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AGE DEPENDENT EFFECTS OF METFORMIN IN WISTAR ALBINO MALE RATS WITH METABOLIC SYNDROME

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Abstract

Background and aims: *Comparative estimation of metformin treatment effectiveness in adult and young rats with metabolic syndrome (MS).* ***Materials and methods:*** *A metabolic syndrome model was induced by full replacement of drinking water with 20% fructose solution in Wistar albino male rats of two age categories (young animals of 21 days age (50-70g) and adults (160-180g)). After 60 days of MS modelling and metformin treatment, hematological, biochemical, blood pressure, chromatin DNA fragmentation investigations, as well as morphological macroscopic and microscopic studies were carried out.* ***Results:*** *In young rats, effects of metformin on blood clotting time, lipid metabolism and DNA fragmentation were more pronounced. Mature rats showed greater susceptibility to this drug as for influence on pancreas and visceral fat relative weights.* ***Conclusions:*** *In our experiment with young and adult rats with MS and metformin treatment we showed that this preparation effect was age-dependent for lipid metabolism indices, blood clotting time, nuclear DNA fragmentation parameters, as well as for changes of relative organs weights and target organs morphological structure. Metformin treatment allowed a partial normalization of serum levels of lowdensity lipoproteins (LDLP) and ratio high lowdensity lipoproteins / lowdensity lipoproteins (HDLP/LDLP), hemoglobin contents, hematocrit percentage, DNA fragmentation rates, with simultaneous worsening in blood clotting time, blood pressure levels, liver and pancreas relative organs weights (of young rats).*

key words: *metabolic syndrome, young, adult, rats.*

Background and aims

The concept of "metabolic syndrome" (MS) combines a cluster of clinical and paraclinical features, which manifestations are based on the same pathogenic mechanism – a decrease in the sensitivity of target tissues to insulin-mediated glucose assimilation [1]. Presently, there is no doubt that initiation of metabolic disorders is in childhood and adolescence [2,3]. However, the MS specificity in the pediatric population is studied fragmentary. Adult patients` data cannot replace the investigation of metabolic syndrome peculiar properties in childhood. Children and adults differ significantly in such parameters as *p*H of gastric and duodenal juice, terms of

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gastric emptying and intestinal passage, secretion and activity of bile acids and secretions of the pancreas, bacterial intestinal contents, transport proteins such as P-glycoprotein (P-gp), organs sizes, permeability of membranes, concentration and composition of proteins in plasma, water content, fat content, and so on [4]. Data on the combined effect of the MS itself, as a pathological process, in the child or adolescent age are also fragmentary.

Pharmacotherapy of MS in childhood is rarely used. Its effectiveness and safety were not specially assessed for the adolescent population. But, the drugs used for pharmacological treatment of the MS can have a notable effect on young organisms, which differs from its action in adults.

Metformin is often used for MS pharmacotherapy in pediatric population due to its low (as considered) level of side effects. Among the effects on carbohydrate and lipid metabolism, metformin can reduce the level of the inhibitor of plasminogen-1 activator, von Willebrand factor, platelet aggregation and adhesion, simultaneously increasing the tissue plasminogen activator activity and improving the relaxation of smooth muscle vessels [5,6]. The results obtained by other authors also indicate that metformin directly affects the production of androgens [7]. This preparation, aside from the anticancer effect, mediated by reduction of insulin resistance, may directly suppress cancer cells' growth and proliferation via stimulation of AMP kinase (AMPK) [8].

All these data indicate a much stronger and more diverse effects of metformin on the body than previously thought. However, the literature does not provide data for a detailed analysis of its possible long-term consequences in children and adolescents. A comparative investigation of metformin biological effects in young organisms and adults could be of great importance for its side effects mechanisms understanding, as well as its safety and efficacy prediction and monitoring.

The aim of our present study was to carry out a complex estimation of metabolic syndrome and metformin mediated age-dependent changes in rats.

Materials and methods

A total of 48 Wistar albino male rats of two age categories (young animals of 21 days age (50-70g) and adults (160-180g)) were used in the study. They were kept under a controlled temperature (from 22°C to 24°C), relative humidity of 40% to 70%, lighting (12 h light-dark cycle), and on a standard pellet feed diet ("Phoenix" Ltd., Ukraine). The study was performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committee. The model of MS was reproduced according to the protocol of Abdulla et al. [9]. Young and adult animals were divided into 6 groups (6 animals in each group): 1 – Control 1 (intact young rats), 2 – Control 2 (intact adults), 3 – MS3 (young rats with MS), 4 – MS4 (adult rats with MS), 5 – MS5+metformin (young rats with MS and metformin (266,0 mg/kg of body weight)), 6 – MS6+metformin (adult rats with MS and metformin treatment). MS was induced by full replacing of drinking water with 20% fructose solution (200g/l). Crystalline D-fructose >99% (Khimlaborreactiv, Ukraine, series 072000897834, batch XW 130105) was used in experiments. 20% fructose (instead of drinking water) was prepared daily and was given every day for two month ad libitum. In the studies, the following test item was used: metformin (Metformin, metformin hydrochloride) - tablets manufactured by SANDOZ, Lek S.A., Poland,

series CN8407. Metformin dosing was determined taking into account the interspecies sensitivity coefficient [9].

