrian hamsters with the experimental metabolic syndrome

Our study of lipid and lipoprotein metabolism in the blood serum and liver under modeling MS in Syrian hamsters of different sex and age also indicates the significant changes in lipid metabolism as well as sex and age differences of lipid and lipoprotein metabolism in the health animals and under the experimental MS.

Age	Group	Indices			
		Triacylglycerols, g/L	Total cholesterol, mmol/L	apoB-containing lipoproteins, g/L	High density lipoproteins, g/L
4 weeks	Intact	1.06±0.07	2.93±0.19	4.72±0.23	1.11±0.05
	MS	1.56±0.09*	3.56±0.10*	6.68±0.15*	0.98±0.07
20 weeks	Intact	1.57±0.22	2.84±0.15	5.66±0.34	1.01±0.02
	MS	2.00±0.13*	3.71±0.18*	6.68±0.21*	0.85±0.08
1 year	Intact	1.50±0.10	2.73±0.02	5.21±0.06	1.74±0.13
	MS	2.27±0.13*	3.15±0.08*	7.00±0.22*	2.32±0.13*

Intact groups – animals fed standard normal diet aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. Each group was composed of six animals.

Mean±S.D. \* – p≤0.05 vs the same age intact group.

**Table 2.** Some indices of lipid metabolism in blood serum of male Syrian hamsters with the experimental metabolic syndrome

Atherogenic dyslipidemia develops independently of age in males fed high-calorie diet (Table 2). As it can be seen from the data presented, increase of total lipids content in the blood serum of animals is mediated by the increase of apoB-LP level because the HDL content did not change. Herewith, the serum TAG level rose by 47% and 30% relative to intact groups in young and adult animals, respectively (Table 2).

According to our data the accumulation of apoB-LP in the blood and increasing of these class lipoproteins content in the liver undergo simultaneously (Table 3).

Age	Group	Indices				
		Total lipids (TL), mg/g	apoB-containing lipoproteins, mg/g	High density lipoproteins, mg/g	Glucose-6-phosphate dehydrogenase, nmol / mg of protein×min	Lysosomal acid lipase, nmol / mg of protein× min
4 weeks	Intact	104.24±2.52	11.46±0.37	1.25±0.14	3.74±0.33	0.67±0.03
	MS	124.16±2.05*	15.16±0.54*	1.11±0.07	2.80±0.17*	1.09±0.07*
20 weeks	Intact	112.62±2.66	13.03±0.50	0.94±0.10	4.44±0.28	0.54±0.03
	MS	143.59±2.65*	15.69±0.36*	1.10±0.20	3.13±0.28*	1.27±0.09*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* – p≤0.05 vs the same age intact group.

**Table 3.** Some indices of lipid metabolism in the liver of male Syrian hamsters with the experimental metabolic syndrome (for the damp tissue)

The significant changes in the lipid and lipoprotein metabolism were observed in the liver of adult male hamsters (1 year old) fed high-calorie diet (Tables 4 and 5). In particular, the changes of apoB-LP composition in the liver (Table 4) led to lipid depletion by lipoproteins.

Indices	Group	
	Intact	MS
Total cholesterol, % of the total content of fraction	9.46±0.81	7.18±0.06*
Triacylglycerols, % of the total content of fraction	45.33±1.39	42.00±1.29*

Intact group – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS group – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment. Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group.

**Table 4.** The composition of apoB-containing lipoproteins post mitochondrial fraction in liver of the 1-year-old male Syrian hamsters with the experimental metabolic syndrome

The low lipid content, predominantly TAGs, in the hepatic apoB-LP composition observed in our experiments (Table 4) indicated the lipolysis activation through the triacylglycerol lipases action (e.g., HL) under experimental MS.

Herewith, the lipoprotein uptake by the liver under experimental MS, obviously, is enhanced, as the content of the apoB-LP in this organ was increased (Table 5).

The liver G6PDH activity in experimental animals of this age group declined by 35% compared to the intact group (Table 5).

As can be seen from the obtained data, the 6PGD that is less than G6PDH is sensitive to damage by free radicals, retained its activity, and the NADP-dependent malate dehydrogenase activity even increased under experimental MS (Table 5).

Indices	Group	
	Intact	MS
Glucose-6-phosphate dehydrogenase, nmol NADPH(H <sup>+</sup> )/min× mg of protein	4.02±0.17	2.62±0.28*
6-phosphogluconate dehydrogenase, nmol NADPH(H <sup>+</sup> )/min× mg of protein	1.98±0.15	2.20±0.15
Malate dehydrogenase, nmol NADPH(H <sup>+</sup> )/min× mg of protein	14.57±0.40	15.09±0.03*
Total lipids, g/100 g for the fresh tissue	11.36±0.69	14.97±0.75*
apoB-containing lipoproteins, mg/g for the fresh tissue	14.71±0.46	18.92±0.84*

Intact group – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS group – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment. Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group.

**Table 5.** Some indices of lipid metabolism in the liver of 1-year-old male Syrian hamsters with the experimental metabolic syndrome

The lowering HDL-cholesterol level is apparently associated with an increased rate of CE transfer from HDL to apoB-LP. According to our data, the CE transfer rate from HDL in animals fed high-calorie diet was increased by 166% and 199% relative to young and adult intact animals, respectively (Table 6).

We recorded the decrease of serum LPL activity and increase in HL activity in young males fed high-calorie diet (Table 7). This may be an additional factor for TAG accumulation in the blood and decrease of the HDL-cholesterol level that we observed in our experiments (see Tables 4 and 6).

Some age-related features in the serum lipid profile were found in the healthy male hamsters with aging (from 4 to 20 weeks). Thus, levels of serum FFA (60%), TAGs (48%), and apoB-LP (20%) increased in 4 weeks old intact males, but the HDL level tended to decrease in 4 weeks

old intact males with the unchanged total lipid and lipoprotein content in the blood serum. All this testifies that lipidosis develops with aging. Also we found out that in adult males, the free cholesterol and cholesterol ester levels were lower than in young animals (20% and 25%, respectively), and the cholesterol ester transfer rate from HDL in adult animals exceeded this index in young animals 191% (Table 6).

Age	Group	Indices			
		HDL cholesterol, mcmole / L	HDL cholesterol esters, in mcmole / L	Cholesterol etherification, mcmole / L × hour	Transfer of cholesterol esters, mcmole / L ×hour
4 weeks	Intact	174.17±18.99	1028.33±12.76	54.92±0.58	20.42±1.76
	MS	80.83±9.17*	810.00±22.78*	49.00±2.50	33.83±1.56*
20 weeks	Intact	138.00±8.00	770.00±32.56	45.50±2.55	59.50±5.39
	MS	164.50±9.97	512.50±0.01*	20.25±2.28*	116.88±9.43*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* – p≤0.05 vs the same age intact group.

**Table 6.** The HDL-cholesterol and HDL-cholesterol esters content, cholesterol esterification rate, and cholesterol esters transfer in blood serum of male Syrian hamsters with experimental metabolic syndrome

Age	Group	Indices	
		Lipoprotein lipase (U/ml)	Hepatic triglyceride lipase(U/ ml)
4 weeks	Intact	8±2	51±4
	MS	4±1*	91±3*
20 weeks	Intact	83±2	3±1
	MS	129±3*	2±1

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* – p≤0.05 vs the same age intact groups.

**Table 7.** The post-heparin plasma lipase activities in male Syrian hamsters with experimental metabolic syndrome

The atherogenic dyslipidemia development significantly depends on the age in females in contrast to males (Table 8).

Age	Group	Indices				
		Glucose-6-phosphate dehydrogenase, nmol/min×mg of protein	Total lipids (TL), mg/g	apoB-containing lipoproteins, mg/g	HDL, mg/g	Lysosomal lipase, nmol/min×mg of protein
4 weeks	Intact	4.72±0.17*	117.67±4.72	8.87±0.24	1.27±0.08	0.34±0.03
	MS	5.38±0.13*	144.34±5.00*	10.24±0.25*	0.65±0.05*	1.24±0.05*
10 weeks	Intact	5.15±0.22	137.54±3.91	10.65±0.46	0.89±0.07	0.83±0.04
	MS	5.80±0.15	179.22±3.44*	13.44±0.30*	0.46±0.06*	1.33±0.08*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* – p<0.05 vs the same age intact groups.

**Table 8.** The indices of lipid metabolism in liver homogenate of female Syrian hamsters with experimental metabolic syndrome (for the damp tissue)

In particular, while significant changes in liver apoB-LP content in males was not observed with aging, this index in females increased at growing up in intact animals by 20%, and in animals with experimental MS by 31%. This indicates intensification of liver lipolytic processes in females with aging and may be a manifestation of the lipid metabolism activation that is proved by similar changes in TL content (Table 8).

Moreover, the female liver contains more lipids than male, especially in adulthood – in intact by 22%, while the MS by 24%. This can be explained by the well-known more pronounced effect of estradiol on liver lipid metabolism intensity compared to testosterone.

However, the G6PDH activity in females was significantly higher than in males, especially under MS by 92% in the young ones, and 85% in the adults (Table 8). In addition, this enzyme activity increased with aging. It can also indicate a significant dependence of the liver lipid metabolism rate from hormonal background. As can be seen from the obtained data (Table 9), feeding high-calorie diet did not lead to pronounced atherogenic changes in serum lipid and lipoprotein spectrum in young hamsters-females. The fact that young females had a more favorable serum lipid profile compared to males of the same age group also attracts attention (Tables 9, 10). Thus, the serum total lipid level in young females was 35% lower compared to males, and the total lipoprotein level lower by 32% (in young intact females 4.01±0.31 mg/ml).

Thus, lower serum total lipoprotein level in females may be associated with the decrease of the apoB-LP content (the content of this lipoprotein atherogenic fraction was at 39% lower in females compared to males, and the HDL content was similar in animals of both sexes).

As we can see in Tables 9 and 10, in females cholesterol metabolism also was changed in the blood stream, and these changes clearly depended on age: in young females the activity of CE transfer and the cholesterol esterification rate was increased, in the adults was increased only CE transfer, at the same time, the activity of cholesterol esterification rate decreased.

Age	Group	Indices			
		Triacylglycerols, g/L	Total cholesterol, mmol/L	apoB-containing lipoproteins, g/L	HDL, g/L
4 weeks	Intact	0.79±0.04	2.32±0.34	2.92±0.34	1.17±0.07
	MS	0.81±0.04	2.00±0.07	3.24±0.25	1.22±0.06
20 weeks	Intact	0.97±0.03	2.09±0.07	4.40±0.25	0.99±0.01
	MS	2.14±0.06*	1.91±0.17	3.57±0.12*	0.68±0.06*
1 year	Intact	1.48±0.14	2.54±0.08	4.03±0.07	1.85±0.23
	MS	2.20±0.09*	2.40±0.04	3.50±0.10	0.75±0.06*

Intact groups – animals fed standard normal diet aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* – p≤0.05 vs the same age intact groups.

