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The effect of age and a high-fat, high-carbohydrate diet on the development of arterial hypertension and kidney disease in the experiment

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ABSTRACT

Aim. To identify the structural foundations of the pathogenesis of arterial hypertension and kidney disease associated with a high-fat, high-carbohydrate diet and age.

Materials and methods. The study was carried out on male Wistar rats aged 60 and 450 days. The animals were divided into 4 groups: group 1 (n = 14) – intact rats (60 days old) fed with a standard diet for 90 days; group 2 (n = 14) – rats (aged 60 days) receiving a high-fat, high-carbohydrate diet for 90 days; group 3 (n = 14) – intact rats (aged 450 days) receiving a standard diet for 90 days; group 4 (n = 14) – rats (aged 450 days) fed with a high-fat, high-carbohydrate diet for 90 days. Clinical and instrumental research methods, enzyme-linked immunosorbent assay, and immunohistochemistry and histology techniques were used in the study.

Results. Feeding 60-day-old animals with a high-fat, high-carbohydrate diet resulted in an increase in body weight and abdominal fat, a rise in systolic blood pressure, and moderately pronounced histologic changes in the kidneys. In intact 450-day-old rats, age-related changes prevailed: changes in the myocardial mass, an increase in TGF-β1, morphological changes in the renal tubules and glomeruli. In 450-day-old rats receiving a high-fat, high-carbohydrate diet, the most pronounced increase in both systolic and diastolic blood pressure, a significant rise in serum fibronectin, and destructive changes in the renal tissue were noted.

Conclusion. Functional and biochemical signs of arterial hypertension and morphological changes in the kidneys were the most pronounced in 450-day-old rats fed with a high-fat, high-carbohydrate diet.

Keywords: arterial hypertension, age-related changes in the kidneys, high-fat, high-carbohydrate diet

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Влияние возраста, высокоуглеводной и высокожировой диеты на развитие артериальной гипертензии и поражения почек в эксперименте

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РЕЗЮМЕ

Цель: выявить структурные основы патогенеза артериальной гипертензии и поражения почек, связанных с высокоуглеводной высокожировой диетой (ВУСЖД) и возрастом у крыс линии Wistar.

Материал и методы. Исследование проводили на самцах крыс линии Wistar в возрасте 60 и 450 сут. Животных распределяли на четыре группы: 1-я (n = 14) – интактные крысы (возраст 60 сут), содержащиеся на стандартном рационе в течение 90 сут; 2-я (n = 14) – крысы (возраст 60 сут), содержащиеся на ВУВЖД в течение 90 сут; 3-я (n = 14) – интактные крысы (возраст 450 сут), содержащиеся на стандартном рационе в течение 90 сут; 4-я (n = 14) – крысы (возраст 450 сут), содержащиеся на ВУВЖД в течение 90 сут. Использовались клинико-инструментальный, иммуноферментный, иммуногистохимический и гистологический методы исследования.

Результаты. Высокоуглеводная высокожировая диета приводила у 60-дневных животных к увеличению массы тела и абдоминального жира, нарастанию систолического артериального давления, появлению умеренно выраженных гистологических изменений почек. У интактных 450-дневных крыс преобладали изменения, связанные с возрастом: увеличение массы миокарда, увеличение TGF-β1 в сыворотке крови, морфологические изменения почечных канальцев и клубочков. У 450-дневных крыс, содержащихся на ВУВЖД, отмечалось наиболее выраженное нарастание как систолического, так и диастолического артериального давления, значительное увеличение концентрации фибронектина в сыворотке крови, выраженные деструктивные изменения в почечной паренхиме.

Заключение. Функциональные и биохимические признаки артериальной гипертензии и морфологические изменения в почках были наиболее выражены у 450-дневных крыс, содержавшихся на ВУВЖД.

Ключевые слова: артериальная гипертензия, возрастные изменения почек, высокоуглеводная высокожировая диета

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

Risk factors for arterial hypertension include age, obesity, and associated pathologies, such as diabetes mellitus, diabetic nephropathy, and atherogenic dyslipidemia. Overweightness and obesity are currently equated with a global epidemic and are the main factors underlying a number of metabolic disorders in organs and tissues, which contribute to progression of insulin resistance and development of metabolic syndrome, increasing the risk of diabetes, cardiovascular diseases, hyperlipidemia, non-alcoholic fatty liver disease, and kidney disease [1, 2].

