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**CHANGES IN THE MICROBIOME OF A HUMAN AND  
IN THE SIMULATOR OF HUMAN INTESTINAL MICROBIAL  
ECOSYSTEM (SHIME®) IN RESPONSE TO A DIET  
AND PROBIOTIC SUPPLEMENTATION**

S u m m a r y

**Background.** The Simulator of Human Intestinal Microbial Ecosystem (SHIME®) is used to study the behavior of a human microbiome in response to various factors. The aim of this study was to simultaneously demonstrate changes in the microbiome of a human volunteer and in the SHIME® system in response to a change in a diet and probiotic therapy due to a scarcity of published research with similar design.

**Results and conclusions.** The probiotic therapy resulted in a decrease in fasting insulin and glucose (by 18 % and 13 %, respectively), while the increased fiber intake in the experimental diet seemed to reduce triglyceride, total and LDL cholesterol levels (by 27 %, 15 % and 14 %, respectively) in the volunteer's blood. Due to the improvement of the volunteer's metabolic status, changes in her microbiome were observed. Namely, the improvement of glucose homeostasis led to the occurrence of bacteria of the genus *Akkermansia* in the feces, while the improvement of lipid homeostasis resulted in an increase in the abun-

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dance of bacteria of the genus *Bacteroides* and *Bifidobacterium* (approx. two and four times, respectively). While changes in the microbiome diversity in the SHIME® luminal fluid (L-SHIME) and in the faeces microbiome were partially similar, the microbiome of SHIME's intestinal wall (M-SHIME) behaved differently. The characteristic feature of both L- and M-SHIME was a microbiome that differed in its composition compared to the volunteer's microbiome at parallel sampling points. The results of this study indicate that adjustments to the standard SHIME® experimental protocol should be made to enable the replication of the human microbiome diversity and its changes in the system.

**Key words:** microbiome, validation, artificial digestive tract, SHIME, probiotics, food

## Introduction

The Simulator of Human Intestinal Microbial Ecosystem (SHIME®, ProDigest, Belgium) is an artificial digestive system used increasingly in scientific research to determine changes in the gut microbiome in response to various factors, including, for example, dietary supplements or probiotic preparations [29]. Although the number of investigations applying SHIME® increases, not much is known about how microbiome changes in SHIME® follow microbiome changes in humans.

There are several studies that refer to the validation of the SHIME® system. For example, in one of the recent studies, Duysburgh and colleagues [6] demonstrated that a novel oat ingredient was able to increase the abundance of *Lactobacillaceae* and *Bifidobacteriaceae* in SHIME® in both, simulated intestinal lumen (L-SHIME) and mucosal layer (M-SHIME). In addition, an increase in the short-chain fatty acids (SCFAs) production was observed [6]. Following the SHIME® experiment, the authors also performed a human trial in which they confirmed that the tested substance was able to increase the abundance of *Lactobacilli*.

In another study, the potential of a microbiome from two distinct human metabolotypes to produce urolithin from ellagitannins and ellagic acid contained in a pomegranate extract supplement was demonstrated in SHIME® [9]. The authors validated findings through a discussion with *in vivo* studies, in which these metabolites were reported in humans and animals.

One of the most broadly cited and pioneering studies on the subject investigated the fate of the ulcerative colitis treatment substance, pro-drug sulphasalazine along with enzyme activities and fermentation of polysaccharides in SHIME® [18]. The authors compared the microbial activity of the fecal microbiome in fresh fecal samples taken from human volunteers and from SHIME®. They found that the fermentation patterns of pectins, arabinogalactan and xylan, were not significantly different in terms of SCFAs production between microbiome from the respective SHIME® compartments and fecal samples, and the only difference was reported for the fermentation of starch. Also, enzyme activities were not significantly different between the fecal material and samples from SHIME®. The fate of sulphasalazine was in agreement with the

literature findings of *in vivo* studies. An earlier work of the same group of authors leading to the development of SHIME® demonstrated that the obtained logarithmic counts of various groups of microorganisms (total anaerobes, total aerobes, fecal streptococci, *Enterobacteriaceae* and *Lactobacillus* spp.) cultured in SHIME® were consistent with the reported findings of *in vivo* studies [17].

Critical for the development of the system and its validation was work by Possemiers et al. (2004), in which the authors demonstrated the length of the stabilization period for the microbial community in SHIME®. By means of a polymerase chain reaction coupled with denaturing gradient gel electrophoresis (PCR-DGGE) and a moving window correlation analysis, the authors demonstrated that the system achieved stability in terms of the composition of the microbial community (overall bacteria, *Lactobacillus* sp. and *Bacteroides/Prevotella*) after 12 days [24]. Nevertheless, the functional stability, evaluated based on the concentration of SCFAs was reached after 17 days.

In all current works, therefore a period of two weeks is respected as the time required for the stabilization of the microbial community in the SHIME® system. The majority of publications, in which the focus is on microbial metabolites, also add another week or two to evaluate the stability of the microbial community and reach the functional stability of the system. During the stabilization period, a standard protocol applied through the literature uses a standard SHIME® medium composed of undigested, complex carbohydrates (pectin, arabinogalactan, xylan and starch), protein, minerals and vitamins (yeast extract and special peptone), glucose, mucin and l-cysteine [25]. This medium simulates food residues that reach the colon after digestion and absorption of nutrients in the small intestine. Interestingly, its composition differs very little among studies and it is modified in relation to the content of nutrients which are replaced by active substances researched in a particular study. The most commonly replaced nutrients are starch (for studying the effects of complex carbohydrates) and glucose (for studying fermentable sugars) e.g. [16, 21]. While the use of a standard SHIME® feed medium helps to reproduce experimental conditions across the studies, it is not clear how it may influence the initial microbiome, which was supplied with the fecal inoculum to the system. It could be assumed that the shape of the microbial community of the inoculum should be preserved, since the system was validated in the multiple studies mentioned above, however, this element was not discussed by the authors. In fact, if as recommended, SHIME® is inoculated with a fecal sample from a single human donor [25], then the expectation that the standard SHIME® medium will mimic residues from the diet of this particular donor is not plausible. It is with no doubt that the failure to match the residues of the diet of the donor with the nutritional SHIME® medium may affect the composition of the microbiome in the SHIME® sys-

tem. To what extent and whether this change may affect the fate of the microbiome in response to researched factors remains to be answered.

