

ADIPOKINES AND MYOKINES AS INDICATORS OF OBESE PHENOTYPES AND THEIR ASSOCIATION WITH THE GUT MICROBIOME DIVERSITY INDICES

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Today, metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) are distinguished. Adipose and muscle tissues can determine the obese phenotype due to adipokine and myokine production. Gut microbial community is also involved in MHO. The study was aimed to reveal the features of adipokine and myokine levels and their association with the gut microbiome alpha diversity in patients with MHO and MUO. A total of 265 subjects were divided into two groups: healthy individuals and obese patients. The latter were divided into two subgroups: patients with MHO and patients with MUO. Body mass index, waist circumference, HOMA-IR, adipokine and myokine levels, gut microbiome taxonomic composition, alpha diversity indices were defined in all the surveyed individuals, lipid and carbohydrate metabolism was also assessed. Significant differences in the adipokine and myokine levels and their association with the gut microbiome diversity indicators were revealed in patients with different obese phenotypes. Patients with MHO and MUO showed significantly lower adiponectin levels ($p < 0.05$) and significantly higher leptin and asprosin levels ($p < 0.05$) than healthy individuals. Patients with MUO had lower adiponectin and leptin levels ($p < 0.05$) than patients with MHO. Significantly higher FGF21 levels were observed in patients with MUO. Large-scale correlation analysis revealed the relationship between the glucose levels and the gut microbiome diversity indices that was missing in patients with MUO. This indicated the loss of the microbiota diversity effects on the blood glucose control in individuals with MUO, as well as different regulatory roles in the gut microbiome–liver–muscle/adipose tissue axes of individuals with MHO and MUO played by gut microbiota. The findings show the relationship between the gut microbiome diversity and the obese phenotype.

Keywords: obesity, metabolically healthy obesity, adipokines, myokines, gut microbiome alpha diversity

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АДИПОКИНЫ И МИОКИНЫ КАК ИНДИКАТОРЫ ФЕНОТИПОВ ОЖИРЕНИЯ И ИХ СВЯЗЬ С ПОКАЗАТЕЛЯМИ РАЗНООБРАЗИЯ МИКРОБИОМА КИШЕЧНИКА

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В настоящее время выделяют метаболически здоровое ожирение (МЗО) и метаболически нездоровое ожирение (МНЗО). Жировая и мышечная ткани могут определять фенотип ожирения за счет выработки адипокинов и миокинов. В формировании МЗО участвует и микробное сообщество кишечника. Целью работы было изучить уровни адипокинов и миокинов и их связь с альфа-разнообразием микробиома кишечника при МЗО и МНЗО. Из 265 обследуемых сформированы две группы: здоровые лица и пациенты с ожирением. Последние были разделены на две подгруппы: пациенты с МЗО и пациенты с МНЗО. У обследуемых определяли индекс массы тела, окружность талии, индекс HOMA-IR, уровень адипокинов и миокинов, таксономический состав микробиома кишечника и индексы альфа-разнообразия, оценивали липидный и углеводный обмен. Выявлены статистически значимые различия уровней адипокинов и миокинов, а также их взаимосвязь с показателями разнообразия микробиома кишечника у пациентов с разными фенотипами ожирения. При МЗО и МНЗО уровень адипонектина был значимо ниже ($p < 0,05$), а лептина и аспросина — выше ($p < 0,05$) по сравнению со здоровыми лицами. Пациенты с МНЗО имели уровни адипонектина и лептина более низкие ($p < 0,05$), чем пациенты с МЗО. При МНЗО статистически значимо выше уровень FGF21. Массовый корреляционный анализ при МЗО показал связь между уровнем глюкозы с индексами разнообразия микробиома кишечника, которая отсутствовала при МНЗО, что указывает на утрату влияния микробиоты кишечника на регуляцию уровня глюкозы крови при МНЗО и о различной регуляторной роли микробиоты кишечника в оси «микробиом кишечника — печень — жировая и мышечная ткани» при МЗО и МНЗО. Полученные данные указывают на взаимосвязь между разнообразием микробиома кишечника и фенотипом ожирения.

Ключевые слова: ожирение, метаболически здоровое ожирение, адипокины, миокины, альфа-разнообразие микробиома кишечника

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Complications of obesity reduce the quality of life and life expectancy [1]. However, obesity is not always associated with metabolic disorders, that is why metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) are distinguished [2]. The prevalence of MHO among obese patients is 6–60% depending on the criteria of MHO [3]. Obesity classification that is based on BMI does not provide a full picture of the health status and potential risk of adverse outcome. This has been documented in the AACE/ACE (American Association of Clinical Endocrinologists and American College of Endocrinology) guidelines (USA, 2014) suggesting to take into account metabolic status when classifying obesity [4]. The adipose and muscle tissues play an important role in maintaining metabolic homeostasis via secretion of biologically active peptides: adipokines and myokines [5]. Imbalance between these insulin sensitivity and adipogenesis increasing and reducing adipokines and myokines may contribute to metabolic complications, such as insulin resistance, slow inflammatory adipose tissue remodeling, extracellular lipid deposition, atherosclerosis, etc. [6]. It is known that adiponectin exerts insulin sensitizing, anti-atherogenic and anti-inflammatory effects [7]. In contrast, such adipokines as leptin and resistin are potential factors of insulin resistance that have pleiotropic effects on food intake, neuroendocrine control of hypothalamus, reproductive function, and energy metabolism [8]. Information about the recently discovered adipokine, asprosin, is extremely fragmented: it is believed that asprosin can affect glucose metabolism, that it is probably associated with obesity, and that asprosin levels can be used as a marker for early diagnosis of diabetes mellitus [9]. Currently, information about the role of myokine (myostatin) in the pathogenesis of obesity and insulin resistance is limited. Some authors suggest using myostatin for identification of MUO [10]. The insulin-responsive myokine, FGF21, functions as a metabolic factor involved in the glucose homeostasis control [11]. Recently the interest of researchers has been drawn to the osteocrin peptide that is secreted by fibroblasts and osteoblast-like cells. Its pathophysiological role is poorly understood [12]. Sporadic results obtained in obese patients with insulin resistance showed that had higher osteocrin levels than healthy individuals [13]. In the last decade, gut microbiota has been considered as a factor affecting the development of obesity. Microbiota can influence the metabolic phenotype via both direct effects on the energy and nutrient availability and modulation of the microorganisms' signalling and metabolic pathways. There is growing evidence that the balance and diversity of microorganisms are essential for both optimal interaction with macroorganism and prevention of extraintestinal diseases [14]. The diversity of microbiome reflects the abundance of various bacterial species. Two types of diversity are distinguished: alpha diversity (within the same biotope) and beta diversity (between distinct biotopes). Alpha diversity is assessed based on the Chao 1, Shannon, Simpson (direct and inverse), Gini-Simpson, and Berger-Parker indices. The Chao 1 index is used to estimate the observed/hidden diversity, the Shannon index is an estimator of the microbiome diversity and evenness (higher index values correspond to wider diversity); the Simpson index describes the probability that any two specimens randomly selected in the indefinitely large community belong to different species (the diversity decreases with the increasing index); the Berger-Parker index is a measure of the species dominance in the microbiome (the larger is the index, the lower is alpha diversity) [15]. Previous studies have shown that the stool microbiome of obese individuals is less diverse than that of lean people [16]. The decrease in microbial diversity in 75% of patients with morbid obesity

and 40% of patients with class I and II obesity has been also shown [17, 18]. Some authors have found a positive correlation between the decrease in the gut microbiome diversity and MUO [19]. Despite the fact that the number of studies aimed at assessing the role of adipokines and myokines in MHO, as well as the relationship between adipokines and myokines and the gut microbiome indicators, is limited, the associations of adiponectin, resistin, FGF21 with MHO reported in the literature [5, 6] have provided the basis for the scientific hypothesis used in our study: various profiles of adipokines and myokines may contribute to phenotypic dissociation of obesity. The study was aimed to reveal the features of adipokine and myokine levels and their association with the gut microbiome diversity indicators in patients with different obese phenotypes.

