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ORIGINAL CONTRIBUTION

Association of dietary type with fecal microbiota in vegetarians and omnivores in Slovenia

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Abstract
Purpose The purpose of this study was to discover differences in the human fecal microbiota composition driven by long-term omnivore versus vegan/lacto-vegetarian dietary pattern. In addition, the possible association of demographic characteristics and dietary habits such as consumption of particular foods with the fecal microbiota was examined.
Methods This study was conducted on a Slovenian population comprising 31 vegetarian participants (11 lacto-vegetarians and 20 vegans) and 29 omnivore participants. Bacterial DNA was extracted from the frozen fecal samples by Maxwell 16 Tissue DNA Purification Kit (Promega). Relative quantification of selected bacterial groups was performed by real-time PCR. Differences in fecal microbiota composition were evaluated by PCR–DGGE fingerprinting of the V3 16S rRNA region. Participants' demographic characteristics, dietary habits and health status information were collected through a questionnaire.
Results Vegetarian diet was associated with higher ratio (% of group-specific DNA in relation to all bacterial DNA) of *Bacteroides–Prevotella*, *Bacteroides thetaiotaomicron*, *Clostridium clostridioforme* and *Faecalibacterium prausnitzii*, but with lower ratio (%) of *Clostridium* cluster XIVa. Real-time PCR also showed a higher concentration and ratio of *Enterobacteriaceae* (*16S rDNA* copies/g and %) in female participants ($p < 0.05$ and $p < 0.01$) and decrease in *Bifidobacterium* with age ($p < 0.01$). DGGE analysis of the 16S rRNA V3 region showed that relative quantity of DGGE bands from certain bacterial groups was lower (*Bifidobacterium*, *Streptococus*, *Collinsella* and *Lachnospiraceae*) or higher (*Subdoligranulum*) among vegetarians, indicating the association of dietary type with bacterial community composition. Sequencing of selected DGGE bands revealed the presence of common representatives of fecal microbiota: *Bacteroides*, *Eubacterium*, *Faecalibacterium*, *Ruminococcaceae*, *Bifidobacterium* and *Lachnospiraceae*. Up to 4 % of variance in microbial community analyzed by DGGE could be explained by the vegetarian type of diet.
Conclusions Long-term vegetarian diet contributed to quantity and associated bacterial community shifts in fecal microbiota composition. Consumption of foods of animal origin (eggs, red meat, white meat, milk, yoghurt, other dairy products, fish and seafood) and vegetarian type of diet explained the largest share of variance in microbial community structure. Fecal microbiota composition was also associated with participants' age, gender and body mass.

Keywords Vegetarian diet · Fecal microbiota · PCR–DGGE · Real-time PCR

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Introduction

The gastrointestinal tract of humans contains a huge number of microorganisms including bacteria, archaea, viruses, parasites and fungi referred to as human gut

microbiota. The positive role of the commensal gut microbiota may be attributed mainly to prevention of infections by pathogenic bacteria, maintaining gut barrier integrity, protection against epithelial cell injury, supplying the colonocytes with short-chain fatty acids, synthesis of vitamins and bioactive compounds, maintenance of acidic pH in the intestines, promoting the development of intestinal lymphoid tissues and enhancing the immune function and balancing the pro-inflammatory against anti-inflammatory signals [1]. In addition, intestinal microbiota is hypothesized to modulate lipid and glucose homeostasis and to influence the weight gain [2].

Recent advances in next generation sequencing technologies enabled studies of gut microbiome on unprecedented levels and in a large number of individuals which made possible the proposal of the core human microbiota existence, i.e., those microorganisms which are present in all or in majority of humans in a given habitat [3–5]. However, the core microbiota presents only a part of total microbiota, since the composition of the large majority of gut microbiota is host specific and highly variable, influenced by different genetic and environmental factors [6].

Besides host genotype, age and sex, diet also appears to be an important factor affecting the gut microbiota in terms of abundance, composition and activity. As gut microbiota interacts with the host, e.g., influences gene expression in various tissues and affects different metabolic processes, it may contribute to the occurrence/development of various metabolic disorders such as obesity or type 2 diabetes [1, 2]. Interactions between the gut microbiota and hosts seem to be involved also in the pathogenesis of irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) like ulcerative colitis and Crohn's disease, although it is still not clear whether changes in fecal and mucosal microbiota commonly observed in patients with such chronic intestinal diseases (described as gut microbiota "dysbiosis") are causative factors of the disease or its consequence [7–9].

The results of several studies suggest that dietary shifts toward reduced or omitted meat intake could favorably contribute to the prevention of chronic diseases such as type 2 diabetes [10], obesity and cardiovascular diseases [11–13], diverticular disease [9], inflammatory bowel disease (IBD) and certain cancers [12–14]. Clinical study on Crohn's disease patients reverting to semi-vegetarian diet conducted by Chiba et al. [14] showed encouraging results, suggesting that limited ingestion of foods of animal origin, fats and sugars and their coordinated substitution with grains, vegetables and fruits could represent the basis for a systematic approach in disease prevention. Brathwalte et al. [15] reported that the benefits of vegetarian diet regarding obesity and body mass index (BMI) accrue over time, and finally, the actual evidence is manifested only after years of diet adherence.

The influence of different dietary types such as vegetarian or vegan diet on the gut microbiota has, however, not been thoroughly studied in larger groups of individuals so far. Few observational studies reported the dietary-induced shifts in fecal microbiota composition [5, 16–19]. Some of these studies included a limited number of participants, however, or suffered from a lack of powerful microbiological methodology such as culture-independent techniques. Inadequately defined dietary regimes (vegetarian subjects were consuming fish) or short length of diet adherence may reduce the significance of these studies, too.

In the present study, the differences in the fecal microbiota composition of 29 volunteers on an omnivore diet and 31 subjects on a lacto-vegetarian (plant-based diet avoiding all foods of animal origin except for milk and dairy products) or vegan diet (plant-based diet excluding all foods of animal origin) were evaluated by quantitative and qualitative molecular methods (real-time PCR and DGGE). All volunteers adhered to the specific diet for at least a year before taking part in our study. The primary purpose of the study was to establish whether dietary type has a significant impact on the fecal microbiota composition. Beside this, possible associations of demographic characteristics and dietary habits such as consumption of particular foods with fecal microbiota of participants were investigated.

Materials and methods

Study design and sampling

This study was approved by The National Medical Ethics Committee of the Republic of Slovenia (number 36/12/11), and all participants or their parents/guardians (for the participants under age of 18) have signed a written informed consent. Prior to signing a written consent, the participants or their parents/guardians were given detailed information on the course, purpose and methods of the research. All information collected in the survey was confidential. All participating subjects were given a code number so that the identification of subjects was not possible.

