FFHD
Functional Foods in Health and Disease

Influence of a food supplement on the gut microbiome in healthy overweight women

Peter Joller[1], Sophie Cabaset[2], Susanne Maurer[3]

[1]Dr. Joller BioMedical Consulting, Zurich, Switzerland; [2]Bio- Strath® AG, Zurich, Switzerland; [3]Adimed-Zentrum für Adipositas- und Stoffwechselmedizin Winterthur, Switzerland

Corresponding author: Peter Joller, PhD, Spitzackerstrasse 8, 8057 Zurich, Switzerland

ABSTRACT

Objectives: We attempted to influence the gut microbiome in 26 women with a BMI (Body Mass Index: kg/m^2) between 30 and 35, and aged 25 to 35 years with an herbal yeast-based dietary supplement (3 x 5ml/d) during 3 weeks in a first pilot trial.

Methods: Shotgun Metagenomic Sequencing in 2 stool samples of each participant.

Results: The majority of the bacteria in the gut microbiome were altered to an extent, which could have an impact on health. Five bacterial species found were identical to probiotic species known as good butyrate producers and described as beneficial for the gut.

Seventy percent of our study cohort showed an increase in the majority of these beneficial microbes during the
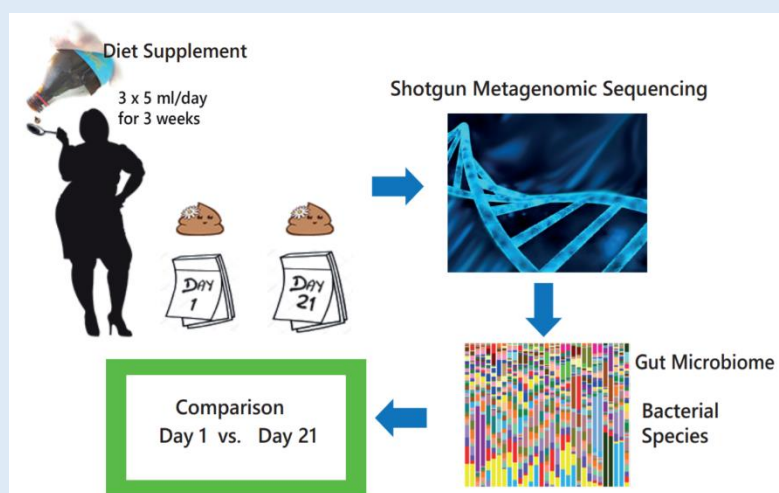
study. The ratio of Firmicutes to Bacteroidetes is an important parameter in analyzing overweight persons. Twelve of the participants initially showed a Firmicutes to Bacteroidetes ratio above 1.6. After three weeks, five of these women normalized. Overall, 14 of the 26 participants had a reduction of their F/B ratios.

Conclusions: A baker's yeast-based food supplement can modulate the gut microbiome of overweight young women within 3 weeks to an extent described in the literature as influencing well-being and health.

INTRODUCTION

The gut microbiota is a highly important entity of the human body [1]. The human intestine contains the largest community of roughly 100 trillion commensal and symbiotic bacteria. It influences the immune system [2], autoimmunity, allergies, inflammatory bowel disease, depression [3], wellbeing, and BMI [4]. The gut-associated lymphoid tissue (GALT) is our largest immunologic organ with over 70% of the total immune system [5]. Over the *Nervus vagus,* there is a direct bi-directional connection between the gut and the brain [6]. Newer publications find a link between the gut microbiome and obesity [7], Attention Deficiency Syndrome [8], personalized medicine, Alzheimer's disease [9] and even autism [10]. It was found that sex hormones, BMI and dietary fiber contribute to shaping the gut microbiome in humans [11]. The gut microbiome is considered as a human organ with its own specific functions and complexity [12]. The microbiome produces hormones, neurotransmitters, immunologic signal molecules, SCFA, and mucus. A human or a mammalian life without the gut microbiome is not conceivable. But it would be wrong to attribute only beneficial effects to the gut bacteria [13]. Depending on the number of bacteria and the composition of the microbiome it can also produce dysbiosis, toxic effects, inflammation, and infectious diseases. The gut bacteria exhibit more than three million genes, producing thousands of metabolites, whereas the human genome has only about 23,000 genes [14]. In contrast to the microbiomes of children or elderly people, in persons between 20 and 65 years of age, the microbiome is stable [15]. This temporal stability, however, can be broken by disease, antibiotics, or diet [16]. Until now there is a lack of knowledge about a "normal" microbiome. Therefore, the Human Microbiome Project (http://www.hmpdacc.org) was instituted in 2007 [17].