The aim of this study was to evaluate the effect of the modification of the diet and the introduction of probiotic treatment on the composition of the microbiome in SHIME® and human volunteer. A novel element of this study, apart from an attempt to modify the standard SHIME® feed medium to match the diet of the volunteer, was also the design of the parallel sampling of fecal microbiome from human and distal colonic microbiome from SHIME®. So far, the majority of the validation studies for SHIME® have supported findings with time-separated human trials or data available in the literature.

In addition, the metabolic health data for the volunteer was also reported and evaluated in this work, as these factors are known to influence changes in the microbiome and vice-versa.

## Materials and methods

### *The human study*

The volunteer was a 39-year-old female with no diagnosed diseases and no antibiotic treatment for up to 12 months prior to the commencement of the study. The volunteer supplied researchers with a record of dietary intake for 28 days prior to the experiment. These records were collected using the Fitatu mobile application (Poznań, Polska), where the quality and portion size of all consumed products were included.

The experiment lasted 28 days. During the whole experiment, the volunteer received a dietary intervention-nutritionally balanced standard diet covering needs for all macronutrients and energy, which was supplied by a professional dietary catering service (Dietering.com®, Stara Wieś, Poland). The diet of the volunteer, 28 days prior to and during the whole experiment, was deprived of fermented foods containing live bacterial cultures.

During the first 14 days of the experiment, the volunteer received also a probiotic *Lactobacillus rhamnosus* GG provided with a commercially available supplement twice a day, with each portion containing  $6 \times 10^9$  bacterial cells. The volunteer was fully aware of the experimental design and subjected herself to blood testing three times (the beginning, the middle-end of the probiotic therapy and the end of the experiment) using commercial laboratory services (ALAB, Legionowo, Polska). The blood test results allowed to assess the metabolic health and nutritional status of the volunteer. The tests included fasting glucose and insulin levels, lipid profile, the status of Fe, Ca, Mg, vitamins D and B12, as well as blood morphology.

In addition, the volunteer was subjected to a body composition analysis by means of bioimpedance (Tanita MC-780, Amsterdam, the Netherlands) at the time points of blood testing.

The volunteer supplied fresh fecal sample for the inoculation of the SHIME® system and thereafter 11 additional samples during the study, as outlined in the sampling section.

The nutritional value of meals was calculated based on DietetykPro online service (Wrocław, Polska). For commercially available, processed foods, data from the Fitatu application was obtained, as this service contained records of nutritional labels. The experimental diet, with a balanced content of nutrients, was designed by a dietitian by means of a system for managing dietary catering and the dietary module for creating menus MasterLife CRM (Warsaw, Poland).

The nutritional intake of the volunteer with experimental and routine diet was compared by means of an independent sample t-test. The analysis was carried out by means of Excel 2013 (Microsoft, Washington, USA) and assumed a significance level  $\alpha = 0.05$ .

The study design, prior to its commencement, was approved by the Research Ethics Committee at Jan Dlugosz University in Czestochowa (decision KE-U/12/2022).

#### *The Simulator of Human Microbial Intestinal Ecosystem (SHIME®)*

An instrumental model SHIME® (ProDigest, Gent, Belgium) was applied in the study. The study was run in a Multi-SHIME setup, which was focused on the distal colon. The nutritional medium in this design was sequentially flowing through three separated digestive compartments 1. Stomach/duodenum joint vessels (2 pieces), 2. Proximal colon bioreactors (2 pieces) and 3. Distal colon bioreactors (6 pieces, three bioreactors connected to a single proximal colon). In three distal colon bioreactors, L-SHIME and another three M-SHIME setups were simulated.

The procedure of setting up the instrument was based on the manufacturer's manual [25] and mimicked closely procedures in available literature e.g. [14]. Briefly, the instrument was inoculated with fecal microbiome prepared by suspending fresh fecal sample in phosphate buffer (1:5 ratio), homogenization and centrifugation for two minutes at 500 x g. Then, the supernatant was injected into the instrumental compartments filled with a standard nutritional medium (proximal and distal colon) and left overnight to stabilize during the start-up phase. Afterwards, a two-week stabilization period, in which the standard nutritional medium (210 cm<sup>3</sup> per one stomach compartment, see Table 1 for composition) and pancreatic liquid (90 cm<sup>3</sup> per one stomach compartment) were dozed three times a day to the system, was followed.

During the experiment, the proportion of the nutrients in the standard nutritional medium was changed to mimic the residues that could reach the colon of the volunteer.

The calculation was based on data from one of the most recent studies on the nutritional content of ileostomies from humans fed with a diet of known nutritional value [12]. To estimate the content of nutritional residues, the content of nutrients consumed with the volunteer's diet was multiplied by the percentage fraction of expected residue (Table 1) and normalized to the content of total fiber residue in the standard medium. The nutritional content of the experimental medium was given in Table 1.

The same probiotic supplement as the one used for the human study was applied directly to the stomach of SHIME® twice a day, at time points at which feeding the vessel with the experimental nutritional medium ceased. Samples were taken from six distal colon compartments of the system as soon as the volunteer supplied a fecal sample for analysis.