The study population comprised 60 healthy individuals living in Slovenia. Demographic characteristics of the participants are listed in Table 1. The participants of a broad age range (1.5–67 years) adhering to their regular dietary type for at least 1 year before this research commenced were recruited through acquaintanceships. In the case of participants under age of 18, parents responded on their behalf. Vegan and vegetarian participants included in our study were highly morally and ethically motivated individuals strictly refraining from harming any sentient organisms this being applied also in their plant-based

Table 1 Demographic characteristics of 60 subjects included in the study

	All subjects ($n = 60$)	Vegetarians ($n = 31$)	Vegans ($n = 20$)	Lacto-vegetarians ($n = 11$)	Omnivores ($n = 29$)	p^a	p^b
Gender (M/F)	31/29 (51.6/48.4)	19/12 (59.4/40.6)	12/8 (60.0/40.0)	7/4 (58.3/41.7)	12/17 (43.3/56.7)	0.199	0.299
Age (years)	33.5 (1.5–67)	35.0 (2–67)	35.0 (2–63)	34 (30–67)	30.0 (1.5–61)	0.041*	0.114
Body mass (kg)	68 (10–110)	70 (11–110)	65 (11–110)	78 (50–100)	66 (10–95)	0.371	0.101
Height (cm)	170 (80–193)	170 (85–193)	171 (85–189)	170 (160–193)	168 (80–185)	0.630	0.843
BMI (kg/m^2)	23.2 (12.8–35.9)	23.5 (12.8–35.9)	21.71 (12.8–31.8)	24.22 (19.5–35.9)	22.99 (15.6–31.0)	0.412	0.071

Continuous data are expressed as median (range), categorical as number (%) of subjects

p^a, Mann–Whitney U test (vegetarians vs. omnivores)

p^b, Kruskal–Wallis test (vegans vs. lacto-vegetarians vs. omnivores)

* $p < 0.05$

dietary adherence. As revealed by detailed interviewing vegan and vegetarian subjects were already instructed how to maintain strict and balanced vegetarian diet. Origin of food ingredients was carefully selected, and the food products' labels were regularly checked to avoid any possible animal-based contaminants. In vegan diet, the foods of animal origin were mostly replaced by lentils, beans, nuts, rice, seeds, gluten, meat substitute, potatoes, some greens, etc. and processed plant foods (tofu, non-dairy milks, cereals, plant oils, etc.). In lacto-vegetarian diet, meat, fish and eggs consumption was compensated with dairy and plant foods.

The exclusion criterion for subject's participation was the use of antibiotics or chemotherapy in a period of 1 month prior to sampling. All participants or their parents/guardians (for the participants under age of 18) have agreed to fill out a two-part questionnaire and to collect one stool sample.

The first part of self-administered questionnaire disclosed their age, gender, height, body weight, health status and dietary type, where participants could choose among the following categories: vegans as subjects who reported consuming no animal products (red meat, poultry, fish, eggs, milk and dairy products <1 time/month), lacto-ovo vegetarians as consuming dairy products and/or eggs ≥1 time/month but no fish or meat (red meat, poultry and fish <1 time/month), pescovegetarians as consuming fish ≥1 time/month and dairy products and/or eggs but no red meat or poultry (red meat and poultry <1 time/month), semi-vegetarians as consuming dairy products and/or eggs and meat (red meat and poultry ≥1 time/month and <1 time/week) and omnivores (non-vegetarians) as consuming animal products (red meat, poultry, fish, eggs, milk and dairy products >1 time/week) [20]. Non-vegetarians were asked also about the ratio of food of animal origin versus plant-based food in their diet (alternatives: mostly plant food, mostly food of animal origin or equally represented plant food and food of animal origin). Adherence to vegan/lacto-vegetarian was reassured in an interview.

A long-term dietary intake was assessed by a second part of in-house questionnaire (usual intake, self-administered, not validated) asking about inclusion/exclusion of particular foods, alcohol, additional salt, multivitamin dietary supplements during the last year (categories (24) are listed in Supplemental Table 1), with the exception of liquid consumption, where participants had to choose between consumption of more or less than 1 l/day. Inclusion of particular categories was based on the consumption of ≥1 time/month, with the exception of alcohol, where consumption of any amount during the past 12 months was considered as positive answer.

Participants were provided with sterile containers for stool samples and instructed to store the samples immediately after collection in household freezer. Frozen samples were transferred on ice within a week to the laboratory, where they were stored at −80 °C and analyzed within 10 months.

Sample preparation and DNA extraction

0.1 g of feces was homogenized in 9.9 g of anaerobic diluent (1 g/l peptone, 2 g/l porcine gelatine, 8.5 g/l NaCl and 0.557 g/l L-cysteine hydrochloride monohydrate); 1 ml of suspension was pelleted by centrifugation (3,600 g/10 min), lysed by 2-h incubation with lysozyme (5 mg/ml) and mutanolysine (5 U/ml) and sonicated (Soniprep 150 plus, MSE (UK) Limited). Finally, samples were transferred to the Maxwell 16 Tissue DNA Purification Kit (Promega) cartridge, and DNA was extracted according to automated Maxwell 16 System (Promega) protocol. DNA concentration was measured by NanoVue Spectrophotometer (Fisher Scientific).

DNA for real-time PCR standard curves was isolated from overnight bacterial cultures of *Bifidobacterium*

Table 2 Cultivation conditions, media and estimated number of copies of 16S rRNA operons of bacterial strains used for standard curves in the real-time PCR analyses of fecal samples

Bacterial strain[a]	Medium[b]	Cultivation conditions	Number of 16S rRNA operon copies[c]
Bifidobacterium animalis subsp. *lactis* BB-12 (Chr. Hansen, Denmark)	MRS + 1 % cysteine hydrochloride	Aerobic/ 37 °C/ 18 h	4
Bacteroides thetaiotaomicron DSM 2079	Modified M2 (Hobson, 1969)	Anaerobic/ 37 °C/ 18 h	6
Clostridium clostridioforme DSM 933	RCM	Anaerobic/ 37 °C/ 48 h	9
Clostridium leptum DSM 753	RCM	Anaerobic/ 37 °C/ 48 h	9
Escherichia coli K12	BHI	Aerobic/ 37 °C/ 24 h	7
Faecalibacterium prausnitzii DSM 17677	M330	Anaerobic/ 37 °C/ 20 h	n.d.

