Adipocytokines in metabolically healthy obesity

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ABSTRACT

The aim of the investigation was to study the relationship among adipokines, markers of subclinical inflammation and endothelial dysfunction in patients with metabolic healthy obesity (MHO). The study included 50 persons aged 25-50 years with obesity in the absence of metabolic disorders (International Diabetes Federation criteria, 2005, marked as MHO), the control group consisted of 50 healthy respondents without obesity. We studied clinical and biochemical parameters, insulin resistance index (HOMA-IR), levels of leptin, soluble leptin receptors (sLR), resistin, adiponectin, C-reactive protein (CRP-hs), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), endothelin-1 (ET-1), von Willebrand factor, free leptin index was calculated in a formula (FLI = leptin × 100 / sLR). In MHO patients, independently of HOMA-IR index, there was an increase in leptin, FLI, resistin, VEGF, and IL-6 parameters. The concentration of CRP-hs and TNF-α in MHO group with HOMA-IR ≥2.7 was increased. Systolic blood pressure correlated with leptin level (r=0.43, P<0.05), FLI (r=0.54; P=0.01), TNF-α (r=0.44; P<0.05) and IL-6 (r=0.33; P<0.05); diastolic blood pressure - with leptin level (r=0.35, P<0.05). Links between high density lipoproteins and leptin (r=–0.55 and r=–0.60; P<0.01), resistin (r=0.32; P<0.05 and r=0.60; P<0.01) and VEGF (r=–0.70, P<0.01) were established. The VEGF level correlated with HOMA-IR (r=0.62; P<0.01), leptin (r=0.29; P<0.05), FLI (r=0.50; P<0.05), resistin (r=0.70; P<0.01), IL-6 (r=0.74, P<0.01) and ET-1 (r=0.29; P<0.05). Obese patients without metabolic disorders, having normotension and normal insulin sensitivity, are less influenced to adverse cardiovascular risks due to less expressed hormonal and inflammatory activation of adipose tissue and, as a result, less pronounced endothelial dysfunction. While insulin resistance develops, cardiovascular risk increases due to activation of subclinical inflammation, angiogenic endothelial dysfunction and leptin resistance.

Introduction

The last decade is characterized by a steady increase in non-communicable diseases, especially obesity, thus according to ESSE-RF study (2013) about a quarter of the Russian population aged 35-44 years are obese.[1] Currently, various obesity phenotypes have been established, but the most discussed is a metabolically healthy phenotype - metabolically healthy obesity (MHO), a condition characterized by low cardio-metabolic risk. Data on the prevalence of MHO are very diverse and depend on the selected diagnostic criteria plus, according to different researchers, they may average between 12 and 45% of the population.[2] Studies concerning MHO, are mainly focused on the study of the prevalence, depending on the selected criteria of diagnostics and the prediction of cardiovascular risk.[3,4] The works devoted to the peculiarities of adipokine status, systemic inflammation and endothelial dysfunction within the MHO group are isolated, and their results are contradictory.[2,5,6]

The objective of the research is to study the relationship of adipokines with the markers of subclinical inflammation and endothelial dysfunction (ED) in patients with metabolically healthy obesity.

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Materials and Methods

Fifty people aged 25-50 years (women - 65%) with obesity without metabolic disorders (International Diabetes Federation criteria, 2005) and arterial hypertension (AH) (European recommendations for AH 2013) - MHO group were examined. The MHO group was divided into subgroups depending on the degree of insulin resistance (IR) by the level of HOMA-IR. 1st MHO sub-

group with HOMA-IR <2.7 (n=35), 2nd MHO subgroup with HOMA-IR ≥2.7 (n=15). The comparison group included 50 healthy respondents with body mass index (BMI) <25 kg/m^2. *Exclusion criteria*: endocrine forms of obesity, obesity-associated diseases and conditions. All patients underwent clinical and laboratory examination, according to medical and economic standards. The concentration of biomarkers in serum was investigated by enzyme immunoassay (ELISA) using reagent sets: leptin - the DBC (Canada), insulin - ELISA Monobind Inc. (Germany), adiponectin, soluble leptin receptors (sLR) and resistin - BioVender (Czech Republic), endothelin-1 (ET-1) Biomedica ENDOTELIN (1-21) (Germany), C-reactive protein (CRP-hs), vascular endothelial growth factor (VEGF), tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) - Vector-best company (Novosibirsk, Russia). Von Willebrand factor (vWF) was determined in blood plasma by a set of TECHNOZYM vWF: Ag ELISA (Austria), insulin resistance index (HOMA-IR) was calculated in a small homeostasis model, free leptin index (FLI = leptin × 100 / sLR).