It is well documented that that long-term diet can directly influence the composition of the gut, and was inspired by publications stating that yeast β-glucan and α-mannan are considered beneficial components of food [2]. We designed a study to determine whether a baker's yeast-based dietary supplement can alter the composition of the gut microbiome in test persons in a clinically relevant dimension within three weeks. David et al. [18] and Turnbaugh et al. [19] established that a rapid alteration from under -0.5 to over +0.5-fold log2 in the gut microbiome can be achieved with a short-term change in the overall diet. These studies included a radical change of the entire diet from "low-fat, plant polysaccharide–rich" to "high-fat, high-sugar" nutrition, or composed entirely of animal or plant products. Additionally, Matsumoto [20] described a fermented milk beverage that increased the healthy butyric acid by more than 20% within 2 weeks, and Magnusson [21] showed that after a 2 weeks diet the changes in Bacteroidetes and Firmicutes exceeded 20% and influenced the behavior significantly. As we aimed at testing the changes driven by a food supplement (3x5ml/d), given to test persons without them changing their everyday diet, we estimated that

based on the above cited literature a shift of 20% or more in the bacterial species constitutive of the microbiome could be expected. We also wanted to investigate if   the putative shift in the bacterial composition could be considered beneficial or not.

The necessary number of test persons for our study was determined accordingly. Jacobo de la Cuesta-Zuluaga, reported that gender and age critically influence the composition of the microbiome [22]. We therefore decided on a sample that is as homogeneous as possible, recruiting only women of a specific age group.

METHODS

Study design

Participants: 26 healthy women aged 25-35 with a measured body BMI between 30 and 35 were recruited to a specialized medical center (Adimed - Zentrum für Adipositas- und Stoffwechselmedizin Winterthur, Switzerland) for this pilot trial. After approval of the competent ethics committee (BASEC (Business Administration System for Ethics Committees, Switzerland) ID 2016-02162; ClinicalTrials.gov NCT03223987, BS2016.1) and written informed consent by the participants, the test persons were assessed for the inclusion and exclusion criteria. The inclusion criteria were female gender; age (between 25 and 35 years); BMI (between 30 and 35 kg/m^2); fluency in German language and smartphone users. The exclusion criteria were the presence of nutrition therapy dependent diseases and other serious diseases requiring continuous drug therapy; women who underwent formal dietary alterations or therapeutic dietary interventions during the last 6 months, took drugs for weight loss at any time before the study, or were enrolled in any weight loss program; regular consumption of pre- or probiotics. For statistical reasons and in an effort to minimize bias we attempted a homogenous group comprising only women 25-35 years of age. All 26 test persons fulfilled all criteria, visits and provided the necessary samples. There were no drop-outs during the study.

Study conduct: This pilot study was executed as a 5-week, single-arm, pre/post intervention. Recruited participants attended three visits at Adimed. During their first visit, the terms and conduct of the study were explained to the study participants and screened for suitability. Qualifying participants were then asked to sign an informed consent form before undergoing a physical examination. At the end of the consultation, participants were given the required collection tubes for stool sampling. The second visit took place immediately before the start of the intervention: the participating women brought back their first stool sample (pre-treatment) and were given the dietary supplement, which they were asked to take for three weeks (5ml, three times daily, at least 30 Minutes before the meals). Finally, the third visit took place at the end of the intervention. Participating women brought back their second stool sample (post-treatment) and the remaining quantity of their dietary supplements. Compliance was measured by assessing the quantity of dietary supplement remaining after three weeks of intervention. No deviation from compliance was registered. At the beginning participants were further instructed not to deviate from their normal diet during the entire study period; this was monitored using a smartphone-based nutrition monitoring application (Oviva Schweiz, https://oviva.com/ch).

Stool Sample collection: Participants were instructed at first visit on how to collect the stool samples. Samples were collected at home in an OMNIgene®•GUT tube (DNA Genotek Inc. 3000 - 500 Palladium Drive, Ottawa, ON, Canada)) [23].

Dietary supplement: Strath® liquid is a plasmolyzed herbal yeast preparation with over 60 vital substances (such as vitamins, minerals, enzymes, amino-acids, oligo-elements), sold worldwide for almost 60 years (Bio-Strath® AG, Zurich, Switzerland, https://bio-strath.com/). Strath® comprises 83% Plasmolyzed Herbal Yeast (Saccharomyces cerevisiae MEYEN), 9% Malt extract, 5% Orange syrup and 3% Honey. The prescribed daily intake was 3 x 5ml each at 15 minutes before meals for three weeks. Batch Number 010916301.

Shotgun Metagenome Sequencing (SMS): The extracted stool samples were analyzed by shotgun sequencing (CoreBiome, St. Paul, MN, USA).

DNA Extractions were made using Qiagen DNeasy 96 PowerSoil Pro automated for high throughput on QiaCube (Qiagen, Germantown, MD, USA). DNA Quantification was performed using Qiant-iT Picogreen dsDNA Assay (Life Technologies Corporation, Grand Island, NY, USA). All samples passed the internal quality control.