[a] ATCC *American Type Culture Collection*, Rockville, USA. DSM *Deutsche Sammlung von Mikroorganismen und Zellkulturen*, Braunschweig, Germany

[b] RCM Reinforced Clostridial Medium (Merck, Darmstadt, Germany). BHI Brain Heart Infusion Broth (Merck, Darmstadt, Germany). MRS De Man0. Rogosa and Sharpe Medium (Merck, Darmstadt, Germany)

[c] Estimated 16S rRNA operon copy numbers of individual species were derived from *rrn*DB database (http://rrndb.mmg.msu.edu/search.php); n.d. no data available in *rrn*DB database

animalis subsp. *lactis* BB-12, *Bacteroides thetaiotaomicron* DSM 2079, *Clostridium clostridioforme* DSM 933, *Clostridium leptum* DSM 753, *Escherichia coli* K12 and *Faecalibacterium prausnitzii* DSM 17677. Cultivation conditions and media are listed in Table 2. Bacteria were enumerated by phase-contrast microscopy using Petroff-Hausser Counting Chamber (Hausser Scientific) according to the manufacturer's instructions. Bacterial numbers for relative abundance quantification were recalculated (except for *F. prausnitzii*) to *16S rDNA* copy number estimates (Table 2) according to the data available in the *rrn*DB database (http://rrndb.mmg.msu.edu/search.php). For *F. prausnitzii*, the number of *16S rDNA* copies in the genome is not available in the *rrn*DB database; therefore, the results are presented as the number of cells/g feces. In order to simulate the possible effect of fecal matrix on the DNA extraction efficiency and on the real-time PCR efficiency, pure cultures were spiked into the fecal matrix (1 ml of 1:100 diluted fecal samples) which was previously autoclaved twice and UV treated in order to degrade the bacterial DNA already present in the sample. Following centrifugation, the DNA was extracted from the pellets as described above.

Table 3 Oligonucleotide primers and efficiency of qPCR reactions

Target bacteria (species, genus or group)	Primer set	References (modifications in annealing step)	qPCR efficiency of two analyses
Bacteroides–Prevotella group	Bac303F Bac 708R	[27, 51] (62 °C/ 30 s)	90.1 %, 90.8 %
Clostridium leptum group (cluster IV)	S-*-Clos-0561-a-S-17 S-*-Clept-1129-a-A-17	[52]	90.8 %, 91.3 %
Clostridium coccoides group (cluster XIVa)	g-Ccoc-F g-Ccoc-R	[53]	87.8 %, 87.7 %
Enterobacteriaceae group	Eco 1457f Eco 1652r	[27]	92.9 %, 95.8 %
Bifidobacterium genus	Bif-F Bif-R	[26] (59 °C/15 s)	90.0 %, 90.8 %
Clostridium clostridioforme	CC-1 CC-2	[54]	87.5 %, 87.2 %
Bacteroides thetaiotaomicron	BT-1 BT-2	[54]	91.7 %, 93 %
Faecalibacterium prausnitzii	Fprau223F Fprau420R	[27, 54]	95.0 %, 95.0 %
All bacteria	Eub338F Eub518R	[55]	91.8 %, 91.7 %

Quantitative PCR

Real-time PCR reactions were accomplished in a total volume of 20 µl consisting of Maxima SYBR Green qPCR Master Mix (Fermentas, Life Science) or Express SybrGreenER qPCR SuperMix Universal (Invitrogen, Life Technologies) (for *Enterobacteriaceae* group and *F. prausnitzii* only), 0.2 µM of each of the two oligonucleotide primers (listed in Table 3) and 1 µl of the isolated DNA (10- or 100-fold diluted). Stratagene Mx3000P (Stratagene, La Jolla, CA92037, USA) real-time PCR cycler was used. Samples were run in duplicates, and for each primer set, two runs were conducted. In each experiment, at least 4 dilutions of standard DNA were included. Standard curves representing the correlation between Ct values and bacterial concentration (number of *16S rDNA* copies/g feces or number of cells/g feces for *F. prausnitzii*, respectively) were generated by the Stratagene software.

PCR–DGGE fingerprinting

Differences in fecal microbiota composition were evaluated using PCR–DGGE fingerprinting of 16S rRNA V3 region which was amplified with universal bacterial primers HDA1-GC and HDA2 [21]. PCR mixture (25 µl) was composed of 0.625U of *Taq* DNA polymerase (GoTaq Flexi, Promega, Madison, WI, USA), 1× Colorless GoTaq Flexi buffer, each deoxynucleoside triphosphate at a concentration of 200 µM, 2.5 mM $MgCl_2$, primers HDA1-GC and HDA2 [21] at a concentration of 0.5 µM each and 1 µl of DNA template. The PCR was carried out with Gene Amp 2700 (Applied Biosistems, Carlsbad, CA, USA). The program consisted of initial denaturation for 2 min at 95 °C, 35 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 40 s and final elongation at 72 °C for 5 min.

PCR products were processed with D GENE denaturating gel electrophoresis system (Biorad, Hercules, CA, USA). Denaturating gradient gel was composed of 8 % polyacrylamide (acrylamide/bis-acrylamide = 37:1, Sigma-Aldrich, Saint Louis, MO, USA) and 30–65 % denaturants (urea, formamide). The electrophoresis was run at 60 °C and 75 V for 16 h. Gels were stained with Sybr Safe (Invitrogen, Carlsbad, CA, USA) and visualized with UV transillumination and short-wave band pass filter on ChemiGenius2 (Syngene, Cambridge, United Kingdom). Origin of 26 selected bands was determined through multiple consecutive excisions of band from DGGE gel and PCR amplifications and subsequent sequencing of single-band PCR products (Microsynth, Balgach, Switzerland). Sequence identity was established using RDP Classifier tool [22] and with phylogenetic trees including similar sequences from SILVA project [23].

Statistical analysis

Statistical analysis was carried out using SPSS version 20 (IBM SPSS, Chicago, IL, USA). For descriptive purposes, continuous variables were presented as medians (ranges), and frequencies (percentages) were used to describe categorical variables. Significance level was set at $p < 0.05$. Kruskal–Wallis test was used for bivariate analyses of the influence of subject's dietary type (vegan vs. lacto-vegetarian vs. omnivorous) on fecal microbiota composition. Mann–Whitney *U* test was used in subsequent pair-wise comparisons and to assess differences between two groups (vegetarian vs. omnivorous) of continuous data. Holm's method was used for control of type I error in post hoc tests. Group differences on categorical variables were assessed by Pearson's chi-square test applying Yates' correction, and non-parametric Spearman's rank correlation coefficient corrected for ties was used to examine the associations between continuous variables.

DGGE gel images were analyzed in Bionumerics (Applied Maths, Sint-Martens-Latem, Belgium). Band migration was normalized with standard samples that were present in four lanes of each gel. Standard samples were prepared from ten excised bands distributed evenly throughout the gel gradient. Further normalization was achieved using bands from samples that evidently represented same migration distance. Pearson correlation coefficient was used as a sample similarity measure, and UPGMA dendrograms were analyzed. Bands were classified into band migration categories with band-matching algorithm within Bionumerics, and relative peak area of the sample was calculated for all peaks. Table of samples with relative peak area for all band migration categories was exported as "Species Table" for use with Canoco 4.5 (Microcomputer Power, Ithaca, NY, USA). Redundancy analysis (RDA) was used for multivariate exploration of microbial community structure (DGGE) in relation to subjects' individual characteristics and dietary habits that were gathered through questionnaire. RDA was also used for exploration of real-time PCR results and data obtained with questionnaire.

