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Correlation of Vascular Endothelial Growth Factor Receptor-2 Expression and Morphological Changes in the Myocardium of Rats on a High-Carbohydrate High-fat Diet

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ABSTRACT

Aim. To evaluate the relationship between the expression of vascular endothelial growth factor receptor 2 (VEGFR2) in the myocardium and its association with morphological changes in cardiac muscle cells in rats on a high-carbohydrate high-fat diet with regard to the age using the immunohistochemical method.

Materials and methods. The study was conducted on male Wistar rats aged 5 and 18 months, some of which were fed with a standard diet, while the other previously received a high-carbohydrate and high-fat diet (HCHFD) for 90 days. VEGFR2 was detected by immunohistochemical staining of myocardial sections, signs of myocardial damage were assessed by the presence of perinuclear depletion (edema) of the sarcoplasm and contracture changes in cardiac muscle cells, karyopyknosis, and changes in the specific volumes of the stroma.

Results. An increase in the specific volume of VEGFR2 positive cardiomyocytes occurs in young (5 months old) rats on HCHFD, in old (18 months old) rats on a standard diet, and, to the greatest extent, in aged animals receiving HCHFD. The change in the proportion of cardiomyocytes expressing VEGFR2 correlates with the content of cardiomyocytes with morphological signs of damage in the form of karyopyknosis, contracture, and depletion of the perinuclear zone of sarcoplasm. According to multiple regression analysis, karyopyknotic disorders made the greatest contribution to the change in VEGFR2 expression in cardiomyocytes in older animals.

Conclusion. HCHFD induces predictable changes in VEGFR2 expression in cardiac muscle cells, depending on age and the severity of myocardial damage. The study results suggest that the protective effect of VEGFR2 expression may be disrupted in HCHFD and with age.

Keywords: age-related myocardial changes, high-carbohydrate high-fat diet, vascular endothelial growth factor receptor-2 (VEGFR2), contracture changes in cardiac muscle cells

Conflict of interest. The authors declare no obvious or potential conflicts of interest related to the publication of this article.

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Корреляция экспрессии рецептора-2 сосудистого эндотелиального фактора роста и морфологических изменений миокарда крыс на высокоуглеводной высокожировой диете

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РЕЗЮМЕ

Цель: с помощью иммуногистохимического метода оценить взаимосвязь экспрессии рецептора-2 сосудистого эндотелиального фактора роста (VEGFR2) в миокарде и с морфологическими изменениями кардиомиоцитов у крыс на высокоуглеводной высокожировой диете в возрастном аспекте.

Материалы и методы. Исследование проведено на самцах крыс линии Вистар в возрасте 5 и 18 мес, одна часть которых содержалась на стандартном пищевом рационе, другая – предварительно находилась на высокоуглеводной и высокожировой диете (ВУВЖД) в течение 90 дней. VEGFR2 выявляли при иммуногистохимическом окрашивании срезов миокарда, признаки повреждения миокарда оценивали по наличию перинуклеарного опустошения (отека) саркоплазмы и контрактурных изменений кардиомиоцитов, кариопикноза, изменений удельных объемов стромы.

Результаты. Увеличение удельного объема VEGFR2 иммуногистохимически позитивных кардиомиоцитов возникает у молодых (5 мес) крыс на ВУВЖД, у старых крыс (18 мес) на стандартной диете и, в наибольшей степени, у возрастных животных, содержавшихся на ВУВЖД. Изменение доли кардиомиоцитов, экспрессирующих VEGFR2, коррелирует с содержанием кардиомиоцитов с морфологическими признаками повреждения в виде кариопикноза, контрактуры и опустошения перинуклеарной зоны саркоплазмы. По данным множественного регрессионного анализа, у старых животных наибольший вклад во влияние на изменение экспрессии VEGFR2 в кардиомиоцитах оказали кариопикнотические нарушения.