Table 1. The nutritional content of the standard and experimental SHIME feed media and the fractions of nutrients reaching the colon based on [12]

Tabela 1. Zawartość składników odżywczych w standardowej i eksperymentalnej pożywce SHIME, a także frakcje składników odżywczych docierających do okrężnicy według [12]

Component / Składnik	Standard feed Pożywka standardowa (g/dm <sup>3</sup> )	Experimental feed mean ± s.d. Pożywka eksperymentalna średnia ± o.s. (g/dm <sup>3</sup> )	Fraction reaching the colon from the dietary intake Frakcja docierająca do okrężnicy z pożywienia (%)
Arabinogalactan / Arabinogalaktan	1.2	1.8 ± 0.8	83 (fiber / błonnik)
Pectin / Pektyny	2	3.5 ± 0.9	
Xylan / Ksylan	0.5	0.7 ± 0.3	
Starch / Skrobia	4	2.3 ± 1.0	
Glucose / Glukoza	0.4	0.3 ± 0.1	1.1 (sugars / cukry)
Yeast extract / Ekstrakt drożdżowy	3	5.5 ± 1.5	16.0 (protein / białko)
Special pepton / Pepton specjalny	1	3.5 ± 2.1	
L-cystein-HCl / L-cysteiny HCl	0.5	0.5	Substances present in the intestines Substancje obecne w jelitach
Mucin / Mucyna	3 (L-SHIME), 2 (M-SHIME)	3 (L-SHIME), 2 (M-SHIME)	

### Sampling

The sampling of feces, M-SHIME mucosa and L-SHIME liquid was carried out 11 times during the experimental period on days outlined in Fig. 1. In addition, a sample of inoculum-feces used to populate the SHIME® system with human intestinal microbiome was acquired. Sampling for the analysis of the microbiome composition was carried out in three replicates.

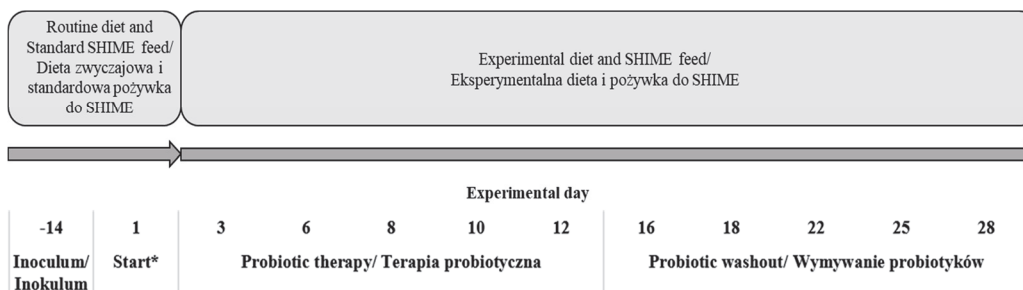


Fig. 1. The sampling scheme and experimental design

Rys. 1. Próbkobranie oraz projekt eksperymentu

### Bacterial DNA Extraction and metabarcoding

Total genomic DNA was extracted using Genomic Mini AX Stool (mod.1) (cat. no. 065-60-M1; A & A Biotechnology, Poland) and purified with Anty-Inhibitor Kit (cat. no. 1015-50; A & A Biotechnology, Poland) according to the manufacturer's suggestions. Briefly, up to 100 mg of feces, microbial pellet from 2 cm<sup>3</sup> of L-SHIME content (centrifuged at 5,000 x g for 10 min) and 200 mg of mucin taken from M-SHIME beads was subjected to extraction and purification using the same kit. The quality of each DNA extract was confirmed spectrophotometrically and by gel electrophoresis. Triplicates of extracted DNA were pooled prior to sequencing.

Bacterial relative abundance and taxonomic diversity identified in the samples were revealed using high throughput sequencing (HTS) of 16S rRNA gene amplicons. For amplification of the V3-V4 regions of 16S rDNA, the following primers were applied: 341F 5'-CCTACGGGNGGCWGCAG-3' and 785R 5'-GACTACHVGGGTATCTAATCC-3. 34 amplicons (12 samples of stool, 11 of L-SHIME and 11 of M-SHIME) were prepared using high-fidelity KAPA HiFi DNA Polymerase (Roche, Basel, Switzerland). Amplicons were sequenced on an Illumina MiSeq platform (Illumina, California, USA) by the Genomed company (Warsaw, Poland) using a v3 MiSeq chemistry kit in the paired-end mode (read lengths 2 x 250 bp).

The quality estimation of sequences was performed using FastQC software [1]. Qiime2 [2] with dada2 pipeline and taxonomic assignment based on Naive Bayes classifier trained on Silva database v. 138, as downloaded in April 2022, was used to assign accepted 16S rDNA sequences. Taxonomic profiles were used to characterize the microbiome community of the studied samples.

## Results and discussion

### *Nutritional intake and metabolic and nutritional status of the human volunteer*

The nutritional intake of the volunteer before and during the experiment was shown in Fig. 2 and her metabolic and nutritional status was summarized in Table 2.

The intake of protein, fiber, cholesterol and energy was significantly greater with the experimental diet compared to the routine diet of the volunteer ( $p < 0.05$ ). On the other hand, the intake of remaining macronutrients (carbohydrates, sugars and fats) did not significantly differ between the diets ( $p > 0.05$ ).

