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# «Enzymatic Landscape» of the Gut Microbiome in Obese Patients

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**Abstract:** Indole and indole-3-lactate are known dominant microbial tryptophan catabolites (MICT). In obesity, the fecal indole concentration corresponds to the normal one, and that of indole-3-lactate significantly decreases along with other MICT, while it increases in blood plasma. During the analysis of the «enzymatic landscape» of the intestinal microbiota we find an almost twofold increase in the correlation between the concentrations of fecal MICT and the «enzymatic landscape», with indole-3-lactate having the closest relationships with the "enzymatic landscape" of all MICT. Here, we report statistically significant correlations of indole-3-lactate and the gut microbial enzymes for fructose, amino sugars, nucleotides, polyamines metabolism, and sulfoglycolysis. We also demonstrate that indole-3-lactate producing microbiota representatives increase three-fold in obesity. The phenotype of the microbiotic population is thus represented by completely different genera and species of microorganisms in obese individuals compared to healthy donors.

**Keywords:** tryptophan metabolites; microbiome; obesity; enzymatic landscape

## Introduction

In recent decades the investigations on understanding the role of hologenome effervesce owing to the progress in Next-generation Sequencing (NGS), which allowed to identify microbial species and associated metabolic pathways to a high degree of accuracy. Thus, a hologenome describes a genetic aggregate of host genes and those of symbiotic/mutualistic microbes [1].

Lately, there appeared a large number of studies dedicated to the analysis of taxonomic representation of gut microbiota in different physiological and pathological conditions. Many researchers place high emphasis on investigation of the role of dysbiosis in the development of

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obesity, diabetes mellitus [2], ulcerative colitis [3] and other bowel diseases [4], psoriasis [5], and immunodeficiencies [6].

It was also demonstrated that during aging the amount of butyrate-producing organisms decreases [6], and that the bone mass loss depends just on the development of an activated immune phenotype against a background of gut dysbiosis [7]. More and more studies evidence the participance of microbiome in the progression of neurodegenerative diseases and cancers [8] of different origin and localization.

Undoubtedly, gut microbiota is a key player in the development of metabolic disease states and «metabolic inflammation» [9]. However, the issue of what can be considered a metabolic disease is not perfectly defined to date. The scientific interest to metabolomics steadily grows [10], as it became obvious that metabolites can serve as signaling molecules. Thus, it was revealed that tryptophan (Trp) metabolites take part both in the regulation of bacterial population and in human organism functioning [11]. Indole-3-ethanol, indole-3-pyruvate, and indole-3-aldehyde are known to act through aryl hydrocarbon receptors (AhR) of enterocytes, supporting the integrity of apical junctional complexes (tight junctions) and providing normal gut permeability [12]. Besides, other indole metabolites, such as indole-3-acetate, indole-3-propionate, and indole-3-lactates, have their regulatory effects through AhR as well [13]. AhR itself is a ligand-activated transcription factor expressed in immune cells including those of intestines. So, bacteria-derived Trp metabolites modulate gut barrier function and resistance to intestinal pathogens [10, 14]. One of the metabolites of kynureine pathway of Trp, namely, kynurenic acid, plays a signaling role through a G-protein coupled receptor (GPCR) called GPR35, which is mainly overexpressed in cells of nervous tissue, and one of the main effects of kynurenic acid is decreased synaptic activity [15].

Many researchers analyze the peculiarities of gut microbiota taxonomic composition in a normal state and during different pathologies [16], including obesity, or investigate fecal metabolites produced with high probability exclusively by microbiota [11]. Although, this issue is to be demonstrated and proved, for gut microbiota composition in patients with obesity remains unexplored so far [16]. Some authors believe that indole-3-acetate is an exclusively microbial metabolite [17], on the other hand, human cells express an enzyme potentially able to synthesize this molecule. The enzyme converting indole-3-acetaldehyde to indole-acetate is aldehyde dehydrogenase (EC: 1.2.1.3) [18]. Other gut microbial catabolites of tryptophan (MICT) include indole, tryptamine, skatole, indole-3-pyruvate, indole-3-acrylate, indole-3-propionate, indole-3-acetamide, indole-3-ethanol, indole-3-aldehyde, and indole-3-acetaldehyde [11]. It is worth to note that out of all the MICT the highest concentration in the intestines was demonstrated for indole, which is both a *quorum sensing* molecule for microbiota and a signaling molecule in human body [19]. Moreover, we propose considering signaling role of MICT as a systemic *quorum sensing* molecule of an organism, for gut-derived indole can perfectly participate in quorum sensing of vagina ecosystem, endobronchial tree, and even blood microbiome.

It is of note that indole-3-acetate and indole-3-lactate are considered main MICT [20]. Tryptophan from food can be metabolized to indole-3-acetate by gut microbiota via indole-3-acetamide pathway catalyzed by tryptophan monooxygenase (EC:1.13.12.3) and indole-3-acetamid

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hydrolase (EC:3.5.1.4). Indole-3-lactate is produced from indole-3-pyruvate in the reduction reaction catalyzed by aromatic indole-lactate dehydrogenase (EC: 1.1.1.110), while indole-3-pyruvate, in turn, is produced by three enzymes: tryptophan transaminase (EC: 2.6.1.27), L-tryptophan-pyruvate aminotransferase (EC: 2.6.1.99), and L-amino acids oxidase (EC: 1.4.2.2). Further conversion of indole-3-lactate to indole-3-acrylate is possible due to 3-(aryl) acryloyl-CoA:(R)-3-(aryl) lactate CoA-transferase (EC:2.8.3.17) [21]. Interestingly, the metabolite produced from indole-3-lactate (i.e., indole-3-acrylate) activates the enzyme (indole-pyruvate decarboxylase, EC: 4.1.1.74) converting indole-3-pyruvate to indole-3-acetate [21] (Fig.1)