The software program Statistica 10.0 was used for statistical data processing. Mean value (M), standard deviation (SD) and Student *t*-test were used to evaluate data with normal distribution. Data with abnormal distribution were presented as median (Me) and interquartile range [25;75]. For multiple comparisons between groups we used the criterion Kruskal-Wallis, a pairwise comparison within the same unit using the Mann-Whitney test. Differences between the samples were significant at P<0.05. The correlation of features was evaluated by regression analysis with the determination of Spearman rank correlation coefficient.

Results

The mean age of the examined patients was 44.8±7.7 years, mean BMI: 36.0±5.5 kg/m^2. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) did not differ from the control group (SBP: 125±7.4 mmHg and 125±5.1 mmHg; DBP: 74±8.5 mmHg and 74.1±4.3 mmHg; P=0.9). In the MHO subgroup with HOMA-IR ≥2.7 DBP level was significantly higher than in the subgroup with HOMA-IR <2.7 (71.6±8.1 mmHg and 79.1±6.6 mmHg; P=0.02). Analysis of metabolic parameters revealed no significant differences between the MHO and control groups. It should be noted that in the MHO subgroup with HOMA-IR ≥2.7 in the absence of metabolic syndrome criteria, the levels of postprandial glycemia (5.3±1.1 mmol/L and 4.8±1.0 mmol/L; P<0.05), low-density lipoprotein (LDL) cholesterol (1.3±0.2 mmol/L and 1.6±0.1 mmol /L; P<0.05), triglycerides (TG) (1.4±0.5 mmol/L and 1.0±0.5 mmol/L; P<0.05) and uric acid (300±43.1 μmol/L and 226.2±36.8 μmol/L; P<0.05) were significantly higher than in healthy individuals.

In the MHO group, high-density lipoprotein (HDL) cholesterol reduction was associated with an increase in fasting glycemia (r=–0.68; P<0.01), and SBP had a direct relationship with TG (r=0.37; P<0.05). This trend was observed in both MHO subgroups, but in the subgroup with HOMA-IR ≥2.7 this relationship was statistically stronger (r=–0.71 and r=0.57, respectively; P<0.01).

The study of the hormonal activity of adipose tissue revealed no differences. The level of adiponectin was lower in the subgroup with HOMA-IR ≥2.7 both with respect to the control group and the MHO subgroup with HOMA-IR <2.7 (Table 1).

The level of resistin in the MHO group was higher than in the control group (3.4 [2.5;3.9] ng/mL and 2.2 [1.7;2.8] ng/mL; P<0.05). The analysis revealed a significant increase in the level of resistin in the subgroup with HOMA ≥2.7 in comparison with the control group, while in the subgroup with normal IR there was only a tendency to increase the concentration of the marker (Figure 1).

In the MHO group, there was a relationship between leptin, FLI and resistin with HOMA-IR (r=0.40; P<0.05; r=0.54; P=0.01; r=0.62; P=0.001), and leptin with the level and FLI of SBP (r=0.43, P<0.05; r=0.60; P=0.01) and DBP (r=0.35, P<0.05; r=0.70; P=0.01). Note that in the subgroup of metabolically non-healthy obesity group with HOMA-IR <2.7 correlations between SBP and DBP levels were obtained only through FLI (r=0.30 and r=0.35; P<0.05). Positive correlations between the level of FLI, resistin, and TG (r=0.35 and r=0.45; P<0.05) were obtained in the subgroup of international normalized ratio with HOMA-IR> 2.7. Note that in the subgroup of MHO with HOMA-IR<2.7 correlations between SBP and DBP levels were obtained only with FLI (r=0.30 and r=0.35; P<0.05). Positive correlations between the level of FLI and resistin with TG (r=0.35 and r=0.45; P<0.05) were obtained in the subgroup of MHO with HOMA-IR >2.7.