The prevalence of metabolic syndrome and its components has alarmingly increased over the past decade, becoming a public health problem. According to numerous studies, the incidence of metabolic syndrome ranges from 7.5 to 42.2% in different countries [3, 4], which is associated with increased prevalence of bad habits (smoking, overeating, etc.) and a sedentary lifestyle.

Powerful risk factors for the development of kidney disease in metabolic syndrome include not only diabetic nephropathy and arterial hypertension, but also obesity, which, due to the direct effect of oxidative stress on the renal parenchyma, is believed to lead to chronic kidney disease and end-stage renal failure [5]. The latter is a serious health problem, which may result in such cardiovascular complications as arterial hypertension, heart failure, myocardial infarction, and sudden cardiac death [6]. However, the mechanisms of kidney disease in obesity are still unclear.

Therefore, the aim of the study was to identify the structural foundations of the pathogenesis of arterial hypertension and kidney disease associated with a high-fat, high-carbohydrate diet and age in the experiment.

MATERIALS AND METHODS

The study was carried out on male Wistar rats aged 60 and 450 days weighing 350–400 g and 400–600 g, respectively. All procedures were carried out in accordance with the Directive 2010/63 / EU of the European Parliament and the FASEB statement on the principles for the use of animals in research and education. The animals were divided into 4 groups: group 1 (n = 14) – intact rats (aged 60 days) fed with a standard diet for 90 days; group 2 (n = 14) – rats (aged 60 days) receiving a high-fat, high-carbohydrate diet (HFHCD) for 90 days; group 3 (n = 14) – intact rats (aged 450 days) receiving a standard diet for 90 days; group 4 (n = 14) – rats (aged 450 days) fed with HFHCD for 90 days.

HFHCD contained 16% proteins, 21% fats, and 46% carbohydrates, including 17% fructose and 0.125% cholesterol [7]. Water was replaced with a 20% fructose solution. The rats of groups 1 and 3 (intact animals) were fed with standard rodent food (24% proteins, 6% fats, 44% carbohydrates) and pure water *ad libitum*. After the end of HFHCD, the animals received a standard diet and regular drinking water for one week in order to exclude osmotic load due to the consumption of fructose.

Body weight, as well as systolic and diastolic blood pressure (BP) were assessed weekly by tail cuff plethysmography using the MP35 data acquisition system (Biopac Systems Inc., USA). The animals were removed from the experiment by decapitation following anesthesia with chloralose (100 mg / kg intraperitoneally). Before the decapitation, blood samples were taken from the common carotid artery. Immediately after the sacrifice, the heart, both kidneys, and visceral fat were removed and weighed. The study was approved by the Ethics Committee at the Cardiology Research Institute of Tomsk NRMC (Protocol No. 201 of 30.07.2020).

The blood samples were centrifuged (15 min 3,000 rpm), the serum samples were stored in a freezer at –70 °C. Fibronectin and TGFβ-1 in the serum were determined by enzyme-linked immunosorbent assay (ELISA) using the rat fibronectin (ab108850) and rat TGFβ (ab119558) ELISA kits (Abcam, USA), respectively. Sample measurements were performed using the Infinite 200 PRO microplate reader (Tecan GmbH, Salzburg, Austria).

For a histologic examination, kidney samples were taken, which were fixed in 10% neutral buffered formalin and embedded in paraffin according to the standard technique. The sections were stained with hematoxylin and eosin, Van Gieson's stain, and periodic acid – Schiff (PAS) stain with nuclear counterstaining with hematoxylin. Immunohistochemistry (IHC) was performed using monoclonal antibodies Ki-67 (Abcam, USA). The histologic sections were visualized and photographed using the Axiostar plus light microscope (Carl Zeiss, Germany) at 400x and 1000x magnification. The percentage of Ki-67$^+$ positive cells was counted in each glomerulus. A morphometric analysis was performed using the ImageJ software for image analysis and processing (National Institutes of Health, USA); the area of the renal glomeruli and the width of the Bowman's space were calculated.