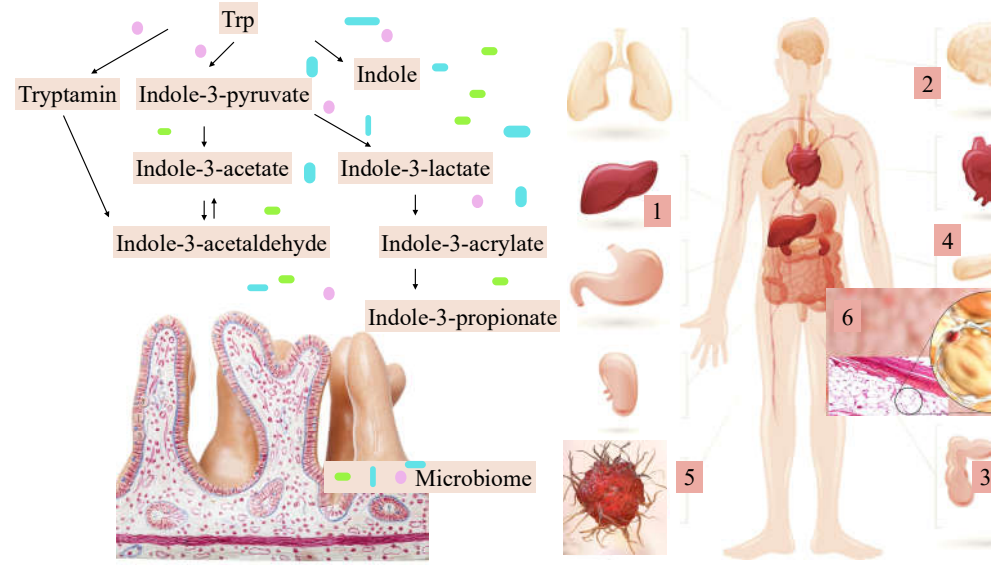


Figure 1. Intestinal microbiota produce various MICT. The figure shows some of the MICT which play a critical role in human organism: (1) indole-3-acetate and indole-3-propionate regulate lipogenesis in liver and prevent hepatic steatosis; (2) indole-3-propionate has neuroprotective function; indole-3-lactate is in charge with axon growth and the development of cognitive functions; (3) indole-3-propionate regulates mucosal barrier permeability by increasing the synthesis of tight junctions proteins, by decreasing tumor necrosis factor $\alpha$ (TNF-$\alpha$) production, and by acting as an anti-oxidant; (4) MICT suppress autoimmune diabetes development in pancreas, moreover, indole-3-acetate was demonstrated to suppress cancer cells proliferation in pancreas; (5) indole-3-acetate decreases the production of proinflammatory cytokines in macrophages; indole-3-acetaldehyde stimulates the production of interleukin-22 in immune cells, including those of intestines; (6) indole-3-acetate and indole-3-propionate have anti-inflammatory action in adipose tissue, thus preventing insulin resistance development.

In the works dedicated to the investigation of obesity the increasing of indole-3-acetate, indole-3-lactate, and indole concentrations was demonstrated in patients' blood [11, 22]. At the same time, many authors report the impoverishment of the microbiota in patients with obesity [23, 31]. Indole-3-acetate and indole-3-propionate were proved to suppress inflammation, thus preventing adipose tissue remodeling and the

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development of insulin resistance and protumorigenic cytokine background [24]. Without doubt, diet correction significantly changes gut microbial phenotype. However, it is very difficult to define so far if these changes remain stable and how fast the phenotype converts back to dysbiosis state characteristic to obesity, as these parameters are frequently individual for each specific patient [25]. Unambiguously, metabolomics of so-called «microbiotic predators» should be studied, for these are their number and metabolic pathways which define taxonomic diversity and *quorum sensing* [23]. In such a way, the issues of the composition of producers of individual tryptophan metabolites and its homeostatic stability during normal state and obesity, as well as of the role of these metabolites in overall metabolic landscape of intestinal microbial community remain opened to date.

**The goal of the current study was to** analyze the content of tryptophan metabolites in fecal extracts and their interconnections with the taxonomic composition of microbiota and its «enzymatic landscape» in patients with obesity.

## Materials and methods

*Subjects*

223 patients mean aged 39.9±4.2 y.o. were examined, and 2 clinical groups were formed. The first (control) group (n=109) comprised of healthy subjects without obesity and\or metabolic syndrome and with average body mass index (BMI) of 22.7kg/m$^2$, waist 79.8sm. The second group (n= 114) consisted of patients diagnosed with obesity and/or metabolic syndrome and with average BMI of 32.96kg/m$^2$, waist 108.98sm.

*Chromatography*

The study of the concentration of tryptophan 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. The samples were transported and stored at -40°C.

Quantitative analysis of tryptophan 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. Mobile phase gradient was 1-10% for 4 min, followed by 90% by the 9$^{th}$ min of the analysis, the speed of the mobile phase flow 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).

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Metabolite concentrations were determined by the method of internal standards (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.

For blood serum samples preparation, the internal standard (2-hydroxynicotinic acid) was added to 100µl of serum, proteins were precipitated by acetonitrile, and the supernatant was evaporated and redissolved in 10% methanol water solution with the addition of ascorbic acid to prevent analytes oxidation.

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 microbiota composition determination 16S rRNA sequencing was performed. DNA extraction from feces was carried out according to standard procedures. DNA libraries were prepared using 16S Metagenomic Sequencing Library Preparation (Part #15044223 Rev. B) according to manufacturer's instructions. The v3-v4 region of 16S rRNA was amplified using gene-specific primers containing additional adapter sequences (Table 1).

**Table 1.** Gene-specific primers for V3-V4 region of 16S rRNA.

| Primer name | Sequence (the additional adapter sequence is italicized) |
|---|---|
| 337F | 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′ |
| 805R | 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′ |

PCR was carried out using Q5® High-Fidelity DNA Polymerase (NEB) with the following run protocol: (1) 98°C – 30 sec; (2) 25 cycles: 98°C – 30 sec; 55°C – 20 sec; 72°C – 20 sec; (3) 72°C – 2 min; (4) holding at 4°C.