The study of proinflammatory status and ED markers in the groups did not reveal any differences in the values of vWF and ET-1 between the groups. The levels of CRP-hs and TNF-α in the MHO group with HOMA-IR ≥2.7 were higher than in the control group (Table 2).

The level of IL-6 in the MHO group was higher than in the control group, regardless of the level of HOMA-IR. It should be emphasized that IL-6 level increased with the increase of IR (Figure 2).

In the MHO group, TNF-α and IL-6 were associated with SBP (r=0.44 and r=0.33; P<0.05), HDL cholesterol (r=−0.55 and r=−0.60; P<0.01), FLI (r=0.30 and r=0.39; P<0.05) and resistin (r=0.32; P<0.05 and r=0.60; P<0.01). The values of IL-6 and CRP-hs were

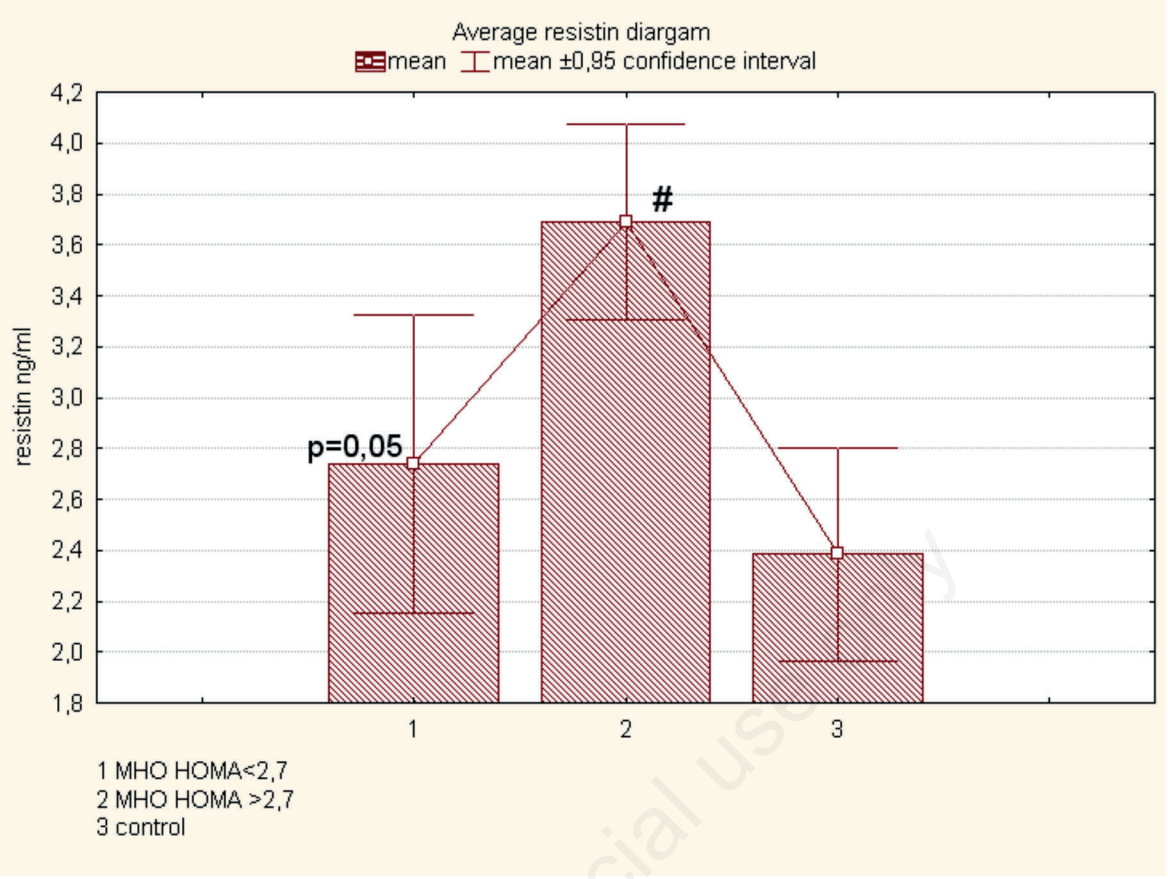

Figure 1. The level of resistin in subgroups of metabolically healthy obesity (MHO) in comparison with the control group. Note: *P<0.05; #P<0.01.

