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Morphological and functional characteristics of retrosternal adipose tissue and their relation to arterial stiffness parameters in patients after coronary artery bypass grafting

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ABSTRACT

Background. The attention of many researchers is focused on studying the role of adipokines secreted by subcutaneous, visceral, epicardial, and perivascular adipose tissues in the pathogenesis of diseases of the cardiovascular system. At the same time, adipose tissue of retrosternal localization remains out of research focus. This pool of fat cells is formed at the site of the thymic involution and has a significant volume. However, their functional activity and participation in the development of cardiovascular pathology remain unexplored.

Aim. To study the morphological characteristics of adipocytes of the retrosternal adipose tissue (RSAT) and their production of adipokines in comparison with epicardial (EAT) and subcutaneous adipose tissue (SCAT) and to investigate their relationships with arterial stiffness parameters in patients who underwent coronary artery bypass grafting.

Materials and methods. The study included 17 patients (12 men / 5 women aged 40 – 70 years) with the diagnosed coronary artery disease (CAD) who underwent coronary artery bypass grafting (CABG). Each patient underwent measurement of carotid-femoral pulse wave velocity (PWV) and aortic augmentation index (AIx) with the oscillometric device. Isolated adipocytes were obtained enzymatically from explants of SCAT, EAT and RSAT during coronary artery bypass grafting. The adipocytes were analyzed under the microscope at magnification 200. The release of adiponectin, leptin and insulin was studied in the adipocyte supernatant after 1 hour incubation using ELISA.

Results. It was found that adipocytes of the RSAT are smaller than adipocytes of SCAT: 83.96 ± 2.21 vs 98.62 ± 2.67 μm ($p = 0.00002$), respectively, and comparable in size to adipocytes of EAT: 86.65 ± 1.33 μm. The release of adiponectin by adipocytes of the RSAT turned out to be comparable to the production of this adipokine in SCAT and EAT, however, adipocytes of the RSAT produce less leptin than SCAT and EAT: 0.26 (0.19; 0.27) ng/l vs 0.37 (0.28; 0.55) ($p = 0.01$) and vs 0.32 (0.28; 0.44) ($p = 0.006$) ng/ml, respectively. Furthermore, RSAT produce less insulin than SCAT and EAT: 1.56 (1.03; 2.08) vs 1.70 (0.99; 2.18) ng/ml, ($p = 0.0022$) and 1.76 (1.16; 2.40) ng/ml ($p = 0.006$), respectively.

A positive correlation was found between the secretion of leptin by adipocytes of the RSAT and the AIx ($r_s = 0.52$, $p = 0.046$). An inverse relationship was found between insulin secretion by retrosternal adipocytes and PWV ($r_s = –0.55$, $p = 0.035$). There was no relationship between the size of the retrosternal adipocyte or hypertrophy of the thymic adipocytes (more than 100 μm) and the production of leptin and insulin and arterial stiffness parameters.

Conclusions. The data of our pilot study show that adipocyte hypertrophy of the retrosternal AT is not a significant marker of adipokine production disturbance. The observed relationships suggest that an increase in leptin production and reduced insulin secretion by retrosternal AT may contribute to the formation of adipokine-related

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arterial stiffness. Based on the data obtained, it can be assumed that adipokines produced by the retrosternal AT can participate in the formation of arterial stiffness in patients with coronary artery disease.

Key words: epicardial, retrosternal and subcutaneous adipose tissue, arterial stiffness, adipocyte, adipokines, coronary artery disease.

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

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Conformity with the principles of ethics. The study was performed in accordance with the Helsinki Declaration of the World Medical Association "Ethical Principles for Conducting Scientific Medical Research with Human Participation" as amended in 2000 and the "Rules of Clinical Practice in the Russian Federation" approved by the Order No. 266 of the Ministry of Health of the Russian Federation of June 19, 2003. The research was approved by the local Ethics Committee of the Cardiology Research Institute of Tomsk National Research Center (Protocol No.146 of 16.06.2016). All individuals included in the study signed an informed consent.