Amplicons were detected in 1% agarose gel followed by extraction with AMPure XP beads (Beckman Coulter) according to manufacturer's instructions for DNA libraries preparation. Purified amplicons were then indexed by amplification with Q5® High-Fidelity DNA Polymerase (NEB) and specific primers from Nextera XT Index Kit, the run protocol being the following: (1) 98°C – 30 sec; (2) 8 cycles: 98°C – 15 sec; 55°C – 30 sec; 72°C – 30 sec; 3) 72°C – 2 min; 4) holding at 4°C. After that amplicons were purified using AMPure XP beads according to manufacturer's instructions. Concentrations of purified DNA were measured using Qubit HS Assay Kit and Qubit 2.0 fluorometer (Invitrogen). The libraries were then mixed in equimolar concentrations and assessed using 2100 Bioanalyzer (Agilent Technologies), followed by additional purification using AMPure XP beads if necessary. The prepared library pool was denatured with the help of MiSeq Reagent kit v3 and sequenced using MiSeq (Illumina) according to manufacturer's instructions.

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*Sequencing data processing*

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 [26]. 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 [27].

The analysis of microbiota functional role based on 16S rRNA was carried out according to the published protocol [28] with the help of Microbiome Analyst [29] and Calypso [30]. The *enzymatic landscape* was studied using bio statistical 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* (enzymatic representation) are presented in relative units allowing to compare samples and the cohorts within the frames of the project. Here, we consider the *enzymatic landscape* (enzymatic representation) as a presence and a content (in relative units) of genes encoding different enzymes, in other words, a possible presence of an enzyme in conformity with the presence of DNA of microorganisms potentially capable to express one or another enzyme.

**Statistical analysis** of the results of the study was performed in STATISTICA 12.0 (StatSoft Inc, USA). T-test was carried out to determine the median and the sigma. In the tables below Spearman's rank correlation coefficient is presented. Correlation analysis was carried out with the use of statistical significance of correlation coefficient. The difference was considered statistically significant if $p < 0,05$.

**Results and discussion**

During the analysis of the content of tryptophan catabolites in feces we found *indole ad indole-3-lactate to be dominant MICT* (Table 2).

In feces of adult patients with obesity the concentration of eight Trp metabolites, namely, indole-3-lactate, quinolinic acid, 5-hydroxyindole-3-acetic acid, anthranilic acid, xanthurenic acid, indole-3-carboaldehyde, indole-3-acrylate, and indole-3-propionate, was significantly decreased compared to healthy donors. It is worth to note that the content of indole-3-lactate in feces from patients with obesity was dramatically decreased with the mean concentration being 78% lower than normal one.

**Table 2.** Fecal tryptophan metabolites concentrations in adults, nmol/g.

| Metabolite | Healthy adults (n=109) | Patients with obesity (n=114) |
|---|---|---|
| Indole | 464±462 | 389±361 |
| Indole-3-lactate | 454±18,6 | 101±218* |
| Indole-3-acetate | 21.9±40.6 | 21.9±42.9 |
| Indole-3-propionate | 21.51±29.9 | 8.28±11.3* |
| Kynurenic acid | 7.68±9.15 | 5.59±9.71 |
| Indole-3-carboaldehyde | 5.14±4.31 | 2.60±2.24* |
| Quinolinic acid | 5.13±4.71 | 2.23±2.26* |

| | | |
|---|---|---|
| Tryptamine | 2.59±7.42 | 2.11±8.52 |
| Xanthurenic acid | 2.36±4.18 | 1.06±2.22* |
| 5-hydroxyindole-3-acetic acid | 2.35±4.28 | 1.45±2.13* |
| Kynurenine | 0.65±0.96 | 0.55±0.57 |
| Anthranilic acid | 0.32±0.23 | 0.19±0.26* |
| Indole-3-acrylate | 0.16±0.21 | 0.05±0.08* |

* – significant difference, $p < 0.05$.

With the help of bio statistical tools for the analysis of taxonomic composition diversity we managed to assess the "*enzymatic landscape*" of normal gut microbiota and of those in obesity state, and then conducted a correlation analysis of enzymes representation of gut microbiota and the content of Trp catabolites in feces. We observed a more pronounced coupling between the "*enzymatic landscape*" and tryptophan metabolites in patients with obesity. Thus, in healthy donors 251 statistically significant correlations were found (significance level=3, p≤0,001), while 479 statistically significant correlations appeared in patients with obesity with the same level of significance (Table 3).

**Table 3.** Quantitative analysis of «Trp catabolite – gut microbiota enzyme representation» correlation.

| **Metabolites** | **Enzymatic representation** | | | |
|---|---|---|---|---|
| | **Adults norm** | | **Adults obesity** | |
| | **Number of statistically significant correlations** | **Correlation value** | **Number of statistically significant correlations** | **Correlation value** |
| 3-Hydroxy-indole-acetoacetic acid | 1 | 0,30 | 5 | 0,24-0,23 |
| Anthranilic acid | **84** | 0,37-0,26 | 27 | 0,29-0,22 |
| Indole | 2 | 0,28-0,27 | **22** | 0,29-0,22 |
| Indole-3-lactate | 5 | 0,31-0,27 | **214** | 0,40-0,22 |
| Indole-3-acrylate | **117** | 0,38-0,26 | 7 | 0,27-0,23 |
| Indole-3-carboxaldehyde | - | - | 22 | 0,27-0,22 |
| Indole-3-acetate | 1 | 0,26 | 4 | 0,26-0,24 |
| Indole-3-propionate | 2 | 0,33-0,29 | 9 | 0,27-0,22 |
| Kynurenic acid | 17 | 0,30-0,28 | 114 | 0,31-0,22 |
| Quinolinic acid | 4 | 0,30-0,26 | 54 | 0,31-0,22 |
| Xanthurenic acid | 1 | 0,25 | 1 | 0,25 |
| Tryptamine | 17 | 0,31-0,26 | - | - |

It was determined that in obesity fecal indole concentration did not change significantly (Table 2), while the number of genes encoding for

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enzymes which indole concentration correlated with was increased 10-fold (Table 3).