**Table 9.** Some indices of lipid metabolism in blood serum of female Syrian hamsters with experimental metabolic syndrome

Serum TAG level and total cholesterol in young females was lower by 26% and 21% as compared to the corresponding values of these parameters in males, and the HDL-cholesterol in females exceeded the value of this index in males by 32% (Tables 9 and 10).

Age	Group	Indices			
		HDL-cholesterol, mcmol/L	HDL-cholesterol esters, mcmol/L	Cholesterol esterification, mcmole / L × hour	Cholesterol esters transfer, mcmole / L ×hour
4 weeks	Intact	230.83±7.46	1004.17±3.75	45.58±4.56	10.75±0.80
	MS	208.83±5.19*	835.50±20.53*	64.33±4.92*	23.83±3.53*
20 weeks	Intact	258.33±13.08	715.83±48.14	80.17±5.02	18.67±1.30
	MS	123.33±7.60*	650.00±22.36	34.25±3.14*	32.08±1.50*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* – p≤0.05 vs the same age intact groups.

**Table 10.** The HDL-cholesterol and HDL-cholesterol esters content, the cholesterol etherification activity and cholesterol ester transfer rate in blood serum of female Syrian hamsters with experimental metabolic syndrome

According to our data, the CE transfer rate in young females was 48% less than it was in the serum of males of the same age group (Table 10). The HL activity in intact young females was 28% less in comparison with males (Table 11).

Age	Group	Indices	
		Lipoprotein lipase (U/ml)	Hepatic triglyceride lipase (U/ml)
4 weeks	Intact	12±1	37±2
	MS	7±1*	66±7*
20 weeks	Intact	9±1	47±2
	MS	5±2*	94±6*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. \* –  $p \leq 0.05$  vs the same age intact groups.

**Table 11.** The post-heparin plasma lipase activities in female Syrian hamsters with experimental metabolic syndrome

However, it is interesting that female hamsters have more favorable (antiatherogenic) initial lipid serum profile than males. Thus, the total lipid content in intact young females was lower than in males by 35%, and it was almost 25% in adults. This index was almost two times lower than in males even with the MS in young females. Only adult females and males with experimental pathology hardly differed from each other. The same tendency is typical for the total lipoprotein content and TAG level.

The serum cholesterol content increased by 22% in juvenile age and by 31% in male adults with MS. However, the corresponding index in females remained practically unchanged, which confirms the absence of a direct correlation between the MS development and hypercholesterolemia. At the same time, there were the changes in lipoprotein fractions: in males with the MS the level of apo-B-LP increased (almost by 20% in adulthood), and the HDL content did not change. On the contrary, HDL level with MS decreased by 32% in adult females, and the content of the apoB-LP remained unchanged.

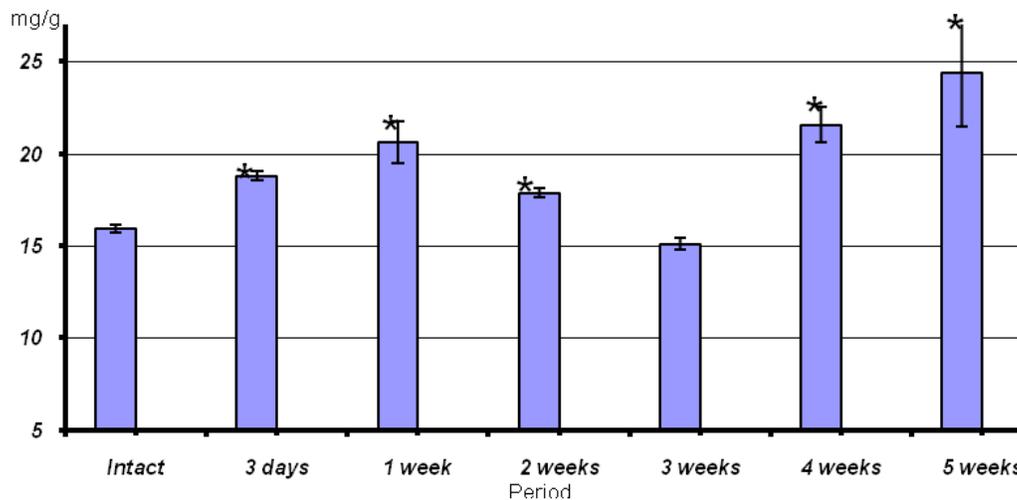
In intact males aged 4–20 weeks under unchanged total serum lipid and lipoprotein content, the levels of following values increased: TAG (by 48%), FFA (by 60%), and apoB-LP (by 19%). However, the HDL level tended to decrease. There were almost the same changes under experimental MS: the TAG level in adult animals with MS was higher by 28% as compared to the young animals with experimental pathology, FFA level – by 59%, HDL level – decreased by 14%, although the level of apoB-LP remained the same (see Tables 1 and 2).

All these data indicate to the hyperlipidemia increase due to age, which is further enhanced by the hyperinsulinemia and IR development. In females these changes were even more pronounced, although not quite so dramatic. In intact females the total lipid content grew with age by 37% (compared with 24% for males), lipoprotein by 32% (although it was lower than 21% for males), TAG by 22% (less than 39% for males), and apoB-LP by 50% (in males it was more than 33%), and the HDL content decreased by 16% (see Tables 1 and 9).

The situation under MS became worse: increase of the TL level in females having MS with aging was 88%, total lipoproteins was 81%, and TAGs was 164%, which was slightly higher than the corresponding indices in males. However, increased apoB-LP content in females with aging under MS was smaller than in the intact animals and 47% less than males in the older age group (see Tables 8 and 9).

For the next series of experiments we used 1-year-old male and female Syrian hamsters that are most likely to MS development and study pathological changes caused by high-calorie diet during 5 weeks in dynamics.

The liver TAG content in the animals fed high-calorie diet increased after 3 days of experiment and remained at a high level in further periods (see Figure 1). The presence of a significant positive correlation between the liver and serum TAG content in the experimental animals (correlation coefficient 0.9) confirms the leading role of the intracellular TAG content increase in the formation of hypertriglycerolemia in our experiments.



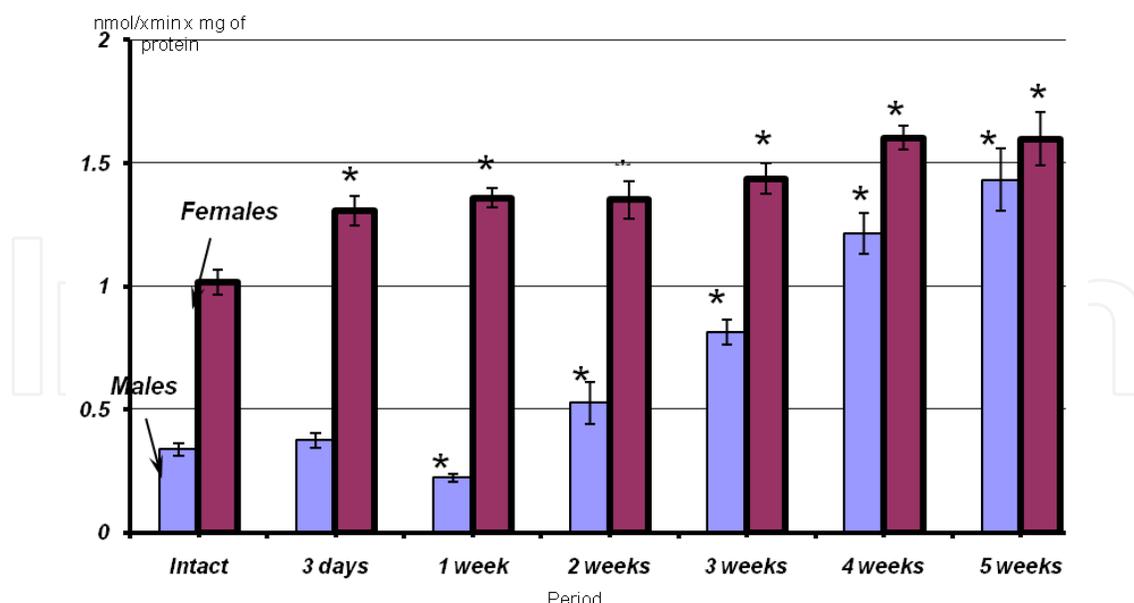
Each group was composed of seven animals. Mean±S.D. \* –  $p \leq 0.05$  vs intact group.

Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 1.** The liver triglyceride content in male 1-year-old Syrian hamsters under the experimental metabolic syndrome development (mg/g fresh tissue).

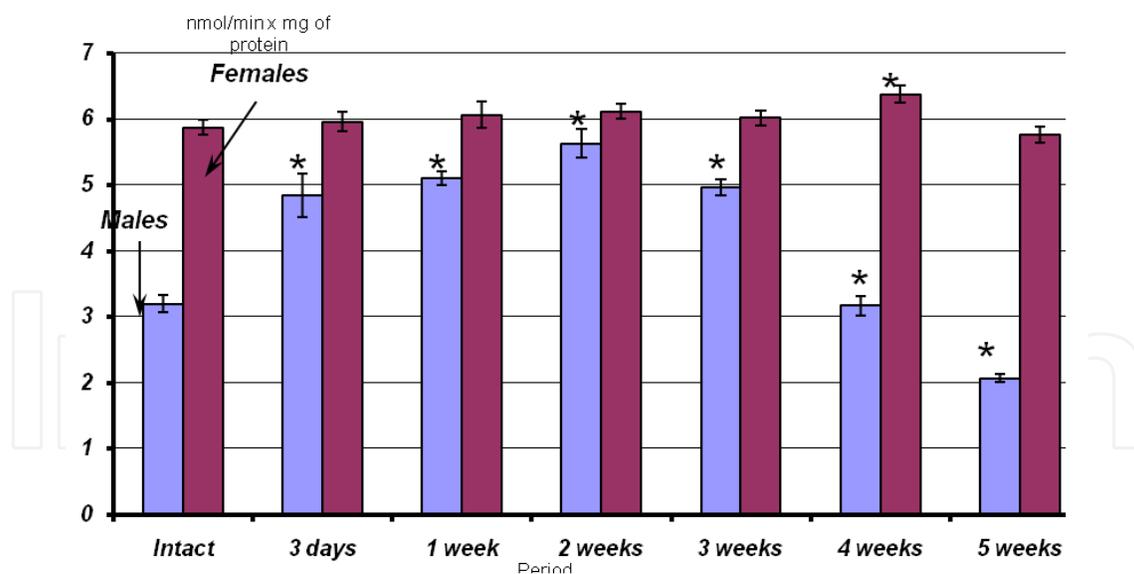
Based on our data, the LAL activity, which reflects the lipoproteins absorption intensity from the blood, decreased in the liver of experimental males at the beginning of our experiments, and it did not change significantly in females, and the enzyme activity increase was observed only after 2 weeks (Figure 2).

As can be seen from the data shown in the Figure 3, the G6PDH activity in males was increasing at the beginning of the experiment, probably because of pentose phosphate pathway activation, and after that it was decreasing, probably due to the lipid peroxidation (LPO) activation. As for females this rate was not changed significantly during the experiment.