Compared with dietary reference values for the European population and women aged 30 ÷ 39 [7], both diets provided adequate amounts of fat (on average 30 and 34 % for experimental and routine diets, respectively, with reference intake 20 ÷ 35 %) and carbohydrates (on average 47 and 50 % for experimental and routine diet, respectively, reference intake 45 ÷ 60 %). Both diets also covered on average the requirement of the volunteer for protein, exceeding the population reference intake of 0.83 g/kg or body weight by 50 % for the experimental diet and 1 % for the routine diet. On the other hand, the intake of fiber exceeded the adequate intake value of 25 g per day only in the experimental diet (average 38 g per day) and was much lower in the routine diet (average 14 g per day). The intake of energy was slightly lower in the routine and experimental diets (on average 1,647 and 1,771 kcal/day, respectively) compared to the average requirement for women with low physical activity (1,811 kcal/day) and could be termed as sufficient, since the volunteer's body weight remained quite stable prior to and during the experiment. On the other hand, the mean intake of cholesterol in both diets could be considered too high in view of the recommendations of the Polish Diabetes Society, where levels  $< 300$  mg/day are recommended for the general population and  $< 200$  mg/day for dyslipidaemia [23].

While in the experimental diet, the intake of cholesterol was on average at 437 mg/day, in the routine diet it was only 244 mg/day, however, considering that the levels of total cholesterol in the volunteer's blood were elevated (240 mg/100 cm<sup>3</sup> compared to recommended  $< 190$  mg/100 cm<sup>3</sup> [19]), then an intake of  $< 200$  mg/day could be referred to. It should be noted, however, that not all experts agree with the recommendations for cholesterol intake and some of them even state that "Cholesterol is not a nutrient of concern for overconsumption." [5]. The results of this study support the above-mentioned statement, since despite an increased cholesterol intake with the experimental diet the level of cholesterol in the participant's blood did not increase through the experiment.

In terms of the metabolic health and nutritional status of the volunteer (Table 2), values for total cholesterol, LDL cholesterol and body fat % were considered too high compared to the accepted norms [19, 28], while the level of vitamin D too low



throughout the entire experiment [3]. Other parameters, including body weight (with the volunteer's height of 164 cm) were considered normal.

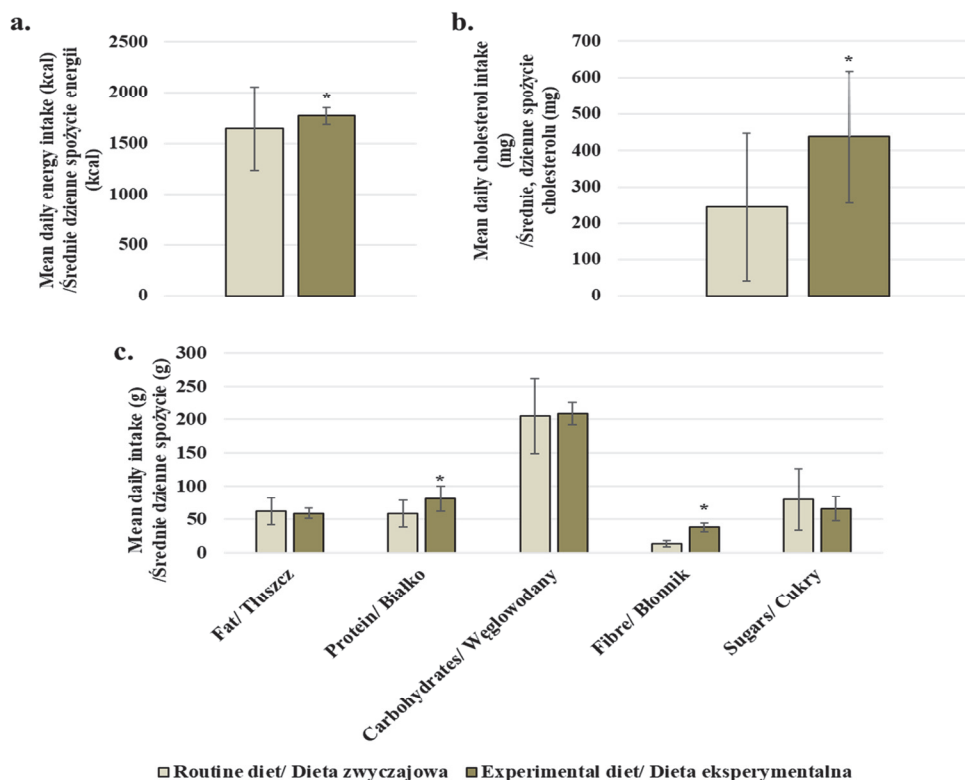


Fig. 2. Energy value (a.), intake of cholesterol (b.) and macronutrients (c.) with experimental and routine diet. \*denotes a statistically significant difference in nutrient or energy intake between the experimental and routine diet (t-test,  $p < 0.05$ ).

Rys. 2. Wartość energetyczna (a.), spożycie cholesterolu (b.) oraz makroskładników pokarmowych (c.) z diety eksperymentalnej i zwyczajowej. \*oznacza statystycznie istotną różnicę w spożyciu składnika pokarmowego lub energii pomiędzy dietą eksperymentalną a zwyczajową (test-t,  $p < 0,05$ ).

Despite no replication for blood testing and body analysis on test days, there were some differences in the values of several studied parameters that exceeded 10% between test days, hence could not be explained by the measurement uncertainty alone, and were termed considerable. A considerable decrease in fasting blood glucose and insulin levels was noted after two weeks of probiotic therapy, while at the end of the experiment, the levels of total, LDL and HDL cholesterol fell down along with the levels of triglycerides and levels of body fat. A decrease in the blood iron concentration through the experiment was also noted. Overall, all these changes, apart from the

decrease in iron concentration, were positive and indicated that the probiotic therapy could have improved the tolerance of glucose in the volunteer, while the diet contributed mainly to improved lipid homeostasis.