Another dominant intestinal Trp metabolite, namely, indole-3-lactate, was demonstrated to significantly decrease in obesity (Table 2). At the same time, the number of statistically significant correlations between this metabolite and different enzyme-coding genes increased from 5 pairs showed for healthy donors to 214 pairs in patients with obesity (Figure 2). It is worth to note that the concentration of indole-3-lactate significantly increased in blood serum of patients with obesity from 459±180 nmol/l to 683±378 nmol/l. 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 tryptophan correlating 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 these key signaling molecules.

Further analysis revealed the particular enzymes the presence of which more tightly correlated (Spearman's rank correlation coefficient=0.407-0.340) with fecal indole-3-lactate concentration (Table 4).

**Table 4.** Fecal indole-3-lactate concentration correlation with the enzymatic landscape of gut microbiome in patients with obesity.

| Enzymes | Spearman's rank correlation coefficient | Metabolic pathways |
|---|---|---|
| **Allose kinase EC: 2.7.1.55** | **0.407** | Fructose and mannose metabolism |
| UDP-4-amino-4-deoxy-L-arabinose formyltransferase EC: 2.1.2.13 | 0.372 | Amino sugars and nucleotide sugars metabolism |
| UDP-glucuronic acid dehydrogenase EC: 1.1.1.305 | 0.372 | Amino sugars and nucleotide sugars metabolism |
| UDP-4-amino-4-deoxy-L-arabinose-oxoglutarate aminotransferase EC: 2.6.1.87 | 0.371 | Amino sugars and nucleotide sugars metabolism |
| *Sulfoquinovose isomerase EC: 5.3.1.31* | *0.370* | *Sulfoquinovose catabolism (sulphoglycolysis)* |
| Oxalyl-CoA decarboxylase EC: 4.1.1.8 | 0.363 | Glyoxylate and dicarboxylate metabolism |
| Glucose-1-phosphatase EC: 3.1.3.10 | 0.358 | Gluconeogenesis |
| 1,4-dihydroxy-2-naphthoyl-CoA hydrolase EC: 3.1.2.28 | 0.351 | Vitamin K metabolism, secondary metabolites biosynthesis |

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| | | |
|---|---|---|
| N-hydroxyarylamine O-acetyltransferase EC: 2.3.1.118 | 0.351 | N-acetylation of arylamines and acetylation of aryl hydroxamic acids |
| Delta3-Delta2-enoyl-CoA isomerase EC: 5.3.3.8 | 0.350 | Lipid metabolism |
| *Sulfofructose kinase EC: 2.7.1.184* | *0.349* | *Sulfoquinovose catabolism (sulphoglycolysis)* |
| *Formate dehydrogenase-N EC: 1.1.5.6 (1.17.5.3)* | *0.347* | *Sulfoquinovose catabolism (sulphoglycolysis)* |
| Succinylornithine aminotransferase EC: 2.6.1.81 | 0.347 | Metabolism of arginine |
| Ribonuclease I (enterobacter ribonuclease) EC: 3.1.27.6 | 0.346 | RNA degradation |
| *Sulfofructosephosphate aldolase EC: 4.1.2.57* | *0.346* | *Sulfoquinovose catabolism (sulphoglycolysis)* |
| Ferric-chelate reductase (NADPH) EC: 1.16.1.9 | 0.345 | Reduction of iron (III) bound to different iron chelators (siderophores) |
| Succinate-semialdehyde dehydrogenase EC: 1.2.1.24 | 0.344 | Metabolism of glutamic acid and glutamine |
| (d)CTP diphosphatase EC: 3.6.1.65 | 0.343 | Metabolism of pyrimidine nucleotides |
| dTDP-4-amino-4,6-dideoxy-D-galactose acyltransferase EC: 2.3.1.210 | 0.343 | Nucleotide sugars biosynthesis |
| Inosine kinase EC: 2.7.1.73 | 0.343 | Metabolism of purine nucleotides |
| dTDP-N-acetylfucosamine:lipid II N-acetylfucosaminyltransferase EC: 2.4.1.325 | 0.343 | Biosynthesis of enterobacterial common antigen (ECA) |
| KDO2-lipid IV(A) palmitoleoyltransferase EC: 2.3.1.242 | 0.342 | Lipid A and lipopolysaccharides biosynthesis |
| 2-ketogluconate reductase EC: 1.1.1.215 | 0.342 | Ketogluconate metabolism, pentose phosphate pathway |
| *Sulfolactaldehyde 3-reductase EC: 1.1.1.373* | *0.341* | *Sulfoquinovose catabolism (sulphoglycolysis)* |
| alpha-D-ribose 1-methylphosphonate 5-triphosphate synthase EC: 2.7.8.37 | 0.340 | Phosphonate and phosphinate metabolism |

Among the microbial enzymes which presence correlated with the intestinal indole-3-lactate concentration in patients with obesity there can be found those of carbohydrate, amino acids, nucleotides, and sulfosugars metabolism (Table 4). Thus, we conclude that the patients possess the «*enzymatic landscape*» of gut microbiota producing nucleotides

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which is connected to indole-3-lactate in intestines. 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 in charge with polyamines synthesis is of great importance. Polyamines are known to be produced by gut microbiota from arginine and its catabolite, i.e. ornithine [31]. 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. Besides, polyamines inhibit proinflammatory cytokines production and possess antioxidant activity [32]. 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 [32]. Moreover, it is 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 [33]. 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 [34]. Polyamines metabolites are also known to participate in adipogenesis [35]. In addition, it was shown that the treatment with exogenous spermine effectively decreased the body mass and fasting blood glucose level, as well as improved glucose tolerance in mice with diet-caused obesity [36]. Finally, spermine affects insulin perception and sensitivity to insulin [37]. 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. The coupled reduction of the two tolerogenic mechanisms may lead to an 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 nearly 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, thus the pathway is analogous to the classical glycolysis [38]. Some published works identify microorganisms performing sulphoglycolysis [39-40]. However, there are 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 sulfoglycolysis enzymes and the concentration of indole-3-lactate in patients with obesity (Figure 2). 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 protumorigenic

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phenotype formation characteristic for patients with sarcopenic obesity [24].