Each group was composed of seven animals. Mean±S.D. \* -  $p \leq 0.05$  vs intact group. Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

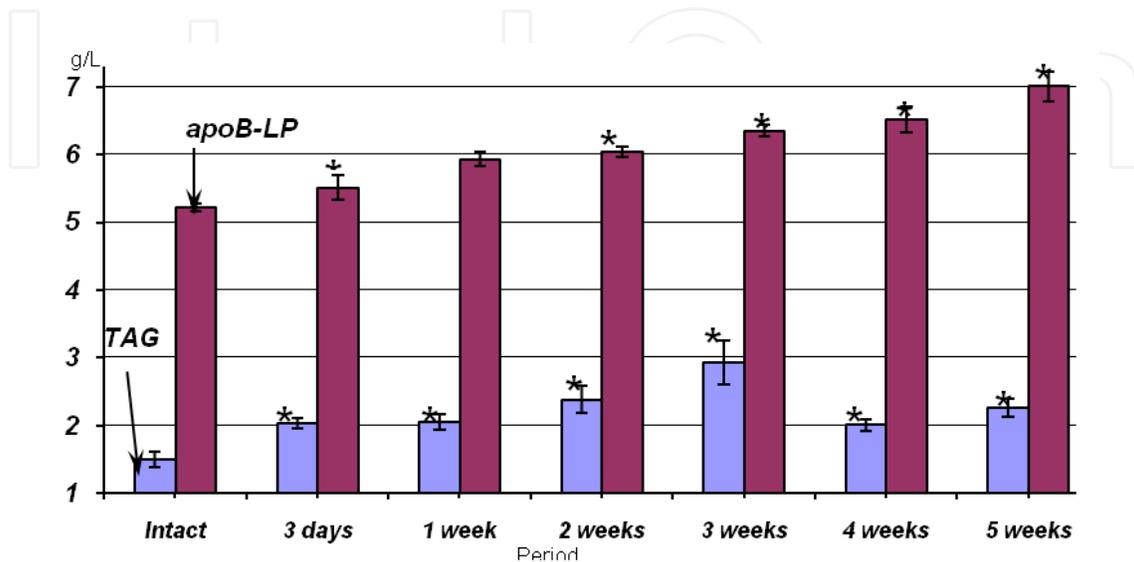
**Figure 2.** The liver lysosomal acid lipase activity in male and female hamsters under the experimental metabolic syndrome.



Each group was composed of seven animals. Mean±S.D. \* -  $p \leq 0.05$  vs intact group. Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 3.** The liver glucose-6-phosphate dehydrogenase activity in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.

As can be seen from the data obtained (Figure 4), the severe hypertriacylglycerolemia was developing fairly quickly in males fed high-calorie diet during the whole study period. Serum TAG content in the experimental animals increased after 3 days from the beginning of the experiment and reached its maximum value after 3 weeks (197% in regards to intact), and remained at a high level in the subsequent periods (Figure 4).



Each group was composed of seven animals. Mean±S.D. \* –  $p \leq 0.05$  vs intact group.

Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

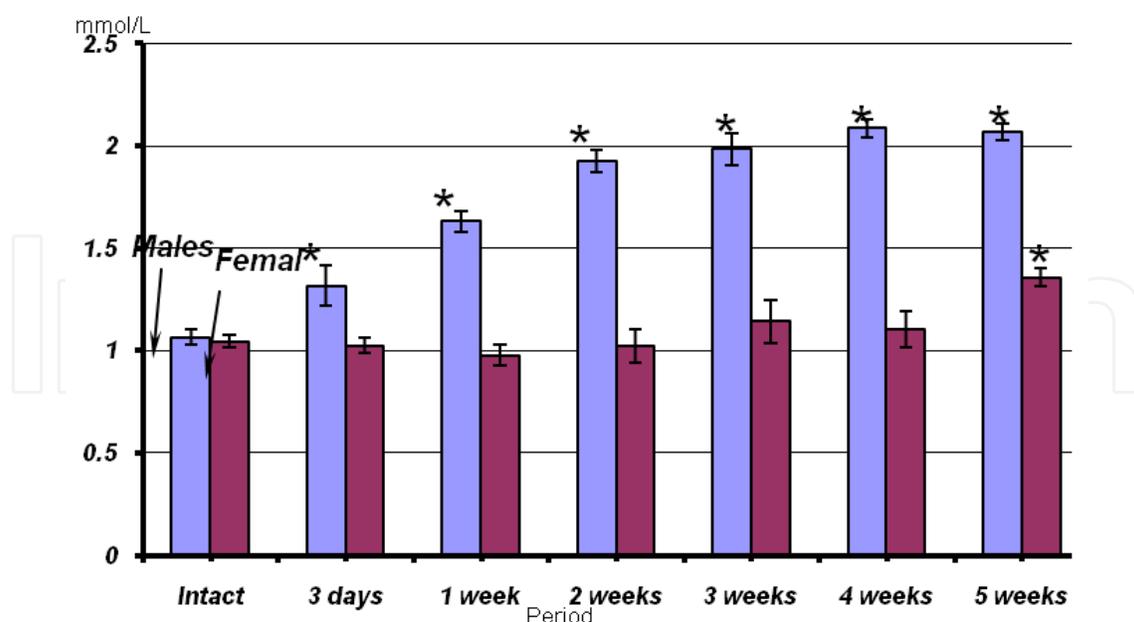
**Figure 4.** The serum triacylglycerols and apoB-containing lipoproteins content in 1-year-old male Syrian hamsters under the experimental metabolic syndrome development.

At the same time, according to our data, feeding high-calorie diet increased serum apoB-LP content in experimental animals, but the elevated levels of these lipoprotein fractions was observed at later time periods and was relatively less pronounced in comparison with the increased serum TAG levels (Figure 4).

The serum FFA content in animals also was increased after 3 days of the experiment and was increasing in subsequent periods as well (Figure 5).

In our experiments, the LPL activity was decreased rapidly since 3 days, during all the study periods (Figure 6), which indicates the stable disorders in VLDL utilization, and may be an additional factor that contributes to the hypertriacylglycerolemia development.

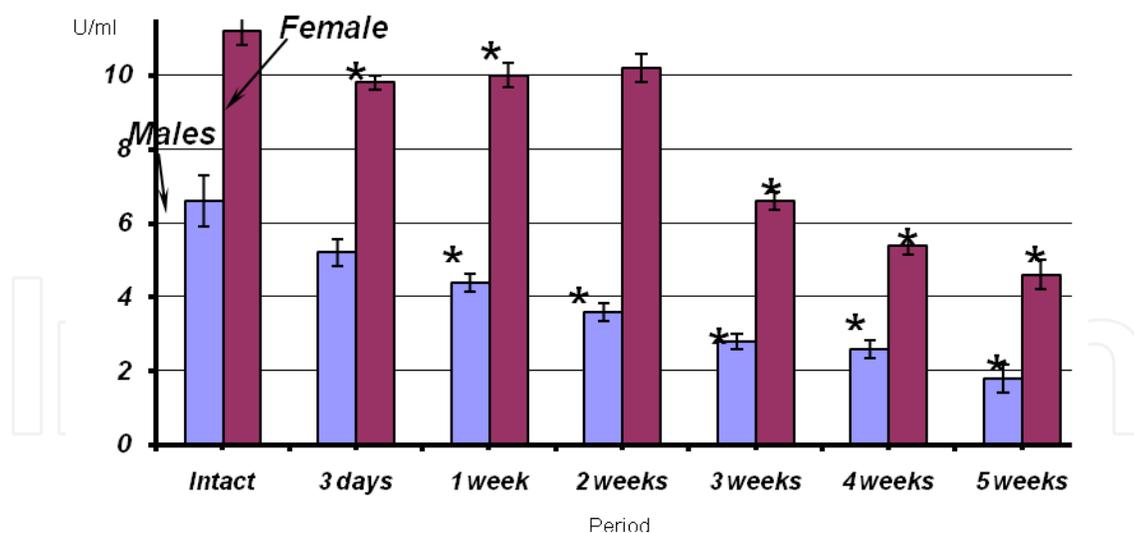
The abnormal cholesterol transport between different subfractions of lipoprotein particles, which leads to the blood atherogenic profile formation, is under discussion. As we have already noted, in our experiments the CE transfer rate was enhanced and this was already observed in the early stages of MS developing (Figure 7). It correlates with the serum TAG content increasing (the correlation coefficient is 0.77) and suggests that changes in apoB-LP morphology is one of the earliest manifestations of MS proatherogenic process.



Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group.

Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

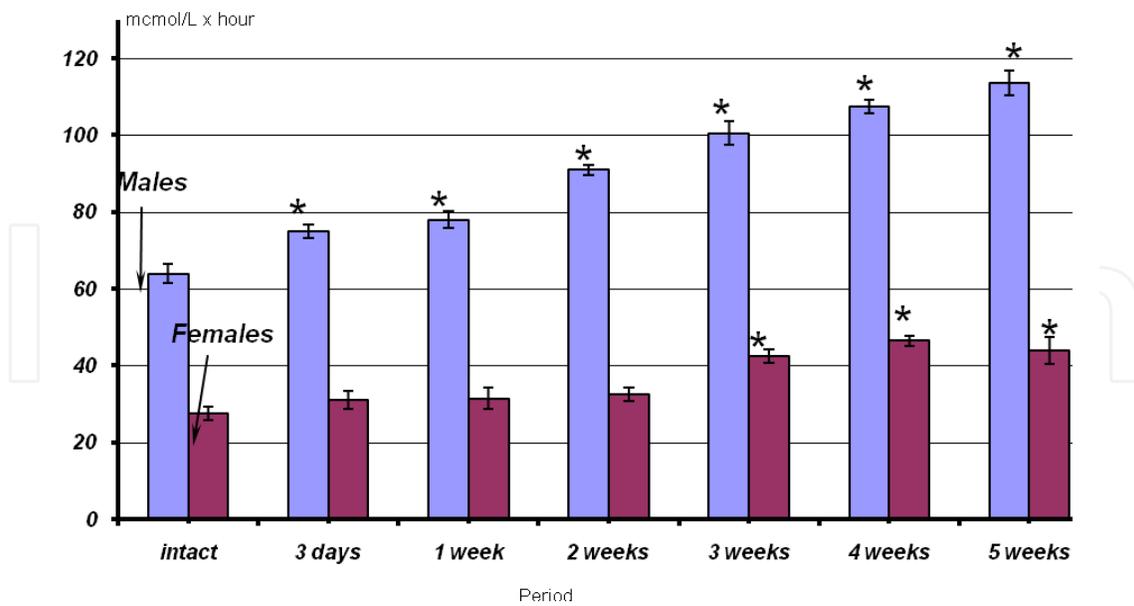
**Figure 5.** The serum free fatty acids content in 1-year-old male Syrian hamsters under the experimental metabolic syndrome development.



Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group.