METHODS

The study was performed in the Rostov State Medical University, Kazan Federal University, and the Center for Molecular Health at the Institute of Digital and Translational Biomedicine. The patients were enrolled between 2019 and 2020. All the subjects were divided into two main groups based on the body mass index (BMI) and waist circumference (WC): healthy individuals (controls) were included in group 1, while obese patients were included in group 2. Then group 2 was divided into two subgroups depending on whether or not metabolic complications were present: subgroup 2a included patients with MHO and subgroup 2b included patients with MUO. Inclusion criteria: age over 18 years; taking no antibiotics, pre- or probiotics within three months before enrollment. Additional inclusion criteria for group 1: $18.5 \text{ kg/m}^2 < \text{BMI} \leq 24.9 \text{ kg/m}^2$; no dyslipidemia, hyperglycemia or arterial hypertension (AH). Additional inclusion criteria for group 2: $\text{BMI} \geq 30 \text{ kg/m}^2$, $\text{WC} > 102 \text{ cm}$ in males and $> 88 \text{ cm}$ in females. Subgroup 2a included patients who met more than three criteria, while subgroup 2b included those who met \geq three criteria of the National Cholesterol Education Program (NCEP), Adult Treatment Panel III (ATPIII) (NCEP-ATP III) including the following indicators: systolic blood pressure (SBP) > 130 and/or diastolic blood pressure (DBP) $> 85 \text{ mm Hg}$, fasting plasma glucose (FPG) $\geq 6.1 \text{ mmol/L}$, triglycerides (TG) $\geq 1.7 \text{ mmol/L}$, high density lipoprotein cholesterol (HDL-C) < 1.29 (females) or < 1.03 (males), waist circumference (WC) $> 102 \text{ cm}$ (males) or $> 88 \text{ cm}$ (females). Exclusion criteria: severe somatic disease, any gastrointestinal disorder, acute condition, depression, alcohol abuse, pregnancy.

Study design

A cohort study was performed. Clinical interview and examination, as well as assessment of anthropometric indicators were performed in all subjects; all of them were through questionnaire survey and the laboratory tests: fasting plasma glucose (FPG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), adiponectin, leptin, asprosin, resistin, vascular endothelial growth factor (VEGF), irisin, fibroblast growth factor 21 (FGF21), myostatin, osteocrin, and insulin. The insulin resistance index (HOMA-IR) was calculated, the composition of gut microbiome was defined, and the alpha diversity indices of bacterial phylotypes were computed. Blood pressure was measured using the manual blood pressure monitor (Little Doctor LD-60; China) using the standard method by N.S. Korotkov. Biochemical parameters, such as FPG, HDL-C, LDL-C, TG, TC (mmol/L), were assessed by photometry using the Hitachi U-2900 spectrophotometer

Table 1. Clinical and laboratory characteristics of the surveyed individuals of groups 1 and 2

Parameters	Group 1 <i>n</i> = 129	Group 2 <i>n</i> = 136	<i>p</i>
Males	15 (11.6%)	28 (20.6%)	0.621
Females	114 (88.3%)	108 (79.4%)	0.613
Age/years	39.6 ± 4.2	54.6 ± 4.7	0.032
BMI, kg/m ²	20.8 [19; 23]	34 [31; 36]	0.024
WC, cm	74 [69; 75.5]	100 [95; 103]	0.011
SBP, mm Hg	120.5 [90; 125]	135 [125; 145]	0.034
DBP, mm Hg	74.5 [60; 90]	85 [80; 90]	0.001
FPG, mmol/L	3.96 [4.05; 5.1]	5.57 [5.1; 6.93]	0.0001
TC, mmol/L	4.5 [4.1; 5.0]	5.42 [4.62; 6.2]	0.624
LDL-C, mmol/L	3.11 [2.4; 3.21]	3.19 [2.6; 3.64]	0.745
HDL-C, mmol/L	1.93 [1.49; 2.24]	1.23 [1.11; 1.39]	0.031
TG, mmol/L	0.79 [0.57; 1.13]	1.65 [1.33; 2.34]	0.001
Insulin, pg/mL	262 [133.6; 418.2]	390 [170.02; 678.78]	0.001
HOMA-IR	1.86 [1.47; 2.82]	11.7 [5.08; 19.3]	0.001

Note: BMI — body mass index, WC — waist circumference, SBP — systolic blood pressure, DBP — diastolic blood pressure, TC — total cholesterol, LDL-C — low density lipoprotein cholesterol, HDL-C — high density lipoprotein cholesterol, TG — triglycerides, HOMA-IR — Homeostasis Model Assessment of Insulin Resistance.

(Hitachi; Japan) and the Olvex Diagnosticum kits (Russia). Adiponectin, leptin, resistin, asprosin, VEGF, irisin, myostatin, FGF21, osteocrin, and insulin (ng/mL) were quantified by multiplex immunoassay using the Magpix analyzer (BioRad; USA) and the kits manufactured by Milliplex (USA). The concentration of asprosin was determined by ELISA using the ELISA KitForAsprosin (Cloud-Clone.; USA). Faecal samples were collected in accordance with the handbook edited by V.V. Menshikov [20]. Gut microbiome composition was defined by Next-Generation Sequencing (NGS) of the bacterial 16S rRNA gene (V3-V4 variable region) on the MiSeq platform (Illumina; USA) in the paired-end reading mode (2 × 300). Various alpha diversity indices of the bacterial community were used to characterize microbiota.

Statistical analysis

R environment (version 3.2, R Foundation for Statistical Computing; Vienna, Austria) was used for statistical processing. The following was calculated for quantitative indicators: mean ± standard deviation; median and quartiles (25%, 75%); minimum and maximum values of the sample. The average group levels were compared using the Mann–Whitney U test. The median values of the studied parameters were compared using the Kruskal–Wallis test, and the correlation analysis involved the use of Spearman's rank correlation coefficient. The differences were considered significant at $p < 0.05$. Cluster analysis: large-scale correlation analysis was performed in Python v3.8 for each group of patients and the correlations between:

BMI, WC, SBP, DBP, FPG, LDL-C, HDL-C, TG, TC and the levels of adipokines and myokines;

each clinical parameter and microbiota parameters (alpha diversity: species richness (operational taxonomic units (OTUs) with non-zero abundance), Shannon entropy, Berger–Parker dominance index, Simpson index and inverse Simpson index, Gini–Simpson index, Chao1 index;

microbiome parameters and the levels of adipokines and myokines.

The Spearman's rank correlation matrices obtained were clustered together within each group of patients based on the Manhattan distances.

Data availability

The cluster analysis scripts are publicly available from GitHub: https://github.com/ivasilyev/curated_projects/tree/master/ashestopalov/nutrition/obesity_elisa

RESULTS

Clinical and laboratory characteristics of the surveyed individuals of groups 1 and 2 are provided in Table 1.