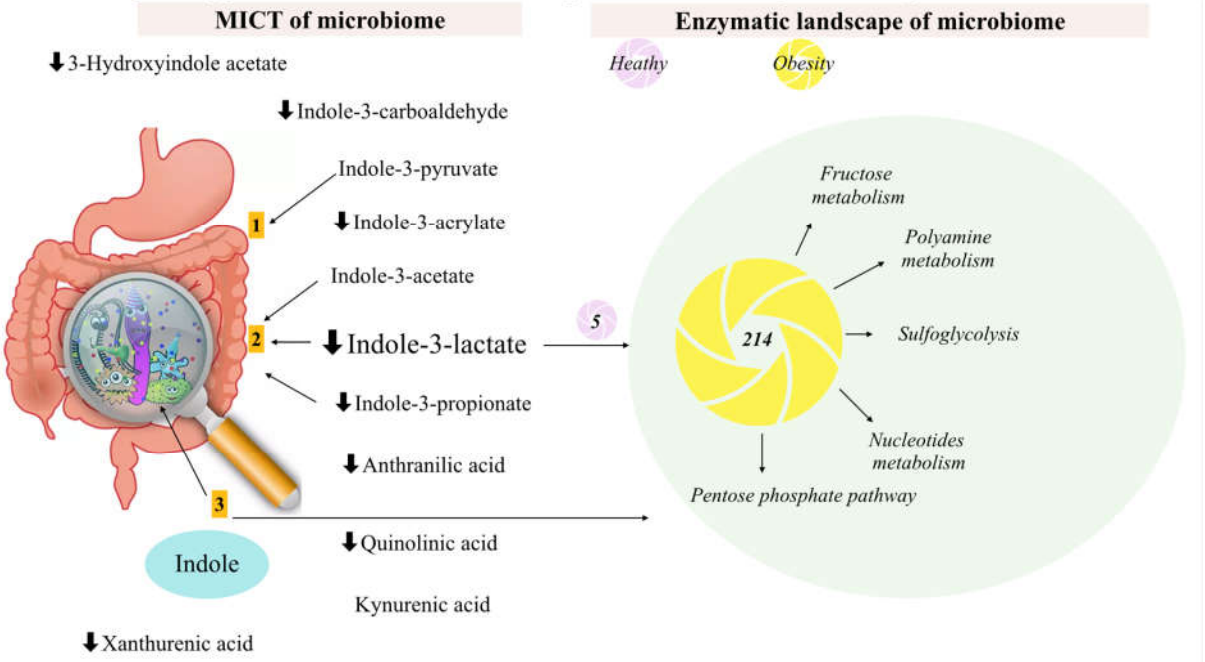


**Figure 2.** A severe deviation in the coupling of indole-3-lactate concentration to the «enzymatic landscape» of gut microbiome is observed in obesity. In healthy donors five statistically significant correlation pairs for indole-3-lactate and the enzymes represented in intestines were indicated, while in obesity a pronounced enrichment of gut microbiome was noted with statistically significant correlation pars for indole-3-lactate concentration in feces and the «*enzymatic landscape*» of various metabolic pathways.

Legend:

⬇– a decrease of fecal MICT concentrations;

**1** – indole-3-pyruvate acts on enterocytes through (AhR) and defines the integrity of tight junctions;

**2** – indole-3-acetate, indole-3-propionate, and indole-3-lactate act on intestinal immune cells (through AhR) and increase the permeability and the resistance to pathogens;

**3** – interspecific QS molecule, indole, with a constant concentration.

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 following microorganisms (Table 5).

**Table 5.** Gut microbial population representatives significantly correlating with the fecal indole-3-lactate concentration in healthy donors (significance level=3, p≤ 0,001).

| № | Gut microbial population representatives | Spearman's rank correlation coefficient |
|---|---|---|
| 1 | f__Ruminococcaceae; g__Faecalibacterium; s__OTU_1 | 0.372 |
| 2 | f__Lachnospiraceae; g__Blautia; s___OTU_1 | 0.369 |
| 3 | f__Lachnospiraceae; g__Lachnospiraceae_ND3007_group; s___OTU_2 | 0.366 |