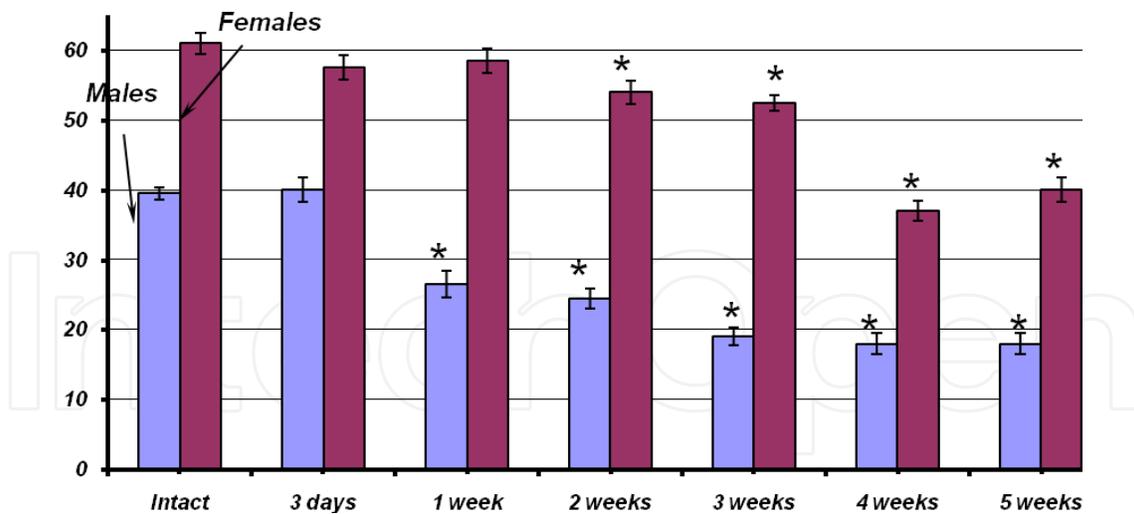
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 6.** The serum lipoprotein lipase activity in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome.



Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group.  
 Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

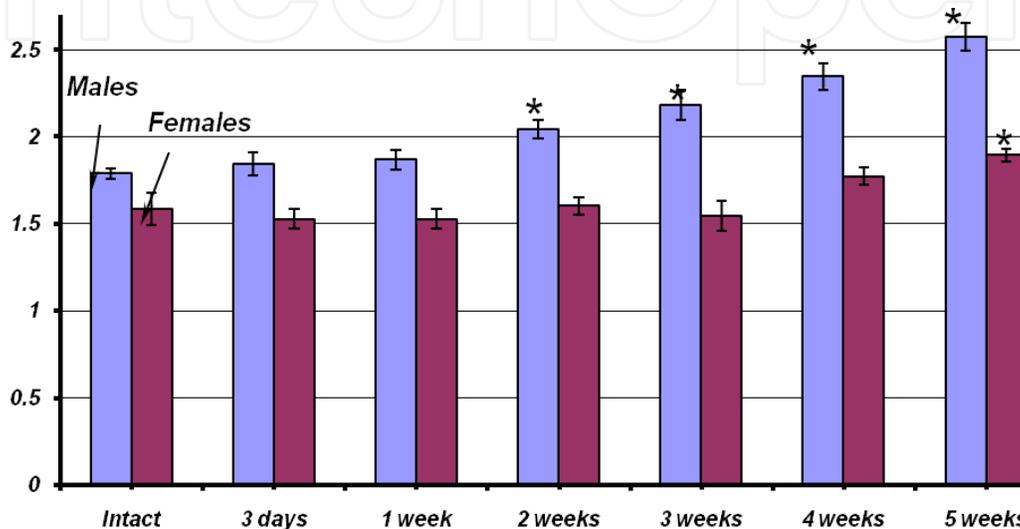
**Figure 7.** The cholesterol ester transfer rate in serum of 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.



Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group.  
 Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 8.** The cholesterol esterification rate in serum of 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome.

The changes in CE transfer activity can be accompanied by cholesterol metabolism changes in the LP composition. In particular, the HDL-cholesterol esterification rate had already decreased to the second week of the experiment in all investigated animals of this age group (Figure 8). However, the overall CE level in these antiatherogenic lipoproteins was decreased (Tables 5, 6, and 10). And this fact again underlines the significant role in CE transfer activation between different classes of lipoproteins in proatherogenic changes of lipid metabolism under the MS.



Each group was composed of seven animals. Mean±S.D. \* –  $p \leq 0.05$  vs intact group.

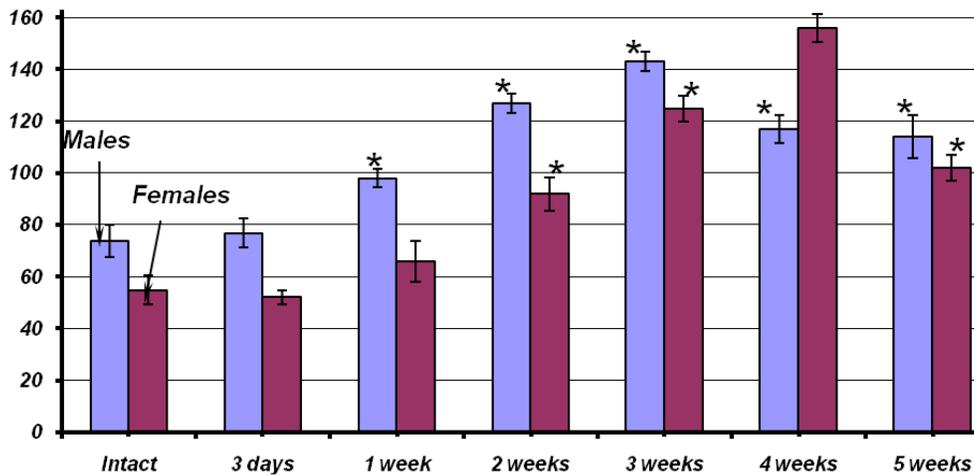
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 9.** The serum esterified cholesterol content in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.

At the same time, the total CE content in the blood of experimental animals was slightly increased (Figure 9), which is the consequence of the overall lipids accumulation in the blood and liver, and may be associated with more active hepatic cholesterol esterification under the growth of FFA absorption by this organ from the blood. It is known that CE along with the TAG is the transport form of FA; therefore, its number increase should lead to the activation of hepatic CE formation.

Compared with changes in cholesterol metabolism and transfer, HL activity was not increased so quickly with just one week since the beginning of the experiment and remains at a high level in subsequent periods (Figure 10).

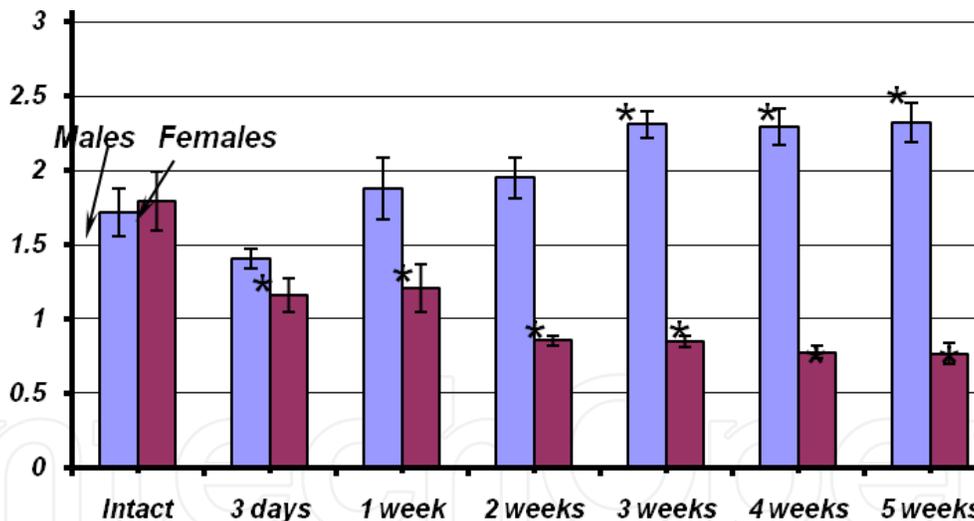
The opposite tendency in the HDL content changes in animals of different sex also attracts attention (Figure 11): we found out that HDL content reduced in females and increased in males in the last stages of MS development, but, as we already pointed out, it was decreased in animals of both sexes in the beginning of the experiment.



Each group was composed of seven animals. Mean±S.D. \* – p<0.05 vs intact group.

Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 10.** The serum hepatic triglyceride lipase activity in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.

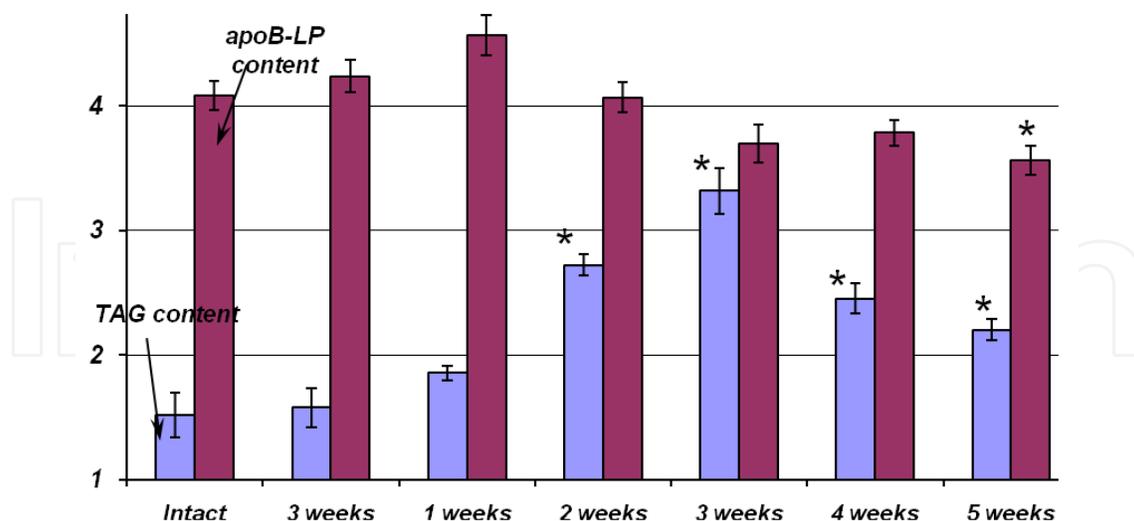


Each group was composed of seven animals. Mean±S.D. \* – p<0.05 vs intact group.

Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 11.** The serum high-density lipoproteins content in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.