After 60 days of 20% fructose solution consumption and metformin treatment rats were sacrificed by decapitation under a mild ether anesthesia.

Determination of rat hematological and clinical biochemistry parameters, hepatocytes' chromatin DNA fragmentation (as one of apoptosis markers), as well as organs' and tissues' morphological macroscopic and microscopic studies were carried out after 60 days of MS modelling. The blood, serum and organs were used for investigation. Blood samples were studied with the hematology analyzer Mythic™ 22 (Switzerland), blood clotting time – by standard clinical Burker's method. The leukocyte formula was calculated in% to make changes in the ratio of white blood cells more demonstrative. Serum levels of total bilirubine, total cholesterol, LDLP and HDLP were measured with a fully automatic biochemical analyzer Prestige 24i (Japan) using kits supplied by "P. Z. Cormay", Poland. Ratio of HDLP/ LDLP was calculated as better risk indicator with greater predictive value than isolated parameters used independently, particularly LDL [10].

Changes of rats' blood pressure were investigated as described by Khromov et al. [11]. Noninvasive investigation of all animals' blood pressure (96-72 hours before euthanasia) was carried out on tail artery with ultrasound sensor of vascular pulsation using a SPHYGMOMANOMETER S-2 (HSE, Germany). Vascular pulsation monitoring was carried out by oscilloscope HM 303-4 (HAMEG GmbH, Germany). Data were analysed using "Chart 5" (ADInstruments, Australia).

Abdominal fat, liver, kidneys and pancreas of all animals were extracted, weighted and used for morphologic investigation. Relative organ weights were calculated per 100 g of body weight. Pieces of extracted organs were fixed in 10% solution of neutral formalin, dehydrated in ethanol solutions of grade concentrations and embedded in paraffin wax. Histological cross-sections (6μm) were made and stained by hematoxilin and eosine. Histological examination was performed under a light microscope (100 x, 200 x and 400 x). In frozen (-20°C) slices of organs, neutral fat was determined by Sudan black B staining [12]. Nucleic acids content (DNA and RNA) was determined histochemically by Shubich method [12], glycogen – by PAS-reaction [13], succinate dehydrogenase activity – by the method of Nachlas et al. [13] and lactate dehydrogenase activity - by the method of Hess et al. [12]. Microscopic studies were carried out with a Cytophan microscope (Leica Microsystems Wetzlar GmbH).

DNA from rat liver was isolated as previously described [14]. Chromatin DNA fragmentation (as one of apoptosis markers), were carried out according to (Kovalenko at al., 2007) [15]. After electrophoresis gels were stained with ethidium bromide and visualized under a UV transilluminator (BIORAD, USA). Electrophoresis data analysis was carried out with Quantity One Software (USA).

Statistical analysis

The obtained data were expressed as the mean ± standard error of the mean (M±SEM) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test using OriginPro 7.5 Software. Differences between groups were considered to be statistically significant at $p < 0.05$.

Results

The results of young and adult rats' serum biochemical parameters investigation with MS and metformin treatment are given in Table 1.

Table 1. Serum biochemical indices of adult and young animals with MS and metformin treatment, (n =6 per group)

Indices	Adult animals			Young animals		
	Control	Metabolic syndrome	Metabolic syndrome + metformin	Control	Metabolic syndrome	Metabolic syndrome + metformin
Glycosylated hemoglobin, mmoles/L	3.66±0.30	3.42±0.26	3.65±0.23	2.66±0.31	2.65±0.25	2.66±0.20
Glucose, mmoles/L	7.07±0.39	6.56±0.50	6.41±0.42	7.53±0.19	7.36±0.39	6.95±0.60
Triglycerides, mmoles/L	0.65±0.08	0.52±0.03	0.50±0.03	0.40±0.03	0.48±0.13	0.48±0.07
Total bilirubine, mmoles/L	0.73±0.19	0.93±0.28	0.99±0.27	0.47±0.16	0.76±0.25	0.82±0.21
Total cholesterol, mmoles/L	1.19±0.10	1.66±0.09*	1.78±0.10*	1.03±0.11	1.45±0.24*	1.68±0.24*
High-density lipoproteins(HDLP), moles/L	0.924±0.047	1.130±0.091	1.310±0.13*	0.875±0.035	1.135±0.007*	1.345±0.088*
Low-density lipoproteins(LDLP), moles/L	0.161±0.060	0.359±0.038*	0.364±0.039*	0.163±0.003	0.250±0.006*	0.234±0.029
Ratio HDLP/LDLP	0.165±0.052	0.326±0.040*	0.293±0.037	0.186±0.009	0.225±0.016*	0.178±0.27

*P<0.05 in comparison with control.

Table 2. Venous blood hematological indices of adult and young animals with MS and metformin treatment, (n =6).