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| | | |
|---|---|---|
| 4 | f__Ruminococcaceae; g__Faecalibacterium; s___OTU_2 | 0.362 |
| 5 | f__Ruminococcaceae; g__Faecalibacterium; s___OTU_3 | 0.328 |
| 6 | f__Lachnospiraceae; g__Lachnoclostridium; s___OTU_1 | 0.321 |
| 7 | f__Lachnospiraceae; g__OTU_1; s___OTU_1 | 0.318 |
| 8 | f__Akkermansiaceae; g__Akkermansia; s___OTU_1 | 0.315 |
| 9 | f__Lachnospiraceae; g__Lachnospiraceae_ND3007_group; s___OTU_1 | 0.315 |
| 10 | f__Barnesiellaceae; g__Barnesiella; s___OTU_1 | 0.312 |
| 11 | f__Lachnospiraceae; g__Lachnospiraceae_UCG-001; s___OTU_1 | 0.306 |
| 12 | f__Lachnospiraceae; g__[Eubacterium]_hallii_group; s___OTU_1 | 0.302 |
| 13 | f__Lachnospiraceae; g__Lachnospiraceae_UCG-010; s___OTU_5 | 0.302 |
| 14 | f__Lachnospiraceae; g__Blautia; s___OTU_2 | 0.302 |
| 15 | f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s___OTU_6 | 0.297 |
| 16 | f__Lachnospiraceae; g__Fusicatenibacter; s___OTU_1 | 0.296 |
| 17 | f__Monoglobaceae; g__Monoglobus; s___OTU_1 | 0.295 |
| 18 | f__Ruminococcaceae; g__Faecalibacterium; s___OTU_4 | 0.295 |
| 19 | f__Lachnospiraceae; g__Roseburia; s___OTU_1 | 0.295 |
| 20 | f__Lachnospiraceae; g__Lachnospiraceae_ND3007_group; s___OTU_2 | 0.293 |
| 21 | f__Tannerellaceae; g__Parabacteroides; s___OTU_1 | 0.289 |
| 22 | f__Lachnospiraceae; g__Blautia; s___OTU_3 | 0.289 |
| 23 | f__Bacteroidaceae; g__Bacteroides; s___OTU_1 | 0.289 |
| 24 | f__Monoglobaceae; g__Monoglobus; s___OTU_2 | 0.286 |
| 25 | f__Lachnospiraceae; g__Lachnospiraceae_NK4A136_group; s___OTU_7 | 0.285 |
| 26 | f__Ruminococcaceae; g__Subdoligranulum; s___OTU_1 | 0.285 |
| 27 | f__Pasteurellaceae; g__Haemophilus; s___OTU_1 | 0.284 |
| 28 | f__Bacteroidaceae; g__Bacteroides; s___OTU_2 | 0.283 |
| 29 | f__Monoglobaceae; g__Monoglobus; s___OTU_3 | 0.282 |
| 30 | f__Lachnospiraceae; g__Blautia; s___OTU_4 | 0.282 |
| 31 | f__Christensenellaceae; g__Christensenellaceae_R-7_group; s___OTU_1 | 0.280 |
| 32 | f__Lachnospiraceae; g__Blautia; s___OTU_5 | 0.280 |
| 33 | f__Bacteroidaceae; g__Bacteroides; s___OTU_2 | 0.279 |
| 34 | f__Ruminococcaceae; g__Faecalibacterium; s___OTU_5 | 0.278 |
| 35 | f__Lachnospiraceae; g__[Eubacterium]_eligens_group; s___OTU_1 | 0.278 |
| 36 | f__Lachnospiraceae; g__Blautia; s___OTU_6 | 0.276 |
| 37 | f__Lachnospiraceae; g__[Eubacterium]_hallii_group; s___OTU_2 | 0.276 |
| 38 | f__Bacteroidaceae; g__Bacteroides; s___OTU_3 | 0.276 |
| 39 | f__Lachnospiraceae; g__Anaerostipes; s___OTU_1 | 0.275 |

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| | | |
|---|---|---|
| 40 | f__Ruminococcaceae; g__Faecalibacterium; s___OTU_6 | 0.275 |
| 41 | f__Lachnospiraceae; g__Blautia; s___OTU_7 | 0.275 |
| 42 | f__Ruminococcaceae; g__Faecalibacterium; s___OTU_7 | 0.274 |
| 43 | f__Lachnospiraceae; g__Fusicatenibacter; s___OTU_2 | 0.273 |
| 44 | f__Rikenellaceae; g__Alistipes; s___OTU_1 | 0.273 |
| 45 | f__Lachnospiraceae; g__Lachnospiraceae_ND3007_group; s___OTU_3 | 0.273 |
| 46 | f__Lachnospiraceae; g__OTU_1; s___OTU_1 | 0.273 |
| 47 | f__Lachnospiraceae; g__[Ruminococcus]_torques_group; s___OTU_1 | 0.272 |
| 48 | f__Lachnospiraceae; g__Fusicatenibacter; s___OTU_3 | 0.270 |
| 49 | f__Bacteroidaceae; g__Bacteroides; s___OTU_4 | 0.270 |
| 50 | f__Lachnospiraceae; g__Fusicatenibacter; s___OTU_4 | 0.269 |
| 51 | f__Lachnospiraceae; g__Fusicatenibacter; s___OTU_5 | 0.268 |
| 52 | f__Monoglobaceae; g__Monoglobus; s___OTU_4 | 0.268 |
| 53 | f__Lachnospiraceae; g__Lachnospira; s___OTU_1 | 0.268 |
| 54 | f__Lachnospiraceae; g__Lachnospiraceae_ND3007_group; s___OTU_4 | 0.267 |

At the same time, even more correlations, that appeared statistically significant as well, of indole-3-lactate and particular species of gut microbiota were shown in patients with obesity (Table 6). It is worth to note that in obesity microbiota representatives correlating with fecal indole-3-lactate differed markedly from normal ones. Moreover, 54 correlations between various gut microorganisms and indole-3-lactate were demonstrated in healthy donors, while those statistically significant interrelations increased three-fold to 154 in patients with obesity.

**Table 6.** Gut microbial population representatives significantly correlating with the fecal indole-3-lactate concentration in obesity (significance level=3, p≤ 0,001).

| № | Gut microbial population representatives | Spearman's rank correlation coefficient |
|---|---|---|
| 1 | f__Enterobacteriaceae; g__Klebsiella; s__Klebsiella_pneumoniae | 0.378 |
| 2 | f__Pseudomonadaceae; g__Pseudomonas; s__metagenome | 0364 |
| 3 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_1 | 0.351 |
| 4 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_sp. | 0.339 |
| 5 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.334 |
| 6 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__metagenome | 0.331 |
| 7 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_2 | 0.328 |
| 8 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_3 | 0.328 |
| 9 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.326 |
| 10 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_4 | 0.326 |
| 11 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Shigella_dysenteriae | 0.323 |
| 12 | f__Sutterellaceae; g__Parasutterella; OTU_1 | 0.318 |