Заключение. ВУВЖД вызывает закономерные изменения экспрессии VEGFR2 в кардиомиоцитах, зависящие от возраста и степени поражения миокарда. Результаты исследования позволяют предполагать, что протекторная направленность экспрессии VEGFR2 может быть нарушена при ВУВЖД и с возрастом.

Ключевые слова: возрастные изменения миокарда, высокоуглеводная высокожировая диета, рецептор-2 сосудистого эндотелиального фактора роста (VEGFR2), контрактурные изменения кардиомиоцитов

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

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INTRODUCTION

Cardiovascular diseases are largely the result of an unhealthy lifestyle, including an unbalanced diet with high levels of fats and carbohydrates, which affects both general metabolism and endothelial metabolism in particular [1, 2]. A key link in this process is impaired angiogenesis and endothelial dysfunction, which has been shown in experiments with metabolic disorders in animals: a decrease in microvessel density and VEGFR2 expression underlies the process of disruption of signaling along the VEGF/VEGFR pathway in cardiac endothelial cells [2, 3]. Moreover, an imbalance in the VEGF system, which acts as a key regulator of angiogenesis and cardiomyocyte survival, can aggravate the course of cardiovascular diseases [4]. The importance of the VEGF/VEGFR pathway is highlighted by the fact that bidirectional signaling between endothelial cells and cardiac muscle cells via VEGFR2 coordinates physiological cardiac growth, and its disruption contributes to the transition to pathological hypertrophy and heart failure [2].

In the myocardium, cardiac muscle cells are a source of VEGF, along with the endothelium [4]. Saturated fatty acid overload increases cardiac muscle cells apoptosis *in vitro* [5], while lipotoxic effects on the myocardium are mitigated by VEGF overexpression, significantly restoring cardiac muscle cells viability. The protective effect of VEGF in combating cardiac muscle cell apoptosis may identify therapeutic targets in diseases caused by fatty acid stress [6].

However, the effect of a high-carbohydrate high-fat diet (HCHFD) on VEGFR2 expression in the myocardium remains unstudied. Identification of this link is critical for complete understanding of the pathogenesis and the development of new strategies for the treatment and prevention of cardiovascular complications in metabolic disorders associated with excessive consumption of saturated fatty acids and carbohydrates, which determines the high relevance of this study.

The aim of this work is to evaluate, using the immunohistochemical method, the relationship between VEGFR2 expression in the myocardium and morphological changes in cardiac muscle cells in rats on HCHFD with regard to age.

MATERIALS AND METHODS

The study was conducted on male Wistar rats aged 5 and 18 months. All procedures complied with Directive 2010/63/EU of the European Parliament and the FASEB statement on the use of animals in research and education. The study was approved by the Ethics Committee of Cardiology Research Institute of Tomsk NRMC (Protocol No. 201 dated July 30, 2020).

The experimental groups were formed as follows: group 1 (n = 14) – intact 150-day-old (5-month-old) rats receiving a standard diet for 90 days (starting at 60 days of age); group 2 (n = 14) – 150-day-old rats receiving a HCHFD for 90 days (starting at 60 days of age); group 3 (n = 14) – intact 540-day-old (18-month-old) rats fed with a standard diet for 90 days (starting at 450 days of age). Group 4 (n = 14) included 540-day-old rats maintained on a high-frequency intrauterine fluid (HIF) for 90 days (starting at 450 days of age). The HCHFD contained 16% protein, 21% fat, 46% carbohydrates, including 17% fructose, and 0.125% cholesterol. Water was replaced with a 20% fructose solution. Rats from groups 1 and 3 (intact animals) were given standard rodent chow (24% protein, 6% fat, 44% carbohydrates) and clean water *ad libitum*.

Animals were sacrificed from the experiment by decapitation after preliminary anesthesia with chloralose (100 mg/kg intraperitoneally). For histologic examination, the heart wall was cut into 2-3-mm-thick slices, fixed in 10% buffered formalin for 24 h, washed in running water, and dehydrated in Izoprep (BioVitrum, St. Petersburg), a solution for histological processing based on absolute isopropyl alcohol. After dehydration, the myocardial samples were embedded in BioPlast homogenized paraffin embedding medium (BioOptica, Italy).