The control group and obese patients were matched for gender and the TC and LDL-C levels. Furthermore, the subjects of group 1 were younger ($p = 0.032$), they had lower BMI ($p = 0.024$) and WC ($p = 0.011$). Obese patients had higher SBP, DBP, TG, FPG, insulin levels, HOMA ($p < 0.05$) and lower HDL-C ($p < 0.05$) than the control group. Clinical and laboratory characteristics of the surveyed individuals of subgroups 2a and 2b are provided in Table 2.

Subgroups 2a and 2b were matched for the number of males and females, subjects' age, BMI, and WC. When comparing patients with different obese phenotypes, significant differences in various indicators were revealed: SBP, DBP, TG, FPG, insulin, and HOMA were higher ($p < 0.05$) and HDL-C was lower ($p < 0.05$) in the MUO subgroup than in the MHO subgroup. Particularly noteworthy, however, was hyperglycemia in patients of the subgroup 2b that corresponded not to prediabetes, the early carbohydrate metabolism disorder, but to diabetes mellitus. By comparison, normal blood glucose levels were observed in the subgroup 2a, despite insulin resistance revealed in both groups. In the subgroup 2b a more than twofold increase in blood levels of triglycerides suggesting triglyceride storage impairment due to the adipose tissue insulin resistance that resulted in lipotoxicity, i.e. extracellular triglyceride accumulation, was noteworthy.

Comparative analysis of the adipokine and myokine levels in the subjects of groups 1 and 2 revealed significant differences in the levels of adiponectin, leptin, and asprosin. Obese patients had lower adiponectin levels ($p < 0.05$) and higher leptin and asprosin levels ($p < 0.05$) than healthy individuals (Table 3). It is known that leptin is a key mediator of interaction between

Table 2. Clinical and laboratory characteristics of the surveyed individuals of subgroups 2a and 2b

Parameters	Subgroup 2a <i>n</i> = 40	Subgroup 2b <i>n</i> = 55	<i>p</i>
Males	6 (15%)	11 (20%)	0.612
Females	34 (85%)	44 (80%)	0.510
Age, years	49.05 ± 5.1	51.3 ± 3.6	0.734
BMI, kg/m ²	33 [31; 36]	33 [31; 36]	0.591
WC, cm	100 [96; 104]	100 [93; 102]	0.112
SBP, mm Hg	120 [110; 125]	145 [136; 150]	< 0.0001
DBP, mm Hg	75 [70; 80]	90 [90; 95]	< 0.0001
FPG, mmol/L	4.88 [4.57; 5.28]	7.2 [6.14; 8.62]	< 0.0001
HOMA-IR	7.48 [3.3; 12]	14.4 [7.57; 27.37]	0.0003
TC, mmol/L	5.08 [4.31; 6.01]	5.74 [4.57; 6.5]	0.102
LDL-C, mmol/L	3.18 [2.78; 3.53]	3.1 [1.95; 3.79]	0.341
HDL-C, mmol/L	1.3 [1.21; 1.47]	1.2 [1.08; 1.45]	0.022
TG, mmol/L	1.19 [0.91; 1.46]	2.39 [1.77; 3.16]	< 0.0001
Insulin, pg/mL	284.92 [142.25; 529.07]	389.96 [221; 721.57]	0.050

Note: BMI — body mass index, WC — waist circumference, SBP — systolic blood pressure, DBP — diastolic blood pressure, TC — total cholesterol, LDL-C — low density lipoprotein cholesterol, HDL-C — high density lipoprotein cholesterol, TG — triglycerides, HOMA-IR — Homeostasis Model Assessment of Insulin Resistance.

the adipose tissue and the hypothalamic-pituitary axis that exerts orexigenic effect. High leptin levels in the group of obese patients suggest the leptin orexigenic effect inhibition due to resistance to appropriate signals developed by the central nervous system, i.e. these suggest the development of insulin resistance characterized by low satiety, excessive intake of nutrients, and weight gain.

When comparing serum levels of adipokines and myokines in patients with MHO, MUO and healthy donors the following earlier detected trends were observed: patients with MHO and MUO had significantly lower adiponectin levels ($p < 0.05$) and significantly higher leptin and asprosin levels ($p < 0.05$) compared to the control group. Furthermore, patients with MUO had lower adiponectin and leptin levels ($p < 0.05$) than patients with MHO. A significant increase in the levels of FGF21 was also observed in patients with MUO (Table 4). Given its insulin sensitizing effect, the decrease in adiponectin levels fits into the general picture of insulin resistance. The lower leptin levels in patients with MUO that seem paradoxical at first glance are likely to result from the adipose tissue insulin resistance that leads to impaired terminal differentiation into adipocytes, abnormal extracellular triglyceride deposition, and lipotoxicity.

The correlation analysis has revealed positive correlations between the levels of adiponectin and resistin ($r = 0.428$;

$p = 0.001$), FGF21 and myostatin ($r = 0.543$; $p = 0.001$), insulin and leptin ($r = 0.429$; $p = 0.0001$) in healthy donors. This suggests normal balance in the insulin sensitivity regulation system and the adipose and muscle tissue endocrine function.

Positive correlations between the adiponectin levels and the levels of insulin ($r = 0.3$; $p = 0.008$), leptin ($r = 0.3$; $p = 0.008$), resistin ($r = 0.5$; $p < 0.0001$), and VEGF ($r = 0.49$; $p < 0.0001$) were revealed in obese patients. Moreover, there were significant correlations between the levels of the FGF21 myokine and myostatin ($r = 0.37$; $p = 0.0001$), as well as between the levels of insulin and leptin ($r = 0.84$; $p < 0.0001$). The leptin levels were also correlated to the VEGF levels ($r = 0.38$; $p = 0.002$), while resistin levels were correlated to the osteocin levels ($r = 0.33$; $p = 0.0006$). Such correlations are indicative of the imbalances in the regulatory system and the involvement of hypoxia-inducible factor in the regulatory system, since there are positive correlations between adiponectin and leptin and between leptin and VEGF.

Patients with MHO showed the following correlations of osteocin levels: negative correlation with asprosin ($r = -0.43$; $p = 0.02$) and resistin ($r = -0.39$; $p = 0.01$), positive correlation with adiponectin ($r = 0.43$; $p = 0.04$). The relationships were revealed between the levels of adiponectin and myostatin ($r = 0.34$; $p = 0.035$), insulin and leptin ($r = 0.59$; $p < 0.0001$), FGF21 and adiponectin ($r = 0.34$; $p = 0.0003$).