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| | | |
|---|---|---|
| 13 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.314 |
| 14 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.311 |
| 15 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_5 | 0.306 |
| 16 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Cronobacter_sp. | 0.305 |
| 17 | f__Ruminococcaceae; g__Faecalibacterium; OTU_1 | 0.304 |
| 18 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_6 | 0.301 |
| 19 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.298 |
| 20 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_7 | 0.295 |
| 21 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_8 | 0.294 |
| 22 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_9 | 0.293 |
| 23 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_10 | 0.292 |
| 24 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_11 | 0.292 |
| 25 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.291 |
| 26 | f__Enterobacteriaceae; g__Enterobacter; s__Enterobacter_sp. | 0.291 |
| 27 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_12 | 0.291 |
| 28 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.290 |
| 29 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_13 | 0.287 |
| 30 | f__Muribaculaceae; g__Muribaculaceae; OTU_1 | 0.286 |
| 31 | f__Streptococcaceae; g__Lactococcus; s__Leuconostoc_pseudomesenteroides | 0.286 |
| 32 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_14 | 0.286 |
| 33 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.285 |
| 34 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__bacterium_enrichment | 0.285 |
| 35 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.284 |
| 36 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__bacterium_B7(2014) | 0.282 |
| 37 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.281 |
| 38 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_15 | 0.280 |
| 39 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_16 | 0.280 |
| 40 | f__Lactobacillaceae; g__Lactobacillus; OTU_17 | 0.279 |
| 41 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_18 | 0.278 |
| 42 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.275 |
| 43 | f__Streptococcaceae; g__Lactococcus; s__Lactococcus_garvieae | 0.274 |
| 44 | f__Streptococcaceae; g__Lactococcus; OTU_1 | 0.273 |
| 45 | f__Eggerthellaceae; g__Senegalimassilia; s__Senegalimassilia_anaerobia | 0.273 |
| 46 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_19 | 0273 |
| 47 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_20 | 0.272 |
| 48 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_21 | 0.271 |

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| | | |
|---|---|---|
| 49 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_22 | 0.271 |
| 50 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_23 | 0.269 |
| 51 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_24 | 0.269 |
| 52 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Oryza_sativa | 0.268 |
| 53 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.268 |
| 54 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.265 |
| 55 | f__Streptococcaceae; g__Lactococcus; OTU_2 | 0.264 |
| 56 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.262 |
| 57 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.260 |
| 58 | f__Enterococcaceae; g__Enterococcus; s__Enterococcus_faecalis | 0.259 |
| 59 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__uncultured_Enterobacteriaceae | 0.259 |
| 60 | f__Bacteroidaceae; g__Bacteroides; OTU_1 | 0.259 |
| 61 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.259 |
| 62 | f__Streptococcaceae; g__Lactococcus; OTU_3 | 0.258 |
| 63 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.258 |
| 64 | f__Muribaculaceae; g__Muribaculaceae; OTU_2 | 0.258 |
| 65 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__bacterium_28W121 | 0.257 |
| 66 | f__Clostridiaceae; g__Clostridium_sensu_stricto_1; OTU_1 | 0.257 |
| 67 | f__Lachnospiraceae; g__Blautia; OTU_1 | 0256 |
| 68 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_25 | 0.256 |
| 69 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_fergusonii | 0.256 |
| 70 | f__Lachnospiraceae; g__Blautia; OTU_2 | 0.255 |
| 71 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.255 |
| 72 | f__Bacteroidaceae; g__Bacteroides; OTU_2 | 0.254 |
| 73 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_26 | 0.254 |
| 74 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_27 | 0.252 |
| 75 | f__Lachnospiraceae; g__Blautia; OTU_3 | 0.251 |
| 76 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_28 | 0.251 |
| 77 | f__Oscillospiraceae; g__Colidextribacter; OTU_1 | 0.250 |
| 78 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_29 | 0.249 |
| 79 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Shigella_flexneri | 0.249 |
| 80 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.249 |
| 81 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU30 | 0.248 |
| 82 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.248 |
| 83 | f__Christensenellaceae; g__Christensenellaceae_R-7_group; OTU_1 | 0.248 |
| 84 | f__Streptococcaceae; g__Streptococcus; OTU_1 | 0.247 |

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| | | |
|---|---|---|
| 85 | f__Lachnospiraceae; g__CAG-56; OTU_1 | 0.247 |
| 86 | f__Lachnospiraceae; g__Blautia; OTU_4 | 0.247 |
| 87 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__uncultured_Klebsiella | 0.247 |
| 88 | f__Lachnospiraceae; g__[Ruminococcus]_torques_group; OTU_1 | 0.247 |
| 89 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_sp. | 0.246 |
| 90 | f__Streptococcaceae; g__Lactococcus; OTU_1 | 0.246 |
| 91 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_31 | 0.246 |
| 92 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.245 |
| 93 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_32 | 0.244 |
| 94 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.244 |
| 95 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_33 | 0.244 |
| 96 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_34 | 0.244 |
| 97 | f__Muribaculaceae; g__Muribaculaceae; OTU_1 | 0.243 |
| 98 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_35 | 0.243 |
| 99 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_36 | 0.242 |
| 100 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_37 | 0.242 |
| 101 | f__Bacteroidaceae; g__Bacteroides; OTU_3 | 0.240 |
| 102 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_38 | 0.240 |
| 103 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.240 |
| 104 | f__Chloroplast; g__Chloroplast; s__uncultured_Oscillatoria | 0.239 |
| 105 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_39 | 0.239 |
| 106 | f__Ruminococcaceae; g__Faecalibacterium; OTU_1 | 0.239 |
| 107 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_40 | 0.239 |
| 108 | f__Lachnospiraceae; g__Blautia; OTU_4 | 0.238 |
| 109 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_41 | 0.238 |
| 110 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Shigella_boydii | 0.238 |
| 111 | f__Lachnospiraceae; g__Fusicatenibacter; OTU_1 | 0.237 |
| 112 | f__Lachnospiraceae; g__Coprococcus; OTU_1 | 0.237 |
| 113 | f__Streptococcaceae; g__Lactococcus; OTU_2 | 0.236 |
| 114 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.236 |
| 115 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.236 |
| 116 | f__Oscillospiraceae; g__UCG-005; OTU_1 | 0.235 |
| 117 | f__Ruminococcaceae; g__Ruminococcus; OTU_1 | 0.235 |
| 118 | f__Lactobacillaceae; g__Lactobacillus; s__Lactobacillus_oris | 0.235 |
| 119 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Shigella_dysenteriae | 0.234 |
| 120 | f__Tannerellaceae; g__Parabacteroides; s__Parabacteroides_gordonii | 0.234 |