Table 2. The metabolic and nutritional status of the volunteer during the experiment  
Tabela 2. Stan metaboliczny i odżywienie ochotnika w czasie trwania eksperymentu

Parameter/ Parametr	Before the experiment Przed eksperymentem	Last day of the probiotic treatment Ostatni dzień probiotykoterapii	Last day of the experiment Ostatni dzień eksperymentu
Total Cholesterol / Cholesterol całkowity (mg/100 cm <sup>3</sup> )	240	234	205
Cholesterol HDL (mg/100 cm <sup>3</sup> )	63	61	55
Cholesterol LDL (mg/100 cm <sup>3</sup> )	159	153	137
Triglycerides / Trójglicerydy (mg/100 cm <sup>3</sup> )	90	100	66
Fasting insulin / Insulina na czczo (mU/dm <sup>3</sup> )	4.82	3.93	4.87
Fasting glucose / Glukoza na czczo (mg/100 cm <sup>3</sup> )	97	84	90
Iron / Żelazo (ug/100 cm <sup>3</sup> )	113	79	65
Calcium / Wapń (mg/100 cm <sup>3</sup> )	9.7	9.8	9.2
Magnesium / Magnez (mg/100 cm <sup>3</sup> )	2.2	2.11	2.14
Vitamin / Witamina B12 (pg/cm <sup>3</sup> )	427	375	389
Vitamin / Witamina D (ng/cm <sup>3</sup> )	23	23	23.9
Body weight / Masa ciała (kg)	65.9	65.9	65.4
Fat weight / Masa tłuszczu (kg)	21.2	21.6	19.1

Some studies confirm the beneficial influence of *Lactobacillus rhamnosus* GG on glucose homeostasis. While in human studies authors applied this probiotic along with *Bifidobacterium animalis* spp. *lactis* BB-12 and found it effective in the improvement of insulin sensitivity in pregnant women [8], there are many animal studies showing that *Lactobacillus rhamnosus* GG alone aids the metabolism of sugars in animals [20].

The improvement of lipid homeostasis and a decrease in body fat of the volunteer after 28 days of the experiment was probably attributed to the increased intake of fiber with the experimental diet compared to the routine diet. The limitation of the bioaccessibility of nutrients through the intake of dietary fiber is a known fact. Nevertheless, in relation to cholesterol, a recent meta-analysis of observational studies on the development of cardiovascular diseases (CVD) and controlled trials, where a fiber intake was increased in people with CVD and hypertension, found only weak evidence confirming this hypothesis [26]. On the other hand, not only quantity, but also the quality of the fiber could be an important factor to be considered while reviewing similar studies. Old, but convincing evidence comes from the study on ileostomised humans, in which

Table 3. Expected changes in the abundance of specific intestinal microbial taxa associated with human metabolism

Tabela 3. Spodziewane zmiany w liczebności specyficznych taksonów drobnoustrojów jelitowych powiązane z metabolizmem człowieka

Microorganism Mikroorganizm	Expected behaviour Oczekiwana reakcja	Observation in the literature Obserwacja w literaturze	Source Źródło
<i>Akkermansia</i> in particular <i>Akkermansia muciniphila</i>	Increase Wzrost	Inversely correlated with impaired glucose homeostasis, it increases with a decrease in cholesterol levels. Odwrótnie skorelowany z zaburzoną homeostazą glukozy, wzrasta wraz ze spadkiem poziomu cholesterolu.	[11, 13, 15]
<i>Bacteroides</i>	Increase Wzrost	Linked to the Western diet, higher in people consuming high amounts of animal protein and fat, it increases with an increased fiber intake. / Związany z dietą zachodnią, wyższy u osób spożywających duże ilości białka i tłuszczu zwierzęcego, wzrasta wraz ze wzrostem spożycia błonnika.	[4, 11, 15, 31]
<i>Bifidobacterium</i>	Increase Wzrost	It may increase due to a probiotic therapy, inversely correlated with type 2 diabetes, and negative relationship with the level of visceral fat, levels of LDL cholesterol and triglycerides. / Może wzrosnąć w związku z terapią probiotykami, odwrótnie skorelowaną z cukrzycą typu 2 oraz ujemnym związkiem z poziomem trzewnej tkanki tłuszczowej, poziomem cholesterolu LDL i trójglicerydów.	[10, 11, 15]
<i>Blautia</i>	Increase Wzrost	Lower levels in humans with type 2 diabetes. / Niższe poziomy u ludzi z cukrzycą typu 2.	[15]
<i>Christensenellaceae</i>	Increase Wzrost	Negatively correlated with cholesterol levels in children. Ujemnie skorelowany z poziomem cholesterolu u dzieci.	[13]
<i>Enterobacteriaceae</i>	Decrease Spadek	Higher abundance in metabolic diseases. / Większa obfitość w chorobach metabolicznych.	[15]
<i>Eubacterium</i>	Increase Wzrost	Potential probiotic, it may decrease cholesterol levels, metabolizes plant polysaccharides. / Potencjalny probiotyk, może obniżać poziom cholesterolu, metabolizuje roślinne polisacharydy.	[4, 13]
<i>Faecalibacterium</i>	Increase Wzrost	Negatively correlated with type 2 diabetes and blood cholesterol levels, increased levels associated with the Mediterranean diet. / Ujemnie skorelowany z cukrzycą typu 2 i poziomem cholesterolu we krwi, podwyższony poziom związany z dietą śródziemnomorską.	[11, 13]
<i>Prevotella</i>	Increase Wzrost	It increases with a high intake of fiber and plant foods. Zwiększa się wraz z wysokim spożyciem błonnika i pokarmów roślinnych.	[4, 15, 31]
<i>Roseburia</i>	Increase Wzrost	Negatively correlated with type 2 diabetes, it metabolizes plant polysaccharides. / Ujemnie skorelowany z cukrzycą typu 2, metabolizuje polisacharydy roślinne.	[4, 11]

it was demonstrated that the addition of pectin from citrus fruits allowed to increase the excretion of fat, but unfortunately, also the absorption of some of the minerals, including iron, was affected [27]. This is in line with our observation, especially, given that a dietary intake of pectin from fruit and vegetables increased the most from among studied fractions of soluble dietary fiber (by 240 %, other fractions being arabinogalactan, xylans and starch, the intake of which changed by -1, -32 and 151 %, respectively) during the experiment.