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Морфофункциональные характеристики загрудинной жировой ткани и их связь с артериальной жесткостью у пациентов с коронарным атеросклерозом, подвергшихся операции аортокоронарного шунтирования

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РЕЗЮМЕ

Цель – исследование морфофункциональных характеристик адипоцитов загрудинной жировой ткани (ЗЖТ) в сравнении с другими типами жировой ткани (ЖТ), а также изучение их связи с показателями артериальной жесткости у пациентов с коронарным атеросклерозом, подвергшихся операции аортокоронарного шунтирования (АКШ).

Материалы и методы. В настоящее пилотное исследование включены 17 пациентов (12 мужчин и 5 женщин) в возрасте 40–70 лет со стабильной ишемической болезнью сердца и документированным коронарным атеросклерозом, которым была проведена операция АКШ и которые подписали информированное согласие на участие в исследовании. Материалом для исследования явились эксплантаты эпикардиальной, подкожной и загрудинной жировой ткани (ЗЖТ), их забор осуществлялся в ходе операции. Для изучения состояния регионарной артериальной жесткости использовали осциллометрическую артериографию (TensioMed, Венгрия). Определяли уровень адипонектина, лептина, инсулина в супернатантах адипоцитов.

Результаты. Обнаружено, что адипоциты ЗЖТ имели меньшие размеры, чем адипоциты подкожной ЖТ, и были сопоставимы по размеру с эпикардиальными адипоцитами. Выброс адипонектина адипоцитами ЗЖТ не имел различий с таковым в подкожной и эпикардиальной ЖТ, однако адипоциты ЗЖТ вырабатывали существенно меньше лептина и инсулина. Впервые продемонстрирована взаимосвязь выработки адипоци-

тами ЗЖТ лептина и инсулина с показателями регионарной артериальной жесткости: прямая корреляционная связь – между секрецией лептина адипоцитами ЗЖТ и аортальным индексом аугментации и обратная – между секрецией инсулина адипоцитами ЗЖТ и скоростью пульсовой волны. Линейных корреляций между размерами адипоцитов ЗЖТ, наличием адипоцитов >100 мкм ЗЖТ и выработкой адипоцитами ЗЖТ лептина, инсулина, а также параметрами регионарной артериальной жесткости выявлено не было.

Заключение. Продемонстрировано наличие у ЗЖТ секреторной активности, интенсивность которой не имеет линейных ассоциаций с размером адипоцитов и их гипертрофией. Полученные нами данные впервые указывают на взаимосвязь адипокинов, вырабатываемых ЗЖТ, с процессами нарушения эластических свойств магистральных артерий у пациентов с коронарным атеросклерозом.

Ключевые слова: эпикардиальная, загрудинная и подкожная жировая ткань, артериальная жесткость, адипоциты, адипокины, коронарный атеросклероз.

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

Источник финансирования. Статья подготовлена в рамках темы фундаментальных исследований № АААА-А15-115123110026-3.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование было одобрено локальным этическим комитетом НИИ кардиологии Томского НИМЦ (протокол № 146 от 16.06.2016).

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INTRODUCTION

Currently, the attention of a large number of researchers is focused on studying the role of adipokines secreted by visceral adipose tissue and its ectopic depots in the pathogenesis of cardiovascular diseases [1–3]. Numerous studies have shown the pathological role of epicardial obesity, while adipose tissue of the retrosternal localization remains out of research focus. This pool of adipose cells has a significant volume and is formed mainly due to age-related involution of the key organ of the immune system – the thymus, when it is almost completely replaced by adipose tissue [4]. The morphological characteristics of adipocytes of the retrosternal adipose tissue (RSAT), their functional activity and possible participation in the development of cardiovascular pathology have not been studied yet. However, it is the structural and functional features of fat depots that are the most important pathological factor in development of a high cardiometabolic risk.

It is known that both obesity and arterial stiffness are independent predictors of cardiovascular morbidity and mortality [5]. Recent publications have reported a close relationship between the epicardial fat depots and an increase in arterial stiffness, which is presumably associated with adipocyte dysfunction and impaired adipokine production [6], while there is no information on this with respect to RSAT.

The aim of this work was to study the morphological and functional characteristics of RSAT adipocytes in comparison with other types of adipose tissue (epicardial and subcutaneous) as well as to investigate their potential relationship with arterial stiffness in patients with coronary atherosclerosis after coronary artery bypass grafting (CABG).