Indices	Adult animals			Young animals		
	Control	Metabolic syndrome	Metabolic syndrome + metformin	Control	Metabolic syndrome	Metabolic syndrome + metformin
Leukocytes,10^3μL	4.45±0.48	5.15±0.64	4.57±0.34	2.97±0.55	2.67±0.22	3.20±0.28
Lymphocytes, %	65.95±2.40	68.33±4.37	65.03±3.34	69.52±2.96	73.47±1.38	73.23±2.50
Monocytes, %	4.10±0.45	4.42±0.68	5.20±0.52	2.93±0.34	3.10±0.25	4.07±0.93
Neutrophils, %	25.13±2.35	22.33±3.02	24.67±2.65	22.60±2.26	18.97±1.39	18.45±1.95
Eosinophils, %	0.40±0.10	0.42±0.21	0.30±0.12	0.97±0.37	0.60±0.34	0.28±0.06
Basophils, %	4.42±0.39	5.50±1.10	4.80±0.45	3.98±0.44	3.87±0.30	3.97±0.45
Erythrocytes,10^6 μL	8.24±0.19	8.43±0.13	8.73±0.18	8.54±0.11	7.98±0.28	8.27±0.14
Hemoglobin,g/dLl	14.88±0.29	14.88±0.17	15.38±0.29	15.82±0.27	14.23±0.51*	14.93±0.32
Hematocrit, %	41.03±0.78	38.45±0.63*	42.83±0.81	43.12±0.60	39.82±0.94*	41.47±0.60
Mean corpuscular volume, fL	49.82±0.31	48.92±0.90	49.10±0.56	50.55±0.76	50.08±0.99	50.17±0.65
Mature RBC cellular hemoglobin content, pg	18.05±0.11	17.67±0.30	17.63±0.23	18.57±0.41	17.85±0.16	18.07±0.33
Platelets, 10^3 μL	592.33±34.03	543.67±28.51	560.83±21.04	549.83±50.08	858.17±318.40	567.67±71.19
Peripheral blood clotting time, s	444.50±9.29	260.00±7.19*	128.43±5.24*,#	311.38±6.10	141.12±10.50*	146.88±6.26*

*P<0.05 in comparison with control.
#P<0.05 in comparison with MS.

We did not find any changes in glycosylated hemoglobin, glucose, triglycerides, total bilirubine contents in young and adult animals, both with the MS and with metformin treatment, in comparison with control (Table 1). Simultaneously levels of total cholesterol, HDLP, LDLP and ratio HDLP/LDLP were increased in all experimental groups independently of age. It should be noted that with MS changes in total cholesterol and HDLP levels were stronger in the group of young rats, while LDLP and ratio HDLP/LDLP levels – in

adults. Metformin treatment of adult rats did not cause any correction of these lipid metabolism indices. In the group of young animals total cholesterol, HDLP, LDLP levels remained high, but the ratio HDLP/LDLP – normalized with metformin treatment.

The results of the hematological parameters investigation (Table 2) show, that there were no changes in the number of platelets, erythrocytes and leukocytes, also leukocyte count, in the rat blood of both age groups with MS. However, in blood of adult rats with MS, there was a decrease in hemoglobin content. In blood of young animals we noted, not only a decrease in hemoglobin content, but also hematocrit percentage lowering, which might indicate a change in body's hemorheological profile. Metformin treatment led to the normalization of these parameters both in adult and young animals.

As the development of obesity and diabetes leads to increased rates of blood coagulation and hypercoagulability development [16,17], it was of particular interest to study these parameters under conditions of MS at different ages.

In our experiments, there was a significant decrease in the time of blood clotting, both in young and adult rats with MS. These changes were more profound in younger age: in adult rats the decrease was 1.7 times, while in young animals – 2.2 times. Metformin treatment in young and adult rats did not lead to this parameter normalization. At the same time, there was a multidirectional effect of metformin on blood clotting in animals at different age. In young rats, it caused a slight increase in peripheral blood clotting time as compared with the MS group. In adult animals group the opposite process was noted.

The relative organ weights in these experimental groups were also changed as compared to control (Table 3). Adult animals' liver tissues state with metformin treatment largely resembled such of animals with MS (Figure 1a). There were dystrophic changes in hepatocytes. Also, isolated acidophilic necroses, isolated lympho-histiocytic infiltrates of varying degrees of severity and activated stellate reticuloendoteliocytes of the elongated form with hyperchromic nuclei were observed. However, there were no circulatory abnormalities. Investigation of histochemical reaction to glycogen in the liver demonstrated that its content increased in comparison to MS group. But this parameter did not reach full normalization. Neutral fat accumulation was mainly concentrated in hepatocytes at the periphery of the liver lobules (Figure 1b).

Table 3. Relative organ weights of adult and young animals with MS and metformin treatment, g/100g b.w. (n =6).

Organs	Adult animals			Young animals		
	Control	Metabolic syndrome	Metabolic syndrome + metformin	Control	Metabolic syndrome	Metabolic syndrome + metformin
Liver	3.33±0.18	3.18±0.10	3.58±0.12	3.17±0.10	3.46±0.07*	3.76±0.10*
Pancreas	0.21±0.01	0.17±0.009*	0.19±0.01	0.18±0.007	0.16±0.004*	0.148±0.01*
Kidneys	0.61±0.022	0.63±0.015	0.67±0.016	0.59±0.091	0.65±0.044	0.62±0.01
Visceral fat	3.58± 0.25	4.81± 0.40*	3.71± 0.23	3.30±0.08	3.92±0.17*	3.47±0.13

*P<0.05 in comparison with control.