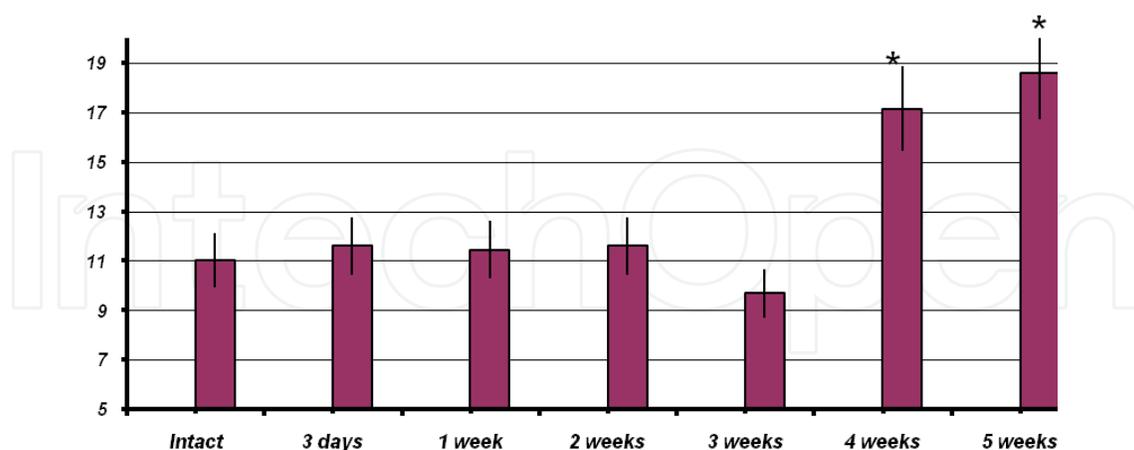
Unlike in males, serum TAG content did not change during the first week in females fed high-calorie diet, and the increase of this index values was observed only after 2 weeks from the beginning of the experiment (Figure 12).



Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group. Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 12.** The serum triacylglycerols and apoB-containing lipoproteins content in 1-year-old female Syrian hamsters under the experimental metabolic syndrome development.

At the same time, in the serum of females fed high-calorie diet the apoB-LP content did not differ from the intact level during the first 4 weeks of the experiment, and after 5 weeks we found out lower values of this index (Figure 12).



Each group was composed of seven animals. Mean±S.D. \* – p≤0.05 vs intact group. Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

**Figure 13.** The liver triacylglycerol content in female Syrian hamsters under the experimental metabolic syndrome development.

The hepatic TAG content did not change during the first three weeks of feeding high-calorie diet and after 4 and 5 weeks since the beginning of the experiment, we found increased values of this index relative to intact 55% and 69%, respectively (Figure 13).

#### 4. Discussion

The numerous experimental and clinical studies suggest that excessive body weight gain is associated with reduction of insulin potency to block lipolysis in adipose tissue [18, 32, 65, 92]. Suppression of lipolysis results in raising blood FFA level and intensifies their intake by insulin-dependent tissues, especially by the liver and muscles [36, 48]. Excessive FFA disrupts insulin binding to hepatocyte receptors and leads to the liver IR development. Such conditions cause gluconeogenesis activation, increase of glucose production by the liver, reduction of the insulin excretion rate, and as the result hyperglycemia and hyperinsulinemia development [48].

The intensive FFA uptake into the muscle cells disturbs the utilization and intracellular glucose metabolism in this tissue [8, 69]. The rates of glycolysis and glycogen synthesis are decreased in muscle cells, and also the uptake of glucose from the blood is considerably reduced. This enhances hyperglycemia and hyperinsulinemia and contributes to IR development.

Thus, raising blood FFA levels due to metabolic activity disorders in the adipose tissue can cause IR under obesity.

Numerous clinical studies proved [40, 72] that the FFA release rate from adipose tissue in women is lower compared with men. This regularity was also observed in obesity. For example, it was found [9] that the rate of FFA release from adipose tissue in men is twice higher compared with women under obesity.

Observed increasing of serum FFA content could be a result of lipid hydrolysis activation in adipose tissue under the body weight gain of animals in our experiment. Thereby (see Table 1), the data of our experiments indicated that the low serum FFA level maintained in young females fed high-calorie diet could be explained by gender differences involving estrogens in the regulation of adipose tissue lipolysis.

The molecular mechanisms that are the basis of these differences should be dependent on the different adipose tissue receptor activity in males and females. It is known [70] that the lipolysis regulation in adipose tissue is carried out mainly at the level of modulation of hormone-dependent lipases activity, particularly, by the insulin and catecholamines action. The activity of lipases is inhibited by insulin action. Catecholamines stimulate the activity of hormone-sensitive lipases indirectly via the  $\beta$ -adrenoreceptors ( $\beta$ -AR), and inhibit enzyme activity via  $\alpha$ 2-adrenoreceptors ( $\alpha$ 2-AR). Female sex hormones increase the number of  $\alpha$ 2-AR in women adipose tissue [58, 70]. It was found out that in women adipose tissue number of  $\alpha$ 2-AR is greater compared to men, while in men preponderate  $\beta$ -AR [16, 19, 77]. There is evidence that in women adipose tissue hormone-sensitive lipase has low sensitivity to the increased intracellular cAMP that is related to the lower protein kinase A (PKA) activity. It is also known

that estrogens are able to suppress lipolysis even at lower sensitivity of adipocytes to insulin. All of this evidence can determine the lower lipolytic activity in women adipose tissue compared to men.

The sex differences in the regulation of adipose tissue lipolysis become less pronounced with aging [43]. These differences can be associated with significant hormonal changes in women with aging and specifically depend on the decrease in the sex hormone levels [97], increased glucocorticoid hormones secretion, and reduced sensitivity to insulin. These changes increase the risk of MS development in women under obesity with aging.

According to our data, serum FFA level in adult females fed the high-calorie diet increased to the same level as that in same-age males (see Table 1). The latter can be associated with the age-related hormonal changes in the female body (correlation coefficient between age-related changes of FFA content and estradiol is 0.75).

Despite the differences in the release rate of FFA from adipose tissue, the feeding high-calorie diet leads to hyperglycemia and hyperinsulinemia development and aggravates insulin resistance in experimental animals, regardless of gender or age [96, 97]. This indicates that MS development in females is independent to adipose tissue lipolytic activity.

Thus, one of the main features of metabolic proatherogenic changes that we observed under experimental MS was a significant increase in serum FFA level. FFA overabundance could not affect the rest of the lipid metabolism links that leads to general lipid and lipoprotein metabolism disorders and is one of the key components of MS.

Clinical studies [15, 39, 76] show that dyslipidemia developed under MS is characterized by serum TAG level increase, HDL-cholesterol level decrease, and accumulation of LDL that have a high atherogenicity (LDLB).

The blood TAG content increase under MS is considered to be the key factor in the atherogenic dyslipidemia formation. A clear correlation between hypertriacylglycerolemia, HDL-cholesterol level decrease, and LDLB accumulation in the blood plasma demonstrated in numerous experimental and clinical studies [4, 39, 45, 54, 55, 74].

It is known that hepatic VLDL hyperproduction plays the leading role in the TAG and apoB-LP accumulation in blood under the MS development [62].

So we can suppose that VLDL hepatic production is activated in animals fed a high-calorie diet during our experiments.

Based on these data, we can suppose that lipolysis activation and FFA accumulation in the blood lead to morphological changes of lipoproteins that are secreted by the liver under the MS development.

Mechanisms of VLDL hyperproduction in the liver under FFA intensive uptake by hepatocytes still remains not fully understood. This activation may occur both using FFA, which intensively enter from the blood, or via the stimulation of *de novo* fatty acids synthesis that is caused by hyperglycemia.

It is known that FFA, which enters into the liver cells from the blood, is mainly used for TAG resynthesis under IR. This leads to increased intracellular TAG content and correlated with increased VLDL secretion rate to the blood [22, 29]. The VLDL composition, which is determined primarily on the second stage of their formation, significantly depends on the intracellular TAG content and hepatocytes sensitivity to insulin [33]. The intense pre-VLDL lipidation involving phospholipase D takes place under the conditions of intracellular TAG content increase and hepatic insulin resistance [7, 64]. Insulin blocks the VLDL formation in the liver [7]. These changes, combined with the TAG intracellular content increase under the IR, determine mainly the VLDL1 formation and secretion by the liver.

In our experiments, the activation of *de novo* fatty acids synthesis in the liver, obviously, did not occur. The G6PDH activity decrease in this organ proved this suggestion (see Table 3). It is known that the activity of G6PDH, which is the main donator of NADP reduced, directly correlates with the lipogenesis activity [38]. A certain contribution to the VLDL formation activation in the liver of animals that was fed high-calorie diet probably makes remnant lipoproteins (RLPs) uptake by the hepatocytes from the blood stream. The increasing LAL activity in the liver of experimental animals (see Table 3) is the evidence of this process. LAL is involved in the RLPs degradation that enters hepatocytes by receptor-mediated transport.

We found the direct correlation between the serum FFA content in animals fed high-calorie diet and apoB-LP content in the liver (correlation coefficient is 0.77). The FFA content is also correlated with the content of the TAG and apoB-LP in the blood serum of the studied animals (coefficient of correlation between the content of FFA and apoB-LP and FFA and TAG is 0.9). Hence, we can suggest that the main cause of TAG-rich lipoproteins hyperproduction by the liver is really the flow of FFA large amounts to this organ from the blood under feeding high-calorie diet.

Therefore, the increase of apoB-LP content in the liver is obviously linked to the activation of TAG synthesis using FFA that undergo to this organ from adipose tissue due to the activation of lipolysis. The high serum FFA content proved the lipolysis activation (see Table 1).

A number of studies have been shown that hypertriacylglycerolemia is always accompanied by the HDL-cholesterol content decrease and LDLB accumulation in blood [11]. LDLB are highly atherogenic, because of their small size, high sensitivity to oxidative damage, and low affinity to selective LDL receptors.

A clear correlation between blood serum TAG content and LDLB was demonstrated in many studies, indicating a prominent role of the TAG content in the blood for the formation of the LDL morphology. It is also known that LDL morphology is determined primarily by the morphology of their precursors – VLDL. VLDL1 has relatively high triacylglycerol content, slowly metabolized and remained for a long time in the blood stream. Increased hepatic VLDL1 secretion leads to the LDLB formation, whereas VLDL2 are precursors of LDLA that have a low level of atherogenicity and are dominated in the normal state.

Thus, the probable reason for activation of the apoB-LP formation in the liver in males under MS development is the FFA intake from adipose tissue to this organ. However, high serum apoB-LP level registered in our experiments is the evidence for increased hepatic secretion of

these lipoproteins (see Table 2). As mentioned above, increased serum cholesterol level was also observed (see Table 2). Herewith, the HDL level did not change. These changes indicate that the increased cholesterol level rose due to apoB-LP cholesterol content. The determined changes are typical for many people with MS and have specific proatherogenic character.

It is well known that hyperinsulinemia and insulin resistance contribute to the lipolysis activation and lipogenesis suppression under MS. So, the biggest part of the liver post-mitochondrial fraction should be composed from lipoproteins that absorbed from the blood stream, but not those that synthesized in the liver. And presence of lipid-depleted particles also confirmed the intensification of lipoprotein metabolism in the blood stream.

