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«Enzymatic Landscape» of the Gut Microbiome

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Olga P Shatova[1]*, AA Zabolotneva[1,2], VV Makarov[3], SM Yudin[3], SA Roumiantsev[1,2] and AV Shestopalov[1,2]

[1]Pirogov Russian National Research Medical University, Academic Chair of Biochemistry and Molecular Biology, Moscow, Russia

[2]National Medical Research Center of Endocrinology, Moscow, Russia

[3]Federal State Budgetary Institution "Centre for Strategic Planning and Management of Biomedical Health Risks" of the Federal Medical Biological Agency, Moscow, Russia

***Corresponding Author:** Olga P Shatova, Pirogov Russian National Research Medical University, Academic Chair of Biochemistry and Molecular Biology, Faculty of General Medicine, 117997 Moscow, Russia.

Abstract

It is known that obesity is traceable to gut dysbiosis. Changes in the microbial ecosystem in the gut have major implications for the content of microbial signaling molecules in the host organism. The representation of tryptophan (Trp) signaling molecules in the gut will have a significant impact on intestinal microbiota diversity. Using metagenomic analysis, we investigated the taxonomic representation of the intestinal microbiota in healthy individuals and obese patients, which allowed us to reconstruct its potential metabolic activity depending on the level of Trp metabolites using PICRUSt software. We found that the key microbial Trp catabolite (MICT) are indole-3-lactate. Using the data of metagenomic sequencing analysis and bioinformatic tools we have analyzed a potential metabolic activity of the microbial communities or so-called «enzymatic landscape». We have found a twofold increase in the correlation between the content of Trp metabolites in feces and the «enzymatic landscape» of microbiome. The maximum number of statistically significant correlations between Trp metabolites and the potential metabolic activity of the microbial communities were established for indole-3-lactate. We have shown statistically significant relationships for indole-3-lactate and the abundance of genes for the metabolism of monosaccharides, nucleotides, amino acids, polyamines, and sulfosaccharides. It has been established that in obese patients there is a threefold increase in indole-3-lactate-producing microbiota. The phenotype of the microbiotic population is represented by completely different genera and species of microorganisms in obese individuals. The content of indole-3-lactate in the feces of obese patients mainly depends on the taxonomic representation of *Escherichia-Shigella*.

Keywords: tryptophan metabolites; indole-3-lactate; microbiome; obesity; enzymatic landscape

Introduction

For a long time, obesity and metabolic syndrome have taken a leading position among dysmetabolic disorders [1, 2]. The pathogenesis of obesity is extremely complex and depends on multiple factors, including the influence of the metabolites of gut microbiota [3, 4]. The intestinal microbiota is a community of microorganisms reside the gut and participating in the regulation of food intake and production of multiplicity of signaling molecules which on the one hand serve as *quorum sensing* regulators, and on the other hand can affect host homeostasis [5]. During evolution microbial and host metabolic processes co-evolved in mutualistic manner [6]. Rich microbial ecosystem of the gut contributes to a large number of physiological functions: catabolism of indigestible food components, synthesis of essential amino acids and vitamins production, protection against pathogens, the host immune system maturation, maintenance of intestinal barrier function, regulation of nervous system development, etc. [7, 8]. The repertoire of molecules synthesized by microorganisms from endogenous metabolites or exogenous components coming from food is extremely diverse [9]. These microbial metabolites are key players in the crosstalk between host cells and the gut microbiota [10, 11]. The beneficial or detrimental effect of specific microbiota-derived metabolites depends on both the composition of the microbial community and the state of the host organism, suggesting an inherently symbiotic nature of the microbiota-host metabolic interaction [12].

Thus, the microbiota, which constitutes a separate «biochemical organ» of a person, plays an important role in maintaining the physiological functions of the host and the homeostasis of its body [13]. However, a significant change in the environment that has occurred over the past century due to scientific and technological progress, a change in diet and a decrease in motor activity have led to the formation of maladaptation (including the development of obesity) and a change in the human microbial community [14]. Dysbiosis is a decrease in microbial diversity with a simultaneous increase in the representation of certain commensal microbial taxa, which will pathologically affect the host metabolism due to the formation of certain regulatory molecules [15]. An example of such a pathological effect is the signaling of tryptophan (Trp) metabolites in the pathogenesis of obesity [15, 16].