Metformin treatment did not greatly improve the young rats' liver tissues state in comparison to MS group. Along with normal hepatocytes, sometimes there were dystrophically altered cells. On the periphery of the liver lobules hepatocytes contained focuses with medium and small vacuoles, which demonstrated positive reaction to fat (Sudan black B).

Also, acidophilic necrosis was detected under the capsule (Figure 1c). In this group, the histochemical reaction to polysaccharide was intensified in comparison with MS without treatment: a considerable number of hepatocytes with an increased number of lilac granules were detected. However, the level of glycogen was not fully normalized (Figure 1d). In general, the positive effect of metformin treatment was more pronounced in young rats.

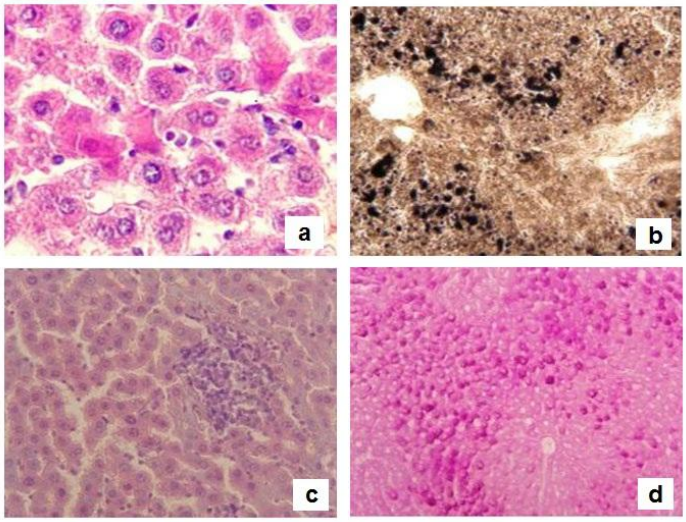

Figure 1. *Wistar albino adult and young rat liver with metformin treatment.*
a – adult rat necrobiotically changed hepatocytes (hematoxilin-eosine staining, x400); b – adult rat hepatocytes neutral fat accumulation (Sudan black B, x400), c – young rat necrobiotically changed hepatocytes (hematoxilin-eosine staining, x400); d – young rat hepatocytes glycogen increased accumulation (hematoxilin-eosine staining, x400)

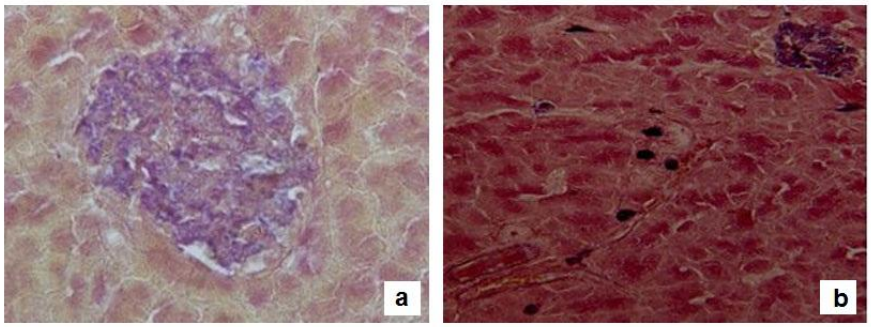

Figure 2. *Pancreas of Wistar albino adult and young rats with metformin treatment.*
a – decreasing number of secretory granules in adult rat β-cells (hematoxilin-eosine staining, x400); b-single β-cells in young rat pancreas (Gomori aldehyde-fuchsine staining, x400).

In the pancreas of young and adult rats with MS and metformin treatment were no hemodynamic disorders and inflammatory reactions. The structure of the exocrine and endocrine parts remained unchanged. But the number of β–cells in some Langerhans islets decreased (Figure 2). In both age groups the number of single β-cells, intensely stained by aldehyde-fuchsine into violet-blue colour, was increased in comparison with control.

In the kidneys of young and adult rats with MS and metformin treatment we detected focuses with plethora of glomerular capillaries (Figure 3). Also, in some proximal tubules there was epitheliocytes` cytoplasm distal part detachment, while the nuclei were located on the basement membrane. In the lumen of some

tubules reticulate, some granular masses were observed. Also (as with MS without treatment), kidneys in both age groups contained reduced quantities of neutral mucopolysaccharides in proximal tubules. Simultaneously, nephrocytes tubules apical parts had a scalloped structure. In the kidneys of young and adult rats morphological studies indicated violations of some nephrons segments functions. These changes were more pronounced in adult animals.