A statistical analysis was performed using the Statistica 13.0 software package (StatSoft Inc., USA).

The analysis of the obtained data was carried out by the methods of descriptive statistics with the calculation of the median and the interquartile range *Me* (Q_1–Q_3) for non-normally distributed variables. For normally distributed variables, the data were presented as the mean and the error of the mean ($M \pm m$). The differences between the groups were determined using the Kruskal – Wallis test. The differences were considered statistically significant at $p < 0.05$; $0.05 \geq p \leq 0.06$ was taken as a trend.

RESULTS

Body weight increased in the experimental animals of groups 2–4 compared with group 1 (Table 1).

Table 1

Weight of the animals, weight of organs and visceral fat of rats of different age fed with HFHCD, g, $M \pm m$

Weight	Group 1	Group 2	Group 3	Group 4
Body	430.3 ± 5.3	481.2 ± 12.4[1]	517.3 ± 13.0[1]	520.0 ± 35.0[1]
Heart	1.39 ± 0.1	1.31 ± 0.053	1.54 ± 0.04[1, 2]	1.44 ± 0.1[3]
Kidneys	2.8 ± 0.1	2.8 ± 0.1	3.51 ± 0.2[1]	3.05 ± 0.2[3]
Visceral fat	8.32 ± 1.1	16.46 ± 1.6[1]	9.91 ± 0.9	17.47 ± 3.8[3]

[1, 2, 3] statistical significance of the differences compared with group 1,2,3,4 (here and in Tables 2, 3).

Note: here and in Tables 2–4: group 1 – intact rats aged 60 days; group 2 – rats aged 60 days receiving HFHCD; group 3 – intact rats aged 450 days; group 4 – rats aged 450 days receiving HFHCD.

At the same time, there was a pronounced increase in the weight of abdominal fat in the groups of animals fed with HFHCD compared with similar parameters in intact animals of different age. When the heart was weighed, its maximum weight was observed in 450-day-old intact rats, which slightly differed from the values in the group of 450-day-old rats fed with HFHCD. The kidney weight was significantly elevated in the animals of group 3 compared with group 1.

Systolic BP in the rats increased in group 2 and showed a trend toward an increase in group 4 after the prescription of HFHCD, while diastolic BP increased only in group 4 (Table 2).

The study of the blood serum showed a two-fold increase in the concentration of fibronectin in the rats of group 4 (Table 3) compared with the animals in groups 1–3. An increase in the serum concentration of TGFβ-1 was observed in the animals of groups 3 and 4 (Table 3).

The histologic examination of the kidneys revealed foci of pronounced perivascular and periductal lymphocyte and monocyte infiltration of the stroma in group 3; in the lumen of some distal convoluted tubules and collecting ducts, PAS-positive casts were observed, while epithelial cells in the nephron tubules flattened toward the lumen or exfoliated in the lumen. In single epithelial cells of the proximal tubules, lipofuscin granules and nuclear damage were noted.

Table 2

Blood pressure in rats of different age fed with HFHCD, mm Hg, *Me* (Q_1–Q_3)

BP	Group 1	Group 2	Group 3	Group 4
Systolic	129 (124–136)	141 (137–143)[1]	133 (129–136)[2]	140 (135–144)[1, 3]
Diastolic	87 (83–89)	85 (77–89)	86 (80–88)	97 (95–101)[1, 2, 3]

Table 3

Concentration of fibronectin and TGFβ-1 in the blood serum of rats of different age fed with HFHCD, $M \pm m$

Parameter	Group 1	Group 2	Group 3	Group 4
Fibronectin, mg / dl	21.23 ± 1.55	27.58 ± 1.78	29.89 ± 2.38	43.00 ± 3.12[1, 2, 3]
TGFβ-1, ng / ml	14.0 ± 3.0	19.1 ± 2.6	35.3 ± 5.2[1]	31.9 ± 4.1

Morphological changes in the rat kidneys in groups 2 and 4 included pronounced plethora in the vessels of both renal glomeruli and stroma with signs of stasis. In group 2, moderate perivascular fibrosis was noted, while in group 4, both perivascular and focal peritubular fibrosis in the stroma with surrounding lymphoid infiltration and thickening of the outer layer of the Bowman's capsule were identified. Damage to the brush border of the epithelial cells in the proximal tubules was observed in both groups fed with HFHCD, which was the most extended in group 4 (Fig. 1). The distal convoluted tubules in group 4 contained PAS-positive casts, which was accompanied by flattening or death of the epithelial cells (Fig. 2).