Libraries were prepared with a procedure adapted from the Nextera Library Prep kit (Illumina, Inc. San Diego, CA, USA) and sequenced on an Illumina NextSeq using single-end 1 x 150 reads with a NextSeq 500/550 High Output v2 kit (Illumina). DNA sequences were filtered for low quality (Q-Score < 20) and length (< 50), and adapter sequences were trimmed using Cutadapt. Fastq files were converted into a single fasta using shi7. Sequences were trimmed to a maximum length of 100 bp prior to alignment.

DNA sequences were aligned to a curated database containing all representative genomes in RefSeq (NCBI Reference Sequence Database) for bacteria with additional manually curated strains. Alignments were made at 97% identity against all reference genomes. Every input sequence was compared to every reference sequence in CoreBiome's Venti database using fully gapped alignment with BURST optimal aligner for mapping large NGS (New Generation Sequencing) data to large genome databases. Ties were broken by minimizing the overall number of unique Operational Taxonomic Units (OTUs). For the taxonomy assignment, each input sequence was assigned the lowest common ancestor that was consistent across at least 80% of all reference sequences tied for the best hit. The number of counts for each OTU was normalized to the average genome length. OTUs accounting for less than one-millionth of all species-level markers and those with less than 0.01% of their unique genome regions covered (and < 1% of the whole genome) were discarded. Samples with fewer than 10,000 sequences were also discarded. Count data were then converted to relative abundance for each sample. The normalized and filtered tables were used for all downstream analyses.

Alpha and Beta Diversity: Bray-Curtis beta diversity metrics were calculated from the filtered species table using QIIME (an open-source bioinformatics pipeline for performing microbiome analysis). The Chao1 index, Shannon Index, and observed OTU count (taxonomic group) were calculated using a rarefied OTU table set to the minimum depth allowed for a sample (10'000) using QIIME 1.9.1.

SMS can sequence the complete collection of microbial genomes present in a microbiome sample, theoretically being able to discriminate between strains that differ by a single nucleotide.

To determine the enterotype we used the enterotype classifier:
http://enterotypes.org/classify.cgi,
http://enterotype.embl.de/enterotypes.html [24].

Statistics: CoreBiome supplied the data for Raw Taxonomy Tables, Filtered Taxonomy Tables, and Strain Coverage Tables as well as Alpha and Beta

Diversity. The statistics for downstream analysis were calculated with Analyse-it® for Excel 5.40 build 7149.20136 (Leeds, United Kingdom). For hypothesis tests Wilcoxon signed-rank test (Paired, Repeated, Hodges-Lehmann Location Shift, posthoc pairwise comparisons with Tukey's Method (False Discovery Rate correction), and significance level 5%) was used. Missing data were handled by Analyse-it®.

RESULTS

Since the personal and healthy core native microbiome can differ between individuals due to the enterotypes [24-26] we analyzed this entity in our cohort. In 1511 reference genomes Arumugam found three robust clusters (enterotypes) that are not nation- or continent-specific [24]. This indicates the existence of a limited number of well-balanced host-microbial symbiotic states that might respond differently to diet and drug intake. Gorvitovskaia

states that the enterotypes of Prevotella and Bacteroides should be interpreted as 'biomarkers' of diet, lifestyle, and disease state [27]. Twenty-one of 26 of our test persons belong to the enterotype 3 (Firmicutes/Ruminococcus enriched), two to the enterotype 1 (Bacteroides enriched), (Test persons: EUC Bacteroides eggerthii 26.5%, CUC Bacteroides stercoris 39.8%) and three to the enterotype 2 (Prevotella enriched), (Test persons: VMA 42.9%, LOH 18.22%, AAC 14.9%).