Results

Demographic characteristics and dietary habits

In the questionnaires, the participants were asked about demographic data (gender, age, body mass, height) and about their consumption of particular foods and drinks (alcohol, liquids, sweets, coffee, extra salt, fruits, vegetables, seeds, etc.) (Supplemental Table 1). Except for the small difference in median age (35.0 years in vegetarians vs. 30.0 years in omnivores, $p = 0.041$), dietary groups compared in our study did not differ significantly in the demographic aspects regardless of whether the vegetarians were considered as one group or were divided into two groups (vegans and lacto-vegetarians) (Table 1). Four of 29 (13.8 %) of omnivorous participants and 3 of 31 (9.7 %) of vegetarian participants included in the study were preschool children. Subgroups of vegetarians, such as lacto-ovo-, ovo-, pescovegetarians, fruitarians or raw vegans, were not identified among the participants of this study. Alcohol consumption was observed only in omnivores (68 % of the group). Other noteworthy differences between vegetarians and omnivores in the consumption of different foods were not observed.

Twenty-five non-vegetarian participants on omnivore diet had their consumption of animal products ingested in equal proportion to plant-based ingredients (cereals, fruits, vegetables, legumes); however, one omnivore reported that the diet was based mainly on plant ingredients, whereas

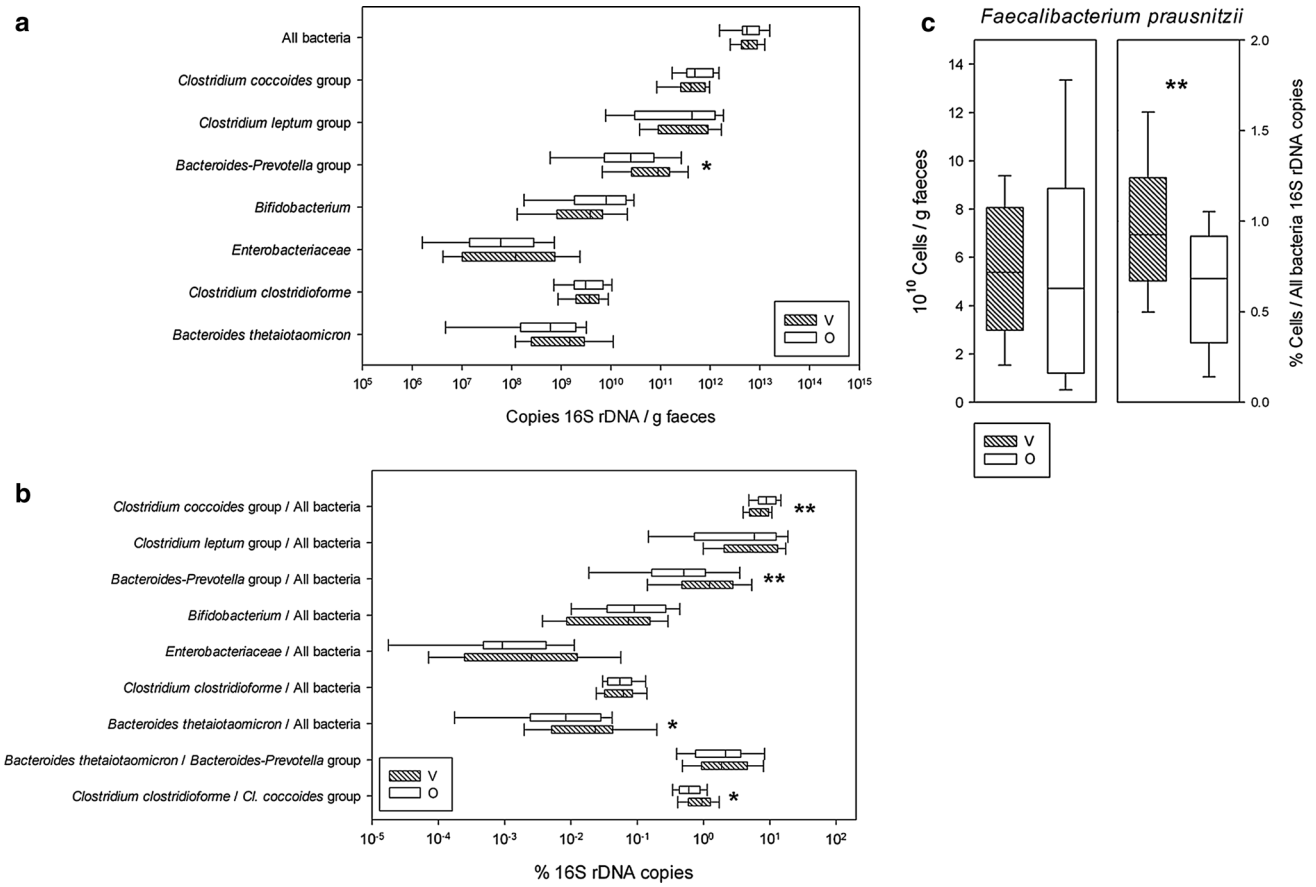

Fig. 1 Relative quantification by real-time PCR of bacteria in the feces of vegetarians V (31) and omnivores O (29). The *box* and *whiskers plots* represent the medians and interquartile ranges; *error bars* 10th and 90th percentiles. *Asterisks* significant differences between vegetarian and omnivore groups (*$p < 0.05$; **$p < 0.01$). The results are presented as a number of *16S rDNA* copies of individual target group of bacteria in g of feces (**a**), as ratio (%) (number of *16S rDNA* copies of each group/g : number of *16S rDNA* copies of all bacteria/g) (**b**), as a number of *Faecalibacterium prausnitzii* cells in g of feces (**c** *left*) or as a ratio (%) of *Faecalibacterium prausnitzii* cells in relation of a number of *16S rDNA* copies of all bacteria (**c** *right*)

three omnivores reported their meals being mostly prepared from animal ingredients (meaty delicacy, dairy). Nevertheless, none of the subjects totally excluded plant-based ingredients from their meals. All of the participants included both cooked and raw food in their meals. Participants reported consuming regularly 2–4 meals of various quantity per day. Majority of the participants (78 % of vegans, 67 % lacto-vegetarians and 75 % omnivores) described their meals as versatile, and meals would not repeat sooner than in 2 weeks.

Relative quantitation of fecal microbiota

The concentration (number of *16S rDNA*/g of feces) of selected groups of bacteria in fecal microbiota of vegetarians and omnivores is presented in Fig. 1a and the ratio (*16S rDNA* of each group vs. *16S rDNA* copies of all bacteria) in Fig. 1b. The concentration and ratio of *F. prausnitzii* are presented in a separate figure (Fig. 1c) since the results are expressed here as the number of cells/g feces. Fecal samples of vegetarians contained significantly more *Bacteroides–Prevotella 16S rDNA*/g ($p < 0.05$) and had a higher ratio (%) of *Bacteroides–Prevotella 16S rDNA*/g versus all bacteria/g ($p < 0.01$) than fecal samples of omnivores. The *16S rDNA* ratio (%) of *C. coccoides* group (*C.* cluster XIVa) was lower ($p < 0.01$) in the feces of vegetarians, however.