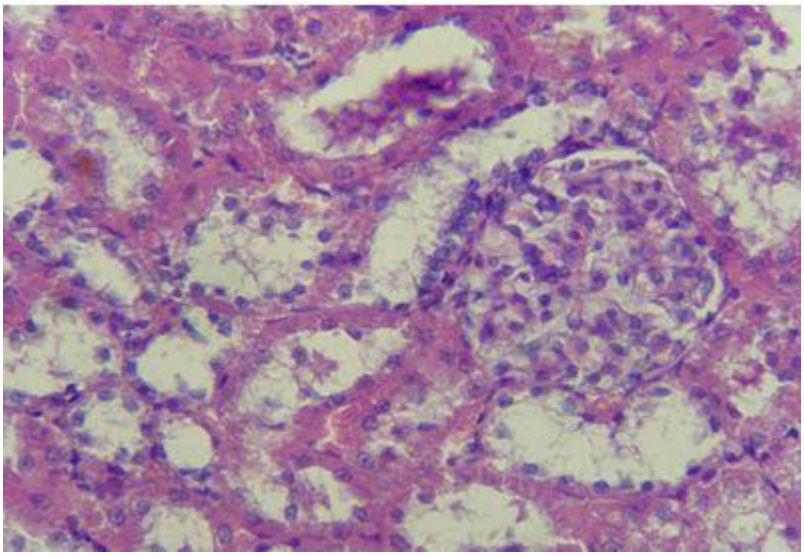

Figure 3. *Kidney of adult rat with metformin treatment.*
a - Plethora of glomerular capillaries, tubular lumens contained reticulate-granular masses (hematoxilin-eosine staining, x 400)

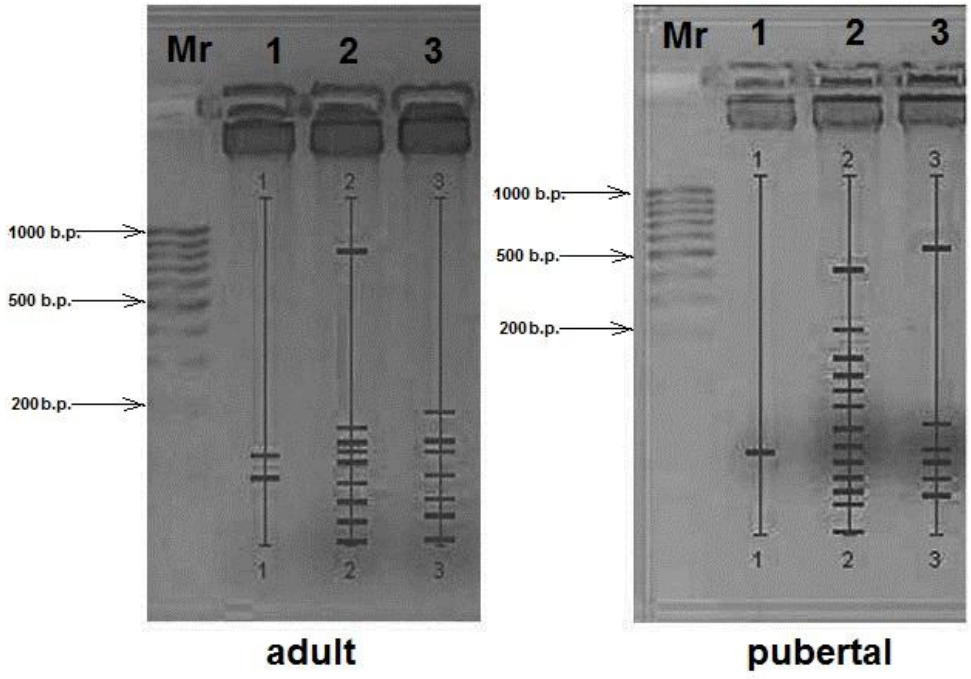

Figure 4. *DNA fragmentation in adult and young rats' hepatocytes with metformin treatment (1-control, 2- metabolic syndrome, 3- metabolic syndrome+metformin).*

In our experiments MS development caused an intensification of DNA fragmentation processes in the liver of adult rats in comparison with control (Figure 4, Table 4). In the group of

adult animals with MS 9 fractions of DNA low molecular weight fragments were detected, whereas in the control there were only two. In adult rats, the percentage of DNA fragmentation for this model of the MS grew almost 5.8 times. The majority of fractions (eight of them) were fragments with masses of 20 to 250 base pairs, whereas relatively longer fragments were represented by only one fraction (750-800 b.p.). In the liver of adult rats with metformin treatment, a marked increase in DNA fragmentation was also observed: 7 fractions (from 20 to 200 b.p.) were formed. It should be noted that the percentage of DNA fragmentation are lower in this group in comparison with MS.

In the liver of young rats with MS, DNA fragmentation also significantly increased in comparison with control (Figure 4, Table 4). The development of MS in young rats caused activation of DNA fragmentation processes, with the formation of 13 fractions of low molecular weight fragments. The percentage of DNA fragmentation increased almost 8 times. At the same time, 11 fractions contained small fragments with weights from 20 to 100 base pairs, whereas relatively longer fragments were represented by only 2 fractions of 450 and 250 pairs of nucleotides.

Table 4. Relative % of rat liver DNA fragmentation in adult and young animals with metformin treatment

Experimental groups	% of DNA fragmentation	
	Adult animals	Young animals
Control	5.57	4.42
Metabolic syndrome	32.50	35.21
Metabolic syndrome+metformin	22.57	20.90

In the liver of young rats with metformin treatment, a profound increase in DNA fragmentation was also observed: 6 fragments with masses from 40 to 550 b.p. were formed. Just as with MS, in this group, most fractions (total – 5) were represented by fragments with 40-100 b.p., whereas relatively longer fragments - by only one fraction of 550 b.p. The percentage of DNA fragmentation lowered in this group in comparison with MS.