Since sequencing can only resolve the taxonomic composition of the gut microbiome and doesn't provide evidence of the biological functions (28) we first concentrated on the positive or negative deviations of the microbiome after three weeks of dietary supplement consumption. Figure 1 displays a stacked histogram of the bacteria found in the test persons at day 0 and day 21.
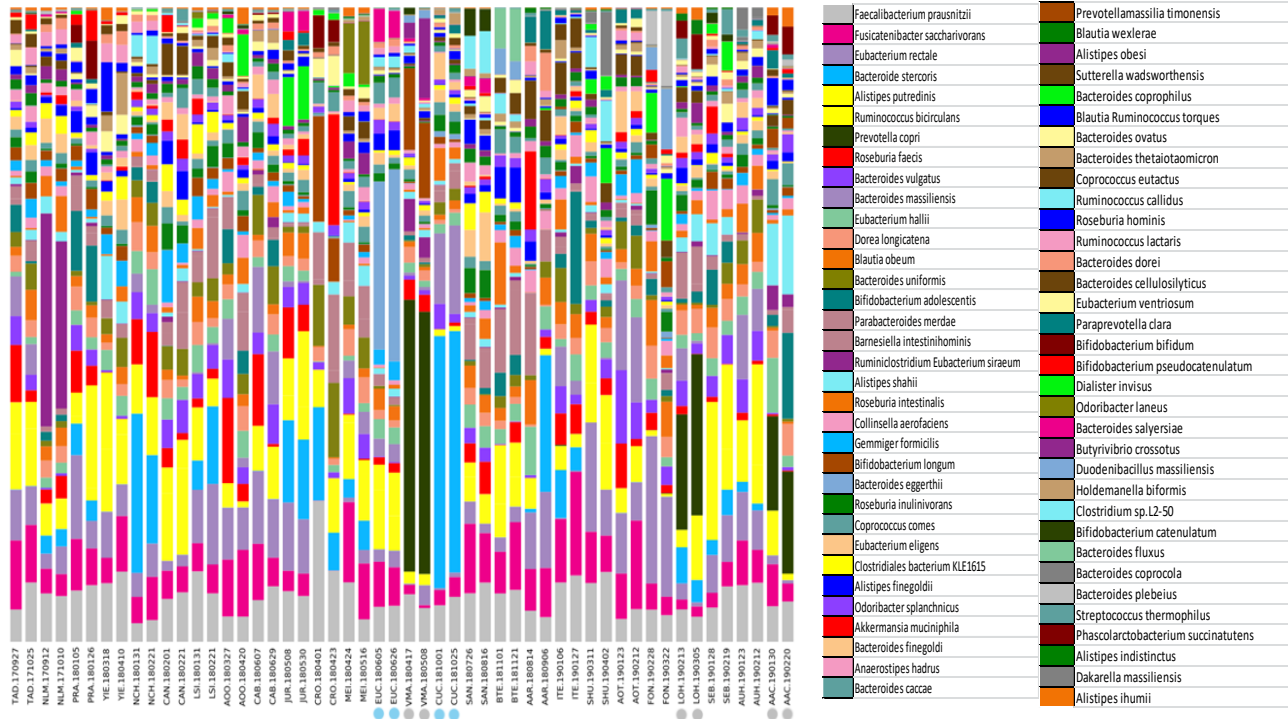

Figure 1. Each test person (3 letter-code at the bottom of the graph) is represented with the bacterial composition at day 0 and day 21 of the study.
The two participants (Code EUC, CUC) belonging to the Bacteroidetes enterotypes (codes with blue points) and the three (Code VMA, LOH, AAC) with the enterotype Prevotella (codes with grey points) are easily discernible. The rest of the group belongs to enterotype 3 Firmicutes/Ruminococcus.

Our working hypothesis was that the dietary supplement based on baker's yeast changes the microbiome by more than 20% within 3 weeks. In Table 1 we calculated the percentual modifications for all participants and all 68 bacteria.

Table 1. The initial value of a specific bacterium for every participant (3 letter code) is set to 100%. The percentage change in all bacteria in the individual test person after 3 weeks is summed-up. This sum is divided by 68 to obtain the mean change per bacterium shown in the table. Mean modification per test person 456.4±1270.4

Mean percentual modification of the bacteria during the study

	Mean change during the study		Mean change during the study
TAD	122%	VMA	130%
NLM	43%	CUC	430%
PRA	58%	SAN	45%
YIE	78%	BTE	133%
NCH	42%	AAR	449%
CAN	72%	ITE	110%
LSI	69%	SHU	80%
AOO	116%	AOT	238%
CAB	93%	FON	88%
JUR	42%	LOH	69%
CRO	5963%	SEB	72%
MEI	57%	AUH	2794%
EUC	52%	AAC	87%