The *16S rDNA* ratio (%) of *C. clostridioforme* species within the *C. coccoides* group and the *16S rDNA* ratio (%) of *B. thetaiotaomicron* species (*B. thetaiotaomicron* vs. all bacteria) were higher ($p < 0.05$) in vegetarians' feces.

In addition, vegans and lacto-vegetarians comprised in vegetarian group were analyzed as two separate groups. The *16S rDNA* ratio (%) of fecal *C. clostridioforme* within the *C. coccoides* group was higher in vegans than in lacto-vegetarians and omnivores ($p < 0.01$) (Supplemental Table 2). Furthermore, the ratio (%) of *F. prausnitzii* was higher in vegan group ($p < 0.05$).

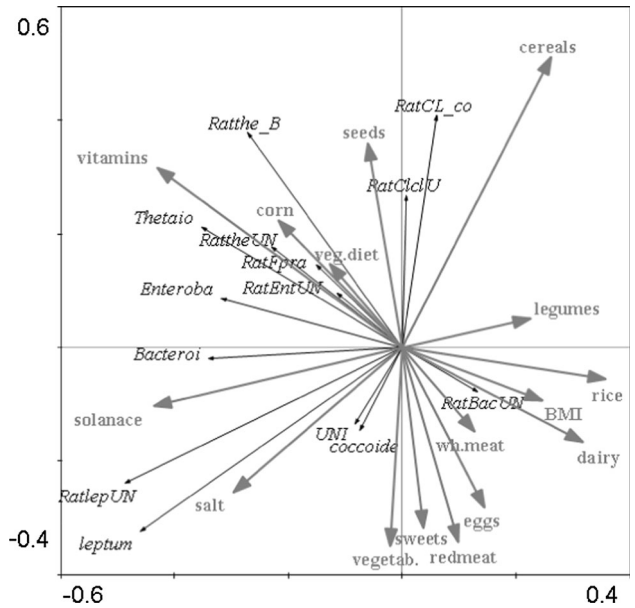

Fig. 2 RDA (redundancy analysis) biplot showing the association of the consumption of particular foods and selected results of real-time PCR analysis of bacteria in the feces of 60 participants. Thetaio *Bacteroides thetaiotaomicron*, Enteroba *Enterobacteriaceae*, Bacteroi *Bacteroides–Prevotella* group, UNI All bacteria, coccoide *Clostridium coccoides* group, leptum *Clostridium leptum* group, *Ratthe_B* Ratio *Bacteroides thetaiotaomicron/Bacteroides–Prevotella* group (%), *RatClcl_co* Ratio *Clostridium clostridioforme/Clostridium coccoides* group (%), RatCL_co Ratio *Clostridium coccoides* group/all bacteria (%), RattheUN ratio *Bacteroides thetaiotaomicron*/all bacteria, RatFpra ratio *Faecalibacterium prausnitzii*/all bacteria, RatEntUN ratio *Enterobacteriaceae*/all bacteria (%), RatBacUN ratio *Bacteroides–Prevotella* group/All bacteria (%), RatLepUN Ratio *Clostridium leptum* group/All bacteria (%), *veg. diet* vegetarian diet, *wh. meat* white meat, *BMI* body mass index, *vegetab.* vegetables, solanace vegetables—family *Solanaceae*, vitamins Multivitamin dietary supplements

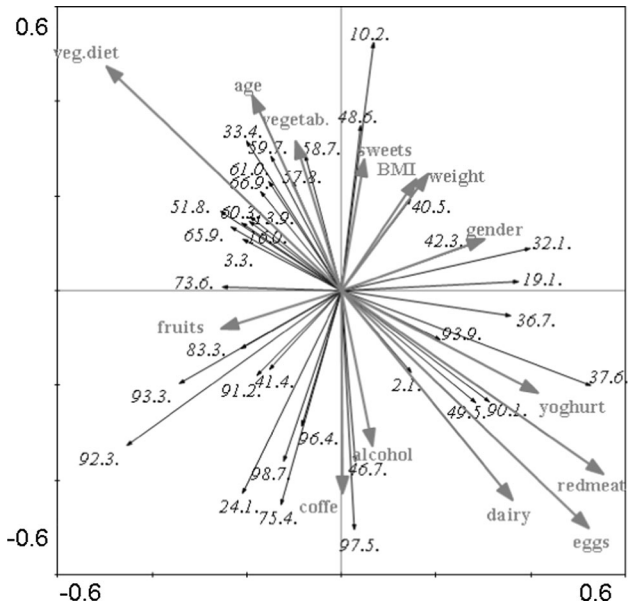

Fig. 3 RDA (redundancy analysis) biplot showing the association of demographic data, consumption of particular foods and selected DGGE bands. The *numerical band* designation indicates the length of the migration (%) in the polyacrylamide gradient gel. *Veg. diet* vegetarian diet, *vegetab.* vegetables, *BMI* body mass index, *redmeat* red meat, *coffe* coffee

Omnivorous dietary type coincided with alcohol consumption; however, no significant shifts in the fecal microbial group quantities and proportions were observed between the respective groups of subjects in the omnivorous group (Supplemental Table 3).

DGGE analysis of fecal microbial community

PCR–DGGE analysis of the 16S rRNA V3 region of bacterial DNA isolated from fecal samples resulted in on average 26.8 bands per sample (min 13, max 35, median 27.5). Electrophoretic curve-based analysis of PCR–DGGE patterns using Pearson correlation identified three major clusters of microbial communities which were not associated with the dietary type (results not shown). Selected bands which appeared to be associated with the dietary habits were extracted from the gel, re-amplified and identified by sequencing (Fig. 4; Table 4). A relative quantity of four bands was significantly higher in omnivores in

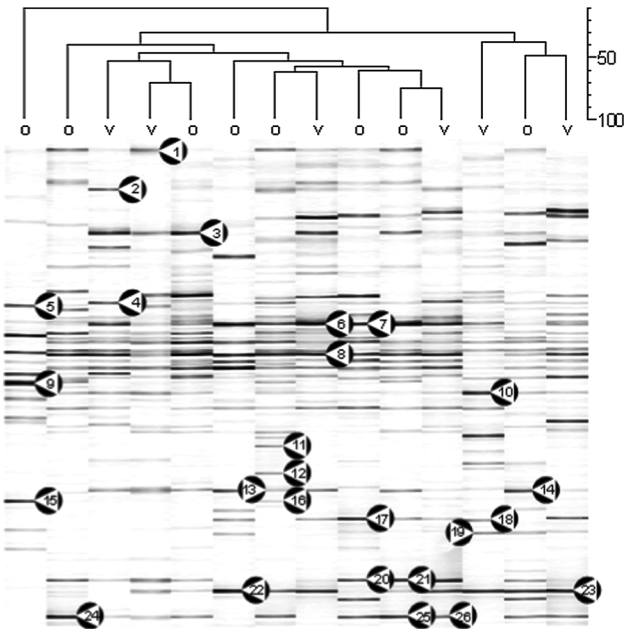

Fig. 4 DGGE profiles of DNA samples which were used for extraction of selected bands and analysis by nucleotide sequencing. All labeled bands together with their taxonomic identification are listed in Supplemental Table 3. *O* omnivorous diet, *V* vegetarian diet

comparison with vegetarians (Table 4). These four bands were identified as 16S rRNA sequences belonging to organisms from family the *Lachnospiraceae* and genera *Streptococus*, *Bifidobacterium* and *Collinsella*. On the