Histological sections (5–7 μm thick) obtained using HM 325 rotary mechanical microtome (Thermo Scientific, USA) were stained with hematoxylin and eosin according to Van Gieson's stain (stains from BioVitrum, St. Petersburg). Expression of VEGFR2 in cardiac muscle cells was assessed by immunohistochemistry using rabbit polyclonal antibodies at a 1:50 dilution (E3712, Spring Bioscience, USA) according to the manufacturer's instructions. 3,3'-Diaminobenzidine was used as a chromogen, and counterstaining was performed with hematoxylin. Stained preparations were embedded in BioMount synthetic mounting medium (BioOptica, Italy) and examined under Axio Lab.A1 light microscope (Carl Zeiss, Germany). Micrographs of histological preparations were obtained using Axiocam 105 color camera (Carl Zeiss, Germany).

In the left ventricular myocardium, the average number of cardiac muscle cells with karyopyknosis, perinuclear depletion (edema) of the sarcoplasm, and contracture changes, as well as the number of

unchanged cardiac muscle cells, were counted per field of view. Cardiac muscle cells were counted in 20 independent fields of view of each left ventricular myocardium section at 400x magnification; the field of view area was 0.196 mm^2. Using the ocular grid proposed by Avtandilov, we determined the specific volumes (%) of myocardial stromal connective tissue stained according to Van Gieson's stain technique and VEGFR2-positive cardiac muscle cells, the expression of which was detected during the immunohistochemical reaction.

Statistical data processing was performed using STATISTICA 13.0 (StatSoft Inc., USA). The obtained data were tested for compliance with the normal distribution using the Shapiro–Wilk test. Data that did not correspond to the normal distribution were presented as the median and interquartile range (Me (Q_1–Q_3)). Homogeneity of variances was tested using Levene's test. The statistical significance of differences in indicators between groups was assessed using the the Mann–Whitney test with Bonferroni correction for multiple comparisons. For six pairwise comparisons, differences were considered statistically significant at $p = 0.05$, if the hypothesis of equality of the mean values of the indicators was rejected at $p = 0.05/6 \approx 0.0127$.

To assess the relationship between the indicators, the Spearman's rank correlation coefficient was calculated. Multiple regression analysis was performed to identify the dependence of VEGFR2 expression in cardiac muscle cells on such variables as karyopyknosis, contracture, perinuclear depletion of the cytoplasm of cardiac muscle cells, and the specific volume of stroma. A standardized regression model was built using indicators pre-transformed so that the mean value of each indicator is 0, and the standard deviation is 1:

$$VEGF = B_1 \times X_1 + B_2 \times X_2 + B_3 \times X_3 + B_4 \times X_4,$$

where X_1 is karyopyknosis, X_2 is perinuclear cytoplasmic depletion (edema), X_3 is contracture, and X_4 is stroma.

In this case, the coefficients B_i allow us to estimate the relative contribution K_i of each independent variable to the prediction of the dependent variable VEGF as a percentage using the formula:

RESULTS

In Group 1 (5 months old, standard diet), immunohistochemical analysis of VEGFR2 expression revealed weak staining of a small proportion of cardiac muscle cells and more intense staining of endothelial cells in some myocardial capillaries (Fig. 1, *a*). The proportion of VEGFR2-positive cardiac muscle cells was 15.3% (13.2–16.3%). In Group 2 rats (5 months old, on HCHFD), immunohistochemical staining was mosaic. Light-brown stained cardiac muscle cells alternated with unstained myocardial cells, with a generally distinct border between them (Fig. 1, *b*).