Positive changes in the metabolism and body composition of the volunteer during the experiment, as mentioned in the literature, should trigger some changes in the composition of the intestinal microbiome. Based on the literature, changes in taxa mentioned in Table 3 were expected.

#### *Microbiome composition of SHIME® and of a human volunteer*

To find out whether the changes in the microbiome of the volunteer followed the improvement in her metabolism during the experiment, data from test days 1 (start of the experiment prior to the probiotic therapy), 12 (the last test day during the probiotic therapy) and 28 (the end of the experiment) was compared. In addition, data from L- and M-SHIME for the same test days was presented to discuss the similarity between the microbiome in SHIME® and feces.

The relative abundance of the dominant bacterial phyla in the microbiome of a human, L- and M-SHIME was presented in Fig. 3.

It can be noticed that the microbiome composition was changing during the experiment, in the volunteer's feces and in the L-SHIME system. In the M-SHIME, the microbiome was much more stable. The stability and the composition of the M-SHIME microbiome were most likely attributed to the affinity of different bacterial strains to the mucosal layer of the intestine, as implied in previous works [30]. However, up to date, there is not much data in the literature regarding the parallel comparison of M-SHIME and the fecal microbiomes which would be similar to that reported in this study.

The relative abundance of the bacterial taxa in volunteer's feces fluctuated over time when looking at the abundance of particular phyla (Fig. 3), whereas in L-SHIME, there was a gradual increase in the relative abundance of *Firmicutes*, as well as a decrease in *Bacteroidetes* and *Actinobacteriota* phyla.

Neither the day 1 L- nor M-SHIME samples was compositionally similar to the microbiome introduced with the inoculum (day -14). On the contrary, the fecal sample from day 1, maintained the proportions of microbial phyla present in the inoculum. The

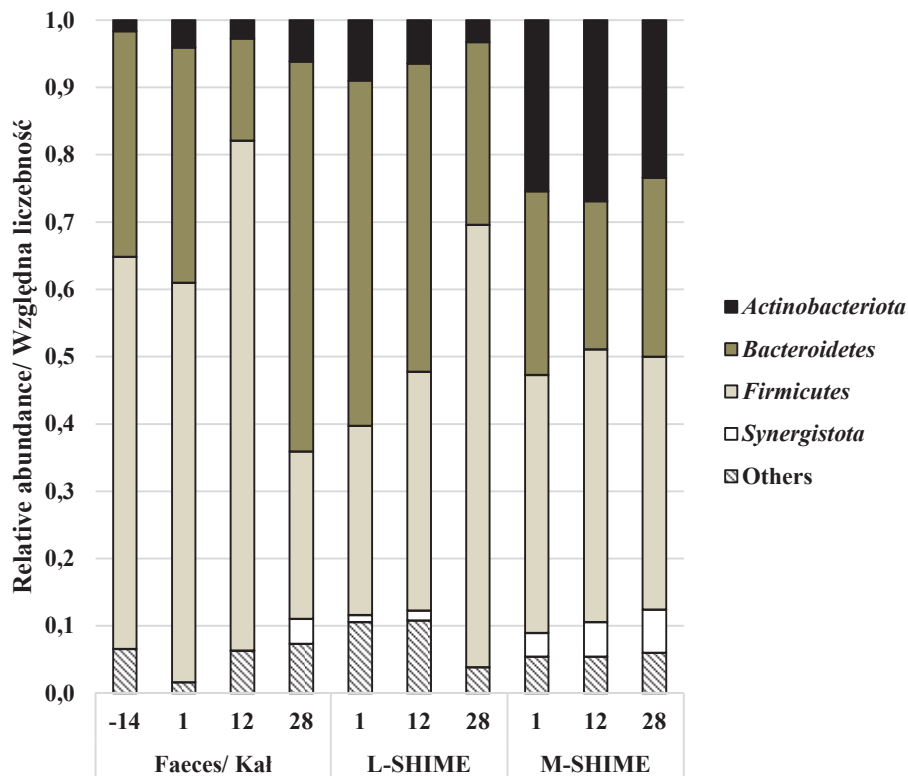


Fig.3. Relative abundance of the dominant microbial phyla in the analyzed samples, others- includes phyla with an abundance of <5%, including unidentified (for each sample <0.1%), L-SHIME (refers to samples taken from SHIME's lumen), M-SHIME (refers to samples taken from SHIME's mucosa)

Rys.3. Względna liczebność dominujących gromad mikroorganizmów w analizowanych próbkach, inne włącza gromady z liczebnością <5%, w tym niezidentyfikowane (których liczebność dla każdej próbki wynosiła <0.1%), L-SHIME (odnosi się do próbek pobranych ze światła jelita SHIME), M-SHIME (odnosi się do próbek pobranych z warstwy mucynowej SHIME)

changes observed in the day 12 and 28 samples could be the result of applied interventions, namely probiotic supplementation (day 12) and diet (day 28). Interestingly, the changes that were observed in L-SHIME led to the final composition of the microbiome (day 28), which was similar to the one that was present in the inoculum. This finding suggests that the fecal microbiome may be compositionally reproduced in L-SHIME. To our best knowledge, the ability of SHIME® to mimic the diversity of the fecal microbiome of the human donor has not been presented so far. One of the reasons why authors in previous studies did not show strong interest in this ability of SHIME® could be the use of fecal cocktails from different donors for SHIME® inoculation instead of single donor samples. One can also speculate that the colonic and

fecal microbiomes could differ considerably, as presumed by authors contributing to the development of SHIME® [24].