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| | | |
|---|---|---|
| 121 | f__Bacteroidaceae; g__Bacteroides; OTU_4 | 0.233 |
| 122 | f__Lachnospiraceae; g__Anaerostipes; OTU_1 | 0.233 |
| 123 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.233 |
| 124 | f__Clostridiaceae; g__Clostridium_sensu_stricto_1; OTU_1 | 0.232 |
| 125 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_42 | 0.231 |
| 126 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.231 |
| 127 | f__Eggerthellaceae; g__Enterorhabdus; OTU_1 | 0.231 |
| 128 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_43 | 0.231 |
| 129 | f__Ruminococcaceae; g__Faecalibacterium; OTU_2 | 0.231 |
| 130 | f__Enterobacteriaceae; g__Klebsiella; s__Klebsiella_sp. | 0.230 |
| 131 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.230 |
| 132 | f__Enterococcaceae; g__Enterococcus; s__Enterococcus_faecalis | 0.230 |
| 133 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.230 |
| 134 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_43 | 0.229 |
| 135 | f__Ruminococcaceae; g__Faecalibacterium; OTU_3 | 0.229 |
| 136 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Escherichia_coli | 0.229 |
| 137 | f__Lachnospiraceae; g__Blautia; OTU_5 | 0.228 |
| 138 | f__Enterobacteriaceae; g__Escherichia-Shigella; s__Citrobacter_freundii | 0.228 |
| 139 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_44 | 0.228 |
| 140 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_45 | 0.228 |
| 141 | f__Streptococcaceae; g__Lactococcus; OTU_2 | 0.228 |
| 142 | f__Enterobacteriaceae; g__Salmonella; s__Salmonella_enterica | 0.228 |
| 143 | f__Prevotellaceae; g__Prevotella; OTU_1 | 0.228 |
| 144 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_46 | 0.227 |
| 145 | f__Enterococcaceae; g__Enterococcus; s__Enterococcus_faecalis | 0.227 |
| 146 | f__Lachnospiraceae; g__Lachnospiraceae_UCG-004; OTU_1 | 0.226 |
| 147 | f__Enterococcaceae; g__Enterococcus; s__Enterococcus_faecalis | 0.226 |
| 148 | f__Enterobacteriaceae; g__Enterobacter; s__Enterobacter_sp. | 0.226 |
| 149 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_46 | 0.226 |
| 150 | f__Lachnospiraceae; g__OTU_1; OTU_1 | 0.226 |
| 151 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_47 | 0.226 |
| 152 | f__Enterobacteriaceae; g__Escherichia-Shigella; OTU_48 | 0.225 |
| 153 | f__Bacteroidaceae; g__Bacteroides; OTU_5 | 0.225 |
| 154 | f__Bacteroidaceae; g__Bacteroides; OTU_6 | 0.225 |

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The analysis conducted resulted in no statistically significant correlations between fecal indole-3-lactate and the presence of *Klebsiella, Pseudomonas, Escherichia-Shigella* in intestines in healthy donors. Apparently, in obesity fecal indole-3-lactate is produced predominantly by *Escherichia-Shigella.* According to published data, obesity is associated with the taxonomic impoverishment of gut microbiota [23]. 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 gut microbiota are transformed in obesity: the number of indole-3-lactate producing species increases three-fold. 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 [23] by means of diet, various signaling molecules, and cytokines. We propose that the enrichment of gut microbiota with the indole-3-lactate producing bacteria occurs possibly as a compensatory mechanism to suppress the synthesis of proinflammatory cytokines which frequently accompanies patients with obesity. For it is known that indole-3-lactate has a pronounced anti-inflammatory action, as it participates in immunoregulatory T-cells induction and proinflammatory T-cells suppression [41]. 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 [40]. In the present work we managed to demonstrate that the concentration of fecal indole-3-lactate in patients with obesity primarily depends on taxonomic representation of *Escherichia-Shigella.*

**Conclusion**

1. *We identify indole and indole-3-lactate as major tryptophan metabolites in both patients with obesity and healthy donors.*
2. *In obesity fecal concentrations of the following metabolites are significantly decreased: indole-3-lactate, quinolinic acid, 5-hydroxyindole-3-acetic acid, anthranilic acid, xanthurenic acid, indole-3-carboaldehyde, indole-3-acrylate, and indole-3-propionate. At that the most dramatic fall is demonstrated particularly for indole-3-lactate.*
3. *We note the more pronounced coupling of MICT and the microbial «enzymatic landscape» in patients with obesity.*
4. *The «enzymatic landscape» of healthy donors depends on the concentrations of indole-3-acrylate and anthranilic acid, while other MICT (indole, quinolinic acid, and, to a greater extent, kynurenic acid and indole-3-lactate) define the gut microbiota «enzymatic landscape» in obesity.*
5. *More tight interrelations between the microbial «enzymatic landscape» and MICT are denoted exactly for indole-3-lactate.*

6. *Fecal indole-3-lactate concentration is shown to correlate with the «enzymatic landscape» of carbohydrate, nucleotides, amino acids, and sulfosugars metabolism.*

7. *In patients with obesity there occurs the pronounced enrichment of gut microbiome with bacteria producing indole-3-lactate*

**Ethic Committee Name:** The Local Public Ethics Committee of the Pirogov Russian National Research Medical University of the Ministry of Health of Russia. **Approval Code:** № 186. **Approval Date:** 26 of June 2019 y. **Informed Consent Statement:** all hospital subscriptions are voluntary consent to the use of biological material for scientific purposes.

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