Trp is an essential aromatic amino acid that, in addition to participating in protein and coenzymes synthesis, is a precursor of important signaling molecules [5]. Dietary Trp is metabolized in the gut via three key pathways: (1) the direct transformation of Trp into indol and its derivatives, most of which are ligands of the aryl hydrocarbon receptor (AhR), by the gut microbiota [17, 18]; (2) the kynurenine pathway occurred in both immune and epithelial cells due to activity of indoleamine 2,3-dioxygenase (IDO) 1; and (3) the serotonin (5-hydroxytryptamine) production pathway in enterochromaffin cells through the activity of Trp hydroxylase 1 (TpH1) [19].

Indole and its derivatives, such as tryptamine, skatole, indole-3-pyruvate, indole-3-acrylate, indole-3-propionate, indole-3-acetamide, indole-3-ethanol, indole-3-aldehyde, and indole-3-acetaldehyde consist a group of tryptophan microbial catabolites (MICT) that significantly affect host metabolism [20] (Fig.1). F.1

For example, MICT suppresses the development of autoimmune diabetes [21]; indole-3-acetate was demonstrated to suppress the proliferation of cancer cells in the pancreas [22]; indole-3-acetate and indole-3-propionate regulate lipogenesis in the liver and prevent liver steatosis [23]; indole-3-acetate decreases the production of pro-inflammatory cytokines in macrophages [24]; indole-3-acetaldehyde stimulates the production of interleukin-22 (IL-22) in immune cells, including those of the intestines [9]; indole-3-acetate and indole-3-propionate have anti-inflammatory action in adipose tissue, thus preventing the development of insulin resistance development [25]; indole-3-propionate regulates mucosal barrier permeability by increasing the synthesis of tight junction proteins, by decreasing the production of tumor necrosis factor α (TNF-α) production, and acting as an anti-oxidant [18]; indole-3-propionate possesses neuroprotective effects [26]; indole-3-lactate is in charge with axon growth and the development of cognitive functions; indole itself has been investigated to induce the production of glucagon-like peptide-1 (GLP-1) by enteroendocrine L-cells [27, 28], which is known to stimulate the secretion of insulin by pancreatic β cells [29].

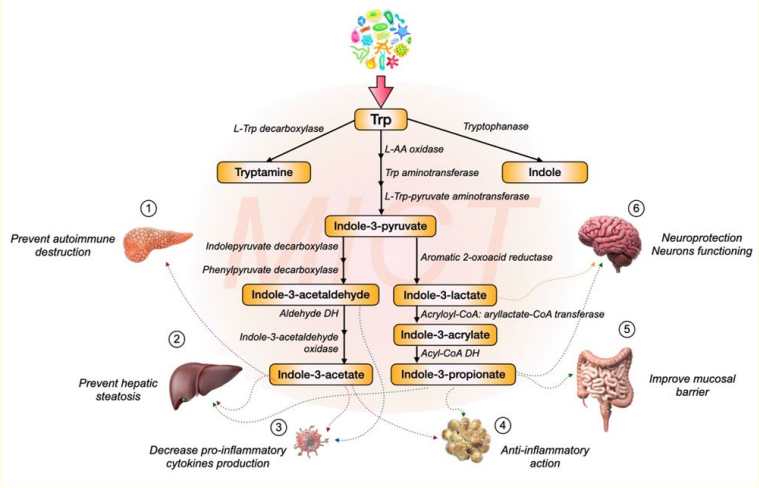

Figure 1: Intestinal microbiotas produce various MICT, which are signaling molecules. The figure shows some of the MICT which play a critical role in human organism.

Increased concentrations of indole-3-acetate, indole-3-lactate, and indole in plasma have been shown to be characteristic features of obese patients [4, 23]. At the same time, indole metabolites may have beneficial effects on host metabolism [30]. Indole-3-acetate and indole-3-propionate were shown to suppress inflammation, thus preventing adipose tissue remodeling and the development of insulin resistance and protumorogenic cytokine background [31]. Without doubt, diet correction significantly changes gut microbial phenotype. However, it is very difficult to define whether these changes remain stable and how quickly the phenotype returns to the dysbiosis state, which is a characteristic of obesity, as these parameters are often individual for each patient [32]. Therefore, to date, the issues of the composition of individual Trp metabolites producers and their homeostatic stability during normal state and obesity, as well as the role of these metabolites in the overall enzymatic landscape of the intestinal microbial community remain open.