Table 1. Hormonal activity of adipose tissue in groups.

Indicator	The median value [25; 75%] Kruskal-Wallis test H (2, N=100) Mann-Whitney U-test				
	1	2	3	4	P
	MHO(n=50)	MHO HOMA-IR <2.7 (n=35)	MHO HOMA-IR ≥2.7 (n=15)	Control (n=50)	
Insulin μIU/mL	12 [11.0;18.0]	8.4 [8.2;10.1]	16.0 [13.4;17.5]	9 [7.0-12.0]	P=0.1 P_{1-2}=0.04 P_{1-3}=0.04 $P_{2-3;3-4}$=0.01
Adiponectinμg/ml	20.5 [16.3-20.7]	20] [7.0;27.1	19.0 [16.3;19.8]	21.7 [17.3-27.7]	P=0.7 $P_{1-2;1-3;1-4;2-3;2-4}$ >0.05 $P_{2-3;3-4}$=0.04
Leptin, ng/mL	36 [29.0;43.0]	39.6 [29;46]	40.1 [36.0;50.1]	8.7 [1.5;12.9]	P=0.01 $P_{1-2;1-3;2-3}$ >0.05 $P_{1-4;2-4;3-4}$=0.0001
sLR, ng/mL	14.2 [12.0;20.0]	14.0 [13.9;21.0]	12.1 [12.1;20.0]	20.4 [16.8;22.2]	P=0.01 $P_{1-2;1-3;2-3}$>0.05 $P_{1-4;2-4;3-4}$=0.0001
FLI	248.5 [192.0;417.0]	252.0 [143.0;350.0]	333.0 [250.0;420.0]	40.6 [25.0;56.0]	P=0.01 $P_{1-2;1-3}$>0.05 P_{2-3}=0.04 $P_{1-4;2-4;3-4}$=0.0001

MHO, metabolic healthy obesity; HOMA-IR, insulin resistance index; sLR, soluble leptin receptors; FLI, free leptin index; P, the accuracy of differences among several groups; $P_{1-2;1-3;1-4;2-3;3-4}$, significance of differences between groups.

associated with LDL-C (r=0.33, P<0.05 and r=0.70; P<0.01) and TG (r=0.29 and r=0.36; P<0.05), and the level of CRP-hs correlated with resistin (r=0.33; P<0.05) and FLI (r=0.40; P<0.05). It should be noted that reliable connections were obtained in the general group of MHO and subgroup with HOMA-IR >2.7, whereas negative relationship in the MHO subgroup with HOMA <2.7 was obtained only between IL-6 and