The proportion of VEGFR2-positive cardiac muscle cells increased compared to Group 1 and was 32.2% (27.2–34.8%) ($p_{1\text{-}2} = 0.048$). In group 2, an increase in the number of cardiac muscle cells with karyopyknosis to 1.3 (1.2–1.5) in the field of view versus 0.4 (0.3–0.4) in group 1 ($p_{1\text{-}2} = 0.0003$) was revealed using histologic examination. It also revealed a rise in the number of cardiac muscle cells with lytic changes in the form of depletion (edema) of the cytoplasm in the perinuclear zone to 1.2 (0.9–1.3) in the field of view versus 0.5 (0.3–0.6) in group 1 ($p = 0.0003$).

These morphological changes were illustrated in one of our previous reports [11]. The number of cardiac muscle cells with contracture changes was 0.1 (0.1–1.0) in the field of view; there were no significant differences in this indicator in groups 1 and 2. In general, the content of cardiac muscle cells with damage to the nucleus and cytoplasm in group 2 increased to a level of 8% of all cardiac muscle cells versus 3% in group 1.

In group 3 (18 months, standard diet), the content, staining pattern, and distribution of VEGFR2-positive cardiac muscle cells were comparable with those observed in group 2, and their specific volume was 36.4% (33.6–38.2%; $p_{1\text{-}3} = 0.018$). In group 3, compared with groups 1 and 2, the content of karyopyknotic cardiac muscle cells significantly increased to 2.5% (2.2–2.7%; $p_{1\text{-}3} = 0.0003$), the proportion of cardiacmuscle cells with lytic changes rose to 1.5% (1.4–1.5%; $p_{1\text{-}3} = 0.0003$), and the content of cells with contracture per field of view increased to 1.1% (1.1–1.5%; $p_{1\text{-}3} = 0.0003$). In total, altered cardiac muscle cells in group 3 accounted for approximately 16%.

In group 4, the specific volume of VEGFR2-positive cardiac muscle cells (Fig. 1, c) increased to 79.4% (62.0–82.2%; $p_{1\text{-}4} < 0.0001$; $p_{2\text{-}4} = 0.00264$; $p_{3\text{-}4} > 0.05$). In this group, the content of karyopyknotic cardiac muscle cells was 3.1 (1.9–6.0; $p_{3\text{-}4} = 0.025$) per field of view, with lytic changes in the cytoplasm – 1.4 (1.2–2.1; $p_{3\text{-}4} = 0.6$), and with contracture changes – 1.3 (0.9–2.5; $p_{3\text{-}4} = 0.3$). Along with cardiac muscle cells, immunopositive staining for VEGFR2 characterizes the cytoplasm of a significant proportion of endothelial

cells in myocardial arterioles, capillaries, and venules (Fig. 1, *d*). Overall, damaged cardiac muscle cells in group 4 accounted for 19% of the total population.

Correlation analysis revealed significant positive and negative associations between various parameters in group 1 (Fig. 2).