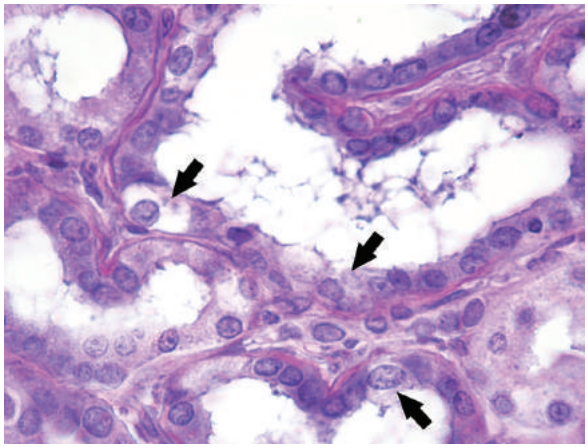

Fig. 1. Damage to the brush border of the epithelial cells in the proximal tubules (indicated by arrows) in the kidneys of 450-day-old rats after the prescription of HFHCD. Staining with PAS and hematoxylin, x400

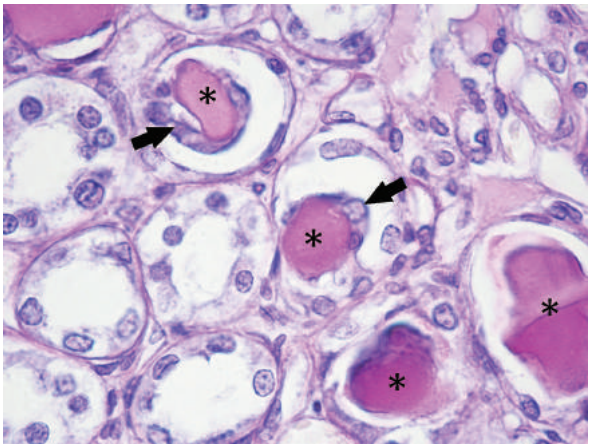

Fig. 2. PAS-positive casts (indicated by asterisks) and exfoliated epithelial cells (indicated by arrows) in the lumen of the distal convoluted tubules of 450-day-old rats after the prescription of HFHCD. Staining with PAS and hematoxylin, x400

A quantitative assessment of changes in the renal glomeruli showed an increase in their size both with age and with the prescription of HFHCD (Table 4). However, the area of the glomeruli in the animals fed with HFHCD was significantly smaller than in the animals receiving a standard diet.

The width of the Bowman's space, located between the inner and outer layers of the Bowman's capsule, also increased with age in all groups, while the urinary space in groups 2 and 4 was significantly wider than that in groups 1 and 3, respectively (Table 4).

Table 4

Area of the renal glomeruli and width of the Bowman's space in rat kidneys, µm 2/ µm, *Me* (Q_1–Q_3)

Parameter	Group 1	Group 2	Group 3	Group 4
The area of the renal glomeruli,×10^3	$23.54^{2,3}$ (19.94–27.21)	$20.41^{1,4}$ (16.98–22.52)	27.04^{1} (23.32–32.03)	24.71^{3} (21.67–29.32)
The width of the Bowman's space	$9.36^{2,3}$ (7.59–11.77)	$12.54^{1,4}$ (10.45–15.10)	$12.21^{1,4}$ (9.88–15.37)	$15.42^{2,3}$ (12.98–17.16)

^{1,2,3,4} significance of the differences compared with group 1, 2, 3, 4.

Ki-67 IHC staining was detected in cells of the renal glomeruli (in mesangial cell processes and flattened endothelial cells) (Fig. 3, 4). At the same time, a rise in the number of Ki-67-positive cells was revealed, which coincided with an increase in the age of the animals fed with HFHCD (Fig. 5).