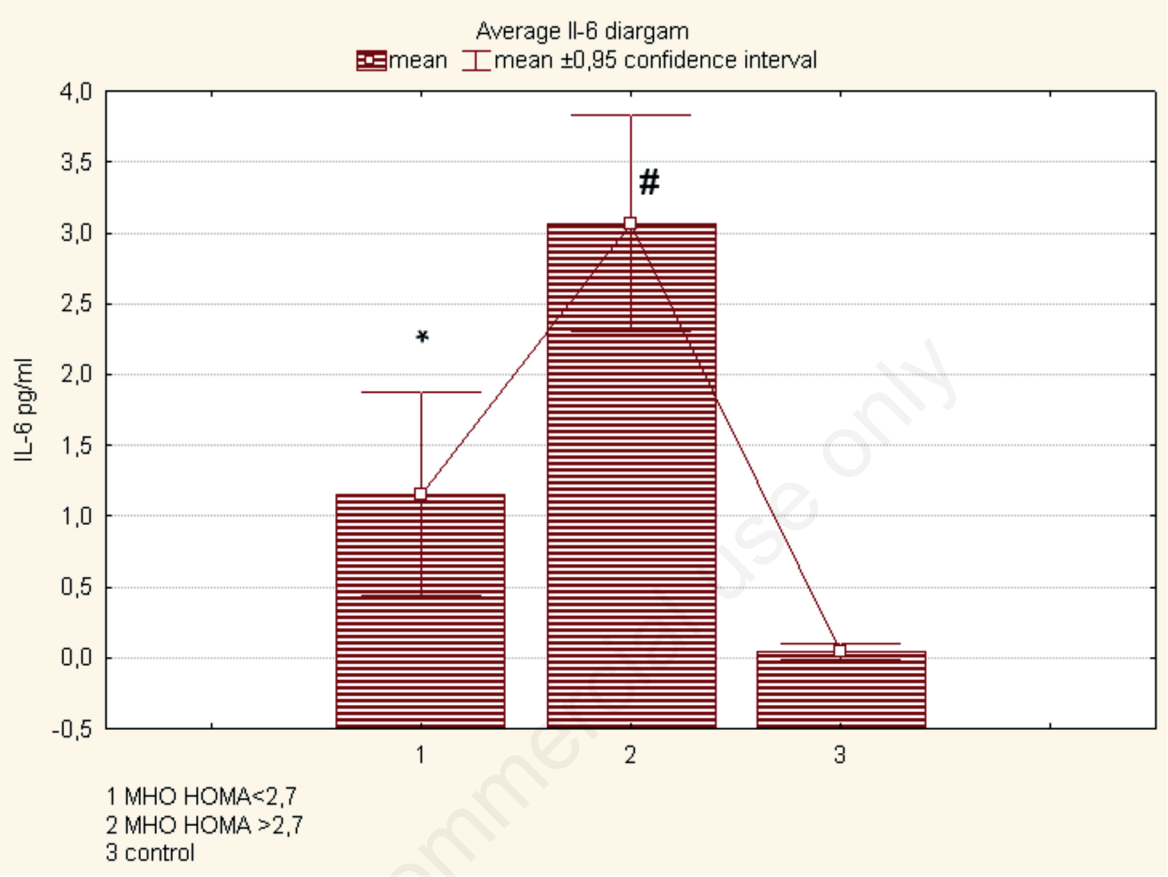

Figure 2. The comparison of the values of interleukin (IL)-6 in metabolically healthy obesity (MHO) subgroups with control group. *P<0.01; #P<0.001.

Table 2. Characteristics of markers of chronic subclinical inflammation and endothelial dysfunction in groups.

Indicator	The median value [25; 75%] Kruskal-Wallis test H (2, N=100) Mann-Whitney U-test				
	1 MHO (n=50)	2 MHO HOMA-IR <2.7 (n=35)	3 MHO HOMA-IR ≥2.7 (n=15)	4 Control (n=50)	P
CRP-hs, mg/L	2.8 [2.9; 4.2]	2.6 [2.2; 3.0]	3.2 [2.4; 4.0]	2.0 [1.0;3.5]	P=0.1 $P_{1-2;1-3;1-4;2-3;2-4}$ >0.05 P_{3-4}=0.03
TNF-α, pg/mL	3.0 [1.9; 3.6]	2.0 [1.0;2.3]	2.8 [2.7;3.2]	1.4 [0.0; 2.1]	P=0.07 $P_{1-2;1-3;2-4}$ >0.05 P_{1-4}=0.01 $P_{2-3;3-4}$=0.04
vWF, %	115 [95.0;125.0]	104 [90.0-120.0]	110 [95.0-125.0]	104 [95.0-120.0]	P=0.8 $P_{1-2;1-3;1-4;2-3;3-4}$ <0.05
ET-1, fmol/mL	0.5 [0.02;0.7]	0.2 [0.01-0.5]	0.4 [0.03;0.7]	0.2 [0.01-0.4]	P=0.8 $P_{1-2;1-3;1-4;2-3;2-4;3-4}$ >0.05

MHO, metabolically healthy obesity; HOMA-IR, insulin resistance index; CRP-hs, C-reactive protein; vWF, Von Willebrand factor; ET-1, endothelin 1; P-confidence of differences in several groups; $P_{1-2;1-3;1-4;2-3;3-4}$, significance of differences between groups.

HDL cholesterol (r=–0.45; P<0.05). The VEGF level in the MHO group was higher than in the control group regardless of the HOMA-IR level. It should be emphasized that with the increase of the IR the level of VEGF increased (Figure 3).