Table 4 A relative quantity, prevalence (%) and identification of selected DGGE bands in relation to dietary type

Band position (% of gel length)	Relative quantity of individual bands, % of samples with detected band						p	Band label in Fig. 4	Band identification (genus or family)[b]
	Vegetarians			Omnivores					
	Mean, %[a]	Median	Maximum	Mean, %	Median	Maximum			
2.1	2.04, 67.7	1.38	8.30	**3.30**, 75.9	2.64	16.80	0.151	1	**Erysipelotrichaceae*
19.1	2.23, 48.4	0.00	12.55	**3.03**, 55.2	1.24	13.54	0.465	3	**Ruminococcaceae*
34.3	0.33, 22.6	0.00	4.51	**0.44**, 31.0	0.00	4.31	0.488	5	**Erysipelotrichaceae*
37.6	5.59, 90.3	4.62	18.41	**8.15**, 96.6	7.87	20.61	**0.040**	6, 7	**Lachnospiraceae*
43.9	**8.33**, 96.8	8.08	16.01	6.72, 100.0	7.77	13.60	0.190	8	*Feacalibacterium*
49.5	0.41, 29.0	0.00	3.15	**1.68**, 51.7	0.48	11.49	**0.031**	9	*Streptococcus*
51.8	**3.34**, 93.5	2.90	13.64	1.74, 89.7	1.52	4.01	**0.009**	10	*Subdoligranulum*
77.6	1.07, 38.7	0.00	5.54	**1.34**, 37. 9	0.00	9.59	0.806	17, 18	*Dialister*
90.1	1.78, 45.2	0.00	8.82	**3.49**, 72.4	2.16	13.12	**0.041**	20, 21	*Bifidobacterium*
92.3	**6.05**, 71.0	5.61	18.29	4.53, 82.8	3.67	19.06	0.592	22, 23	*Bifidobacterium*
97.5	2.50, 64.5	1.86	9.22	**5.14**, 82.8	4.62	15.11	**0.008**	24–26	*Collinsella*

Higher average values for the relative quantity of each band and the p values lower than 0.05 are marked bold

[a] % of samples with individual DGGE band

[b] The same identification of bands at different positions in the DGGE gel indicates that they originated from different taxa belonging to the same genus/family

* The identification at the family level was only relevant

p, Mann–Whitney U test (vegetarians vs. omnivores)

contrary, the band originating from genera *Subdoligranulum* was more abundant in vegetarians (Figs. 2, 3).

Beside the bands presented in Table 4, altogether 26 bands selected on the basis of their quantity or incidence among the studied individuals, were sequenced (Supplemental Table 4). Sixteen of them were identified to genus level, 7 were identified to family level, two of them to class level (*Clostridia*) and one to phylum level (*Actinobacteria*).

Association of demographic data and consumption of particular foods and drinks with fecal microbiota

In addition to linking dietary type and fecal microbiota, various demographic characteristics in regard to fecal microbiota composition were investigated. Observed significant associations were as follows.

Fecal samples of female participants contained higher number of *16S rDNA* copies/g of *Enterobacteria* ($p < 0.05$) and lower number of *16S rDNA* copies/g of all bacteria ($p < 0.05$) (Supplemental Table 5). Consequently also the ratio (%) of *16S rDNA* copies/g of *Enterobacteriaceae* (*Enterobacteriaceae* vs. all bacteria) was higher in samples from female participants ($p < 0.01$). Samples from male participants had higher number of *F. prausnitzii* cells per g of feces ($p < 0.01$).

The fecal bacterial composition was associated also with age (Table 5). Namely, the number of *16S rDNA* copies/g of *Bifidobacteria* and the ratio with regard to the all bacteria (*Bifidobacteria* vs. all bacteria) decreased with age ($p < 0.01$). Interestingly, *Enterobacteriaceae* were negatively correlated with body mass (Table 5) ($p < 0.05$ for *16S rDNA* copies/g and $p < 0.01$ for the ratio of rDNA copies (%) with regard to all bacteria (%)). On the contrary, all bacteria *16S rDNA* copies/g and *F. prausnitzii* cells/g increased with height ($p < 0.05$).

Connections of PCR–DGGE profiles with fecal microbiota, dietary types, selected dietary factors and demographic data obtained by the questionnaire were evaluated using redundancy analysis (RDA). Lacking significant explanatory power, non-dietary choices were excluded from our presentation. Variables listed in Supplemental Table 1 were investigated and discussed in the text if their explanatory power was significant. First stage of the explanatory analysis presumed independence of investigated variables and their share of explained variance is evaluated (marginal effects). The results of this procedure (RDA biplot) are presented in Fig. 3. For the reasons of clarity, the dietary variables showing lower potential impact are presented in Supplemental Fig. 1 in which variable "vegetarian diet" is also included for scale comparison of both figures. From RDA biplot, it is possible to deduct that dietary variables exclusive to omnivore diet are highly correlated (foods of animal origin) in their potential of explaining microbial community structure. This

Table 5 Association of microbiota composition with demographic characteristics of the subjects included in the study