MATERIALS AND METHODS

The present pilot study included 17 patients (12 men and 5 women) aged 40-70 years with stable coronary artery disease (CAD) and documented coronary atherosclerosis, who had indications for CABG.

The study was performed in accordance with the Helsinki Declaration of the World Medical Association “Ethical Principles for Conducting Scientific Medical Research with Human Participation” as amended in 2000 and the “Rules of Clinical Practice in the Russian Federation” approved by the Order No. 266 of the Ministry of Health of the Russian Federation of June 19, 2003. The research was approved by the local Ethics Committee of the Cardiology Research Institute of Tomsk National Research Center (Protocol No. 146 of 16.06.2016). All individuals included in the study signed an informed consent.

All patients received regular drug therapy. The proportion of smokers and patients with metabolic disorders that met the criteria for the metabolic syndrome [7] was high. The clinical characteristics of patients are presented in Table 1.

Exclusion criteria were acute atherosclerotic complications over the past 6 months; any inflammatory disease; chronic kidney disease above C3b; and oncological, hematological and immune diseases.

Table 1

Clinical and demographic characteristics of the studied patients (n = 17)

Parameters	
Gender	12/5
Age, years old	63 (59;66)
History of myocardial infarction, *n* (%)	6 (35.3)
Arterial hypertension, *n* (%)	15 (88.2)
Diabetes mellitus, *n* (%)	4 (23.5)
Duration of arterial hypertension, years, *Me* (Q_{25}; Q_{75})	15 (10; 20)
Duration of coronary artery disease, years, *Me* (Q_{25}; Q_{75})	2 (1.75; 5.5)
Systolic blood pressure, mm Hg, *Me* (Q_{25}; Q_{75})	135 (127; 142)
Diastolic blood pressure, mm Hg, *Me* (Q_{25}; Q_{75})	77.5 (69.5; 84.5)
Smoking, *n* (%)	11 (64.7)
Body mass index, kg/m^2, *Me* (Q_{25}; Q_{75})	29.4 (28.1; 31.2)
Obesity, *n* (%)	8 (47)
Waist circumference, cm	103 (92; 110)
Note: *Me* [Q_1;Q_3]	

All patients underwent selective coronary angiography on the Cardioscop-V angiographic complex and Digitron-3NAC computer system, Siemens (Germany), at the Department of X-ray Diagnosis and Treatment (supervisor – Bayev A.E., Cand. Sci. (Med.)). Anthropometric measurements were performed to assess total obesity according to body mass index (BMI) and abdominal obesity according to the waist circumference. Oscillometric arteriography (TensioMed, Hungary) was used to study the state of regional arterial stiffness. The pulse wave propagation velocity (PWV) and aortic augmentation index were evaluated.

The material for the study was explants of retrosternal (RSAT), subcutaneous (SCAT) and epicardial (EAT) tissues weighing 0.5–1 g. The explants were collected during CABG. The samples were placed in M199 medium and delivered to the laboratory within 15 minutes. The adipose tissue cells were isolated enzymatically, in sterile conditions of the laminar cabinet of the II protection class (BAVp-01 – Laminar-s – 1.5, Laminar systems, Miass, Russia) [8]. The tissue was minced, incubated for 35–40 min at a temperature of 37 ° C and constant gentle stirring (10 rpm) in 5 ml of a sterile solution of type I collagenase (PanEco, Russia) 1 mg/ml in the Krebs – Ringer buffer (2 mM D-glucose, 135 mM NaCl, 2.2 mM CaCl2 · 2H2O, 1.25 mM MgSO4 · 7H2O, 0.45 mM KH2PO4, 2.17 mM Na2HPO4, 25 mM HEPES, 3.5% BSA, 0.2 mM adenosine). To neutralize collagenase, the Krebs – Ringer buffer was added in a 1:1 ratio. The cell suspension was filtered through a nylon filter (Falcon ™ Cell strainer, pore diameter 100 μm), and washed three times with warm Krebs – Ringer buffer. In each sample, 200–600 cells in total were analyzed in non-overlapping visual fields using light microscopy at magnification 200 (Axio Observer Z1 microscope, Carl Zeiss Surgical GmbH, Germany) (Fig. 1). The number and size of the adipocytes were calculated. Adipocytes > 100 μm were classified as hypertrophic. Adipocytes in the amount of 20 × 10^5 were added to a well of a sterile 24-well plate (Greiner, Germany); the volume of the well was adjusted to 1 ml and incubated for 1 hour at 37 °C with constant stirring at 10 rpm.

a *b* *c*

Fig. 1. Light microscopy of isolated adipocytes of subcutaneous (*a*), epicardial (*b*) and retrosternal (*c*) adipose tissue. Magnification 200

Adipocyte supernatants were collected from the bottom of the wells, frozen and stored at –70°C.