The aim of our study was to analyze the content of Trp metabolites in fecal extracts and their interconnections with the taxonomic composition of the microbiota and the representation of microbial enzyme genes responsible for basic metabolism, thus tracing the co-evolutionary relationships between the formation of the intestinal microbial community and the phenotype of the host organism. For this aim, we carried out metagenomic sequencing of microbial DNA isolated from fecal samples of target individuals, and using bioinformatic tools, we have reconstructed the potential metabolic activity of identified microbial communities.

Materials and methods

Subjects

A cross-sectional cohort study was conducted in the period 2018-2022 based on the Department of Internal Diseases No.3 of the Federal State Budgetary Educational Institution of Higher Education of the Rostov State Medical University of the Ministry of Health of Russia, the Center for Digital and Translational Medicine of the Center for Molecular Health LLC and the Kazan Federal University (Volga Region). The study was conducted in accordance with the Declaration of Helsinki, and approved by the Local Ethics Committee of the Federal State Autonomous Educational Institution of Higher Education Pirogov Russian National Research Medical University of the Ministry of Health of Russia (protocol No. 186 of 06.26.2019) and the Local Independent Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education of the Rostov State Medical University of the Ministry of Health of Russia (protocol No. 20/19 of 12.12.2019).

The study involved 223 people who applied to a medical institution as part of a preventive examination and medical examination. Mean age of the patients examined was 39.9±4.2 y.o., and 2 clinical groups were formed. The first group (control) (n=109) consisted of healthy subjects without obesity and/or metabolic syndrome and with an average body mass index (BMI) of 22.7kg/m^2, a waist of 79.8 cm (BMI ≤ 24.9 kg/m^2, WC in women <88cm, in men<102cm). The second group (n=114) consisted of patients diagnosed with obesity and/or metabolic syndrome and with an average BMI of 32.96kg/m^2, waist 108.98 cm (BMI ≥ 30 kg/m^2, WC in women > 88 cm, in men > 102 cm). The group of healthy individuals includes 88% women and 12% men; the group of obese patients includes 79% women and 21% men. Inclusion criteria were: age over 18 years and absence of antibiotics, pre- and probiotics within 3 months prior to inclusion in the study. The exclusion criteria were severe somatic diseases (chronic kidney failure, chronic heart failure, chronic liver failure); any disease of the gastrointestinal tract, including ulcerative colitis, irritable bowel syndrome, Crohn's disease; any acute illness; alcoholism; pregnancy; depression. To minimize the influence of climatic conditions, dietary habits, and ethnic factors on the intestinal microbiome, the study included people living in the same territory (the Rostov region and the city of Rostov-on-Don) in the autumn-summer period. The participants were surveyed according to a specially designed questionnaire, which, in addition to general questions, included specific aspects of the life history, such as the method of birth (natural way, caesarean section), type of feeding (breastfeeding, artificial formula), food regimen (frequency of meals, volume of servings, ratio macronutrients), taking medications. For screening detection of depression and anxiety, the Hospital Anxiety and Depression Scale was used. The patients participating in the study were non-smokers.

Venous whole blood and fecal samples were taken from study participants. Laboratory studies were carried out on the basis of the Central Research Laboratory of the Federal State Budgetary Educational Institution of Higher Education of the Rostov State Medical University of the Ministry of Health of Russia, the Center for Digital and Translational Medicine of the Center for Molecular Health LLC, and the Kazan Federal University (Volga Region).

Chromatography

The study of the concentration of Trp metabolites was carried out using high performance liquid chromatography (HPLC). Feces samples were collected from all of the subjects according to the protocol of the study (Supplementary material: Protocol of the study). Feces were taken once in the hospital, without prior special diet, however, in the questionnaire, each of the study participants indicated food addictions. The samples were transported and stored at -40°C. Quantitative analysis of Trp metabolites in feces was carried out using high performance liquid chromatography and mass-spectrometry (HPLC-MS/MS) with the help of liquid chromatographer Agilent 1200 (Agilent inc., USA) supplied with an autosampler, a column thermostat, and a degasser. For sample preparation, 5mg of lyophilized feces were used for extraction with 50% methanol and the addition of internal standard and ascorbic acid. Following centrifugation, the samples were analyzed by HPLC-MS/MS. Chromatography was carried out using the analytical column Discovery PFP HS F5 (2.1 * 150 mm; 3 μm), with the mobile phase composition being as follows: phase A 0,1% formic acid in deionized water, phase B 100% acetonitrile suitable for HPLC. The mobile phase gradient was 1-10% for 4 min, followed by 90% by the 9th min of the analysis, the mobile phase flow speed was 0.40ml/min. For detection we used mass-spectrometry detector based on triple quadrupole Agilent 6460 (Agilent inc., USA) with MRM and electrospray ion source. For MRM-MS, parent and daughter ions of particular compounds, as well as the ionization and dissociation parameters, were optimized using the standards for the metabolites under study. The data obtained were processed using Masshunter software (Agilent inc., USA). Metabolite concentrations were determined using the internal standards method (with 2-hydroxynicotinic acid). The standards of the substances under investigation were prepared using a solution of bovine serum albumin and sodium chloride, to which the metabolites to be analyzed were added followed by further proceeding according to the protocol of the analysis.