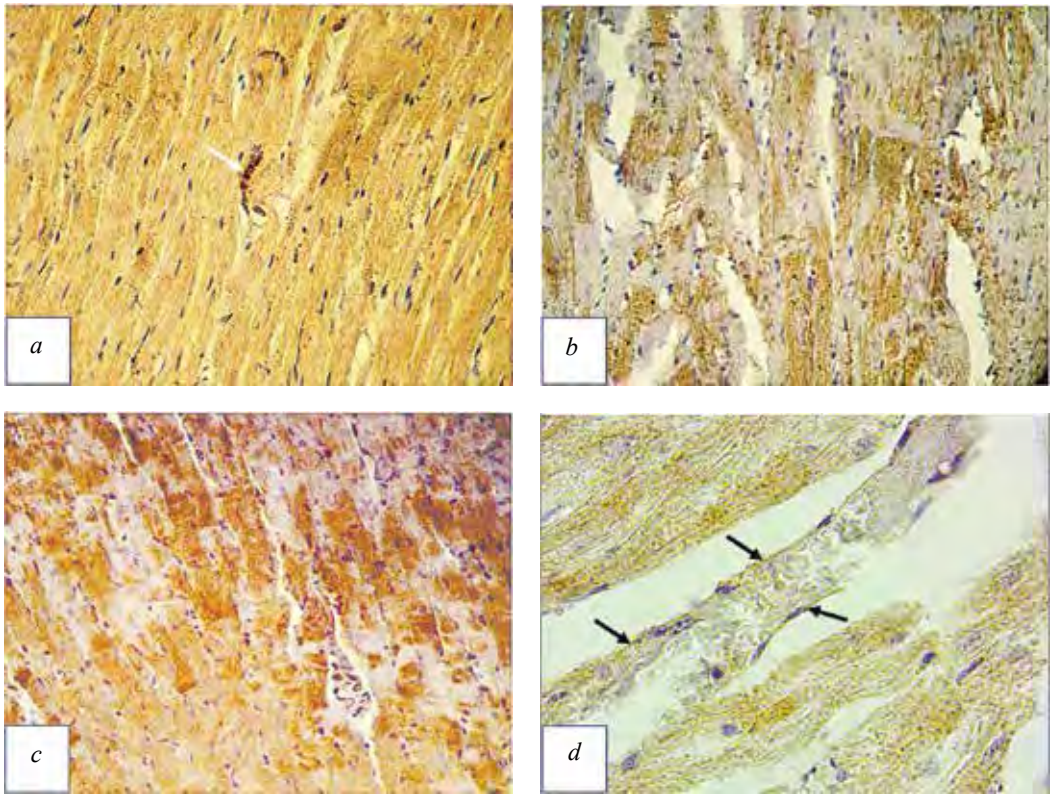

Fig. 1. Immunohistochemical detection of VEGFR2 in rat myocardium: *a* – myocardium of 5-month-old rats receiving a standard diet (group 1), weak VEGFR2 expression in cardiac muscle cells and more intense staining of the hemocapillary endothelium (arrow); *b* – mosaic VEGFR2-positive staining of cardiac muscle cells in 5-month-old rats receiving a HCHFD; *c* – intense VEGFR2 staining of the majority of cardiac muscle cells in 18-month-old rats receiving a HCHFD (group 4); *d* – VEGFR2 expression in cardiac muscle cells and endothelium of blood vessels of the myocardium of 18-month-old rats receiving a HCHFD (group 4). Magnification: x400

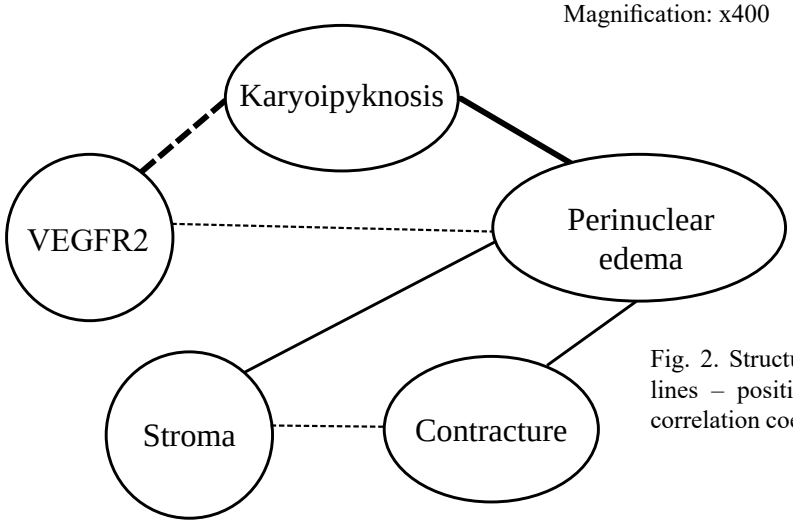

Fig. 2. Structural diagram of correlation links for group 1: solid lines – positive correlation coefficient, dotted lines – negative correlation coefficient; thick lines – strong link $|r| > 0.7$, thin lines – average link $0.3 < |r| < 0.7$.