Probably the activation of free radical oxidation plays the key role because of G6PDH strong sensitivity to the reactive oxygen species (ROS) action. As a result, apoB-LP peroxidation is activated and levels of antioxidants (reduced glutathione (GSH), ascorbic acid, and alpha-tocopherol) are decreased in the liver [97].

These changes should probably contribute in maintaining reduced NADP level, which is necessary for glutathione reductase (GR) [97] and effect the cholesterol synthesis. However, the GR activity decline and GSH level decrease indicates the insufficient antioxidant defense systems activity considering lipidosis and domination of oxidative processes under experimental MS. This fact is also confirmed by the alpha-tocopherol content reduction [94, 95].

Hence, based on our results and literature analysis, we can note that VLDL1 formation is activated in the liver of males fed high-calorie diet independently of age. This is probably one of the reasons for the TAG and apoB-LP accumulation in the blood serum of the experimental animals. The activation of the VLDL formation and secretion by the liver under MS is the result of many changes. In particular, increasing intracellular TAG and CE content, which is mediated by high FFA load into hepatocytes, caused probable activation of microsomal triglyceride transfer protein (MTP) and apolipoprotein B100 (apoB100) synthesis and stabilization of apoB100 [99]. Herewith, TAG-enriched VLDL (VLDL1) secretion is increased under hepatocyte insensitivity to insulin and intrahepatic TAG accumulation.

Thus, summarizing the results, we can state that not only hyperinsulinemia and insulin resistance make an important contribution to the MS development, but other factors that are the result of obesity as well: changes in activity of lipogenesis and lipolysis systems, FA accumulation that leads to lipoproteins metabolism disturbances, etc. Naturally this metabolic situation undoubtedly affects the integral state of the body metabolism.

Hence, the increase of the hepatic VLDL1 secretion should cause the significant changes in lipid and lipoprotein metabolism in the blood stream: an increase of TAG content and LDLB accumulation in the blood, which have a high atherogenicity. These changes are the characteristics of MS and are considered to be the risk factors for the atherosclerosis development.

Therefore, to study the mechanisms of the dependence between LDLB accumulation, serum FFA accumulation, and changes in the VLDL morphology became the next task of our research.

Plasma apoB-LP metabolism is closely linked to HDL metabolism, which carry out the reverse cholesterol transport from peripheral tissues to the liver [9]. The transfer rate of CE from HDL

to apoB-LP involving cholesteryl ester transfer protein (CETP) [91] and hydrolysis of the TAGs in apoB-LP composition involving LPL and HL are important factors of the process of VLDL conversion to LDL in the blood stream [81].

A lot of clinical studies show that increased CETP activity in HDL composition is mostly accompanied by HDL-cholesterol level decrease and blood plasma LDLB accumulation and these changes are correlated with the blood TAG content [51, 83].

According to the literature data, the blood TAG content increase is the factor that leads to disorders in the processes of cholesterol reverse transport, which HDL participate in. The transfer of CE from the HDL to apoB-LP with the participation of CETP is the key component of the cholesterol reverse transport. At that the rate and direction of CE transfer depends primarily on the TAG content in VLDL composition. At the normal state, CETP transfers CE from the HDL to LDL that have a high affinity to hepatic LDL receptors containing apolipoproteins B and E (E/B-LDL), and LDL are rapidly removed from the blood stream. Thus, CETP reveals antiatherogenic action by stimulating the reverse transport of cholesterol. VLDL does not accept the CE and turn into LDL involving LPL. The high TAG content in the VLDL composition increases their ability to accept the EC. It was found that VLDL1 became the main acceptors for CE from HDL under hypertriacylglycerolemia when it is caused by the VLDL1 increase in the blood.

Hence, the CETP activation should be atherogenic for two reasons:

- Firstly, CE-enriched VLDL that are formed converted to LDLB with the HL action.
- Secondly, TAG-enriched HDL are formed and hydrolysis of TAGs in their composition involving HL lead to their rapid removal from the blood stream and result in the HDL-cholesterol level decrease.

A recent study has shown the significant changes in cholesterol and HDL metabolism in the blood serum of animals fed high-calorie diet. These proatherogenic changes are suggested as one of the reasons for LDLB accumulation in the blood. The determined increasing of serum total cholesterol (see Table 2) in hamsters fed high-calorie diet is obviously related to the high cholesterol in apoB-LP composition because the HDL-cholesterol level decreased (see Table 6).

It was found that levels of HDL-cholesterol and HDL-CE lowered in young males, whereas in adults it decreased only CE content. The CE transfer rate from HDL to apoB-LP is activated under increased blood TAG content that was observed at postprandial period [73], as well as at apoB-LP metabolic disorders [23].

In both cases, the CE transfer activation is a consequence of increased TAG-enriched lipoproteins level in blood. This is also confirmed by the increased neutral lipids content of the apoB-LP composition in hamsters with experimental MS [95-97]. These differences are probably based on different rates of HDL cholesterol esterification in males of different age groups (see Table 6), which is mainly determined by the lecithin: cholesterol acyltransferase (LCAT) activity – the enzyme that is associated with HDL.

Increased CE transfer activity from HDL is suggested to be a consequence of the CETP activation. Increased CETP activity under MS was demonstrated in a large number of studies

[14, 75, 79, 91, 99]. It is known that increased activity of CETP in the blood HDL composition is the result of the CETP synthesis activation in the liver, but the mechanisms of this protein induction remain not fully understood.

Thus, the enhanced CE transfer rate from HDL under hypertriacylglycerolemia that was observed in our experimental animals fed high-calorie diet (see Table 6) caused atherogenic changes because CE transfer primarily to TAG-enriched lipoprotein fractions leads to the CE-enriched VLDL1 accumulation, which are the main LDLB precursors. Intensive TAG uptake by HDL in exchange for CE leads to the TAG-enriched HDL accumulation in the blood. TAG-enriched HDL is the preferred substrate for HL and is rapidly removed from the blood stream that leads to the HDL-cholesterol content reduction.

Another factor that can affect the lipoprotein metabolism and atherogenic LDLB formation is the transformation of VLDL to LDL. It takes place in the blood stream involving a number of lipases.

Therefore, changes in the activity of enzymes, which catalyze lipid hydrolysis in lipoproteins in the blood stream, particularly LPL and HP, affect significantly lipoprotein metabolism under MS.

The first enzyme in the vascular lipoprotein transformation cascade is the LPL, which is synthesized mainly in adipocytes and myocytes. TAGs in the TAG-enriched lipoproteins composition (chylomicrons (CM) and VLDL) are substrate for the LPL. FFA released during the hydrolysis by LPL are absorbed by adipocytes and muscle cells where they are involved in TAG synthesis or used as an energy source. TAG hydrolysis in the VLDL composition increases the cholesterol availability to be transferred to HDL, so LPL mediates the reverse cholesterol transport. The LPL activity is regulated via transcription activation, translation, and enzyme transport from the cells [49, 55]. Insulin activates LPL in a healthy state that leads to blood TAG content decrease and reverse cholesterol transport stimulation [28].

According to our data, the LPL activity decreased in the blood serum in young male hamsters fed high-calorie diet (see Table 7). Our results corresponded with the literature data about LPL activity decrease under obesity and IR [60]. Mechanisms of LPL inhibition under these conditions remains to be not fully understood, although IR development may contribute.

The other enzyme – HL, necessary for lipoprotein intravascular transformations is synthesized in hepatocytes, secreted and binds to endothelial cell proteoglycans of hepatic vessels. HL hydrolyzes TAGs and phospholipid content of the different lipoprotein fractions and plays a leading role in their metabolism [41]. It was found that HL mediates selective transport of VLDL remnants (rVLDL) to hepatocytes via the LDL-receptors and participates in the reverse cholesterol transport by stimulating the HDL flow to the liver via scavenger receptors (SR-B1). HL hydrolysis TAGs in apoB-LP content hence plays a significant role in their remodeling in the blood stream. It is known that the HL activity makes a great effect on the lipid composition, size, and properties of LDL [15, 22].

HL activity is regulated mainly at the level of transcription involving sex hormones, glucocorticoids, and adipokines [2]. Intension of HL gene transcription also depends on the lipid

intracellular content in hepatocytes, predominantly cholesterol [20]. In our experiments, the HL activity in the blood serum of males fed high-calorie diet rose irrespective of age (see Table 7), which is consistent with literature data. A number of authors reported that the HL activity was increasing under IR, obesity, and high-calorie diet [30]. HL mRNA content increased in the liver of Syrian hamsters fed high-calorie diet that is the evidence of HL synthesis activation. The authors associated this activation with serum adiponectin content decrease because of the ability to suppress the HL synthesis in hepatocytes.

Taking into consideration these data and the data obtained in our studies [95, 96] that proved the reduction of the serum adiponectin content under obesity, we can suggest that one of the reasons of HL activity increase is reduced adiponectin secretion in adipose tissue under high-calorie diet provided in our experiments.

The increased HL activity is seen as one of the key factors of the atherogenic dyslipidemia development under obesity and MS [10]. Some studies demonstrated a clear correlation between the HL activity and the serum LDLB content [15]. It is considered that the HL activation leads to increased LDLB formation [2]. The latter occurs under the conditions of increasing TAG-enriched VLDL1 content in blood and CETP activation. Moreover, the HL activity increase reduced the HDL-cholesterol level [89]. This happened because the hydrolysis of TAGs in the HDL3 content leads to their transformation into HDL2, which are rapidly removed from the blood stream by the liver. Thus, reducing HDL-cholesterol level observed during our experiments (see Table 6) may be a consequence of HL activity increase.

Hence, we found that serum FFA level increase was accompanied by the activation of the apoB-LP synthesis by the liver in male Syrian hamsters fed high-calorie diet independently of age. The activation of the apoB-LP synthesis causes the increase of TAG and apoB-LP levels in blood. HDL-cholesterol level reduction is obviously a consequence of the cholesterol ester transfer activation from HDL to LDL via the CETP and the HL activation. The development of the atherogenic dyslipidemia, which is the feature of MS, and increased blood atherogenicity are observed as the result of these changes. The received data are agreed with the literature data about the lipid metabolism age-related changes in males that have proatherogenic character [90]. It is known that the level of sex hormones decreases and the level of glucocorticoids secretion increases in men with aging [67]. The blood plasma lipid profile in men is also determined by the level of secretion of sex hormones that have anti-atherogenic properties [93]. A number of studies indicated the direct correlation between blood testosterone and dihydrotestosterone levels and content of HDL-cholesterol [25, 46, 47, 68]. Besides, the high level of sex hormones is correlated with the decrease of TAGs and total cholesterol [12, 47]. Thus, the TAG content increase and HDL-cholesterol content reduction in the male blood serum with aging may be associated with reduced sex hormone secretion [97]. The serum lipid profile changes in males with aging may also be associated with glucocorticoid increased secretion observed in our experiments [95-97].

Thus, in males with aging blood plasma lipid profile undergoes unfavorable changes that are manifested by the FFA and TAG content increase and lowering of HDL-cholesterol level. These changes may be associated with reduced sex hormone levels and increased cortisol secretion. Herewith, atherogenic dyslipidemia develops independently of age under obesity and IR

despite the more favorable blood plasma lipid profile in young males compared with healthy adult animals.