The methods described were validated according to selectivity, linearity, accuracy, reproducibility, matrix effect, and analyte's stability. The validation was conducted in accordance with the FDA guidance for bioanalytical methods validation.

Metagenomic sequencing

For the determination of the microbiota composition, 16S rRNA sequencing was performed. DNA extraction from feces was carried out according to standard procedures. The QIAamp Fast DNA Stool Mini Kit (QIAGEN GmbH, Germany) was used to isolate bacterial DNA from fecal samples.

To perform a polymerase chain reaction (PCR) of the v3-v4 variable region of the 16S rRNA gene, gene-specific primers (337F 5'-*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*CCTACGGGNGGCWGCAG-3' and 805R 5'- *GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG*GACTACHVGGGTATCTAATCC-3') with an additional adapter sequence (italicized) were used.

Amplification was carried out in the standard mode with Q5® High-Fidelity DNA Polymerase (New England Biolabs, USA). The obtained PCR products were detected in 1% agarose gel, after which the reaction mixtures were purified with AMPure XP paramagnetic particles (Beckman Coulter, USA) according to the library preparation protocol. Subsequent amplification of PCR products was carried out using Nextera XT Index Kit index primers (Illumina, Inc., USA) and Q5® High-Fidelity DNA Polymerase (New England Biolabs, USA) and repurified with AMPure XP paramagnetic particles (Beckman Coulter, USA). The concentrations of purified libraries were determined using Qubit HS Assay Kits (Thermo Fisher Scientific, USA) on a Qubit 2.0 fluorimeter (Invitrogen, Thermo Fisher Scientific, USA). The resulting libraries were mixed in an equimolar ratio, and the quality and size of the resulting pool were assessed using a 2100 Bioanalyzer (Agilent Technologies, Inc., USA). If necessary, additional cleaning was performed with AMPure XP paramagnetic particles (Beckman Coulter, USA). The prepared pool of libraries was diluted and denatured using MiSeq Reagent kit v3 reagents (Illumina, Inc., USA) and sequenced on a MiSeq NGS system (next generation sequencing) (Illumina, Inc., USA) according to the manufacturer's protocol.

Sequencing data processing

The resulting reads were analyzed with the QIIME v.1.9.1 program (Knight and Caporaso labs., USA) using the Greengenes v.13.8 reference database (Second Genome, Inc., USA) with a 97% similarity threshold between sequences [33]. The relative representation of the bacterial taxa in the total pool of reads was obtained in proportions (from 0 to 1), which were calculated based on the number of reads mapped for each taxon. Thus, when analyzing the taxonomic affiliation of blood/feces bacterial DNA, data were used, such as the proportion of individual taxa in the total pool of blood/feces bacterial DNA (from 0 to 1), and the frequency of taxon isolation in patients of different study groups. The library pool was diluted and denatured with MiSeq Reagent kit v3 and sequenced on a MiSeq instrument (Illumina) according to the manufacturer's protocol.

For the reads obtained, we performed a quality control using fastQC tool with the following criteria: 1) base quality >25 for 90% of bases; 2) read length 300nt for 90% of reads; 3) 1% or less of Ns (undetermined bases).

Bioinformatics analysis

Primary sequencing data processing and OUT list obtaining was carried our using «QIIME v.1.9.1» open-source bioinformatics pipeline [34]. Next, the analysis on presumable metabolic role of microbiota components was performed by the method of Reconstruction of Unobserved States with the help of PICRUSt software [35]. Raw metagenomics data were translated to representation of the associated enzymatic network using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Ortholog database and the HMP Unified Metabolic Analysis Network 2 (HUMAnN2) software, which is a pipeline for efficient and accurate profiling of abundance in microbial pathways from a community based on metagenomic or metatranscriptomic sequencing data (Fig. 2). F.2

It should be noted that according to literature data there is a work in which a topological analysis of the enzymatic representation of the intestinal microbiota in patients with colorectal cancer was conducted [36].