Thus, a strong positive correlation was noted between the content of cardiac muscle cells with karyopyknosis and their lytic changes in the form of depletion (edema) of the perinuclear zone of the cytoplasm (Spearman's $r = 0.89$; $p = 0.0001$), an average positive correlation was observed between karyopyknotic disorders and contracture of the cytoplasm ($r = 0.69$; $p = 0.0136$), but a strong negative

correlation was observed between the content of cardiac muscle cells with karyopyknosis and the specific volume of cardiac muscle cells with VEGF expression ($r = –0.80$; $p = 0.017$). An average negative correlation was observed between lytic changes in the form of depletion of the perinuclear zone of the cytoplasm and VEGFR2 expression ($r = –0.66$; $p = 0.0193$), as well as between contracture of cardiac muscle cells and the specific volume of myocardial stroma ($r = –0.61$; $p = 0.0349$).

In groups 2–4, the correlations between the parameters were moderate or weak, and they were not statistically significant, often at the trend level. Thus, in group 4, positive correlations of moderate strength were recorded between karyopyknosis and VEGF expression ($r = 0.36$; $p = 0.2769$), and between contracture and VEGFR2 ($r = 0.38$; $p = 0.2466$).

Figure 3 shows the relative contribution of K_i for each independent variable to predicting the dependent variable VEGFR2 in the study groups. If the K_i coefficient is positive, then the higher the parameter, the higher the VEGFR2 value, and vice versa.

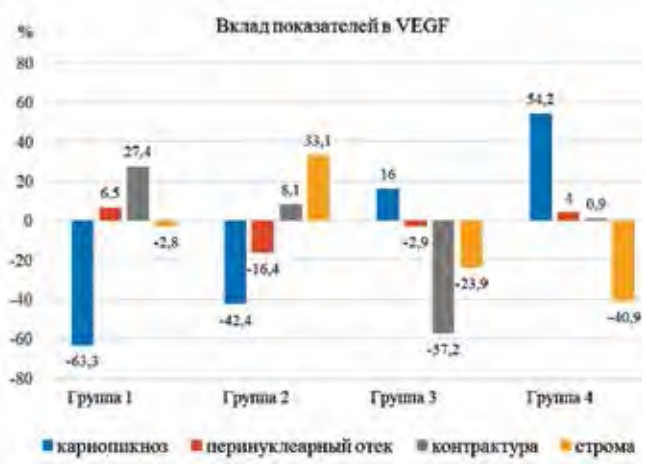

Fig. 3. Multiple regression analysis to identify the dependence of VEGF on the indicators: X_1 – karyopyknosis, X_2 – perinuclear depletion (edema) of the cytoplasm, X_3 – contracture, X_4 – stroma (specific volumes).

Thus, in group 1, contracture of cardiac muscle cells had the greatest positive effect at 27.4%, while a relatively low level of karyopyknosis had a negative effect on VEGFR2 expression. In group 2, the increase in stromal specific volume increased to 33%, while karyopyknosis demonstrated a negative correlation (–42.4%). In groups 3 and 4, with aged (18 months) animals, the positive correlation between karyopyknosis and VEGFR2 expression increased. Thus, in groups 3 and 4, the contribution of karyopyknosis was 16.6 ans 54.2%, respectively. While fibrotic changes in the stroma had a negative effect on VEGFR2 expression, in groups 3 and 4 the stromal contribution was 23.9 and 40.9%, respectively.

DISCUSSION

A large body of evidence has accumulated indicating that VEGF receptor expression is not limited to endothelial cells and that their ligands perform a variety of fundamental functions in other cell types. Non-angiogenic functions of VEGF include the ability to prevent neuronal death due to ischemia and stimulate neurogenesis, stimulate hepatocyte regeneration after liver injury, and stimulate osteoblast migration and differentiation [7–10].