Target bacteria (species, genus or group)[a,b,c]	Age	Body mass	Height	BMI
Bacteroides–Prevotella group[a]	0.116 (0.376)	0.126 (0.339)	0.143 (0.277)	0.064 (0.627)
Clostridium leptum group[a]	0.137 (0.295)	0.063 (0.631)	0.119 (0.367)	0.048 (0.716)
Clostridium coccoides group[a]	−0.004 (0.975)	0.165 (0.207)	0.248 (0.056)	0.113 (0.392)
Enterobacteriaceae[a]	−0.074 (0.575)	−0.274* (0.034)	−0.303* (0.019)	−0.232 (0.075)
Bifidobacterium[a]	−0.333** (0.009)	−0.072 (0.587)	0.030 (0.819)	−0.110 (0.401)
Clostridium clostridioforme[a]	−0.070 (0.593)	0.059 (0.652)	0.203 (0.120)	−0.022 (0.870)
Bacteroides thetaiotaomicron[a]	0.056 (0.672)	0.046 (0.728)	0.168 (0.199)	−0.048 (0.716)
Faecalibacterium prausnitzii[c]	0.056 (0.670)	0.226 (0.082)	0.280* (0.030)	0.141 (0.284)
All bacteria[a]	0.037 (0.781)	0.211 (0.106)	0.293* (0.023)	0.166 (0.205)
Bacteroides–Prevotella group/all bacteria (%)[b]	0.118 (0.370)	0.035 (0.791)	0.012 (0.927)	−0.009 (0.948)
Clostridium leptum group/all bacteria (%)[b]	0.171 (0.191)	−−0.006 (0.962)	0.000 (0.997)	0.013 (0.920)
Clostridium coccoides group/all bacteria (%)[b]	−0.152 (0.247)	−0.116 (0.376)	−0.022 (0.868)	−0.169 (0.197)
Enterobacteriaceae/all bacteria (%)[b]	−0.087 (0.510)	−0.336** (0.009)	−0.386** (0.002)	−0.273* (0.035)
Bifidobacterium/all bacteria (%)[b]	−0.355** (0.005)	−0.199 (0.127)	−0.128 (0.328)	−0.208 (0.110)
Clostridium clostridioforme/all bacteria (%)[b]	−0.139 (0.288)	−0.167 (0.202)	−0.006 (0.964)	−0.249 (0.055)
Clostridium clostridioforme/Clostridium coccoides group (%)[b]	−0.030 (0.818)	−0.125 (0.342)	−0.054 (0.681)	−0.165 (0.209)
Bacteroides thetaiotaomicron/all bacteria (%)[b]	−0.015 (0.910)	−0.121 (0.358)	0.011 (0.932)	−0.188 (0.151)
Bacteroides thetaiotaomicron/Bacteroides–Prevotella group (%)[b]	−0.003 (0.985)	−0.102 (0.438)	−0.026 (0.842)	−0.161 (0.220)
Faecalibacterium prausnitzii/all bacteria (%)[d]	0.067 (0.611)	0.103 (0.435)	0.090 (0.492)	0.050 (0.703)

Spearman's rank correlation coefficient (p value)

[a] A number of *16S rDNA* copies of individual target group of bacteria in g of feces were applied in the analysis

[b] A ratio (%) of *16S rDNA* copies of individual target group of bacteria with regard to the total bacteria was applied in the analysis

[c] A number of cells of *Faecalibacterium prausnitzii* in g of feces was applied in the analysis

[d] A ratio (%) of cells of *Faecalibacterium prausnitzii* with regard to the number of *16S rDNA* copies of total bacteria was applied in the analysis

* $p < 0.05$; ** $p < 0.01$

variables (consumption of eggs, red meat, white meat, milk, yoghurt, other dairy products, fish and seafood) and vegetarian type of diet explained the largest share of variance in microbial community composition. Lambda 1 was the highest for eggs and red meat (0.045) and a bit lower for vegetarian diet as a single variable (0.036) (Fig. 3).

In the second stage of microbial variability explanation, the assumption was that "the same variance cannot be explained by two variables," and the so-called conditional effect of each variable was evaluated. When any of variables with highest marginal effect discussed in the paragraph above (variable "vegetarian diet" and dietary variables exclusive to omnivore diet) was included into explanatory model its explained variance was highly significant ($p < 0.0001$, 5,000 permutations), but none of the other variables explained any further variance ($p > 0.1$). Although cumulative effect of several variables by this approach could not be excluded, variables with the highest share of explained variance were assumed to be the most important. Alcohol consumption, however, was positively correlated with omnivore diet (Fig. 3), but its explanatory power was lower than the explanatory power of other variables discussed in the manuscript. Therefore, alcohol consumption was not recognized as a main variable but could be interpreted as a contributing factor. On the contrary, no correlation with dietary type and even lower explanatory power was found for dietary supplements in our study group.

Additionally, Fig. 3 presents association of the DGGE band identified as genus *Subdoligranulum* specific (51.8 %) with vegetarian diet and association of DGGE bands identified as members of *Collinsella* (97.5 %), *Bifidobacterium* (90.1 %), *Lachnospiraceae* (37.6 %) and *Streptococcus* (49.5 %) wih foods of animal origin. Results of RDA are therefore in agreement with quantitative analysis summarized in Table 4. Furthermore, no significant association with dietary variables was found for the well represented band labeled 92.3 % and identified as genus *Bifidobacterium* representative.

RDA was further used for the assessment of potential associations among real-time PCR results and data obtained by the questionnaire. This analysis revealed that the abundance or ratio (%) of particular bacterial groups of fecal microbiota determined by real-time PCR was not

significantly associated with the consumption of particular foods (Fig. 2). Affiliation of participants to vegetarians was, however, best associated with the higher ratio of *F. prausnitzii*. It is worth mentioning that the *Faecalibacterium*-specific DGGE band (43.9 %) was one of the bands with highest incidence, and the most intense band found in 60 fecal samples, but it was not significantly more abundant in vegetarians' fecal samples (Table 4).

Discussion

In our study, the dietary type, assumed as one of the most important factors shaping gut environment, was examined in relation to fecal microbiota of 60 individuals by real-time PCR and PCR–DGGE.

Real-time PCR relative quantification of fecal microbiota of the participants in this study revealed significant differences in regard to dietary type (vegetarian or omnivorous). In addition to well-known abundant groups of gut bacteria such as *C. coccoides* group (*C.* cluster XIVa), *C. leptum* group (*C.* cluster IV) and *Bacteroides–Prevotella* group, also the family *Enterobacteriaceae* as common human intestines inhabitants and genus *Bifidobacterium* as a typical representative of "beneficial microbes" were investigated. Furthermore, three species common representatives of gut bacteria were also included, i.e., *B. thetaiotaomicron*, *F. prausnitzii* and *C. clostridioforme*.

When the results of bacterial quantification studies are compared, it should be considered that the real-time PCR quantification in this study allows for relative quantification only, since the results are presented as the number of *16S rDNA* copies and cannot be directly translated to the number of cells or cfu of particular microbial groups. This is partly due to the heterogeneities in ribosomal operon number in different microbes and partly due to other factors, such as PCR amplification of DNA originating from non-viable cells, more than one genome equivalent present in exponentially growing cells, limited efficiency and biases of DNA extraction, purification and PCR amplification [24]. Despite these limitations, the results obtained are in accordance with results obtained previously in studies applying the same approach [25–27].

In our study, the vegetarian diet was associated with higher ratio (% of group-specific DNA in relation to all bacterial DNA) of *Bacteroides–Prevotella* and lower ratio (%) of *Clostridium* cluster XIVa. Both observations are in accordance with previous reports; in a study on young women from southern India, the fecal levels of *Clostridium* cluster XIVa were lower in the vegetarians compared with the omnivore group [17]. Higher abundance of *Bacteroides* group in vegetarians compared to omnivores was reported by Liszt et al. [18], but in contrast to our results, no significant differences were observed in *Clostridium* cluster XIVa. De Filippo et al. [28] also reported the significant enrichment in *Bacteroidetes* and depletion in *Firmicutes* in African children whose diet was based on cereals, legumes and vegetables and was rich in carbohydrate, fiber and non-animal proteins. The bacteria belonging to genus *Bacteroides* are known to produce short-chain fatty acids and may thus contribute against gut inflammation [29]. In a recent study of Mai et al. [30] carried out on two human groups with significantly different dietary habits, i.e., African Americans and Caucasian Americans, higher amounts of *Clostridium* cluster XIVa were found to be associated with higher intake of heterocyclic amines, which are typically formed during prolonged cooking or barbequing of meat and are considered as possible carcinogens for humans. The higher intake of heterocyclic amines was found in a group of African Americans which in comparison with Caucasian Americans suffer from an increased incidence and mortality by colorectal cancer. In our study, higher proportion of *Clostridium* cluster XIVa in the feces of omnivores may thus also be associated with meat intake in this group. An increased abundance of *Clostridium* cluster XIVa was observed previously also in fecal samples of IBS patients [8, 31].