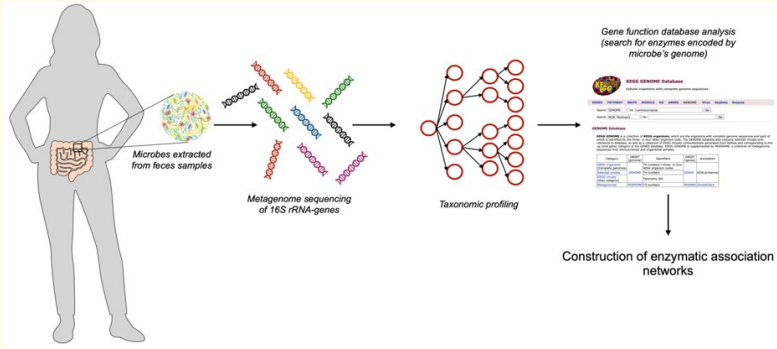

Figure 2: Experiment design.

We believe that it would be correct to use the term «enzymatic landscape» since the data obtained do not at all reflect the level of expression or activity of microbial enzymes but can be useful only for prognostication of the enzymatic capabilities of the microbiota in healthy people or obese patients. It is obvious that it is necessary to evaluate the representation of microbial enzymatic landscape by thoroughly studying the content of the products of specific reactions.

The analysis of the functional role of the microbiota based on 16S rRNA was carried out according to the published protocol [37] with the help of visual and meta-analysis of microbiome databases, Microbiome Analyst [38] and Calypso [39]. Associated enzymatic network identifies enzymatic components of gut microbiota and was studied using biostatistical analysis providing the comparison of the metagenome sequencing data with the KEGG Enzymes database (genome.jp). The results of the study of the enzymatic landscape are presented in relative units, allowing the comparison of samples and the cohorts within the framework of the project. Here, we consider the enzymatic landscape as a presence and a content (in relative units) of genes encoding for different enzymes, in other words, a possible presence of an enzyme in conformity with the presence of DNA of microorganisms potentially capable to express the enzyme.

Statistical analysis of the study results was performed in STATISTICA 12.0 (StatSoft Inc, USA). All obtained data on the content of Trp metabolites in stool analyzes were checked for abnormal distribution using the Shapiro-Wilk test. The distribution was abnormal, and our data are presented as median, 25% and 75% percentile. In the table below, the Spearman rank correlation coefficient is presented. The correlation analysis was carried out with the use of the statistical significance of the correlation coefficient. The difference was considered statistically significant if p <0,05.

Results and Discussion

The main tryptophan catabolite in the intestine is indole. So, its concentration is significantly higher than that of other tryptophan metabolites in both obese and normal BMI individuals (Table 1).

	Healthy adults (n=106)	***Patients with obesity (n=105)***
Indole		
Median	460	379
25-75%	180-577	149-506
Indole-3-acetate		
Median	21.3	21.9*

25-75%	4.69-23.3	2.46-18.7
Indole-3-propionate		
Median	21.4	8.48*
25-75%	4.82-22.5	2.51-9.27
Kynurenic acid		
Median	7.64	5.76*
25-75%	1.43-10.8	0.393-7.46
Indole-3-carboxaldehyde		
Median	5.06	2.57*
25-75%	2.43-6.66	1.09-3.37
Quinolinic acid		
Median	5.18	2.16*
25-75%	1.76-7.55	0.719-2.76
Tryptamine		
Median	2.65	1.49*
25-75%	0.116-1.13	0.047-0.286
Xanthurenic acid		
Median	2.35	1.09*
25-75%	0.261-2.28	0.078-1.24
5-hydroxyindole-3-acetic acid		
Median	2.31	1.42
25-75%	0.298-2.66	0.206-1.72
Indole-3-lactate		
Median	2.23	2.43*
25-75%	1.81-1.06	0.071-0.496
Kynurenine		
Median	0.616	0.523
25-75%	0.227-0.669	0.194-0.579
Anthranilic acid		
Median	0.319	0.189*
25-75%	0.173-0.374	0.095-0.211
Indole-3-acrylate		
Median	0.156	0.048*
25-75%	0.027-0.188	0.011-0.056

* – significant difference, $p<0.05$.