Based on the evidence presented in this study, a probable reason for the difficulties with the replication of microbial diversity of fecal microbiome in SHIME® could be the composition of the standard SHIME® media that differed considerably from the dietary residues expected to reach volunteer's colon. The residues of the volunteer's routine diet should have contained proportionally more protein and less soluble fiber compared to the standard SHIME® feed (average protein-to-fiber ratio 1.1 and 0.5, respectively). On the other hand, the average protein-to-fiber ratio during the experiment in dietary residues and SHIME® media was at the level of 0.9. Since the average protein-to-fiber ratio increased in the SHIME® feed and decreased in the dietary residues reaching the colon of the volunteer during the experiment when compared to the period before the experiment, it could be expected that the changes in the microbiome of SHIME® (especially L-SHIME) and feces would be inverse. Considering the day 1 and 28 samples, the expected reaction of the microbiome was achieved. In L-SHIME, a considerable increase of *Firmicutes* and a decrease of *Bacteroidetes* was noted, while in the human volunteer, an exactly contrary change took place. This also means that at the beginning of the experiment the volunteer's microbiome was characteristic of dysbiosis in obesity [22]. However, during the experiment, bacterial diversity in the microbiome of the volunteer changed into one characteristic of healthy adults. In addition to this positive change, the volunteer experienced weight and fat loss during the experiment, which can be a mutual effect accompanying a positive microbiome shift.

Out of the ten metabolically important microbial taxa mentioned in Table 3, six were present in most of the samples. Changes in the abundance of these six metabolically important microbial taxa were shown in Fig. 4. The abundance of four of these taxa was changing in response to probiotic supplementation and diet, together with improving metabolic health of the volunteer, as could be expected based on the literature summarized in Table 3. The relative abundance of:

1. *Akkermansia* increased during the probiotic therapy (when glucose homeostasis improved), but decreased after the probiotic was discontinued (when it slightly decreased again);
2. *Bacteroides* increased due to a high fiber intake with the experimental diet;
3. *Bifidobacterium* increased on day 28 when lipid homeostasis improved;
4. *Enterobacteriaceae* decreased, presumably due to the improvement of the metabolic status of the volunteer.

The patterns of changes in the relative abundance of six microbial taxa outlined in Fig. 4 were partially similar in the microbiomes of feces and L-SHIME, but not in M-SHIME. For example, in both feces and L-SHIME samples, the relative abundance of *Blautia* and *Enterobacteriaceae* decreased with the progress of the experiment. Like-

wise, the levels of *Eubacterium* were the most abundant in both types of samples on day 12. Nevertheless, some differences were also observed. The levels of *Bacteroides* and *Bifidobacterium* decreased with time in L-SHIME, but were the highest at the end of the experiment in feces.

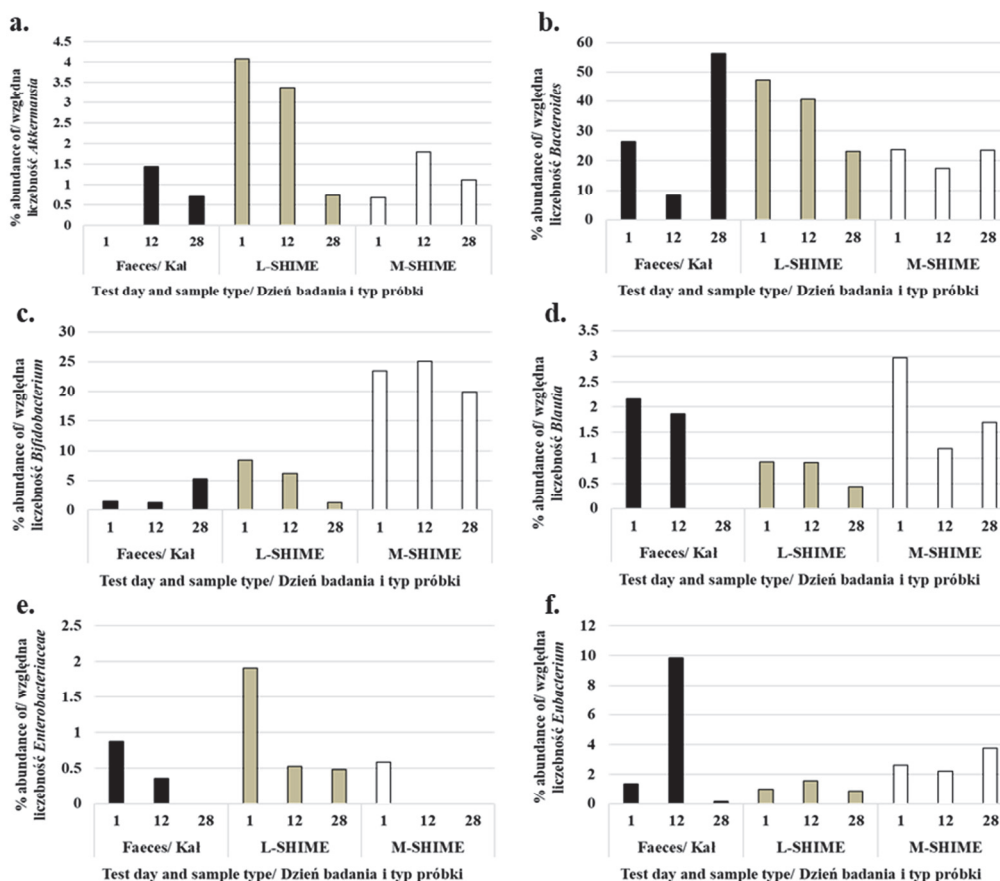


Fig. 4. Relative abundance of metabolically important microbial taxa: *Akkermansia* (a.), *Bacteroides* (b.), *Bifidobacterium* (c.), *Blautia* (d.), *Enterobacteriaceae* (e.), *Eubacterium* (f.). L-SHIME (refers to samples taken from SHIME's lumen), M-SHIME (refers to samples taken from SHIME's mucosa)