Since the shift of a bacterium during the study can be positive or negative, in Fig. 2 maps the number of participants exhibiting a positive or a negative variation above 20% for a specific bacterium.
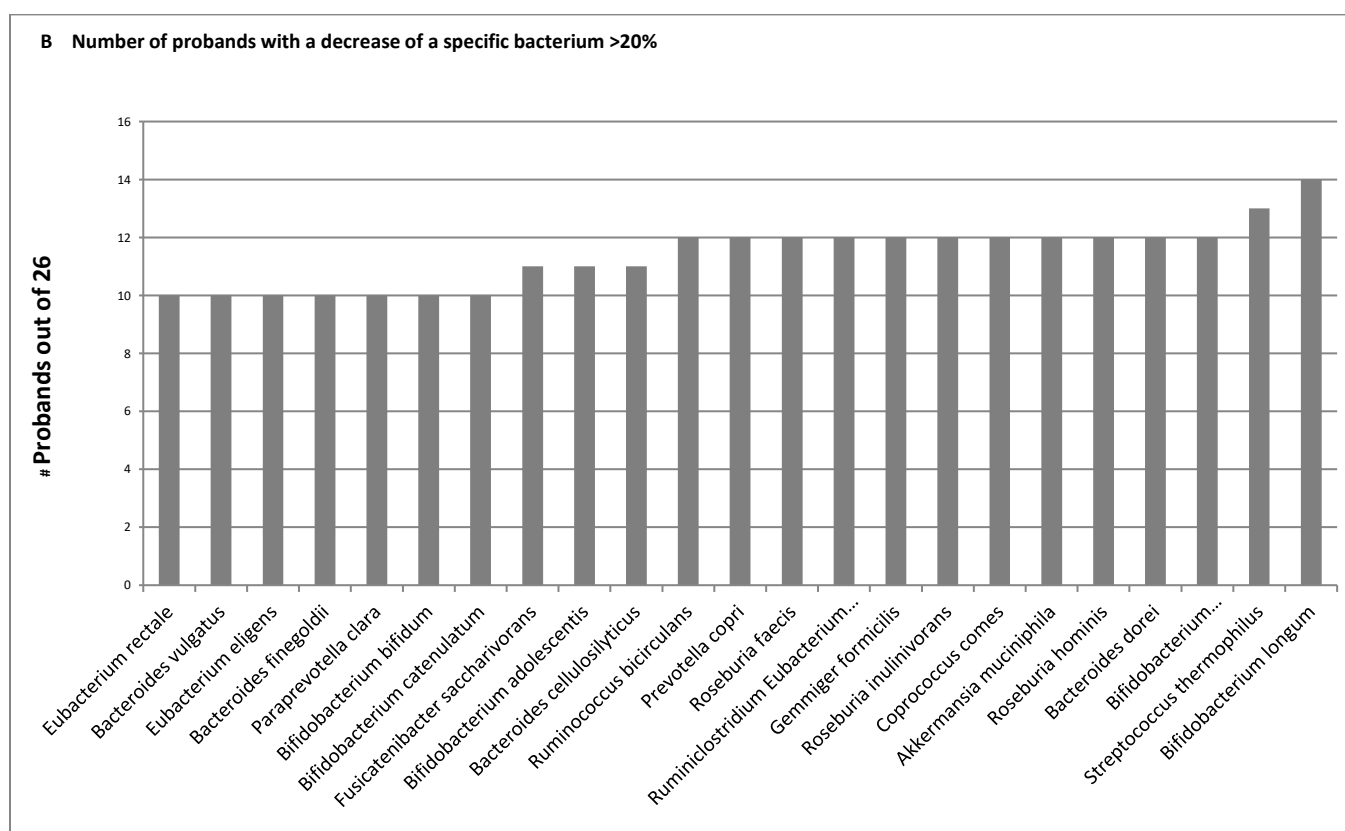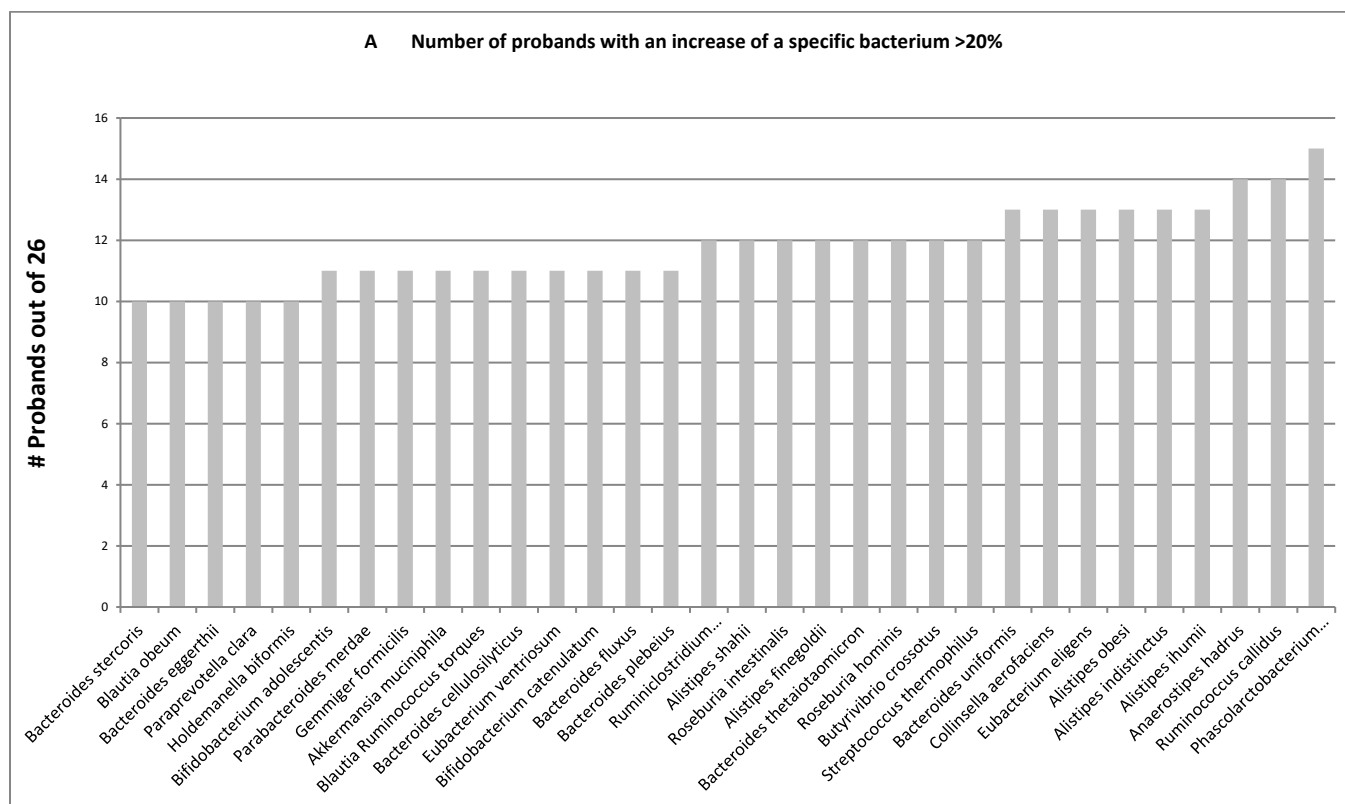
Figure 2. A) 31 out of 68 species detected B) 23 of 68 bacteria found decreased increased in a certain number of by more than 20% during supplement participants in excess of 20% during 3 ingestion weeks of supplement use.