Since metformin is often prescribed as a monotherapy for MS, it had been of interest for us to check whether it has any effect on the hypertension that usually accompanies this pathology. The results on the hypertension state in adult and young rats with MS and metformin treatment are shown in Figure 5.

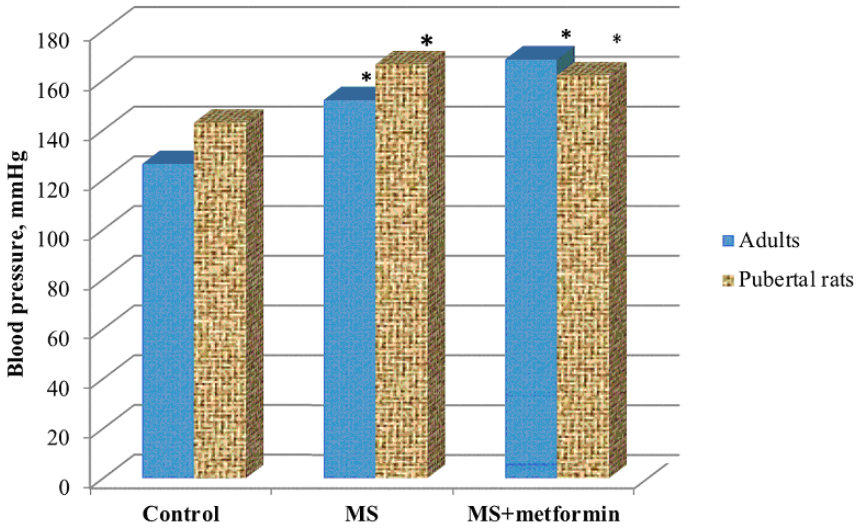

Figure 5. *Adult and young animals blood pressure levels (mmHg) with MS and metformin treatment, n=6.*
*P<0.05 in comparison with control.

Our results demonstrated that with reproducing of the MS experimental model the hypertension developed in both age groups. Increase of blood pressure reached the level of statistical significance in comparison with controls, both in groups of adult and young rats. Metformin treatment with MS did not cause a decrease in blood pressure both in adult and young animals.

Discussion

Dyslipidemia, which is characterized by elevated levels of LDL cholesterol and triglycerides is the most frequent component of the MS phenotype [18]. Our data indicate that the complete replacement of drinking water with 20% fructose solution in rats of both age groups led to an increase in the early markers of MS [19]: the content of cholesterol, LDL, HDLP/LDLP ratio. Metformin treatment was able to correct levels of LDLP and HDLP/LDLP ratio in the group of young animals, while in adults - only HDLP/LDLP decreased. But our data on the metformin effect on the various cholesterol fractions contents are in contradiction with the results of other authors regarding the metformin ability to improve the status of these parameters in clinical trials [20,21]. Such discrepancies, inter alia, may be due to more serious disorders in organism caused by MS experimental model in comparison with the usual noted levels of pathology in the clinic, and/or by interspecies differences in sensitivity to metformin.

Our hematological studies results are in agreement with the data of other authors regarding changes in hematological parameters under conditions of MS in humans [16,17]. Metformin treatment allowed us to partially correct rats' hematological indices in both age groups with the exception of blood clotting time. In this case metformin treatment not only didn't improve this index, but it even deepened the changes noted with MS alone. Effect of metformin was more pronounced in the group of young rats.

Our conclusions concerning the effect of metformin on biochemical and haematological parameters were confirmed by our results of morphological studies. Our previous morphological studies demonstrated, that MS development was accompanied with necrobiotic changes in hepatocytes, neutral fat accumulation, decreased number of β–cells in the islets of Langerhans, degenerative changes in kidney tissues (patchy congestion of glomerular capillaries, partial detachment of nephrocytes` dystal parts in proximal tubules, reduced quantities of neutral mucopolysaccharides in proximal tubules, scalloped structure of some nephrocytes` tubules (apical parts), independently of age [19]. But in young MS rats these changes were less pronounced. Metformin did not normalize morphology of liver, kidneys and pancreas both in young and adult rats. As for relative masses of organs, its effect on young animals was significantly worse than in the case of adult rats. This possibly could happen due to the higher speed of metabolic processes in young animals and the higher rates of this preparation transformation in their organisms. We cannot exclude in this situation the possible direct consequences of metformin's antiproliferative influence on young rapidly developing organism [22-26]. To clarify this assumption, we investigated the effect of metformin treatment on DNA fragmentation in liver cells.

The level and nature of DNA fragmentation is a marker of apoptosis processes [27]. During the last decades, a close relationship was established between the development of MS and the rate of endonuclease NEIL1 involved in DNA repair processes in mammalian cells [28]. Among this, it was shown that during the

development of MS, DNA repairing (by DNA polymerases) could be inhibited and accompanied by simultaneous mitochondrial dysfunctions, vascular wall pathologies and neurodegeneration [29,30]. Previously, we demonstrated [19] that liver DNA fragmentation of adult and young rats with experimentally induced MS was significantly higher than in controls. In this study we have found that metformin treatment had a protective effect on the rate of nucleic DNA fragmentation in liver of both age groups. It should be emphasized that in young animals the corrective effect of metformin was more pronounced than in adult rats. Our data on metformin's ability to inhibit DNA fragmentation acceleration with MS are fully consistent with other authors` results [22-26]. They associated metformin's antigenotoxic and antitumor effects with its ability to affect directly cell proliferation, apoptosis and DNA repair.