Cardiac muscle cells are a source and target of VEGF-A and express its receptors VEGFR1 and VEGFR2 on their cell surface. It should be noted that although VEGFR1 strongly binds VEGF-A, it has a much weaker kinase activity compared to VEGFR2. Therefore, the biological effect of VEGF-A through VEGFR1 activation is much weaker than that exerted through VEGFR2 in metabolic syndrome [1, 3]. We would like to emphasize that one of our previous reports provided data showing that maintenance on HIF a similar experiment modeled the development of metabolic syndrome in old rats with an increase in the weight of animals, the mass of adipose tissue, an increase in serum glucose, insulin, endothelin 1, insulin resistance, and other indicators of carbohydrate metabolism disorders, as well as destructive changes in the aortic wall and persistent hypertension [11, 12].

The function of VEGF-A in the myocardium is multivector. On the one hand, VEGF-A activates cardiac muscle cells, inducing morphogenesis, contractility, and regeneration, causes vasculogenesis [13], recruitment of stem cells [14], reduction of apoptosis [15], and enhancement of vasodilation [16]. On the other hand, VEGF-A is produced by cardiac muscle cells during inflammation, mechanical stress, and cytokine stimulation. High concentrations of VEGF-A have been found in patients with various cardiovascular diseases and often correlate with the prognosis and severity of the disease [4].

In this study, we identified a relationship between VEGFR2 expression, age, and diet. The specific volume of VEGFR2-positive cardiac muscle cells significantly increased in 18-month-old rats compared to 6-month-old animals. The use of HCHFD resulted in an increase in this parameter in both 6-month-old and, to a significantly greater extent, 18-month-old animals.

Using correlation and multiple regression analysis, we established the relationship between VEGFR2 expression and the type of structural damage to cardiac muscle cells and its severity in HCHFD in relation to age. Distinct and quantifiable changes, such as karyopyknosis, contracture, and lytic disorders of cardiac muscle cells, and stromal fibrosis, were used as histological criteria for myocardial alteration. The total content of variously altered cardiac muscle cells across the experimental series was approximately 3% in group 1, 8% in group 2, 16% in group 3, and 19% in group 4. Accordingly, the specific volume of VEGFR2-positive cardiac muscle cells was 15.3 (13.2–16.3)% in group 1, 32.2 (27.2–34.8)% in group 2, 36.4 (33.6–38.2)% in group 3, and 79.4 (62.0–82.2)% in group 4. Consequently, a direct positive relationship is evident between the degree of cardiac muscle cells damage and VEGFR2 expression. At the same time, it is noteworthy that the proportion of VEGFR2-positive cardiac muscle cells in all experimental groups was significantly higher than that of cardiac muscle cells with signs of damage. This indicates that VEGFR2 expression encompasses cardiac muscle cells adjacent to the affected ones. Thus, the proportion of cardiac muscle cells with karyopyknosis, which is an indicator of cell death by both apoptosis and necrosis, in group 4 with the most severe myocardial damage was 3.1 (1.9–6.0) per field of view, or approximately 10.5%, while the specific volume of VEGF-positive cardiac muscle cells was 79.4%.

In 6-month-old animals receiving a standard diet, which can be considered controls, the content of VEGFR2-positive cardiac muscle cells was low – 15.3%. This is consistent with literature data on the low level of VEGF expression in normal cardiac muscle cells and an increase in the content of VEGF-positive cardiac muscle cells in various pathologies, in particular, with VEGFR2 expression in cardiopathy caused by antitumor drugs [18] and in acute myocardial ischemia induced by coronary artery ligation [19]. VEGFR2-immunopositivity of cardiac muscle cells, as a rule, has a spotty mosaic character, and immunopositive cardiac muscle cells alternate with unstained cells.