Thus, activation of the *de novo* fatty acid synthesis in the liver, probably, does not occur as was proved by the G6PDH activity decrease (see Tables 3 and 5). It is known that the G6PDH activity, which is one of the main generators of reduced NADPH, directly correlates with the lipogenesis activity.

There is information about glucose-6-phosphate accumulation, which is utilized by pentose-phosphate pathway, occurs under MS. Therefore, the increase of glucose-6-phosphate content can be an important consequence of reduced NADP accumulation in cells. The close correlation was found between NADPH(H<sup>+</sup>) content and fatty acid synthase activity. Therefore, changes in the activity of dehydrogenases that reduce NADP<sup>+</sup> can be an indicator of the lipogenesis intensity. As we have already pointed out, we found out that MS was accompanied by increased NADP-dependent malate dehydrogenase activity and a reduced activity of pentose-phosphate pathway dehydrogenases (see Tables 5 and 8). So *de novo* lipogenesis activation insignificantly contributes to hyperlipidemia development. Herewith, lipid content in the blood serum and liver homogenate increased significantly. These results are consistent with literature data that under MS mitochondrial lipid oxidation inhibition is primary and is no significant influence on the FA and steroids synthesis rate [53, 75].

Despite the physiological serum apoB-LP level, the apoB-LP content increased in the liver of old females in 10 weeks (see Table 8). This is probably connected with the activation of lipid synthesis using FFA, which is released during the hydrolysis of lipoproteins received from the blood stream. The increased activity of liver LAL that catalyzes the hydrolysis of lipids received via receptor-mediated endocytosis is the confirmation of this suggestion. An increase in the apoB-LP content (see Table 8) in the liver of adult females was also found. However, unlike in young animals, the synthesis of apoB-LP TAGs, probably, involved FFA that undergo to the liver from the blood stream due to lipolysis activation in adipose tissue.

The increased serum FFA level in adult females confirms the lipolysis activation (see Table 1). According to the literature data [26] and our study [96] estrogen secretion decreased with aging, and, as a result lipolysis increased in adipose tissue under the reduced insulin sensitivity. This increases the risk of MS in women with aging. Our results suggest that the risk of atherogenic dyslipidemia development, which increases in females with aging, is probably connected with sex hormones metabolism changes with aging, in particular, with increasing extragonadal estrogen production.

We observed the reduction of HDL level in the liver of females independently of age (see Table 8). Considering the literature data [66, 85] regarding the gender features of lipoprotein metabolism, it can be suggested that the decrease of the liver HDL content in females under the experimental MS is connected not only with changes in their formation, but with active uptake by tissues, including adipose tissue, which is less than characteristically for males. It is known that the abdominal fat accumulation in males occurs due to the TAG-enriched lipoproteins because testosterone increases the tissue sensitivity to insulin [31, 80]. In addition, it is known that phospholipid metabolism in females has the higher rate compared to males [71].

As previously mentioned, the CE transfer activation is proatherogenic, because it leads to the apo-B-LP hydrophobic core growth. This fact was confirmed by the decrease of total HDL-cholesterol content in females with the experimental MS and shows the significant dependence the MS development on aging in females compared to males.

As can be seen from the abovementioned information, the changes in lipid and lipoprotein metabolism in the experimental animals differ greatly depending on the age and sex. We have already pointed out the difference in FFA content and its mobilization by the tissues (see Table 1).

According to our data, the serum FFA content in young females is significantly lower compared to the corresponding value in males, and the feeding high-calorie diet along with the developing IR did not lead to the blood FFA level growth in females (see Table 1). This data is consistent with the literature about the lower FFA release rate from adipose tissue in women is mediated by the antilipolytic estrogen activity [56, 84, 88]. Furthermore, estrogens can suppress lipolysis in adipose tissue in women even with reduced insulin sensitivity in the adipose tissue. This may explain the absence of serum FFA content growth in 4-week-old females fed high-calorie diet.

The absence of lipolysis activation in the adipose tissue in young females fed high-calorie diet is probably the reason for the absence of atherogenic changes in the blood serum in animals of this experimental group even under obesity and insulin resistance.

At the same time, sex differences are revealed in other indices (see Tables 2 and 9). Thus, the total serum lipid level in females with MS was elevated more significantly than in males and especially in adult females (by 58% as compared to the intact animals, while it was only 16% for males of this group).

The obtained data are consistent with literature data about sex differences in the lipid and lipoprotein metabolism [25, 71]. There is sufficient evidence that the serum lipids in women are less favorable for atherosclerosis development as compared to men, which is mainly associated with low serum TAG content in women and high HDL-cholesterol level [42, 56, 84, 88].

These differences are considered to explain the higher risk of CVS disease in men at young age compared with women [46].

Therefore, lipid and lipoprotein metabolism sex differences revealed on a number of key stages, mainly:

- The different lipolytic activity in adipose tissue of men and women
- The differences in the liver lipid and lipoprotein metabolism
- The different CE transfer rate associated with lower CETP activity in women
- The different levels of basal HL activity

It is well known that the HL activity is regulated by hormones via the transcription activation, and estrogens inhibit this enzyme synthesis by binding with estrogen-sensitive areas in the promoter of its gene [44].

According to the literature data, the HL activity in women is approximately 2 times lower compared with men [22], which correlates with a LDL lower level in women in health and obesity and even under MS.

With a feeding high-calorie diet, the CE transfer rate from HDL and HL activity in the serum of young females increased (see Tables 10 and 11). But as it is known, these changes have atherogenic character under the serum TAG level increase, which we did not record during our experiments (see Table 9).

Let us pay our attention to the fact that LPL activity in females was significantly higher than in males, as in intact and under MS, and with aging, the activity of this enzyme was decreased. Furthermore, the HL activity in post-heparin serum was increased in all animals with experimental pathology, and this increasing should reflect the growth of hormone-sensitive lipases activity in response to excessive cortisol production [96, 97] and is primarily adaptive. This increase under MS, on the contrary, leads to the FFA accumulation, dyslipidemia, and dyslipoproteinemia that, finally, can lead to the atherosclerosis development. Moreover, the activity of this enzyme was higher in males (see Tables 7 and 11) and increases with aging indicate the serum lipolysis activation in the animal ontogenesis.

The absence of changes in LPL activity in adult males with MS should be noted (see Table 7). It is well known [22] that males have a higher propensity to atherosclerosis, which also increases with aging, and the given results seem to be paradoxical. However, with a very high (almost 40% higher than in females in the same group) triacylglycerol lipase (TGL) activity and low (60% lower than in females in the same group) LPL activity, even in the absence of changes in this enzyme activity, the ratio between TGL activity and LPL activity in males of this group was 3.5 times higher than in females in the same group. Hence, it is obvious that the high risk of atherogenesis remains. In addition, LPL activity in intact males is 30–35% lower than in females.

The comparison of the obtained data about sex and age features under MS development with the literature evidence indicates that young females have more favorable blood serum lipid profile compared with males due to the lipolysis low rate in adipose tissue in females, low CETP activity, which determines the CE transfer rate from HDL, and low basal HL activity. The expressed atherogenic dyslipidemia was not observed in young females fed high-calorie diet, even under obesity and IR. One of the reasons may be the lack of lipolysis activation in adipose tissue, which is associated with the powerful antilipolytic estrogen activity.

The TAG content increasing was observed in the serum of 20-week-old females fed high-calorie diet. The apoB-LP content did not change (see Tables 5 and 9), which indicates the TAG-enriched VLDL1 accumulation in serum in the animals of this experimental group. According to data about the elevated serum FFA level in adult females fed a high-calorie diet, it can be assumed that the cause of hepatic VLDL1 formation is hepatic absorption of a large number of FFA from the blood, which was released as a result of the lipolysis activation in adipose tissue.

These results are the confirmation of the suggestion that sex differences in the lipolysis regulation in adipose tissue become less pronounced with aging. The latter can be associated

with significant hormonal changes in the body of females with aging. There are changes in the sex hormones secretion – to be more specific, the increase of cortisol secretion (according to our data, the content of cortisol in the serum of adult animals is 54% higher than the value of this indicator in the 4 weeks animals [95, 96]) and decrease of insulin sensitivity. Moreover, we found out that the serum estradiol level in young females was increasing while feeding high-calorie diet, while the serum of adult animals had not changed, which may be an additional factor of age-related differences in lipolytic activity in adipose tissue of females under MS.

We also found a decrease in the serum HDL and HDL-cholesterol content in the adult females fed high-calorie diets (see Table 9). All this gives us the opportunity to state the atherogenic dyslipidemia development in 20-week-old females fed high-calorie diet. However, unlike males, changes in serum lipid profile of adult females in the proatherogenic side may be associated with the TAG-enriched lipoprotein accumulation in the blood and a HDL and HDL-cholesterol content decrease. The latter, obviously, is the result of CE transfer growth rate from HDL (see Table 10) and increased HL activity (see Table 11), which is accompanied by a HDL-cholesterol level decrease under hypertriacylglycerolemia.

Therefore, our data show that by feeding high-calorie diet the expressed atherogenic dyslipidemia in females developed only in adulthood. Probably, this is mediated by different lipolytic activity in adipose tissue in young and adult females and is associated with their hormonal status changes with aging. The favorable cardiovascular risk serum lipid profile that was observed in adult animals fed high-calorie diet is associated with TAG blood content increase and with the HDL decrease. Probably it occurs due to the liver VLDL1 secretion activation and disorders in intravascular lipoprotein remodeling.

To establish mechanisms of atherogenic dyslipidemia development under high-calorie diet, we investigated some lipid and lipoprotein indices dynamics of metabolism in the serum and liver in hamsters during experimental MS development. Also the correlation analysis of the obtained data was conducted. In a series of experiments, we used male and female Syrian hamsters that were 1 year old at the beginning of the experiment (group of animals with predisposition to MS).

As mentioned above, it is known that the liver cells are able to secrete two different VLDL fractions: VLDL1 and VLDL2, which differ in size and density. TAG-enriched VLDL1 have large size and low density. VLDL2 are smaller, denser, and contain smaller amounts of TAG in comparison with VLDL1. It is known that in various pathological conditions, including obesity and hepatic IR, the liver secretes mainly VLDL1, which is the main reason of hypertriacylglycerolemia under these conditions. VLDL morphology essentially depends on the intracellular TAG content and the sensitivity of hepatocytes to insulin.

In our experiment, IR is probably not the main reason of serum TAG increase in the male hamsters in the early stages, as was evidenced by the lack of correlation between these indices, also a later IR development compared with the blood TAG increase (see Figure 1) [96].

Thus, we can suggest that hypertriacylglycerolemia in males developed by feeding a high-calorie diet because of liver preferential VLDL1 secretion due to the TAG accumulation in hepatocytes.