Damage of any intracellular macromolecules (DNA, RNA, proteins, lipids) due to the development of MS could be of fundamental importance to the cell viability. Significant deviations of these molecules contents from the normal levels can lead to cell damage and death. There is a clear correlation between systemic changes in the structure of DNA and rates of MS development indices [31]. Marked in our experiments liver cells apoptosis violations can lead to inhibition of liver tissue regeneration and this organ pathological changes deepening [31,32]. The data of other authors [29,30] also indicate the close correlation between violations of DNA repair processes, apoptosis and MS development.

Differences in the nature and intensity of DNA fragmentation processes between adult animals and young rats noted in our experiments may be due to age-dependent changes in rates of nucleases and other enzymes, which ensure DNA molecules structure stability [33], as well as with the inclusion into functioning of diverse sets of nucleases during ontogenesis [34].

Our findings of changes in DNA fragmentation in liver cells of adult and young rats were more pronounced in comparison with changes in morphological indices of liver necrotic transformations. This is in accordance with the other authors` data, which indicated that DNA fragmentation violations could precede not only apoptosis changes, but also liver cell toxic death, making important contributions to launching liver cell necrosis [32]. As it was previously demonstrated for MS [19] changes of physiological, biochemical and hematological indices, as well as DNA fragmentation violations preceded changes on the level of cells and tissue morphologic structure. As in biochemical, hematological, morphological investigations young animals DNA-fragmentation indices changed more pronounced.

An interesting feature of metformin action revealed the results of its antihypertensive effect studies. In our experiments conducted by generally accepted methods for this experimental animals [35] metformin treatment had not caused any antihypertensive effect in both age groups. This possibly could indicate a lack of a direct relationship between mechanisms of carbohydrate metabolism disturbances and the hypertension development with MS and/or interspecies variations in hypertensive response among mammalian species [36].

Such results (regarding low antihypertensive and antihyperlipidemic effect with profound influence on DNA-fragmentation) could raise questions about the feasibility of metformin use for MS pharmacotherapy, especially in adolescents. Long-term concequences of metformin treatment for normal young organism formation deserve further investigations.

Study limits

Direct extrapolation of obtained results to humans cannot be done due to interspecies differences. The relatively small number of experimental animals is another limitation of our study.

Conclusions

In an animal model with young and adult Wistar albino rats with MS and metformin treatment, we established that this preparation effects was age-dependent for lipid metabolism indices, blood clotting time, nuclear DNA fragmentation parameters, relative organ weight, target organ morphological changes. Metformin treatment demonstrated a partial correction of serum levels of LDLP and ratio HDLP/LDLP, hemoglobin contents, hematocrit percentage, DNA fragmentation rates, with a simultaneous worsening in blood clotting time, blood pressure levels, liver and pancreas relative organ weights (of young rats).

REFERENCES

1. **Aydin S, Aksoy A, Aydin S et al.** Today's and yesterday's of pathophysiology: biochemistry of metabolic syndrome and animal models. *Nutrition* 30: 1-9, 2014.

2. **Weiss R, Dziura J, Burgert TS et al**. Obesity and the *metabolic syndrome* in children and adolescents. *N Engl J Med* 350: 2362-2374, *2004*.

3. **Zimmet P, Alberti G, Kaufman F et al.** The metabolic syndrome in children and adolescents. *Lancet* 369: 2059–2061, 2007.

4. **Strolin Benedetti M, Whomsley R, Baltes EL**. Differences in absorption, distribution, metabolism and excretion of xenobiotics between the paediatric and adult populations. *Expert Opin Drug Metab Toxicol* 1: 447-471, 2005.

5. **Fujita Y, Inagaki N.** Metformin: New Preparations and Nonglycemic Benefits. *Curr Diab Rep* 17:5, 2017.

6. **Scheen AJ, Esser N, Paquot N**. Antidiabetic agents: Potential anti-inflammatory activity beyond glucose control. *Diabetes Metab* 41: 183-194, 2015.

7. **Attia GR, Rainey WE, Carr BR.** Metformin directly inhibits androgen production in human thecal cells. *Fertil Steril* 76: 517-524, 2001.

8. **Zhou G, Myers R, Li Y et al.** Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 108: 1167-1174, 2001.

9. Guidance for Industry and Reviewers Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers. *US Department of Health and Human Services, FDA, CDER and CBER.* –http:// www.fda.gov/cder/guidance/index.htm

10. **Millán J, Pintó X, Muñoz A et al.** Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag* 5: 757–765, 2009.