Thus, in acute myocardial infarction, immunohistochemical staining of cardiac muscle cells was intense in the ischemic zone without necrosis, weak in the area of coagulative necrosis without inflammatory infiltration, and absent in the foci of developed necrosis with inflammatory infiltration. Forensic studies show "patchy" myocardial immunopositivity for VEGF in cases of rapid death due to a stab wound to the heart, limited to the area surrounding the wound, but no VEGF staining in cases of asphyxia, drowning, or in cases of sudden cardiac arrest without prior ischemic-related cardiac pathology. The authors conclude that ischemic myocardial changes precede the development of VEGF immunopositivity in cardiac muscle cells, which is often accompanied by eosinophilia of cardiac muscle cells [20].

In this study, the relationship between VEGFR2 expression in cardiac muscle cells and contracture changes was assessed. Of particular note is the increased sarcoplasmic eosinophilia observed in cardiac muscle cells, typically in the form of wide transverse bands. This is a characteristic type of morphological change in cardiac muscle cells, described in literature as contracture band necrosis, which occurs during reperfusion following hypercontraction and leads to sarcolemmal rupture [21]. The mechanism by which reperfusion of ischemic heart muscle induces contracture necrosis involves the influx of calcium after a period of relative deprivation. A sudden influx of calcium, associated with excessive amounts of locally released norepinephrine, can cause irreversible contractures leading to necrosis.

In the present study, a moderate positive correlation between contracture and VEGFR2 expression in cardiac muscle cells was found in 18-month-old animals fed with HCHFD (group 4). In young rats (6 months) receiving a standard diet, VEGFR2 expression showed the greatest correlation with contracture changes, according to multiple regression analysis. Meanwhile, in aged rats receiving the HCHFD, karyopyknotic changes had the greatest impact on VEGFR2 expression.

The identified clear positive correlation between the specific volume of cardiac muscle cells expressing VEGFR2 and the content of alternatively altered cardiac muscle cells likely indicates an adaptive-compensatory role of VEGFR2 in myocardial injury. Thus, it is known that intramyocardial injection of VEGF165 cDNA can significantly improve cardiac function, stimulate angiogenesis, reduce infarct size and apoptosis of cardiac muscle cells, inhibit the expression of myocardial p53, Fas and Bax, and increase the expression of VEGF and Bcl-2 in the myocardium in an acute infarction model [22–24]. However, the exact mechanism by which VEGF DNA causes these effects is still unclear.

At the same time, a number of studies have shown that VEGF reduces damage to cardiac muscle cells caused by unsaturated fatty acids, in particular

palmitate, via the JNK signaling pathway. High-fat diet induced JNK activation [25], which was abolished by TLR4 knockout [26–28]. Cellular ceramide accumulation activated JNK signaling and apoptosis, which was prevented by ceramide synthase 5 knockout [29, 30]. JNK activation was observed in palmitate-treated cardiac muscle cells and was attenuated by protein kinase R inhibition [31–33]. Enhanced cardiac muscle cells apoptosis upon saturated fatty acid overload may lead to myocardial infarction and cardiac dysfunction. However, viability of cardiac muscle cells was restored by VEGF overexpression during 0.5 mM palmitate treatment. This process was accompanied by a decrease in the apoptosis rate and the expression of caspase 3, Bax, and NF-κB p65. These results indicate protective effects of VEGF in combating lipotoxicity-induced cardiac muscle cells apoptosis and may identify therapeutic targets for cardiovascular protection in combating fatty acid stress [6].

CONCLUSION

HCHFD induces predictable changes in VEGFR2 expression in cardiac muscle cells, depending on age and the degree of myocardial injury. An increase in the proportion of VEGFR2-immunohistochemically positive cardiac muscle cells occurs in young (6 months) rats fed with HCHFD, in old rats (18 months) receiving a standard diet, and, to the greatest extent, in older animals maintained on HCHFD. Changes in the proportion of cardiac muscle cells expressing VEGFR2 correlate with the proportion of cardiac muscle cells with morphological signs of injury, such as karyopyknosis, contracture, and depletion of the perinuclear zone of sarcoplasm. According to multiple regression analysis, karyopyknotic abnormalities had the greatest impact on changes in VEGFR2 expression in cardiac muscle cells in old animals.

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