Table 3. Serum levels of adipokines and myokines in healthy individuals and obese patients

Indicators	Group 1 <i>n</i> = 129	Group 2 <i>n</i> = 136	<i>p</i>
Adiponectin, ng/mL	2149800 [62542; 6457800]	591180 [22366; 6457800]	0.05
Leptin, pg/mL	3999.96 [1120.19; 10099.7]	11291.99 [5541.55; 17827.94]	< 0.0001
Resistin, pg/mL	52364.75 [32515.5; 116732]	46147.46 [27968.18; 104077.3]	0.35
Asprosin, pg/mL	0.18 [0; 0.36]	0.35 [0; 0.54]	0.01
VEGF, pg/mL	73.18 [44.93; 139.3]	58.45 [35.45; 143.28]	0.19
Insulin, pg/mL	262 [133.6; 418.2]	390 [170.02; 678.78]	0.001
Osteocin, pg/mL	55.23 [37.7; 74.28]	45.23 [34.15; 69.60]	0.14
Myostatin, pg/mL	0 [0; 0]	0 [0; 0]	0.6
Irisin, pg/mL	0 [0; 0]	0 [0; 0]	0.58
FGF21, pg/mL	0 [0; 56.86]	0 [0; 83.45]	0.14

Table 4. Serum levels of adipokines and myokines in subjects of the group 1 subgroups 2a and 2b

Parameters	Group 1 <i>n</i> = 129	Subgroup 2a <i>n</i> = 40	P_{1p1-2a}	Subgroup 2b <i>n</i> = 55	P_{1p1-2b}	P_{2a-2b}
Adiponectin, ng/mL	214980 [62542; 6457800]	75 618 [25132; 6457800]	< 0.052	57 891 [22616.75; 2606600]	< 0.054	0.003
Leptin, pg/mL	3999.96 [1120.19; 10099.7]	13808.18 [6332.91; 17956.46]	< 0.051	9942.72 [4664.47; 16802.91]	< 0.055	0.002
Insulin, pg/mL	262 [133.6; 418.2]	284 [142.25; 529.07]	00.1	389 [221.22; 721.57]	< 0.054	0.05
Asprosin, pg/mL	0.18 [0; 0.36]	0.46 [0.23; 0.6]	< 0.05	0.38 [0; 0.57]	< 0.05	0.37
FGF21, pg/mL	0 [0; 56.8]	0 [0; 31.44]	< 0.01	13.98 [0; 90.49]	00.05	0.05
Resistin, pg/mL	52364.75 [32515.5; 116732]	48637.48 [33229.66; 97603.33]	00.5	43421.05 [26433.69; 109927.65]	00.6	0.7
VEGF, pg/mL	73.18 [44.93; 139.3]	56.33 [19.89; 145.32]	< 0.05	58.97 [39.61; 163.05]	00.26	0.34
Osteocrin, pg/mL	55.23 [37.7; 74.28]	44.03 [35.91; 59.08]	00.16	48.96 [32.22; 78.6]	00.17	0.57
Myostatin, pg/mL	0 [0; 0]	0 [0; 0]	0.23	0 [0; 0]	0.3	0.6
Irisin, pg/mL	0 [0; 0]	0 [0; 0]	00.34	0 [0; 0]	00.26	0.7

The correlations of osteocrin with asprosin ($r = -0.38$; $p = 0.04$), resistin ($r = 0.48$; $p = 0.001$), and myostatin ($r = 0.33$; $p = 0.03$) were revealed in patients with MUO. There were significant positive correlations between FGF21 and myostatin ($r = 0.37$; $p = 0.005$), insulin and leptin ($r = 0.37$; $p = 0.005$). Thus, the role of osteocrin in regulation of the adipose and muscle tissue endocrine function becomes more and more important with the development of obesity. No correlations of osteocrin have been found in healthy individuals. There are two correlations of osteocrin with adipokines (asprosin and resistin) in the group with MHO and three such correlations that expand the range to the myostatin myokine in the group with MUO. Therefore, obesity is associated with the interrelated alterations in all three mesenchymal stem cell differentiation branches: adipogenic, myogenic, and osteogenic. It should be noted that positive correlations between insulin and leptin are observed in all studied groups. These are maximal in the group with MHO, which suggests that the effects of insulin on the adipose tissue are preserved, and are minimal in the group with MUO, which could be indirect evidence of the adipose tissue insulin resistance. Also noteworthy is the change of the FGF21 regulatory effects with the development of obesity. In healthy donors and patients with MUO, this myokine positively correlates with the other myokine, myostatin, while in patients with MHO is correlates with adiponectin and myostatin. Perhaps the FGF21–adiponectin regulatory axis is one of the compensatory mechanisms in the adipose tissue–muscle tissue system that allows the adipose tissue to retain insulin sensitivity in obese patients and prevent lipotoxicity.

To assess alpha diversity of the bacterial community in the studied groups, we calculated the Simpson and Shannon phylogenetic diversity indices, Chao1 index, and OTUs. Significant differences in the phylogenetic diversity index and Chao1 index between controls and obese patients have been found, which suggest reduced alpha diversity in fecal samples of obese patients. However, there are no differences in the Shannon index between groups 1 and 2 [21, 22].

It should be noted that the Shannon index, Chao1 index, and OTUs were significantly higher in patients with MHO than in the group with MUO and the group of healthy donors. The increased alpha diversity is likely to be a protective mechanism of the microbial community that prevents metabolic disorders in obese individuals.

The large-scale correlation analysis that revealed positively related ($r \geq 0.3$) clusters typical for each obese phenotype was performed in subgroups 2a and 2b. Thus, the following clustering type was typical for the subgroup of patients with MHO: 1) correlations of glucose levels with inverse gut microbiome diversity indices (Berger–Parker index, Gini–Simpson index, inverse Simpson index); 2) correlations of the levels of leptin, insulin, irisin and HOMA values with the VEGF concentrations; 3) correlations of the FGF21 and HDL-C concentrations with the direct gut microbiome diversity indices (Chao1 index, OTUs, Shannon index, Simpson index) (Fig. 1).

Clustering of the correlation between glucose levels and inverse gut microbiome diversity indices demonstrates the relationship between microbiota and glucose levels, it suggests that gut microbiota of individuals with MHO affects glucose concentration and probably keeps glucose at normal levels, despite the presence of obesity. Clustering of the correlation between VEGF levels and the carbohydrate metabolism indicators together with insulin sensitivity shows that hypoxia has a regulatory effect in individuals with MHO. Clustering of the correlation of the direct gut microbiome diversity indices with the FGF21 and HDL-C levels suggests that microbial diversity has a protective effect in individuals with this obese phenotype, probably due to preservation of the microbiota–liver–adipose tissue regulatory axis.

It is important to note that no clustering of the gut microbiota diversity indicators with the glucose levels typical for MHO was observed in patients with the MUO phenotype. This suggests the loss of gut microbiota influence on the regulation of blood glucose levels (Fig. 2). Furthermore, inverse diversity indices in individuals with MUO were highly correlated to BMI, SBP, DBP, LDL-C, TC, HOMA-IR, and insulin, while direct gut microbiome

Table 5. Characteristics of the stool microbiome alpha diversity in the studied groups of patients

Indices	Group 1 (control)	Group 2 (obesity)	Subgroup 2a (MHO)	Subgroup 2b (MUO)
Simpson index	0.981 [0.979–0.982]	0.977 [0.975–0.981]	0.978 [0.971–0.986]	0.976 [0.973–0.981]
Shannon index	7.80 [7.71–7.90]	7.82 [7.63–8.11]	8.28* [7.67–8.76]	7.63† [7.46–7.90]
Chao1 index	4010.16 [3871.53–4256.37]	4018.56* [3647.52–4492.64]	4899.13* [4139.88–5384.25]	3631.75† [3214.01–4118.26]
OTUs	1974.50 [1865.86–2060.00]	2015.00 [1930.17–2285.94]	2479.00* [1982.88–2727.61]	1930.00† [1743.64–2125.33]

Note: the data are presented as median and 95% confidence interval; * — significant differences from group 1 ($p < 0.05$); † — significant differences from patients with MHO ($p < 0.05$)