Table 1: Concentrations of tryptophan metabolites in feces (adults), nmol/g

We found that in adults with obesity, the content of indole-3-propionate, kynurenic acid, indole-3-carboxaldehyde, quinolinic acid, tryptamine, xanthurenic acid, anthranilic acid and indole-3-acrylate decreases in feces. At the same time, the content of indole-3-acetate and indole-3-lactate tends to increase. Having analyzed a group of metabolically healthy and metabolically unhealthy obese patients, it was found that both groups were statistically significantly different from the control.

With the help of biostatistical tools for the analysis of taxonomic composition diversity, we managed to assess the enzymatic landscape of the normal intestinal microbiota and of those in the obesity state, and then conducted a correlation analysis of the genes representation in the gut microbiome and the content of Trp metabolites in the feces. We observed a more pronounced coupling between the enzymatic landscape and Trp metabolites in obese patients. Thus, in healthy donors 251 statistically significant correlations were found (significance level=3, p≤0.001), while 479 statistically significant correlations appeared in obese patients with the same level of significance (Fig.3).

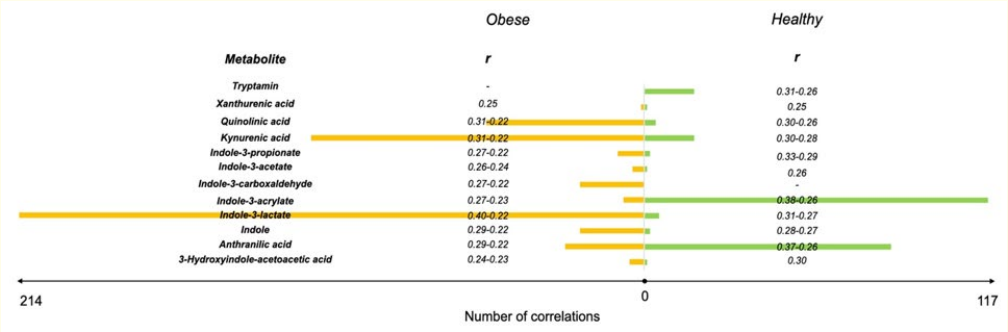

Figure 3: Quantitative analysis of the correlation «Trp catabolites – gut microbiota-associated enzymatic network».

It was determined that in obesity fecal indole concentration did not change significantly (Fig.3), while the number of genes encoding for enzymes which indole concentration correlated with was increased 10-fold (Fig.3).

The number of statistically significant correlations between indole-3-lactate and different enzyme-coding genes increased from 5 pairs shown for healthy donors to 214 pairs in obese patients (Fig. 3). It should be noted that we have previously shown that the concentration of indole-3-lactate in the blood serum of patients with obesity significantly increased [23]. However, it is impossible so far to collate the contribution of gut microbiota and that of microorganisms from other locations, as well as internal synthesis of the metabolite into the overall serum indole-3-lactate concentration.

Indole, indole-3-lactate, kynurenic acid and quinolinic acid were determined as potential key signaling metabolites of Trp correlated with genes coding for microbial enzymes in patients with obesity. On the contrast, in healthy donors indole-3-acrylate and anthranilic acid appeared to be key signaling molecules.

Further analysis revealed particular enzymes, the presence of which was more closely correlated with the fecal indole-3-lactate concentration (Table 2). However, it should be noted that the correlation coefficient does not reflect a strong enough relationship.

Enzyme	***Spearman rank correlation coefficient***	***Pathway of metabolism***
Allose kinase	0.407	Monosaccharides metabolism
UDP-4-amino-4-deoxy-L-arabinose formyltransferase	0.372	Amino sugars and nucleotide sugars metabolism
UDP-glucuronic acid dehydrogenase	0.372	Amino sugars and nucleotide sugars metabolism
UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase	0.371	Amino sugars and nucleotide sugars metabolism