Rys 4. Względna liczebność metabolicznie istotnych taksonów mikrobiologicznych: *Akkermansia* (a.), *Bacteroides* (b.), *Bifidobacterium* (c.), *Blautia* (d.), *Enterobacteriaceae* (e.), *Eubacterium* (f.). L-SHIME (odnosi się do próbek pobranych ze światła jelita SHIME), M-SHIME (odnosi się do próbek pobranych z warstwy mucynowej SHIME)

Overall, the bacterial diversity observed in the three types of the samples was different, however, some of the observed changes in the abundance of selected taxa were

in agreement between fecal and L-SHIME microbiomes. The changes found in the diversity of the human microbiome could perhaps be more accurately reproduced in L-SHIME through the optimization of the inoculation and stabilization process prior to the experiment. In addition, more attention should be paid to the SHIME® media composition, because these media may influence the microbial diversity. Perhaps, if dietary residues that reach the human colon would be precisely applied in SHIME® media, inoculated human fecal microbiome would not undergo dramatic changes during the process of stabilization in SHIME®.

### Conclusions

1. The applied probiotic and dietary interventions were successful in improving the metabolic status of the volunteer participating in the research. While the probiotic *Lactobacillus rhamnosus* GG helped to reduce the levels of fasting glucose (by 13 %) and fasting insulin (by 18 %), the fiber-rich dietary intervention allowed to decrease blood levels of triglycerides (by 27 %), as well as total and LDL cholesterol (by 15 and 14 %, respectively). In addition, the applied experimental diet, despite energy increase compared to the routine diet, seemed to affect % of body fat in the volunteer (a loss of 10 %).
2. The fecal microbiome of the volunteer and the microbiome displayed some changes during the experiment, which were positively related to the improvement of metabolic status and weight loss, such as the occurrence of *Akkermansia* with the improved glucose homeostasis and increased relative abundance of *Bifidobacterium* and *Bacteroides* (approx. four- and two-fold, respectively). Despite some similarities in the behavior of selected microbial taxa in fecal and L-SHIME microbiomes, the general composition of these microbiomes during the same test time points was different. In addition, the microbiome of M-SHIME and its changes were not similar to those obtained for fecal and L-SHIME samples.
3. The SHIME® was found to be a suitable and helpful tool for investigating the changes in the human microbiome. However, a change in the microbiome stabilization protocol, and in particular the feed in L-SHIME, could lead to a better representation of the composition of the fecal microbiome inoculated in the system and its changes in response to the applied interventions.

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**ZMIANY W MIKROBIOMIE CZŁOWIEKA ORAZ W SYMULATORZE LUDZKIEGO  
EKOSYSTEMU DROBNOUSTROJÓW JELITOWYCH (SHIME®) W ODPOWIEDZI  
NA DIETĘ I SUPLEMENTACJĘ PROBIOTYKAMI**

Streszczenie

**Wprowadzenie.** Symulator ekosystemu drobnoustrojów jelitowych człowieka (SHIME®) służy do badania zachowania ludzkiego mikrobiomu w odpowiedzi na różne czynniki. Celem tego badania było jednoczesne zademonstrowanie zmian w mikrobiomie ludzkiego ochotnika oraz w systemie SHIME® w odpowiedzi na zmianę diety i terapii probiotycznej, z powodu niewielkiej liczby opublikowanych badań o podobnym projekcie.

**Wyniki i wnioski.** Terapia probiotykami spowodowała spadek poziomu insuliny i glukozy na czczo (odpowiednio o 18 % i 13 %), podczas gdy zwiększone spożycie błonnika w diecie eksperymentalnej wydawało się obniżać poziom trójglicerydów, cholesterolu całkowitego i LDL (o 27 %, 15 % i 14 %

odpowiednio) we krwi ochotniczki. W związku z poprawą stanu metabolicznego ochotniczki zaobserwowano zmiany w jej mikrobiomie. Mianowicie poprawa homeostazy glukozy doprowadziła do pojawienia się w kale bakterii z rodzaju *Akkermansia*, natomiast poprawa homeostazy lipidów doprowadziła do wzrostu liczebności bakterii z rodzaju *Bacteroides* i *Bifidobacterium* (odpowiednio ok. 2 i 4-krotnego). Podczas gdy zmiany w różnorodności mikrobiomu w płynie luminalnym SHIME® (L-SHIME) i w kale były częściowo podobne, mikrobiom ściany jelita SHIME (M-SHIME) zachowywał się inaczej. Zarówno L, jak i M-SHIME charakteryzowały się mikrobiomem, który różnił się składem w porównaniu z mikrobiomem ochotniczki w równoległych punktach pobierania próbek. Wyniki tego badania wskazują, że należy wprowadzić poprawki do standardowego protokołu eksperymentalnego SHIME®, aby umożliwić odwzorowanie różnorodności ludzkiego mikrobiomu i jego zmian w tym systemie.

**Słowa kluczowe:** mikrobiom, walidacja, sztuczny przewód pokarmowy, probiotyki, pozostałości pokarmowe ☒