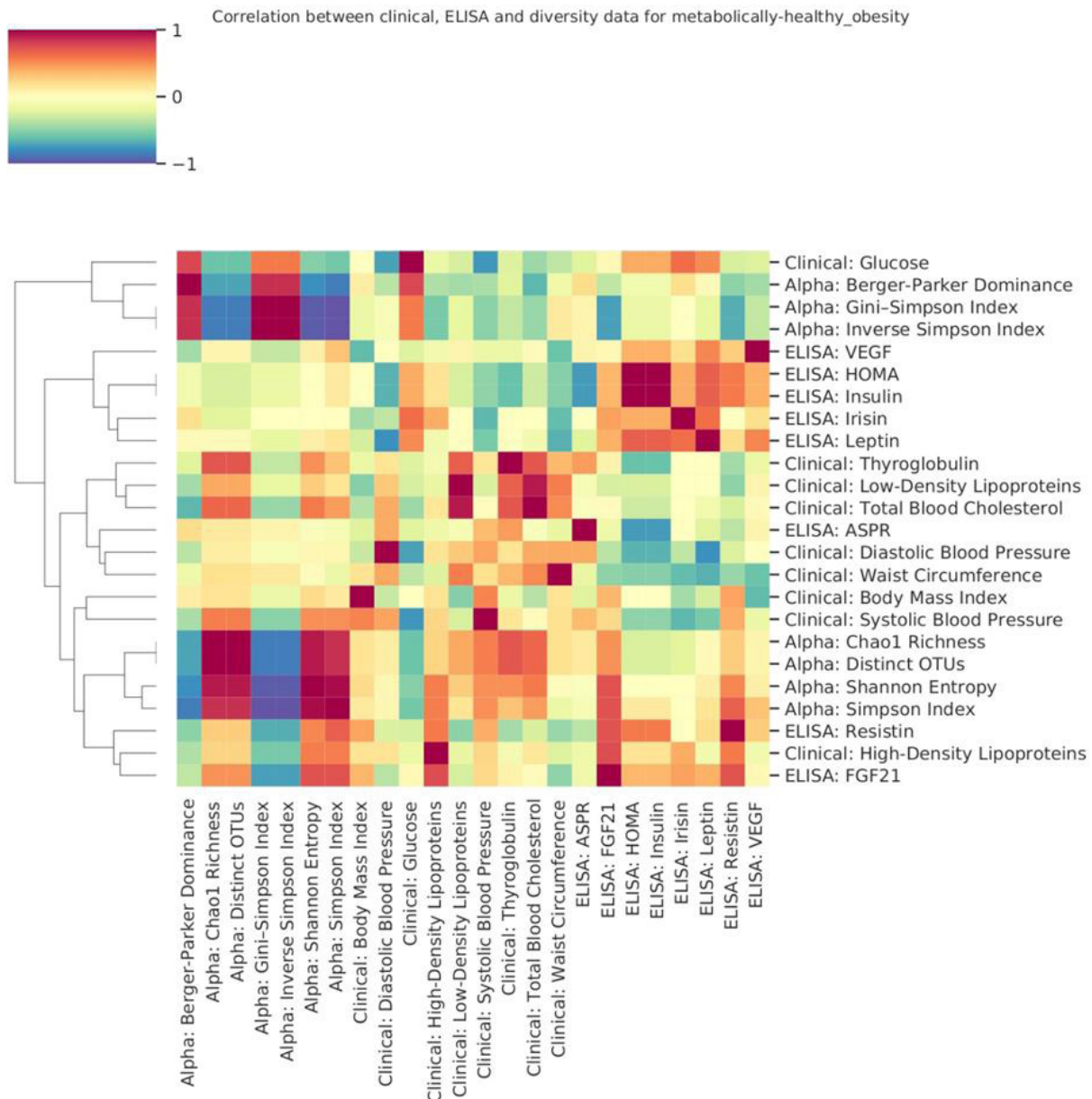


Fig. 1. Clustermap of the correlations between glucose, adipokines, myokines, lipid profile and the gut microbiome diversity indicators for the group of patients with MHO

diversity indices were highly correlated to myostatin, TG, VEGF, FGF21, resistin, and leptin. This suggests that the decrease in the microbial community diversity in individuals with this type of obesity is directly related to the clinical and biochemical manifestations of metabolic syndrome and type 2 diabetes mellitus.

The results obtained suggest that gut microbiota plays different regulatory roles in the gut microbiome–liver–muscle/adipose tissue axis in individuals with various obese phenotypes. The findings also provide evidence of the emergence of abnormal relationships associated with MUO.

DISCUSSION

The roles of microbial community and endocrine factors of adipose and muscle tissues in obesity are quite fully investigated [2, 4, 5]. However, these studies rarely addressed the differences between the obese phenotypes and the correlations of the microbiome characteristics with the adipokine and myokine levels. Our study revealed

alterations in the serum adipokine and myokine profiles consisting of the significant decrease in the adiponectin levels amid the increasing levels of insulin, leptin, and asprosin. This generally reflected the development of insulin resistance, leptin resistance, and, therefore, impaired storage function of adipose tissue and appetite regulation. Downregulation of adiponectin is consistent with the data obtained by other authors [1, 6]. Similar patterns have been previously shown for the levels of leptin and insulin [1, 23]. Higher adiponectin levels and lower leptin levels in healthy individuals are probably due to the lack of terminal adipocyte hypertrophy, preserved adipocyte storage function and metabolic plasticity. That is why such adipocytes must be characterized by high insulin resistance. At the same time, obese individuals may show hyperplasia and hypertrophy of adipocytes, the cytoplasm of which is completely filled with TG. These block further TG deposition in accordance with the negative feedback principle and, consequently, disrupt adipocyte metabolic plasticity [24]. Such remodeling results in ectopic fat accumulation and altered adipocyte morphology, being the triggers of insulin resistance associated with the