The test persons exhibited marked differences in the number of positive and negative divergences as shown in Tab. 2.

Table 2. Allocation of the variations in bacterial species to the individual test persons (3 letter code) (n=26).

Number of bacterial species out of 68 in the investigated cohort with an increase or decrease above 20%

Increase >20%		Decrease >20%	
TAD	27	TAD	19
NLM	19	NLM	24
PRA	25	PRA	21
YIE	27	YIE	14
NCH	24	NCH	16
CAN	26	CAN	24
LSI	26	LSI	29
AOO	32	AOO	17
CAB	28	CAB	19
JUR	21	JUR	18
CRO	20	CRO	21
MEI	34	MEI	16
EUC	22	EUC	20
VMA	19	VMA	27
CUC	27	CUC	20
SAN	25	SAN	18
BTE	22	BTE	14
AAR	25	AAR	32
ITE	26	ITE	31
SHU	29	SHU	21
AOT	28	AOT	21
FON	27	FON	29
LOH	19	LOH	32
SEB	28	SEB	22
AUH	13	AUH	31
AAC	25	AAC	29

From the sequence data, it is difficult to assign a specific function to a bacterium. In order to reduce the 68 bacterial species to a few known beneficial ones we selected five of the probiotic bacteria Geirnaert described: Anaerostipes hadrus, Faecalibacterium prausnitzii, Eubacterium hallii, Roseburia hominis, and Roseburia inulinivorans as good butyrate producers (29). In our 26 participants, 18 (69%) showed an increase in more than 3 of these species (Fig. 3).

An interesting component in the evaluation of the overweight population is the Firmicutes to Bacteroidetes ratio (F/B ratio), the literature suggesting a normal value to be 1.6 or below. In persons with a lower BMI, it can be as low as 0.7

(Confidence Interval 0.6-0.7) [30]. Therefore, we looked at participants with an initial F/B ratio of over 1.6. It was found that 12 persons in the study had an elevated ratio at the beginning. Tab. 3 displays the 5 out of the 12 persons who started with an increased ratio and shifted to normal values after 3 weeks treatment.

Table. 3 Out of 26 test persons (3 letter code), 12 had an initial F/B ratio above 1.6. At the end of the study 5 women normalized their F/B ratio (below 1.6).

Firmicutes to Bacteroidetes Ratio (F/B Ratio)

	F/B Ratio before	F/B Ratio after
CAN	1.83	0.84
LSI	2.65	1.41
AAR	1.63	0.78
SHU	3.77	1.59
FON	3.13	1.24

For the parameters Beta Diversity and Alpha Diversity, we didn't find a statistically significant difference in the cohort within the 3 weeks.

DISCUSSION

To understand the human microbiome new methods such as shotgun metagenome sequencing (SMS) have brought an essential improvement. It is now possible to determine the microbiome down to the species level.