11. **Khromov OS, Dobrelya NV.** Experimental study of phosphatidyl choline liposomes as new hipotensive. *Bulletin of Dnipropetrovsk University (Visnyk Dnipropetrovskogo Universitetu). Biology Ecology*. 1: 197-203, 2008. (in Russian)

12. **Romeis B.** Mikroskopische Technik. In: *Mikroskopische Technik.* Romeis B (ed). Foreign Literature (Inostrannaya literature), Moscow, pp 248-252, 1953 (in Russian).

13. **Piers E.** Histochemistry. In: *Histochemistry.* Piers E (ed). Foreign Literature (Inostrannaya literature), Moscow, pp 962, 1962 (in Russian).

14. **Costa LG, Hodgson E, Lawrence DA, Reed DJ, Greenlee WF.** Unit 2.2. Determination of Apoptosis and Necrosis. Alternative Protocol 2. Detection of DNA fragmentation in Total Genomic DNA. In:*Current Protocols in Toxicology*. Costa LG, Hodgson E, Lawrence DA, Reed DJ, Greenlee WF (eds), John Wiley & Sons Inc. Somerset, pp 2759 , 2005.

15. **Kovalenko VM, Bagnyukova TV, Sergienko OV et al.** Epigenetic changes in the rat livers induced by pyrazinamide treatment. *Toxicology and Applied Pharmacology* 225: 293-299, 2007

16. **Ni S, Wang X, Wang J, Zhao Z, Zeng S.** The Effects of a high-fat and high-energy diet on the hepatic expression of CYP3A in developing female rats. *Xenobiotica* 42: 587-595, 2012.

17. Bulava SV, Zamyshlyaev AV. Hemorheological profile in metabolic disorders. *Yaroslavl Pedagogical Bulletin* 4: 161-164, 2012. (in Russian)

18. de Oliveira J, Hort MA, Moreira EL et al. Positive correlation between elevated plasma cholesterol levels and cognitive impairments in LDL receptor knockout mice: relevance of cortico-cerebral mitochondrial dysfunction and oxidative stress. *Neuroscience* 197: 99–106, 2011.

19. Bondarenko L, Karatzuba TA, Shayakhmetova GM et al. Specificity of metabolic syndrome model reproduction at pubertal and adult male rats. *Rom J Diabetes Nutr Metab Dis* 22: 251-260, 2015.

20. Ayyavoo A, Derraik JGB, Hofman PL et al. Pre-pubertal children born post-term have reduced insulin sensivity and other markers of the metabolic syndrome. *PLoS One* 8(7): 67966, 2013.

21. Mahmood K, Naeem M, Rahimnajaad NA. Metformin: the hidden chronicles of a magic drug. *Eur J Intern Med* 24: 20-26, 2013.

22. Berstein LM, Vasil'ev DA, Kovalenko IG et al. The influence of metformin and N-acetylcysteine on mammographic density in postmenopausal women. *Vopr Onkol* 58: 45-49, 2012.

23. Jiralerspong S, Gonzalez-Angulo AM, Hung MC. Expanding the arsenal: metformin for the treatment of triple-negative breast cancer? *Cell Cycle* 8: 2681, 2009.

24. Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA. The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. *Cell Cycle* 8: 88-96, 2009.

25. Wright JL, Stanford JL. Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. *Cancer Causes Control* 20: 1617-1622, 2009.

26. Gotlieb WH, Saumet J, Beauchamp MC et al. In vitro metformin anti-neoplastic activity in epithelial ovarian cancer. *Gynecol Oncol* 110(2): 246-250, 2008.

27. Wang X, Yongke LuY, Cederbaum AI. Induction of Cytochrome P450 2E1 Increases Hepatotoxicity Caused by Fas agonistic Jo2 antibody in mice. *Hepatology* 42: 400-410, 2005.

28. Vartanian V, Lowell B, Minko IG et al. The metabolic syndrome resulting from a knockout of the NEIL1 DNA glycosylase. *Proc* Natl Acad Sci U S A 103: 1864-1869, 2006.

29. Mercer JR, Cheng KK, Figg N et al. DNA damage links mitochondrial dysfunction to atherosclerosis and the metabolic syndrome. *Circ Res* 107: 1021-1031, 2010.

30. Mattson MP. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. Exp gerontol 44: 625-633, 2009.

31. Erol A. Systemic DNA damage response and metabolic syndrome as a premalignant state. *Curr Mol Med* 10: 321-334, 2010.

32. Ray SD, Kamendulis LM, Gurule MW et al. Ca2+ antagonists inhibit DNA fragmentation and toxic cell death induced by acetaminophen. *FASEB J* 7: 453-463, 1993.

33. Singh DK, Ahn B, Bohr VA. Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging. *Biogerontology* 10: 235-252, 2009.

34. Hakansson A, Zhivotovsky B, Orrenius S, Sabharwal H, Svanborg C. Apoptosis induced by a human milk protein. *Proc Natl Acad Sci U S A* 92: 8064-8068, 1995.

35. Malkoff J. Non-invasive blood pressure for mice and rats. *Animal Lab News, Kent Scientific Corporation*: 1-12, 2005.

36. Tucker A, McMurtry IF, Reeves JT, Alexander AF, Will DH, Grover RF. Lung vascular smooth muscle as a determinant of pulmonary hypertension at high altitude. *Am J Physiol* 228: 762-767, 1975.