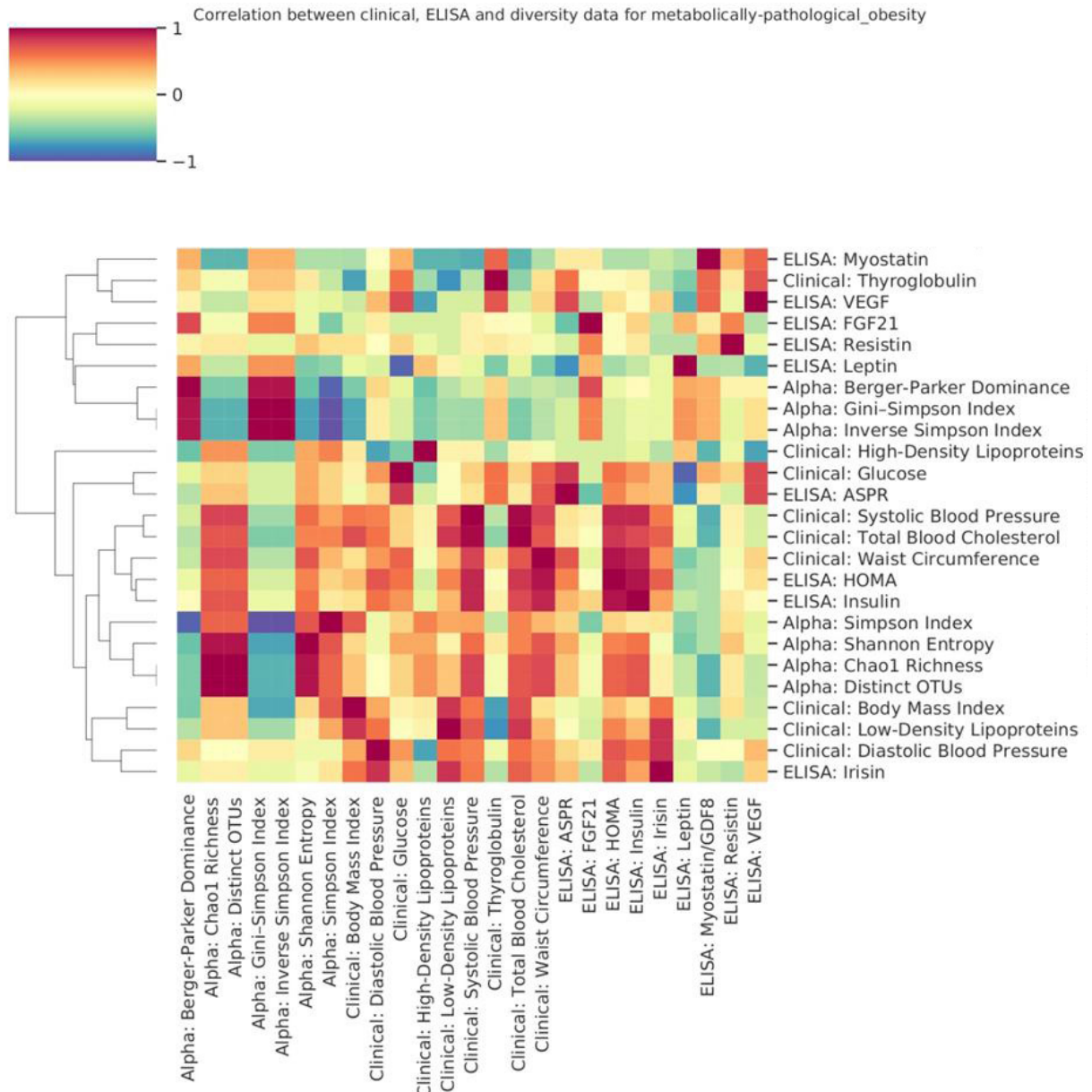


Fig. 2. Clustermap of the correlations between glucose, adipokines, myokines, lipid profile and the gut microbiome diversity indicators for the group of patients with MUO

decreased adiponectin levels and the increased leptin levels. Elevated leptin levels are probably associated with leptin resistance, in which smaller amounts of leptin would reach the brain, thereby decreasing activation of the signaling pathway for body weight regulation. Leptin resistance at the blood-brain barrier (BBB) level is due to the receptor saturation effects caused by excess leptin or reversible inhibition by circulating factors, such as TG [25].

Information about the role of asprosin in obesity is extremely fragmented [26]. The results of this study show higher asprosin levels in obese patients compared to healthy individuals. In the study the levels of asprosin in obese individuals are higher than that in healthy people. It is well-known that asprosin secreted by white adipose tissue increases appetite. Since obesity is associated with the increase in the number of adipocytes, then the asprosin levels increase, which, in turn, results in activation of orexigenic neurons by the agouti-related protein (AgRP) and increased appetite to form a vicious circle [27]. Therefore there are speculations that asprosin can contribute to the development of obesity by affecting food intake.

MHO is characterized by alterations that are similar in all types of obesity, however, lipotoxicity manifestations are minimal. The maximum leptin levels, highest correlation between insulin and leptin, and the relationship between FGF21 and adiponectin are typical for this phenotype. On the one hand, this shows preserved effects of insulin in the adipose tissue, and on the other hand activation of the FGF21–adiponectin regulatory mechanism probably allowing to preserve insulin sensitivity of adipose tissue in individuals with this obese phenotype and to prevent lipotoxicity. The relationship between the gut microbial community diversity and the clinical and laboratory indicators suggests that microbiota is involved in regulation of glucose levels and that the diversity indices have a protective effect on the indicators of cholesterol metabolism, which is probably evidence of preserved microbiota–liver–adipose tissue regulatory axis.

As we have shown earlier, alterations in the share of the main microbiome members, Bacteroidetes and Firmicutes, associated with MHO are less severe than these associated with MUO. Perhaps these alterations are not critical for metabolic

function of the microbial community as a whole, as well as for production of regulatory molecules and metabolites (indoles, kynurenines, short-chain fatty acids, secondary bile acids, vitamins, and essential amino acids), and are compensated by the increased diversity that consequently ensures preservation of the microbiota–liver–adipose tissue regulatory axis.

MUO is characterized by the most severe manifestations of the carbohydrate metabolism disorders, lipotoxicity, and dyslipoproteinemia. Furthermore, patients with MUO have lower adiponectin and leptin levels than patients with MHO. The significantly increased FGF21 levels have been also found in individuals with MUO. Perhaps, elevated FGF21 levels associated with MUO constitute a dysfunctional compensatory mechanism for hyperglycemia, since adipocytes that have reached critical hypertrophy show insulin resistance and are unable to utilize glucose. In this regard, there is a need to form an extra glucose metabolism pathway in order to relieve hyperglycemia. In particular, the increase in the muscle metabolism rate activates glucose utilization by the muscle tissue (myokine FGF21). It is important to note that the missing relationship between the glucose levels and the gut microbiome alpha diversity indicators is a feature of the MUO phenotype. This is indicative of the loss of regulatory effects by the gut microbiome in the microbiota–liver–adipose tissue axis and the emergence of abnormal relationships in individuals with this obese phenotype.

The cause of lower adiponectin concentrations in individuals with MUO compared to MHO is unknown, however, it can be related to the MUO-associated chronic hyperinsulinemia that suppresses adiponectin production by adipose tissue and thereby generates a feedback loop for the decreased insulin secretion caused by insulin resistance [3, 28]. In our study the leptin levels found in patients with MUO were significantly higher than these found in patients with MHO. Such results

were consistent with the data obtained by other authors [29] and were possibly due to abnormal protein synthesis in hypertrophic adipocytes. However, the leptin contribution to the MUO pathogenesis needs to be clarified, since there are studies that have revealed significantly higher leptin levels in patients with MUO [30] or no differences at all [31]. In addition to the common significant correlations, the correlations typical for MUO have been revealed, such as a significant positive correlation between the myostatin and osteocrin levels. In contrast to patients with MHO, the gut microbiome alpha diversity indicators of patients with MUO significantly decreased and no longer correlated with blood glucose levels, while the inverse indices became correlated to the clinical and laboratory indicators of metabolic syndrome and type 2 diabetes mellitus.

CONCLUSIONS

Thus, the levels of leptin, asprosin, and insulin are significantly higher ($p < 0.05$) and adiponectin levels are lower in obese patients than in healthy individuals. Correlation analysis revealed imbalances in the regulatory system involving the hypoxia-inducible factor and interrelated changes in the adipogenic, myogenic, and osteogenic mesenchymal stem cell differentiation. Large-scale correlation analysis allowed us to reveal the loss of the microbiota diversity protective effects on metabolic processes in individuals with MUO. Further study of the relationship between gut microbiota and both lipid metabolism and carbohydrate indicators would contribute to further improvement of the therapeutic strategy for obesity and associated metabolic disorders through the development of the microbiome-based therapy of obesity. Furthermore, the data obtained may be used for early prevention of metabolic disorders and MUO conversion into MHO.