The reasons of the liver TAG content increasing can be as follows:

- The intensive uptake of lipoprotein particles from the blood by hepatocytes
- The enhanced TAG formation in hepatocytes due to the FA *de novo* synthesis or FA uptake from the blood stream

This data suggests that although liver lipolysis activation does occur, it does not play a key role in the MS development.

Moreover, the lack of positive correlation between the TAG content and the liver LAL activity shows that the lipoprotein uptake from the blood stream does not play a key role in the hepatic TAG accumulation in our experiments.

The TAG content increasing in the male hamsters' liver fed high-calorie diet probably occurs because of esterification of FFA coming from the blood. And the presence of a positive correlation between the liver TAG and FFA content (the correlation coefficient at early stages – 0.97) and the liver TAG content and serum FFA level (the correlation coefficient at the early stages – 0.98) demonstrates this fact.

Another evidence of the key role of blood FFA in the formation of MS pathogenic complex is the change in NADPH-generating enzyme G6PDH activity in the liver of the experimental animals. These data indicate the absence of the significant lipogenesis activation within the models used and highlight the key role of extra-hepatic lipolysis activation.

It is well-known that the excessive triacylglycerolemia between the food uptake develops due to the growth of TAG-enriched lipoproteins in blood [50], which may be a result of increased hepatic VLDL secretion and/or changes of its morphology.

A significant serum TAG content increase in the studied animals, with the later and less visible apoB-LP growth, confirms the idea that the main reason of hypertriacylglycerolemia in males under the MS development initial period is the VLDL morphology change toward the TAG enriching.

The main FFA source in serum is the TAG release from adipose tissue due to its hydrolysis. Therefore, the FFA content increase is usually associated with the activation of lipolysis in adipose tissue.

The data that was previously discussed along with the positive correlation between the serum TAG and FFA content (correlation coefficient – 0.85) proved the fact that the activation of lipolysis in adipose tissue is the reason for hypertriacylglycerolemia in the male hamsters fed a high-calorie diet. This leads to an intensive FFA uptake by the liver, growth of intracellular TAG in hepatocytes, and causes the preferential VLDL1 secretion.

Although the lipolysis regulation in adipose tissue is carried out involving many factors including pancreatic hormones, glucocorticoids, and adipokines, its activation may take place

under different conditions including the MS. It is a well-known fact that insulin inhibits lipolysis in adipose tissue under healthy conditions, whereas lipolysis is activated under IR.

The adipose tissue factor adiponectin also has an antilipolytic action, whereas cortisol can increase the lipolytic activity of adipose tissue [98]. We found out the significant positive correlation (coefficient of correlation – 0.87) between the serum FFA and cortisol content in males fed high-calorie diet, and the negative correlation between the FFA and adiponectin content (correlation coefficient – 0.90) [96]. The correlation between the FFA content and IR was not observed at the very beginning of our experiments, which indicates the predominance of hypercortisolemia and hypoadiponectinemia over IR as the reason of lipolysis activation in adipose tissue of males fed high-calorie diet.

Thus, based on the results of our analysis and literature data, we can suggest that the lipolysis activation in adipose tissue is the base of lipid and lipoprotein metabolic disorders in males fed high-calorie diet. And the lipolysis activation is a consequence of the hormonal status disorders, namely, the cortisol increased secretion and adiponectin decreased secretion. The adiponectin decreased secretion is probably a consequence of the adipose tissue rapid growth because of overeating. Lipolysis activation in adipose tissue ultimately leads to impaired lipid metabolism in the liver, in particular the vast VLDL1 secretion that leads to the hypertriacylglycerolemia development.

As we noted above, all these processes led to the excessive formation of the most atherogenic LDLB. It is generally considered that the reasons of LDLB accumulation in blood under hypertriacylglycerolemia are the following:

- The disorders in lipoproteins remodeling in the blood stream [50]
- The disorders in lipoprotein's particle utilization in the hepatocytes and peripheral organs [3, 17, 57]

As already mentioned, LPL plays a significant role in the utilization of TAGs in lipoprotein composition, which is localized predominantly in adipose and muscle tissue. Hydrolysis of TAGs in the VLDL composition catalyzed by this enzyme leads to VLDL transformation into intermediate-density lipoproteins (IDL), which later turns into LDL, and FFA, revealed as a result of hydrolysis, absorbed by adipocytes and muscle cells.

The abnormal cholesterol transport between different lipoprotein subfraction particles that leads to the blood atherogenic profile formation is under discussion.

As we have already noted, in our experiments the CE transfer rate was enhanced and this was already observed in the early stages of MS developing (see Figure 7). It correlates with the serum TAG content increasing (the correlation coefficient is 0.77) and suggests that changes in apoB-LP morphology is one of the earliest manifestations of MS proatherogenic process.

These results are corresponded to the well-known fact from above that the key factor determining the CE transfer rate is the total serum TAG content. Thus, increased cholesterol esterification and CE transfer between lipoprotein particles make a significant contribution to the atherogenic LDLB formation.

Another factor that significantly affects this process is HL activation. As we have already mentioned, HL is associated with proteoglycans of liver blood vessels endothelial cells and hydrolyzes TAGs and phospholipids (PL) in the composition of the various lipoprotein fractions and plays a leading role in their metabolism.

It is known that activation of increased HL activity under CE transfer and growth of blood TAG content determined in our experiments is one of the main reasons of HDL-cholesterol content decrease. This is linked with the fact that TAG hydrolysis in the HDL<sub>3</sub> composition leads to their transformation to HDL<sub>2</sub>, which are rapidly removed from the blood stream by the liver.

According to our data, the HL activity increase is accompanied by LAL activity increase in the liver (compared with Figures 2 and 10), which shows the intense lipoprotein uptake (probably HDL) from the blood stream.

Hence, our results suggest that changes in VLDL secretion are associated with the MS development FFA accumulation in the blood and elevated hepatic FFA uptake then followed changes in the CE transfer activity and after all was HL activation. This leads to the LDLB accumulation and cholesterol reverse transport disorder.

At the same time, it is apparent that changes in lipoprotein enzymatic transformations are led to their abnormal composition. This fact is confirmed by earlier enzymatic changes compared with changes in the blood lipid fractions content. Furthermore, the lipoprotein content changes earlier than their composition, which should reflect the balance disorder of their secretion and absorption. Probably, the latter is related with HDL metabolism in the blood and liver uptake under the condition of EC enrichment.

Thus, the TAG-enriched apoB-LP accumulation, which was accompanied by an increased CE transfer rate and increased HL activity, was found out in blood serum of male Syrian hamsters fed high-calorie die. It is known that such changes have a pronounced proatherogenic character, because they lead to the formation of atherogenic LDL fractions – LDLB and lower of HDL cholesterol.

The reason for hypertriacylglycerolemia development in the experimental animals in our investigation, probably, is the lipolysis activation in adipose tissue due to cortisol secretion elevation and decreased adiponectin secretion, which was observed under body weight gain.

The absence of a positive correlation between the serum TAG content and apoB-LP, as well as the serum and liver TAG content in experimental animals, suggests that the serum TAG content increase in females fed high calorie diet is not associated with increased hepatic VLDL secretion.

Based on these statements we can suggest that the cause of hypertriacylglycerolemia in females in our experiments, probably, is the predominant only liver VLDL1 fraction secretion by liver and/or diminished VLDL utilization because of the LDL activity decrease.

As is mentioned above, the intensive formation of TAG-enriched VLDL1 in liver and their secretion to the blood may occur due to the growth of the intracellular TAG content, including the intensive FFA inflow from the blood, and reducing of the hepatocytes sensitivity to insulin.

In our experiments, the liver TAG content increase in females occurs in later periods as compared to the hypertriacylglycerolemia development in animals fed high-calorie diet (see Figure 13) and these indices are not correlated. In addition, feeding a high-calorie diet did not lead to serum FFA increase in females during the first 4 weeks (see Figure 5), which indicates that there were no significant lipolysis activation in adipose tissue of the experimental animals in the first period of our experiments.

Our current data suggest that lipolysis activation in adipose tissue and intensive FFA flow from the blood and liver cells are not the main reasons of the TAG accumulation in the serum of females when consuming a high-calorie diet.

There is sufficient evidence that hepatic VLDL1 formation may occur under hepatic IR [7, 15, 76]. It is known that the hepatocyte sensitivity to insulin determines the VLDL morphology. Insulin suppresses the pre-VLDL lipidation, and as a result VLDL2 characterized by low TAG content are formed in the liver. The activation of enzymes that transfer TAGs to pre-VLDL leading to the formation of TAG-enriched VLDL1 was recorded under reducing the cell sensitivity to insulin.

Thus, the obtained results allow to make the assumption that the main reason for VLDL morphology defects that lead to hypertriglyceridemia in females fed high-calorie diet is the reduced sensitivity of liver cells to insulin. This IR in females does not cause lipolysis activation in adipose tissue, which probably relates to the ability of female sex hormones to suppress the lipolysis in adipose tissue without dependence on insulin sensitivity.

Another possible reason of hypertriacylglycerolemia in females is diminished TAG-enriched lipoproteins utilization because of the reduced LPL activity.

According to our data, this enzyme activity in the serum of females fed high-calorie diet decreased after 3 days from the beginning of the experiment and it was even lower than in later periods (see Figure 6).

We suppose that in our experiments the reason for serum LPL activity reducing was also the insulin sensitivity decrease in females, which is evidenced by the presence of the significant negative correlation between this enzyme activity and IR index (coefficient of correlation between them makes  $-0.87$ ). Inhibition of LPL activity under IR and obesity diminished the TAG-enriched lipoproteins utilization and can be considered as one of the hypertriacylglycerolemia causes. In the context of our experiments, the LPL activity reduction in the early terms of MS development correlated with the growth of serum TAG content (correlation coefficient  $-0.80$ ).

## 5. Conclusion

The obtained results suggest that the MS begins to develop differently in individuals of different sex. In males, the starting point for MS development is the increase in adipose tissue mass, changes of its endocrine activity, and as a result the hypercortisolemia development,

decreased adiponectin secretion, which is caused by the lipolysis activation in adipose tissue and with time, provokes metabolic and hormonal shifts and the IR development. In females, the MS development begins with the IR appearance, which activates the other pathogenetic factors, although they are delayed by estrogens in the first stages.

Our data are consistent with literature data and demonstrate that feeding high-calorie diet causes the atherogenic dyslipidemia development in experimental animals, which is the consequence of metabolic disorders in adipose tissue and liver as well as lipid and lipoprotein metabolic disorders in the bloodstream.

Our current studies revealed some age and gender features of lipid metabolism disorders mediated by body weight gain. In particular, it was demonstrated that the tendency to atherogenic dyslipidemia in males does not significantly depend on age, but it increases with age in females.

The hormonal disturbances that cause lipolysis activation in adipose tissue in males are the bases of hypertriacylglycerolemia development, which in turn provokes the further blood lipid profile deterioration. The hypertriacylglycerolemia in females is associated with lipid metabolism disorders in the liver due to hepatic IR. The body weight gain of the experimental animals is of great importance as to the formation of these disorders.

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