Sulfoquinovose isomerase	0.370	Sulphoglycolysis
Oxalyl-CoA decarboxylase	0.363	Glyoxylate and dicarboxylate metabolism
Glucose-1-phosphatase	0.358	Glucose metabolism
1,4-dihydroxy-2-naphthoyl-CoA hydrolase	0.351	Naphthoquinone metabolism
N-hydroxyarylamine O-acetyltransferase	0.351	Acetylation of arylamines
Delta3-Delta2-enoyl-CoA isomerase	0.350	Lipid metabolism
Sulfofructose kinase	0.349	Sulphoglycolysis
Formate dehydrogenase-N	0.347	Sulphoglycolysis
Succinylornithine aminotransferase	0.347	Metabolism of arginine
Ribonuclease I (enterobacter ribonuclease)	0.346	RNA degradation
Sulfofructosephosphate aldolase	0.346	Sulphoglycolysis
Ferric-chelate reductase (NADPH)	0.345	Iron metabolism
Succinate-semialdehyde dehydrogenase	0.344	Metabolism of glutamic acid and glutamine
(d)CTP diphosphatase	0.343	Metabolism of pyrimidine nucleotides
dTDP-4-amino-4,6-dideoxy-D-galactose acyltransferase	0.343	Nucleotide sugars biosynthesis
Inosine kinase	0.343	Metabolism of purine nucleotides
dTDP-N-acetylfucosamine:lipid II N-acetylfucosaminyltransferase	0.343	Biosynthesis of enterobacterial common antigen (ECA)
KDO2-lipid IV(A) palmitoleoyltransferase	0.342	Lipid A and lipopolysaccharides biosynthesis
2-ketogluconate reductase	0.342	Ketogluconate metabolism, pentose phosphate pathway
Sulfolactaldehyde 3-reductase	0.341	Sulfoquinovose catabolism (sulphoglycolysis)
Alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase	0.340	Phosphonate and phosphinate metabolism

Table 2: Fecal indole-3-lactate concentration correlation with the gut microbiota-associated enzymatic network in patients with obesity.

Among the microbial genes correlated with intestinal indole-3-lactate concentration in patients with obesity, the majority were responsible for carbohydrate, amino acids, nucleotides, and sulfosugar metabolism (Table 2). Thus, we conclude that patients have the enzymatic landscape of the gut microbiota producing nucleotides that is connected to indole-3-lactate in the intestine. The concentration of indole-3-lactate in feces was also found to correlate with the enzymatic landscape of arginine, glutamic acid, and glutamine metabolism.

In our opinion, the interconnection of indole-3-lactate production and the genes for enzymes responsible for polyamine synthesis is of great importance. Polyamines are known to be produced by gut microbiota from arginine and its catabolite, i.e. ornithine [40]. Metabolism of polyamines plays a central role in the regulation of systemic and mucosal adaptive immunity, with arginine being a significant modulator of macrophages' and T-cells' metabolism able to affect their effector functions. Additionally, polyamines inhibit the production of pro-inflammatory cytokines and possess antioxidant activity [41]. Gut-derived polyamines can decrease cytokine secretion thus providing a better epithelium repair and normal barrier function recovery. Spermine (polyamine) and histamine inhibit the

activation of inflammasome, which is a large protein complex expressed by epitheliocytes and able to regulate interleukin 18 (IL-18) secretion [41]. Moreover, it was demonstrated that the presence of probiotic strain of *Bifidobacterium animalis* may induce resistance to oxidative stress and promote an increasement of life expectancy, which depends on intensified polyamine synthesis by microbes [42]. Data available from the literature indicate that increased polyamine concentration in adipose tissue, liver, or skeletal muscles may stimulate energy metabolism and resistance to the development of alimentary obesity [43]. Polyamines metabolites are also known to participate in adipogenesis [44]. In addition, exogenous spermine treatment was shown to effectively decrease body mass and fasting blood glucose level, as well as improve glucose tolerance in mice with diet-associated obesity [45]. Finally, spermine affects insulin perception and sensitivity to insulin [46]. One can conclude that immunological and metabolic effects of polyamines coincide with those of «indole-AhR» signaling system in many respects. The correlations revealed in the current study indicate a combined action of indole-3-lactate and polyamines in tolerogenicity development or its impairments in patients with obesity. Coupled reduction of the two tolerogenic mechanisms may lead to increased intestinal barrier permeability in obesity.

The connection between the level of intestinal indole-3-lactate and the presence of sulphoglycolysis enzymes in the microbial enzymatic landscape is not well studied so far. Sulfosugar sulfoquinovose (SQ) is produced by almost all photosynthetic organisms on Earth and is metabolized by bacteria in the process of sulphoglycolysis. In the sulfoglycolytic Embden-Meyerhof-Parnas (sulfo-EMP) pathway, SQ is catabolized to dihydroxyacetone phosphate and sulfolactaldehyde, so the pathway is analogous to classical glycolysis [47]. Some published works identify microorganisms performing sulphoglycolysis [48-49]. However, there is no data on the estimation of interconnection between the sulphoglycolysis metabolites and the concentration of indole metabolites of tryptophan. In the current study we managed to establish the statistically significant interrelation of the enzymatic landscape of the sulfoglycolysis enzymes and the indole-3-lactate concentration in obese patients. Noteworthy, sulfosugars are known reservoirs of sulfate which can potentially be used for glycosaminoglycans production and extracellular matrix remodeling. This is of great importance in regard to both chronic inflammation development in obesity, and protumorogenic phenotype formation characteristic for patients with sarcopenic obesity [31].