The adiponectin ELISA kit (Mediagnost, Germany) was used to determine adiponectin in adipocyte supernatants, the Leptin Sensitive ELISA kit (Mediagnost, Germany) was used to determine leptin; insulin was determined using the Insulin Test System kit (Monobind Inc.; USA)

Statistical analysis was performed using the Statistica 10.0 software (StatSoft Inc., USA). The data distribution was tested the by Shapiro – Wilk test. The median and interquartile range of the 25th and 75th percentiles were used to describe data with non-standard distribution. A mean and a standard error of the mean (*M* ± *SEM*) were used for description of data with standard distribution. The differences between groups with non-standard distribution were determined according to the Wilcoxon signed rank test. In cases with standard data distribution, the paired sample t-test was used. To evaluate the relationship between parameters, the Spearman's rank correlation coefficient (*Rs*) was used. All statistical hypotheses were accepted at $p < 0.05$.

RESULTS

A morphometric study showed no difference in the size of adipocytes of RSAT and EAT, while the size of adipocytes of RSAT was significantly smaller than that of SCAT (Table 2). The part of hypertrophied adipocytes, defined as the percentage of cells >100 μm, was more than two times less in the RSAT than in the SCAT. The part of hypertrophied adipocytes of EAT did not differ from the same indicator for RSAT, and the part of small adipocytes (< 50 microns) in all three tissues was comparable. For RSAT, this indicator amounted to 2.23 (1.08; 4.40) %, for EAT – 2.07 (0.83; 3.87)%, and for SCAT – 1.92 (0.23; 4.68)%. Therefore, RSAT adipocytes are smaller and less hypertrophied than SCAT adipocytes. At the same time, adipocytes of RSAT are comparable in size to epicardial adipocytes. The size of RSAT adipocytes did not correlate with the body mass index.

Table 2

The size of adipocytes in subcutaneous, epicardial and retrosternal adipose tissue

Parameters	Subcutaneous adipose tissue	Epicardial adipose tissue	Retrosternal adipose tissue
Adipocyte size, μm, *M* ± *SEM*	98.62 ± 2.67	86.65 ± 1.33	83.96 ± 2.21 $p_1 = 0.00002$ $p_2 = 0.23$
Percentage of hypertrophied adipocytes, %, *Me* (Q_{25}; Q_{75})	47.66 (31.78; 55.50)	17.59 (9.84; 25.53)	16.11 (11.73; 30.20) $p_1 = 0.000062$ $p_2 = 0.81$

Note. Adipocytes >100 μm were considered hypertrophied; p_1 – comparison of retrosternal adipose tissue with subcutaneus adipose tissue, p_2 – comparison of retrosternal adipose tissue with epicardial adipose tissue (paired sample t-test); p_1 – comparison of retrosternal adipose tissue with subcutaneous adipose tissue, p_2 – comparison of retrosternal adipose tissue with epicardial adipose tissue (using Wilcoxon signed-rank test).

Adiponectin release by adipocytes of RSAT was found to be comparable with its production by adipocytes of EAT and SCAT (Table 3). It was found that the level of leptin in the incubation medium of RSAT adipocytes was 30% and 20% lower than this indicator for SCAT and EAT, respectively.