References

- Frühbeck G, Catalán V, Rodríguez A, Gómez-Ambrosi J. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. *Adipocyte*. 2018; 7 (1): 57–62. DOI: 10.1080/21623945.2017.1402151.
- Iacobini C, Pugliese G, Blasetti Fantauzzi C, et al. Metabolically healthy versus metabolically unhealthy obesity. *Metabolism*. 2019; 92: 51–60. DOI: 10.1016/j.metabol.2018.11.009.
- Smith GI, Mittendorfer B, Klein S. Metabolically healthy obesity: facts and fantasies. *J Clin Invest*. 2019; 129 (10): 3978–89. DOI: 10.1172/JCI129186.
- Garvey WT, Garber AJ, Mechanick JL, et al. The AACE Obesity Scientific Committee. American association of clinical endocrinologists and American college of endocrinology position statement on the 2014 advanced framework for a new diagnosis of obesity as a chronic disease. *Endocr Pract*. 2014; 20 (9): 977–89. DOI: 10.4158/EP14280.PS.
- Graf C, Ferrari N. Metabolic Health—The Role of Adipo-Myokines. *Int J Mol Sci*. 2019; 20 (24): 6159. DOI: 10.3390/ijms20246159.
- Bakhal A. Adipokines—targeting a root cause of cardiometabolic risk. *QJM*. 2008; 101 (10): 767–76. DOI: 10.1093/qjmed/hcn066.
- Doumatey AP, Bentley AR, Zhou J, Huang H, Adeyemo A, Rotimi CN. Paradoxical Hyperadiponectinemia is Associated With the Metabolically Healthy Obese (MHO) Phenotype in African Americans. *J Endocrinol Metab*. 2012; 2 (2): 51–65. DOI: 10.4021/jem95W.
- Blüher S. Metabolically healthy obesity from childhood to adulthood — does weight status alone matter? *Metabolism*. 2014; 63: 1084–109.
- Wang HY. Plasma asprosin concentrations are increased in individuals with glucose dysregulation and correlated with insulin resistance and first-phase insulin secretion. *Mediators of Inflammation*. 2018; ID 9471583.
- Amor M, Itariu BK, Moreno-Viedma V, Keindl M, Jürets A, Prager G, et al. Serum Myostatin is Upregulated in Obesity and Correlates with Insulin Resistance in Humans. *Exp Clin Endocrinol Diabetes*. 2019; 127 (8): 550–6.
- Staiger H, Keuper M, Berti L, Hrabe de Angelis M, Häring HU. Fibroblast Growth Factor 21—Metabolic Role in Mice and Men. *Endocr Rev*. 2017; 38 (5): 468–88.
- Hu C, Zhang X, Zhang N, Wei WY, Li LL, Ma ZG, et al. Osteocrin attenuates inflammation, oxidative stress, apoptosis, and cardiac dysfunction in doxorubicin-induced cardiotoxicity. *Clin Transl Med*. 2020; 10 (3): e124.
- Fu J, Li Y, Esangbedo IC, Li G, Feng D, Li L, et al. Circulating Osteonectin and Adipokine Profiles in Relation to Metabolically Healthy Obesity in Chinese Children: Findings From BCAMS. *J Am Heart Assoc*. 2018; 7 (23): e009169. DOI: 10.1161/JAHA.118.009169.
- Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*. 2019; 7 (1): 14. DOI: 10.3390/microorganisms7010014.
- Stoma IO, Karpov IA. *Мікробіом чело́века*. Минск: Доктор Дізајн, 2018; 122 s. Russian.
- Bombin A, Yan S, Bombin S, Mosley JD, Ferguson JF. Obesity influences composition of salivary and fecal microbiota and

- impacts the interactions between bacterial taxa. *Physiological Reports*. 2022; 10: e15254. Available from: <https://doi.org/10.14814/phy2.15254>.
17. Aron-Wisnewsky J, Prifti E, Belda E, et al. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. *Gut*. 2019; 68 (1): 70–82. DOI: 10.1136/gutjnl-2018-316103.
 18. Cotillard A, Kennedy SP, Kong LC, Edi Prifti, Nicolas Pons, Emmanuelle Le Chatelier, et al. Dietary intervention impact on gut microbial gene richness. *Nature*. 2013; 500 (7464): 585–8. DOI: 10.1038/nature12480. Erratum in: *Nature*. 2013; 502 (7472) 580.
 19. Everard A, Belzer C, Geurts L, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA*. 2013; 110 (22): 9066–71. DOI: 10.1073/pnas.1219451110.
 20. Menshikov VV, redaktor. *Metodiki klinicheskix laboratornyx issledovanij. Spravochnoe posobie. Tom 3. M.: Labora, 2009; 880 s. Russian.*
 21. Gaponov AM, Volkova NI, Ganenko LA, Naboka YuL, Markelova MI, Sinyagina MN, i dr. Osobennosti mikrobioma tolstoj kishki u pacientov s ozhireniem pri ego razlichnyx fenotipax. *Zhurnal mikrobiologii, ehpidemiologii i immunobiologii*. 2021; 98 (2): 144–155. Dostupno po ssylke: <https://doi.org/10.36233/0372-9311-66>. Russian.
 22. Kolesnikova IM, Gaponov AM, Ganenko LA, Volkova NI, Grigoreva TV, Lajkov AV, i dr. Vzaimosvyaz' soderzhaniya nejrotrofov kishhechnogo mikrobioma pri razlichnyx metabolicheskix tipax ozhireniya. *Zhurnal ehvolyucionnoj bioximii i fiziologii*. 2022; 58 (54): 298–310. Russian.
 23. Yosae S, Khodadost M, Esteghamati A, et al. Adiponectin: An Indicator for Metabolic Syndrome. *Iran J Public Health*. 2019; 48 (6): 1106–15.
 24. Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. *Trends Endocrinol Metab*. 2015; 26 (4): 193–200. DOI: 10.1016/j.tem.2015.01.006.
 25. Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC. Leptin, obesity, and leptin resistance: where are we 25 years later? *Nutrients*. 2019; 11 (11): 2704. DOI: 10.3390/nu11112704.
 26. Yuan M, Li W, Zhu Y, et al. Asprosin: A novel player in metabolic diseases. *Front Endocrinol (Lausanne)*. 2020; 11: 64. DOI: 10.3389/fendo.2020.00064.
 27. Ugur K, Aydin S. Saliva and blood asprosin hormone concentration associated with obesity. *Int J Endocrinol*. 2019; 2019: 2521096. DOI: 10.1155/2019/2521096.
 28. Elisha B, Karelis AD, Imbeault P, et al. Effects of acute hyperinsulinaemia on total and high-molecular-weight adiponectin concentration in metabolically healthy but obese postmenopausal women: a Montreal-Ottawa New Emerging Team (MONET) study. *Diabetes Metab*. 2010; 36 (4): 319–21. DOI: 10.1016/j.diabet.2010.03.004.
 29. Ung UJ, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *International Journal of Molecular Sciences*. 2014; 15 (4): 6184–223. Available from: <https://doi.org/10.3390/ijms15046184111,112>.
 30. Jamar G, Caranti DA, de Cassia Cesar H, et al. Leptin as a cardiovascular risk marker in metabolically healthy obese: Hyperleptinemia in metabolically healthy obese. *Appetite*. 2017; 108: 477–82. DOI: 10.1016/j.appet.2016.11.013.
 31. Fu J, Li Y, Esangbedo IC, et al. Circulating osteonectin and adipokine profiles in relation to metabolically healthy obesity in chinese children: findings from BCAMS. *J Am Heart Assoc*. 2018; 7 (23): e009169. DOI: 10.1161/JAHA.118.009169.