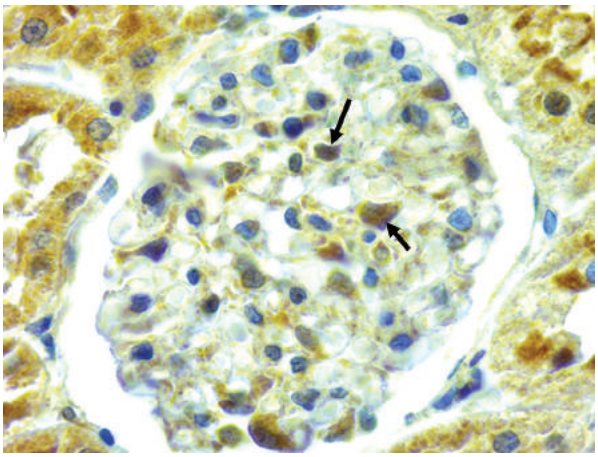

Fig. 3. Ki-67$^+$ cells (indicated by arrows) in the glomeruli of 60-day-old intact rats. IHC staining with Ki67 antibodies and hematoxylin, x1000

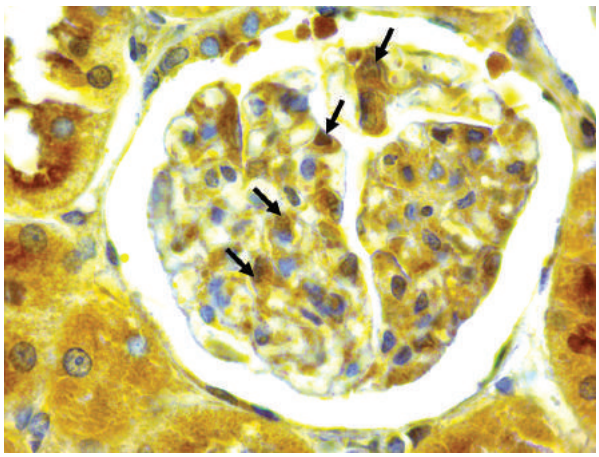

Fig. 4. Ki-67$^+$ cells (indicated by arrows) in the glomeruli of 450-day-old intact rats after the prescription of HFHCD. IHC staining with Ki67 antibodies and hematoxylin, x1000

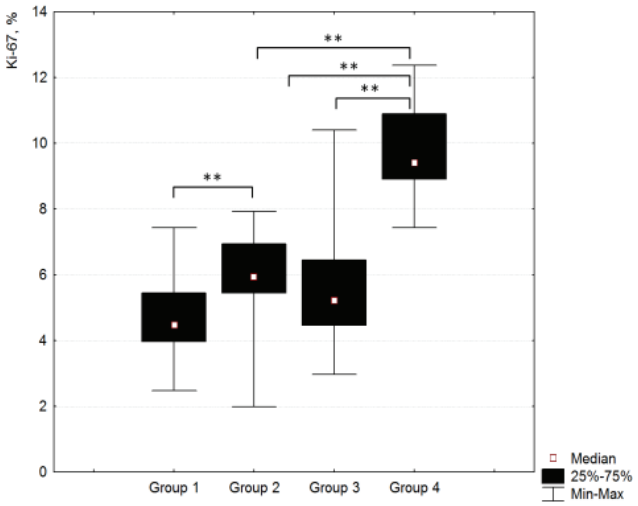

Fig. 5. The percentage of Ki-67+ cells in the renal glomeruli: ** the differences between the groups

DISCUSSION

The HFHCD used in this study corresponds to the so-called cafeteria diet, which, along with obesity, causes a decrease in glucose and insulin tolerance, dyslipidemia, an increase in lipid peroxidation, a decrease in antioxidant activity of the liver, kidneys, and brain, as well as interstitial nephritis [8]. The increase in body, heart, and kidney weight in 450-day-old rats fed with HFHCD and a standard diet could be a consequence of not only a diet, but also an age-related increase in organ mass, while a significant increase in the weight of visceral fat in the rats receiving HFHCD, obviously, was the result of only HFHCD.