The remaining challenge is understanding the cooperation of the entirety of the microbiome system. It is impossible to find direct evidence from sequencing data of the biological functions associated with the gut microbial community and there is little information about the mechanism of metabolic interactions [31]. Klemashevich states that to generate a beneficial effect there must be a cooperative interaction with other bacterial strains in the gut [32]; this makes statements about health amelioration solely from the sequence data difficult. Mircea Podar (personal communication) is convinced that only the cultivation of the bacteria can yield insights into their function. The human gut has as many as 1000 microbial species, and the sequences cannot explain whether they contribute to digestion, immune responses, metabolism of drugs, or other processes. Moreover, also environmental exposures, microbial ecology, and human genotype confound straightforward conclusions [19]. Currently, there is

no consensus model of gut microbiota metabolism [33]. It will be necessary to connect parts lists to networks in a spatial and temporal context [34]. In the sequencing results, there is a lack of functional annotation and a large fraction of gut microbial genes is uncharacterized to date [35]. As Amplicon sequencing typically only resolves the taxonomic composition of the gut microbiome, it is to date impossible to provide direct evidence of the biological functions associated with the gut microbial community [28].

Even in the homogenous group we worked with, the taxonomic composition of the gut microbiome varied greatly between individuals, due to both microbiome-intrinsic and microbiome-extrinsic factors [36]. Therefore, the complexity prohibits a straightforward interpretation of our results apart from the genome associated entities. To make some statements concerning the changes during the supplement phase we selected 5 bacteria, which were used as probiotics in Crohn's Disease patients [29]. These butyrate-producing bacteria have beneficial effects on epithelial barrier function and overall gut health. Butyrate is at the same time important to maintain gastrointestinal health because it serves as the main energy source for colonocytes, enhances epithelial barrier integrity, and inhibits inflammation [37]. Furthermore McNabney et al. described that short chain fatty acid and amongst others butyrate play a role in preventing high-fat diet induced obesity and insulin resistance [38]. Seventy percent of our study participants at the end showed an increase in the majority of these probiotic bacteria.

The concept of enterotypes today is controversial. Nevertheless, it is interesting that 21 out of 26 test persons in the studied overweight population belong to the Firmicutes/Ruminococcus enriched group. According to the literature this has an influence on the F/B ratio and therefore on the BMI.

The dietary supplement containing plasmolyzed herbal yeast fulfilled our working hypothesis of shifting the majority of the gut microbiome by more than 20 percent within 3 weeks which according to Turnbaugh et. al. (2009b) is sufficient to significantly influence health and wellbeing. This shift could be linked to the yeast's high content of glucan and mannan, these two substances having been identified to affect bacteria in the gut. Krogfelt found that

mannans inserted in the gut wall facilitate the adhesion of certain bacteria, helping microbes to stay longer in the human intestinal tract by binding to human mucus or mannose sugars present on intestinal surface structures [39]. Despite the microbiome stability described for people in the age group studied [15], it was possible to achieve an alteration within only a three-week course of a diet supplement based on baker's yeast without any alteration in the usual diet of the participants.

CONCLUSIONS

A dietary supplement based on plasmolyzed herbal yeast (*Saccharomyces cerevisiae*, MEYEN) is able to modify the gut microbiome within 3 weeks in an order of magnitude described as influencing well-being and health. Future research should include quantitation of the bacteria by flow cytometry and measurement of metabolites like butyrate, succinic acid and other SCFA in the stool samples at the beginning and the end of the study. Longer studies than 3 weeks should be envisaged.

References:

1. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. *World J Gastroenterol* 2015;21:8836–8847.

2. De Marco Castro E, Calder PC, Roche HM. β-1,3/1,6-Glucans and Immunity: State of the Art and Future

Directions. *Mol Nutr Food Res* 2020;1901071.

3. Liśkiewicz P, Pełka-Wysiecka J, Kaczmarczyk M, *et al.* Fecal Microbiota Analysis in Patients Going through a Depressive Episode during Treatment in a Psychiatric Hospital Setting. *J Clin Med* 2019.

4. Goodrich JK, Waters JL, Poole AC, *et al.* Human genetics shape the gut microbiome. *Cell* 2014;159:789–799.

5. Vighi G, Marcucci F, Sensi L, Di Cara G, Frati F. Allergy and the gastrointestinal system. *Clin Exp Immunol* 2008;153:3–6.

6. Galland L. The Gut Microbiome and the Brain. *J Med Food* 2014;17:1261–1272.

7. Bertram F, Menge D, Andresen V. Die Bedeutung des Mikrobioms für die Adipositas: Machen Darmbakterien dick? *Gynäkologe 50* 2017:111–11.

8. Aarts E, Ederveen THA, Naaijen J, *et al.* Gut microbiome in ADHD and its relation to neural reward anticipation. *PLoS One* 2017;12:1–17.