Table 3

Production of adipokines by adipocytes of subcutaneous, epicardial and retrosternal adipose tissue, *Me* (Q_{25}; Q_{75})

Parameters	Subcutaneous adipose tissue	Epicardial adipose tissue	Retrosternal adipose tissue
Adiponectin, ng/ml	10.59 (8.31; 12.25)	7.93 (6.77; 10.11)	10.21 (8.77; 12.78)
Leptin, ng/ml	0.37 (0.28; 0.55)	0.32 (0.28; 0.44)	0.26 (0.19; 0.27) $p_1 = 0.009$ $p_2 = 0.006$
Leptin/adiponectin	0.038 (0.028; 0.069)	0.033 (0.019; 0.044)	0.022 (0.019; 0.028) $p_1 = 0.001$ $p_2 = 0.004$
Insulin, ng/ml	1.70 (0.99; 2.18)	1.76 (1.16; 2.40)	1.56 (1,03; 2.08) $p_1 = 0.002$ $p_2 = 0.006$

Note. p_1 – comparison of retrosternal adipose tissue with subcutaneus adipose tissue, p_2 – comparison of retrosternal adipose tissue with epicardial adipose tissue (using Wilcoxon signed rank test).

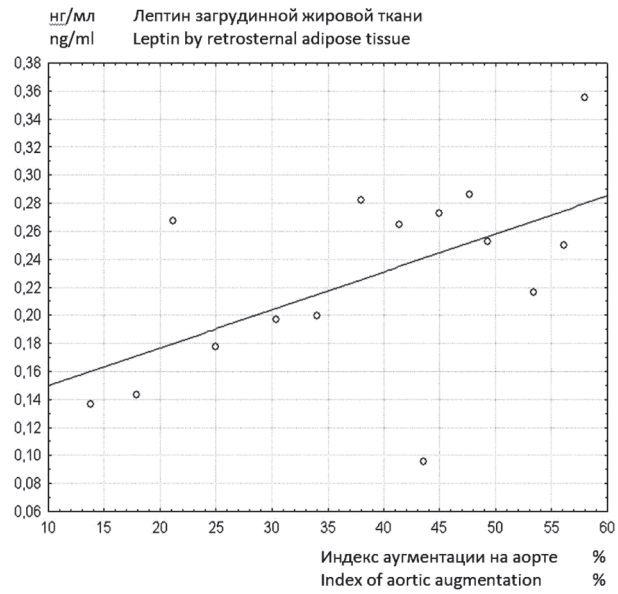

Fig. 2. Correlation between the production of leptin by retrosternal adipose tissue adipocytes and index of aortic augmentation, $r_s = 0.52$, $p = 0.047$

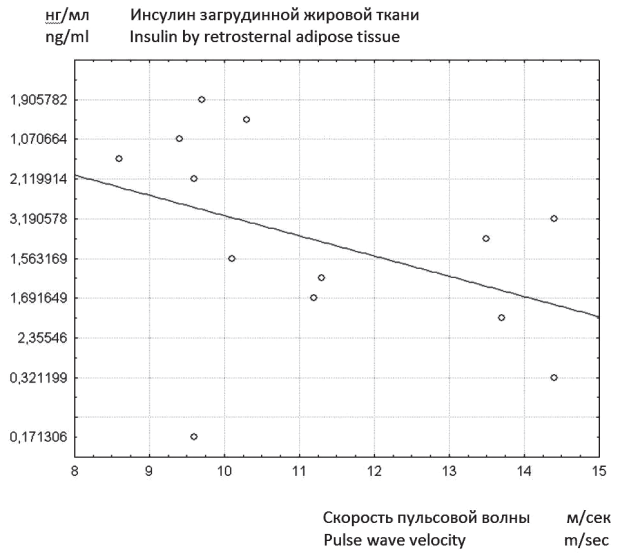

Fig. 3. Correlation between the production of insulin by retrosternal adipose tissue adipocytes and pulse wave velocity, $r_s = –0.55$, $p = 0.035$

Since there were no significant differences in the production of adiponectin by the studied adipose tissue, the RSAT leptin/adiponectin index was one and a half times lower than for SCAT and EAT. The production of insulin by adipocytes of RSAT exceeded that by 8% ($p = 0.022$) for SCAT and by 11% ($p = 0.006$) for EAT.

During correlation analysis, we first discovered the relationship between the secretion of leptin and insulin by RSAT adipocytes and the parameters of regional arterial stiffness. A direct correlation between the secretion of leptin by RSAT adipocytes and the aortic augmentation index was observed (Fig. 2). An indirect relationship was observed between the secretion of insulin by RSAT adipocytes and the pulse wave propagation velocity, PWV (Fig. 3). At the same time, we did not reveal a linear relationship between the sizes or hypertrophy of RSAT adipocytes and the production of leptin, insulin and the leptin / adiponectin ratio.