Changes in the systolic and diastolic BP values in the animals fed with HFHCD might be caused by an increase in the load on the myocardium associated with obesity, while a rise in diastolic BP may be associated with impaired renal function, which might lead to a decrease in the kidney mass in the animals fed with HFHCD. The histologic signs of kidney disease that we established in aged animals were aggravated by the prescription of HFHCD: a decrease in the area of the renal glomeruli combined with an increase in and thickening of the Bowman's capsule might indicate glomerular hyperfiltration.

A similar negative effect on the kidney structure was also demonstrated when studying the effect of long-term intake of fructose with glucose or sucrose [9]. The main pathophysiological mechanism underlying the negative effect of fructose is associated with a by-product of its metabolism – uric acid, which has a direct effect on endothelial cells and vascular smooth muscle cells. In this case, the bioavailability of endothelial nitric oxide is inhibited, and the renin – angiotensin system is activated, which results in glomerular hypertension and tubulointerstitial injury [10]. Subsequently, renal vasoconstriction and systemic hypertension develop. The latter develops due to an increase in the reabsorption of salts and water and may also be accompanied by inflammation and tubulointerstitial injury [11].

Expression of the proliferation marker Ki-67 was observed in mesangial and, probably, endothelial cells of renal glomerular capillaries, since proliferation is not typical of podocytes [12]. The increase in the number of Ki-67$^+$ cells in the group of aged animals that were fed with HFHCD can also be associated with both the age-related accumulation of advanced glycation end products and the damaging effect of uric acid, which causes glomerular hyperfiltration and hyperplasia of mesangial cells after 2 weeks of feeding animals with fructose, as shown in the experiments [13, 14]. The process was accompanied by the accumulation of extracellular matrix proteins, thickening of the glomerular basement membrane, and the development of glomerular hypertension.

Proliferation of mesangial cells and an increase in synthesis of the extracellular matrix are stimulated by α-SMA and TGF-β1, so both factors were identified as the key mediators of glomerular and tubulointerstitial pathology in chronic kidney disease [15]. An increase in the concentration of TGF-β1 was observed in nephropathy caused by induced diabetes mellitus in rats [16]. However, in our study, the increase in the serum concentration of TGF-β1 occurred in all 450-day-old rats, regardless of HFHCD. Therefore, this parameter is more likely associated with age-related changes. Mesangial cells themselves can produce extracellular matrix proteins, including fibronectin, which leads to renal fibrosis in pathological conditions [17]. In our study, in the aged animals fed with HFHCD, the increase in the Ki-67 expression in mesangial cells was combined with the increased serum levels of TGF-β1 and fibronectin, which also supports the hypothesis on the development of glomerular hypertension.

The results obtained are comparable with the studies that established a relationship between an increase in the concentration of fibronectin and diabetic nephropathy [18], increased concentration of fibronectin in the blood plasma and type 1 diabetes mellitus [19], hypertension, nephropathy [20], as well as obesity and triglyceridemia in patients with type 2 diabetes mellitus [21].

CONCLUSION

The revealed histologic changes in the kidneys may indicate an aggravating effect of HFHCD on aging and, subsequently, cause development of renal hypertension associated with disruption of the normal structure and functioning of the kidneys. The results obtained suggest that serum fibronectin level can be used as a marker of kidney disease in diabetes mellitus in elderly patients, however, this assumption needs clinical confirmation.

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Mustafina L.R. – carrying out of the morphological and immunohistochemical studies, morphometry, statistical processing and interpretation of the morphological data. Logvinov S.V. – conception, drafting of the morphology section of the article, substantiation of the manuscript, critical revision of the manuscript for important intellectual content, and final approval of the manuscript for publication. Naryzhnaya N.V. – conception and design, carrying out of the research, drafting of the article, substantiation of the manuscript, critical revision of the manuscript for important intellectual content. Kurbatov B.K. – design of the study, carrying out of the research, statistical analysis and interpretation of the data. Maslov L.N. – conception, drafting of the article, substantiation of the manuscript, critical revision of the manuscript for important intellectual content, and final approval of the manuscript for publication.

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