9. Hu X, Wang T, Jin F. Alzheimer's disease and gut microbiota. *Sci China Life Sci* 2016;59:1006–1023.

10. Li Q, Han Y, Dy ABC, Hagerman RJ. The Gut Microbiota and Autism Spectrum Disorders. *Front Cell Neurosci* 2017;11.

11. Wu GD, Chen J, Hoffmann C, *et al.* Linking long-term dietary patterns with gut microbial enterotypes. *Science (80-)* 2011;334:105–108.

12. Manson JM, Rauch M, Gilmore MS. The commensal microbiology of the gastrointestinal tract. *Adv Exp Med Biol* 2008.

13. Mills S, Stanton C, Lane JA, Smith GJ, Ross RP. Precision nutrition and the microbiome, part I: Current state of the science. *Nutrients* 2019;11:1–45.

14. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. *BMJ* 2018;361:36–44.

15. Faith JJ, Guruge JL, Charbonneau M, *et al.* The long-term stability of the human gut microbiota. *Science (80-)* 2013;341:1–22.

16. David LA, Maurice CF, Carmody RN, *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559–63.

17. Gevers D, Pop M, Schloss PD, Huttenhower C. Bioinformatics for the Human Microbiome Project. *PLoS Comput Biol* 2012;8.

18. David LA, Maurice CF, Carmody RN, *et al.* Diet Rapidly Alters the Human Gut Microbiota. *Nature* 2014;505:559–563.

19. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009;1:6ra14.

20. Matsumoto K, Takada T, Shimizu K, *et al.* Effects of a probiotic fermented milk beverage containing Lactobacillus casei strain Shirota on defecation frequency, intestinal microbiota, and the intestinal environment of healthy individuals with soft stools. *J Biosci Bioeng* 2010;110:547–552.

21. Magnusson KR, Hauck L, Jeffrey BM, *et al.* Relationships between diet-related changes in the gut microbiome and cognitive flexibility. *Neuroscience* 2015;300:128–140.

22. Jacobo de la Cuesta-Zuluaga. Age and sex-dependent patterns of gut microbial diversity in human adults. *bioRxiv* 2019.

23. Ilett EE, Jørgensen M, Noguera-Julian M, *et al.* Gut microbiome comparability of fresh-frozen versus stabilized-frozen samples from hospitalized patients using 16S rRNA gene and shotgun metagenomic sequencing. *Sci Rep* 2019;9:1–11.

24. Costea PI, Hildebrand F, Manimozhiyan A, *et al.* Enterotypes in the landscape of gut microbial community composition. *Nat Microbiol* 2017;3:8–16.

25. Rinninella E, Raoul P, Cintoni M, *et al.* What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. *Microorganisms* 2019;7:14.

26. De Wouters T, Doré J, Lepage P. Does our food (environment) change our gut microbiome ('In-Vironment'): A potential role for inflammatory bowel disease? *Dig Dis* 2013;30:33–39.

27. Gorvitovskaia A, Holmes SP, Huse SM. Interpreting prevotella and bacteroides as biomarkers of diet and lifestyle. *Microbiome* 2016;4:1–12.

28. Ji B, Nielsen J. From next-generation sequencing to systematic modeling of the gut microbiome. *Front Genet* 2015;6:1–9.

29. Geirnaert A, Calatayud M, Grootaert C, *et al.* Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. *Sci Rep* 2017;7:1–14.

30. Koliada A, Syzenko G, Moseiko V, *et al.* Association between body mass index and Firmicutes / Bacteroidetes ratio in an adult Ukrainian population. 2017:4–9.

31. Hornung B, Martins dos Santos VAP, Smidt H, Schaap PJ. Studying microbial functionality within the gut ecosystem by systems biology. *Genes Nutr* 2018;13:1–19.

32. Klemashevich C, Wu C, Howsmon D, Alaniz RC, Lee K, Jayaraman A. Rational identification of diet-derived postbiotics for improving intestinal microbiota function. *Curr Opin Biotechnol* 2014;26:85–90.

33. Sridharan G V., Choi K, Klemashevich C, *et al.* Prediction and quantification of bioactive microbiota metabolites in the mouse gut. *Nat Commun* 2014;5:1–13.

34. Raes J, Bork P. Molecular eco-systems biology: Towards an understanding of community function. *Nat Rev Microbiol* 2008;6:693–699.

35. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C ST 2019. Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers. 2019;10:1–13.

36. Schmidt TSB, Raes J, Bork P. The Human Gut Microbiome: From Association to Modulation. *Cell* 2018;172:1198–1215.

37. Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: The role of butyrate on colonic function. *Aliment Pharmacol Ther* 2008;27:104–119.

38. McNabney SM, Henagan TM. Short chain fatty acids in the colon and peripheral tissues: A focus on butyrate, colon cancer, obesity and insulin resistance. *Nutrients* 2017;9:1–28.

39. Krogfelt KA. Bacterial adhesion: Genetics, biogenesis, and role in pathogenesis of fimbrial adhesins of Escherichia coli. *Rev Infect Dis* 1991.