DISCUSSION

The relationship between the accumulation and dysfunction of visceral adipose tissue and the risk of developing cardiovascular disease was confirmed in numerous studies [9–11]. The key factors of this pathological chain are the excess production of adipokines and reactive oxygen species by adipose tissue [2, 3, 12, 13]. A relationship between dysfunction of adipocytes of small fat depots – perivascular and epicardial localization – and the development of atherosclerosis [14, 15, 16], impaired arterial elasticity [6], arterial hypertension [17], heart failure, and arrhythmia was found [18]. An association of visceral obesity and increased leptin production in adipose tissue with the systemic hypertension and increased arterial stiffness was reported [19–21], while the retrosternal fat depots formed as a result of age-related thymic involution remain out of focus of investigators.

In our work, the morphological characteristics of adipocytes of retrosternal adipose tissue and their production of insulin and adipokines in patients with coronary atherosclerosis who underwent CABG were studied for the first time. According to the results of our study, the size of adipocytes of RSAT corresponds to that in EAT. Our data do not confirm the association of RSAT adipocyte size with body mass index or the intensity of adipokine production by them. That does not allow us to consider RSAT adipocyte hypertrophy as a significant marker of impaired adipokine production. The lack of correlation between the size of adipocytes and BMI has been previously shown for epicardial adipocytes and, possibly, is typical for small fat depots [22]. At the same time, in the literature, there are few data on the correlation between the

size of EAT adipocytes and the leptin and adiponectin level in blood serum [23]. There is no information on the ratio of the size/production of adipokines by EAT adipocytes. Thus, the current opinion about the dependence of the adipocyte secretory activity on its hypertrophy for small fat depots requires additional factual evidence.

Our results showed that the intensity of adiponectin secretion by the RSAT cells does not differ from that in EAT and SCAT, which makes it possible to consider RSAT as a secretory organ. Nevertheless, the production of leptin and insulin by RSAT adipocytes, according to our data, turned out to be significantly lower than in EAT and SCAT. This fact may be associated with the formation of RSAT on the site of a previously functionally active immune regulatory organ – the thymus, which underwent age-related involution.

We found a direct correlation between the production of leptin by RSAT and the aortic augmentation index. An indirect relationship between the production of insulin in RSAT and the pulse wave velocity was observed. Considering the mechanism of the leptin influence on the state of arterial stiffness, it can be assumed that it can be realized through the profibrotic effect of leptin. In an experimental study on a model of isolated smooth muscle cells derived from the aorta of obese rats, it was found that the addition of leptin to the incubation medium led to an increase in the collagen II gene expression, a rise in the cellular content of collagen, fibronectin, a transforming growth factor (TGFβ) and connective tissue growth factor (CTGF) [24]. In addition, the ability of leptin to induce hypertrophy of vascular smooth muscle cells, their osteogenic differentiation and expression of metalloproteinases was reported [25].

Our data on the indirect relationship of insulin produced by RSAT adipocytes with arterial stiffness are consistent with the published data. Thus, experimental studies have shown a decrease in the elastic properties of the aorta in insulin-resistant rats [26] and in rats with diabetes [27]. This fact is confirmed by the results of clinical studies on the inverse dependence of arterial stiffness parameters (pulse wave velocity) on the dosage of insulin administered to patients with metabolic syndrome and type 2 diabetes mellitus [28]. Among the mechanisms mediating the effect of insulin on the elastic properties of the arterial wall, one can consider its effects on the intracellular protective mechanism, including the phosphorylation of Akt, ERK-1/2 and JNK-1/2 kinases followed by activation of the hypoxia-induced factor 1α (HIF-1α) [29], as well as the suppressive effect of insulin on nitric oxide synthase type II (eNOS) [30].

CONCLUSION

Thus, the results of this pilot study demonstrate the presence of secretory activity in the retrosternal adipose tissue, the intensity of which does not have a linear relationship with the size of adipocytes and their hypertrophy. Our data revealed an association between the production of adipokine in the retrosternal adipose tissue and deterioration of the elastic properties of arteries in patients with coronary atherosclerosis.

The limitations of the study are its small volume and a lack of separate studies on the morphological and functional features of RSAT in men, women and patients with diabetes mellitus and obesity.

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