Associations of VEGF content with HOMA-IR (r=0.62; P<0.01), HDL cholesterol (r=–0.70, P<0.01), leptin (r=0.29; P<0.05), FLI (r=0.50; P<0.05), resistin (r=0.70; P<0.01), IL-6 (r=0.74, P<0.01) and ET-1 (r=0.29; P<0.05) were obtained. In the MHO subgroup with HOMA <2.7, VEGF levels were correlated with HOMA-IR (r=0.42; P<0.05), HDL cholesterol (r=−0.29; P<0.05), IL-6 (r=0.34, P<0.05), FLI (r=0.30; P<0.05) and resistin (r=0.40; P<0.05).

Discussion

In recent years, experimental studies have shown the presence of chronic subclinical inflammation in adipose tissue and its role in the formation of many pathological processes in obesity. It is known that up to 40% of all cells of visceral adipose tissue can be macrophages. Macrophage cytokines induce inflammatory shifts in adipocytes, which triggers the synthesis of cytokines by adipocytes themselves.[7] It is known that leptin implements its effects through interaction with leptin receptors, it is believed that not so much hyperleptinemia as leptin resistance at the level of transport to the central nervous system or at the post-receptor level plays an important role in the development of obesity.[8] In terms of leptin resistance, leptin loses the ability to regulate the homeostasis of fatty acids that leads to the development of lipotoxicity and oxidative stress. As a result, TG is deposited between the fibers of skeletal and cardiac muscles, and in liver cells, kidneys and myocytes, triggering the processes of atherogenesis and endothelial dysfunction.[9] The associations between FLI and TG in the subgroup with HOMA-IR >2.7 obtained in our work confirm this position. In light of the significance of metabolic inflammation in the pathogenesis of metabolic diseases, it should be noted that the protein structure of leptin is similar to cytokines, which can cause cross-linking of cytokines with the soluble leptin receptor,[7] which is confirmed by the relationship between FLI and VEGF in all groups of observation. In a number of studies, the association of AH with hyperleptinemia was obtained,[7] the established connection of FLI with the