We also conducted an analysis of taxonomic microbial diversity at the levels of phyla (Phylum - f), genera (Genus - g), and species (Species - s). In healthy donors' indole-3-lactate was indicated to significantly correlate with the presence of the microorganisms presented in Supplementary Table 1 (Supplementary material: Table 1).

At the same time, even more correlations, which also appeared statistically significant, of indole-3-lactate and particular species of intestinal microbiota were shown in obese patients (Supplementary material: Table 2). It is worth to note that in obesity microbiota representatives correlating with fecal indole-3-lactate concentrations differed markedly from normal ones. Moreover, 54 correlations between various gut microorganisms and indole-3-lactate were demonstrated in healthy donors, while the number of statistically significant correlations in obese patients increased three-fold to 154.

We haven't found any statistically significant correlations between fecal indole-3-lactate concentrations and the presence of *Klebsiella, Pseudomonas*, *Escherichia-Shigella* in the intestines of healthy donors. Apparently, in obese donors concentrations of indole-3-lactate in stool samples was found to be produced predominantly by *Escherichia-Shigella*. According to published data, obesity is associated with the taxonomic impoverishment of gut microbiota [30]. However, our study demonstrates that, on the contrary, in obesity there is an enrichment in the species potentially producing indole-3-lactate.

Thus, both the genotype and the phenotype of the gut microbiota are transformed in obesity: the number of indole-3-lactate producing species increases three times. And this is indole-3-lactate concentration that reasons the extension of the connection with the enzymatic landscape of carbohydrate, lipid, nucleotide, and amino acid metabolism, as well as of sulfoglycolysis. It is generally accepted that microbiota, including those of intestines, are selected during evolution and stable at populational level. At the same time, commensal microbiota can be both quantitatively and qualitatively modulated [30] by means of diet, various signaling molecules, and cytokines. We propose that the enrichment of the gut microbiota with indole-3-lactate-producing bacteria occurs possibly as a compensatory mechanism to suppress the synthesis of pro-inflammatory cytokines that often accompany obesity patients. It is known

that indole-3-lactate has a pronounced anti-inflammatory action, since it participates in the induction of immunoregulatory T-cells and the suppression of proinflammatory T-cells [49]. Recent studies report indole-3-lactate being the major tryptophan metabolite for *Bifidobacterium* (*B. longum subsp. infantis*). Besides, quite high indole-3-lactate concentration is characteristic to breast milk [50]. In the present work, we managed to demonstrate that the concentration of fecal indole-3-lactate in patients with obesity depends primarily on the taxonomic representation of *Escherichia-Shigella*.

Conclusion

1. There is a relationship between the content of indole, indole-3-lactate, quinolinic and kynurenic acids and the prognostic enzymatic representation of the intestinal microbiome in obese patients.
2. In individuals with normal body weight, the enzymatic landscape depends on the intestinal content of indole-3-acrylate and anthranilic acid.
3. In obese individuals, we identified 214 pairs of «prognostic representation of the enzyme - metabolite of tryptophan metabolism», while in healthy individuals there were only 5 pairs.
4. Fecal indole-3-lactate concentration is shown to correlate with the enzymatic landscape of carbohydrate, nucleotides, amino acids, and sulfosugars metabolism.
5. The microbiota that potentially produces indole-3-lactate in obese patients is taxonomically different from healthy individuals.

Author Contributions

A.S., S.M.,V.V. and S.R. designed the experiments, O.P. prepared the materials and performed the experiments. O.S., A.Z. and A.S. analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Local Ethics Committee of the Federal State Autonomous Educational Institution of Higher Education Pirogov Russian National Research Medical University of the Ministry of Health of Russia (protocol No. 186 of 06.26.2019) and the Local Independent Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education of the Rostov State Medical University of the Ministry of Health of Russia (protocol No. 20/19 of 12.12.2019).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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