Литература

1. Frühbeck G, Catalán V, Rodríguez A, Gómez-Ambrosi J. Adiponectin-leptin ratio: A promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. *Adipocyte*. 2018; 7 (1): 57–62. DOI: 10.1080/21623945.2017.1402151.
2. Iacobini C, Pugliese G, Blasetti Fantauzzi C, et al. Metabolically healthy versus metabolically unhealthy obesity. *Metabolism*. 2019; 92: 51–60. DOI: 10.1016/j.metabol.2018.11.009.
3. Smith GI, Mittendorfer B, Klein S. Metabolically healthy obesity: facts and fantasies. *J Clin Invest*. 2019; 129 (10): 3978–89. DOI: 10.1172/JCI129186.
4. Garvey WT, Garber AJ, Mechanick JL, et al. The Aace Obesity Scientific Committee. American association of clinical endocrinologists and American college of endocrinology position statement on the 2014 advanced framework for a new diagnosis of obesity as a chronic disease. *Endocr Pract*. 2014; 20 (9): 977–89. DOI: 10.4158/EP14280.PS.
5. Graf C, Ferrari N. Metabolic Health-The Role of Adipo-Myokines. *Int J Mol Sci*. 2019; 20 (24): 6159. DOI: 10.3390/ijms20246159.
6. Bakhai A. Adipokines--targeting a root cause of cardiometabolic risk. *QJM*. 2008; 101 (10): 767–76. DOI: 10.1093/qjmed/hcn066.
7. Doumatey AP, Bentley AR, Zhou J, Huang H, Adeyemo A, Rotimi CN. Paradoxical Hyperadiponectinemia is Associated With the Metabolically Healthy Obese (MHO) Phenotype in African Americans. *J Endocrinol Metab*. 2012; 2 (2): 51–65. DOI: 10.4021/jem95W.
8. Bluher S. Metabolically healthy obesity from childhood to adulthood — does weight status alone matter? *Metabolism*. 2014; 63: 1084–109.
9. Wang HY. Plasma asprosin concentrations are increased in individuals with glucose dysregulation and correlated with insulin resistance and first-phase insulin secretion. *Mediators of Inflammation*. 2018; ID 9471583.
10. Amor M, Itariu BK, Moreno-Viedma V, Keindl M, Jürets A, Prager G, et al. Serum Myostatin is Upregulated in Obesity and Correlates with Insulin Resistance in Humans. *Exp Clin Endocrinol Diabetes*. 2019; 127 (8): 550–6.
11. Staiger H, Keuper M, Berti L, Hrabe de Angelis M, Häring HU. Fibroblast Growth Factor 21-Metabolic Role in Mice and Men. *Endocr Rev*. 2017; 38 (5): 468–88.
12. Hu C, Zhang X, Zhang N, Wei WY, Li LL, Ma ZG, et al. Osteocrin attenuates inflammation, oxidative stress, apoptosis, and cardiac dysfunction in doxorubicin-induced cardiotoxicity. *Clin Transl Med*. 2020; 10 (3): e124.
13. Fu J, Li Y, Esangbedo IC, Li G, Feng D, Li L, et al. Circulating Osteonectin and Adipokine Profiles in Relation to Metabolically Healthy Obesity in Chinese Children: Findings From BCAMS. *J Am Heart Assoc*. 2018; 7 (23): e009169. DOI: 10.1161/JAHA.118.009169.
14. Rinnella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*. 2019; 7 (1): 14. DOI: 10.3390/microorganisms7010014.
15. Стома И. О., Карпов И. А. *Микробиом человека. Минск: Доктор Дизайн, 2018; 122 с.*
16. Bombin A, Yan S, Bombin S, Mosley JD, Ferguson JF. Obesity influences composition of salivary and fecal microbiota and impacts the interactions between bacterial taxa. *Physiological Reports*. 2022; 10: e15254. Available from: <https://doi.org/10.14814/phy2.15254>.
17. Aron-Wisnewsky J, Prifti E, Belda E, et al. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. *Gut*. 2019; 68 (1): 70–82. DOI: 10.1136/gutjnl-2018-316103.
18. Cotillard A, Kennedy SP, Kong LC, Edi Prifti, Nicolas Pons, Emmanuelle Le Chatelier, et al. Dietary intervention impact on gut microbial gene richness. *Nature*. 2013; 500 (7464): 585–8. DOI: 10.1038/nature12480. Erratum in: *Nature*. 2013; 502 (7472) 580.
19. Everard A, Belzer C, Geurts L, et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA*. 2013; 110 (22): 9066–71. DOI: 10.1073/pnas.1219451110.
20. Меньшиков В. В., редактор. *Методики клинических лабораторных исследований. Справочное пособие. Том 3. М.: Лабор, 2009; 880 с.*
21. Гапонов А. М., Волкова Н. И., Ганенко Л. А., Набока Ю. Л., Маркелова М. И., Синагина М. Н., и др. Особенности

- микробиома толстой кишки у пациентов с ожирением при его различных фенотипах. Журнал микробиологии, эпидемиологии и иммунобиологии. 2021; 98 (2): 144–155. Доступно по ссылке: <https://doi.org/10.36233/0372-9311-66>.
22. Колесникова И. М., Гапонов А. М., Ганенко Л. А., Волкова Н. И., Григорьева Т. В., Лайков А. В. и др. Взаимосвязь содержания нейротрофов кишечного микробиома при различных метаболических типах ожирения. Журнал эволюционной биохимии и физиологии. 2022; 58 (54): 298–310.
 23. Yosae S, Khodadost M, Esteghamati A, et al. Adiponectin: An Indicator for Metabolic Syndrome. *Iran J Public Health*. 2019; 48 (6): 1106–15.
 24. Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. *Trends Endocrinol Metab*. 2015; 26 (4): 193–200. DOI: 10.1016/j.tem.2015.01.006.
 25. Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC. Leptin, obesity, and leptin resistance: where are we 25 years later? *Nutrients*. 2019; 11 (11): 2704. DOI: 10.3390/nu11112704.
 26. Yuan M, Li W, Zhu Y, et al. Asprosin: A novel player in metabolic diseases. *Front Endocrinol (Lausanne)*. 2020; 11: 64. DOI: 10.3389/fendo.2020.00064.
 27. Ugur K, Aydin S. Saliva and blood asprosin hormone concentration associated with obesity. *Int J Endocrinol*. 2019; 2019: 2521096. DOI: 10.1155/2019/2521096.
 28. Elisha B, Karelis AD, Imbeault P, et al. Effects of acute hyperinsulinaemia on total and high-molecular-weight adiponectin concentration in metabolically healthy but obese postmenopausal women: a Montreal-Ottawa New Emerging Team (MONET) study. *Diabetes Metab*. 2010; 36 (4): 319–21. DOI: 10.1016/j.diabet.2010.03.004.
 29. Ung UJ, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *International Journal of Molecular Sciences*. 2014; 15 (4): 6184–223. Available from: <https://doi.org/10.3390/ijms15046184111,112>.
 30. Jamar G, Caranti DA, de Cassia Cesar H, et al. Leptin as a cardiovascular risk marker in metabolically healthy obese: Hyperleptinemia in metabolically healthy obese. *Appetite*. 2017; 108: 477–82. DOI: 10.1016/j.appet.2016.11.013.
 31. Fu J, Li Y, Esangbedo IC, et al. Circulating osteonectin and adipokine profiles in relation to metabolically healthy obesity in chinese children: findings from BCAMS. *J Am Heart Assoc*. 2018; 7 (23): e009169. DOI: 10.1161/JAHA.118.009169.