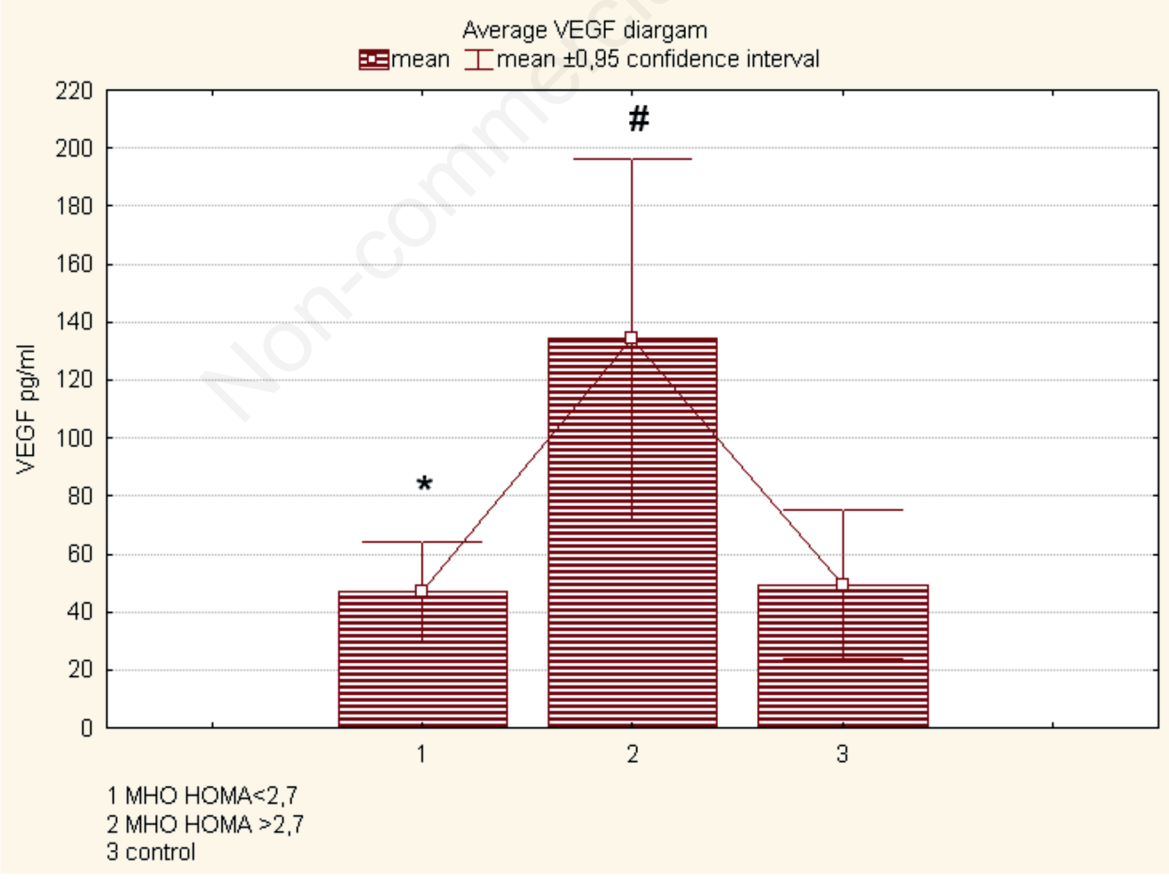

Figure 3. The comparison of vascular endothelial growth factor (VEGF) values in metabolically healthy obesity (MHO) subgroups with control group. *P>0.05; #P<0.01.

level of blood pressure in the subgroup of MHO with HOME-IR<2.7 indicates that in the regulation of blood pressure the main role is played not by the general leptin, but by leptin resistance. It should also be noted that the protein structure of leptin is similar to cytokines, which can cause cross-linking of cytokines with a soluble leptin receptor, contributing to the progression of leptin resistance.[7,8] In support of this theory in our study of TNF-α, CRP-hs and IL-6, a connection with FLI was established.

It is known that resistin, as well as TNF-α and IL-6, are more produced by brown adipose tissue cells, and its metabolic effect is manifested by a decrease in insulin sensitivity in peripheral tissues and the development of atherosclerosis.[10] TNF-α and IL-6 were found to stimulate resistin synthesis additionally.[7,10,11] Thus, the obtained relationships of inflammation markers - TNF-α and IL-6 with resistin, SBP level, lipid spectrum parameters in the MHO group with reduced insulin sensitivity indicate the initiation of mechanisms that contribute to the formation of atherosclerosis and hypertension. The progression of these events is indirectly confirmed by the accompanying decrease in the level of adiponectin and HDL cholesterol. In the subgroup with preserved insulin sensitivity, increased values of IL-6 in the absence of growth levels of CRP-hs and TNF-α can be explained by the fact that in the initial stage of obesity the synthesis of IL-6 contributes to the maintenance of the required number of anti-inflammatory macrophages in adipose tissue, controlling and limiting the inflammatory process.[12] In our study with the progression of IR, activation of the cytokine response was characterized by an increase in CRP-hs, TNF-α, which had a connection with DBP and resistin and, at the same time, the indices of lipid metabolism remained in the optimal range. It is known that IR contributes to the development of ED.[13] The increase in the volume of adipose tissue is accompanied by the growth of the vascular network, one of the factors responsible for angiogenesis is VEGF.[14] This fact explains the relationship of VEGF with adipokines and the IR index in our study. The increase of VEGF synthesis in patients with cardiovascular diseases has been proved, which allows several authors to consider it as a marker of adverse events,[15,16] the obtained association of VEGF with the level of HDL cholesterol also confirms this position. The absence of differences in ET-1 and vWF between the MHO and control groups indicates the preserved vasomotor function of endothelium and normal thrombogenic activity in patients with obesity without metabolic disorders. However, the increased values of VEGF and its relationship with resistin, IL-6 and HDL in patients with MHO with normal insulin sensitivity can indicate both the initiation of processes aimed at the formation of cardio-metabolic disorders, and a possible compensatory reaction aimed at eliminating metabolic disorders.

Conclusions

Patients with a phenotype of healthy obesity, normotension and normal insulin sensitivity are less exposed to adverse cardiovascular risks due to less pronounced hormonal and inflammatory activation of adipose tissue and, as a result, less pronounced endothelial dysfunction. As insulin resistance is formed, even in the conditions of normotension and in the absence of dyslipidemia, the cardiovascular risk increases due to the activation of subclinical inflammation, angiogenic endothelial dysfunction, and progression of leptin resistance.

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