Furthermore, the higher proportion of *F. prausnitzii* ratio (%) (cells vs. all bacteria *16S rDNA* copies) observed in vegetarian group may be related to positive effects on health too, as anti-inflammatory properties of this species have been suggested in previous studies [32]. *F. prausnitzii* is one of the most abundant butyrate producers in the human feces [33]. It is assumed that butyrate, which is produced mainly during colonic fermentation of non-digestible nutrients (dietary fibers) by gut microbiota, serves as an energy source for the colonocytes and may have a protective role against cancer and ulcerative colitis [34]. Benus et al. [35] demonstrated in their study on healthy volunteers consuming normal diet a strong association between *F. prausnitzii* and butyrate production. This species was more abundant in samples from individuals on the raffinose diet and the chickpea diet compared to the control diet [36]. Dietary fiber-free diet applied in the same study was accompanied by lower numbers of butyrate-producing bacteria belonging to *F. prausnitzii* and *Roseburia* groups [35]. Inulin intake was also associated with the increased the numbers of *F. prausnitzii* and genus *Bifidobacterium* [37].

Another observation of our study which may be considered health promoting was increased proportion of *B. thetaiotaomicron* in vegetarian group, as this species has been proposed to represent important fiber degrading members of the gut microbiota due to its use of cell envelope-associated multiprotein systems [38, 39].

Wu et al. [40] discovered a strong association of long-term dietary type to different enterotypes, i.e., different types (clusters) of fecal microbial community. The

enterotype associated with the consumption of protein and animal fat was rather surprisingly characterized primarily by higher levels of members of the genus *Bacteroides*, whereas carbohydrate-rich diet leaded to enterotype characterized by the genus *Prevotella*. Authors highlighted the importance to determine in further studies whether individuals with the *Bacteroides* enterotype have a higher incidence of diseases associated with a Western diet and whether the health may be improved by long-term dietary interventions. The results of the present study cannot be directly compared with observations of Wu et al. [40] since we did not quantify genera *Bacteroides* and *Prevotella* separately, but as a common group comprising the representatives of both genera. Further studies should reveal whether long-term vegetarian diet could lead to development of a particular enterotype which would ideally be protective against diseases associated with Western diet (too much proteins of animal sources, too much saturated fats, too low intake of fibers…).

Some previous studies investigating the effect of vegetarian diet on human fecal gut microbiota have already shown significant shifts in the level of individual bacterial groups associated with diet [16–19, 41]. Zimmer et al. [19] compared the microbial composition of feces of 144 vegetarians and 105 vegans and a similar number of subjects with a mixed diet, using conventional enumeration on selective agar plates. Vegans had in contrast to our study a significantly lower concentration of *Bacteroides* sp., *Bifidobacterium*, *E. coli* and *Enterobacteriaceae* numbers were also lowerin comparison with specimens on a mixed diet. Peltonen et al. [41] evaluated the changes in fecal microbiota of vegans due to consumption of raw vegan food on the basis of measuring the gas–liquid chromatographic fatty acid profiles of bacterial cells. Kabeerdoss et al. [17] reported an increase in relative abundance (quantitative PCR) of clostridial cluster XIVa and butyril-CoA CoA-transferase genes in omnivores (24) compared to vegetarians (32).

A number of studies focused toward revealing the connection between the development of certain gut chronic diseases and diet in recent years, and the results have been accumulating. The so-called Western diet, characterized by high intake of fat and protein but low intake of fruits and vegetables, is apparently associated with the increase in inflammatory bowel diseases. Intake of dietary fibers, fruits and vegetables was associated with the decrease in the risk of ulcerative colitis and Crohn's disease, whereas intake of fats and meat in general increased it [42]. Restricted ingestion of proteins of animal origin, fats and sugars, and their coordinated substitution with grains, vegetables and fruits could thus offer a systematic solution in disease prevention [42]. Protective role of the vegetarian diet can also be attributed to the development of beneficial microbiota which is able to metabolize fermentable fibers and produce lactate and short-chain fatty acids which have been recognized as anti-inflammatory metabolites [29]. Two-year clinical trial with semi-vegetarian diet characterized by a limited ingestion of animal foods (meat restricted to once every 2 weeks, and fish once a week) and foods known to be risk factors for IBD (sweets, bread, cheese, margarine, fast foods, carbonated beverages, juices) gave the encouraging results in preventing relapse in patients with Crohn's disease [14]. The increased proportions of *Bacteroides–Prevotella* group, *B. thetaiotaomicron* and *F. prausnitzii* in the feces of vegetarian subjects in our study are thus in accordance with proposed effects of the vegetarian diet.

The results of relative quantification of fecal microbiota by real-time PCR and by PCR–DGGE in our study were associated not only with the dietary type, but to certain extent also with other characteristics such as gender, age, body mass and consumption of particular foods. At this point of discussion, it should be emphasized that beside the dietary and demographic variables presented, this observational study did not exclude the possibility of additional variables such as particular lifestyle choices to contribute to 4 % of the explained microbial community variance. However, these are the common limitations of human observational studies which cannot be completely avoided. Smoking as lifestyle factor coinciding with omnivorous diet was preliminary investigated (registered in 21 % of omnivores (data not shown)) though not addressed in this paper focused on dietary factors alone since its explanatory power was much lower compared to dietary variables discussed in this manuscript.

Irrespective of the studied dietary groups of volunteers, the abundance of bifidobacteria (*16S rDNA* copies/g and %) decreased with age ($p < 0.01$), too (Table 5). In the past years, a decrease in the abundance of bifidobacteria in the gut as well as a decrease in species diversity in the gut in relation with age has been shown [45, 46]. However, as already pointed out by Biagi et al. [45], more recent studies in which fecal microbiota was analyzed by molecular, i.e., culture-independent techniques are less consistent regarding the prevalence of bifidobacteria in different life stages [8, 47, 48].

One of the observations was a higher concentration and ratio of *Enterobacteriaceae* (*16S rDNA* copies/g and %) in female participants (Supplemental Table 5). The differences in pH of the stool have been observed in omnivorous adults and children [43, 44], and it was suggested that the higher stool pH in women might be the result of a longer bowel transit, and more effective absorption of short-chain fatty acids due to the prolonged time. As for *Enterobacteriaceae* higher pH values are known to be favorable (>6.5), the higher amount of *Enterobacteriaceae* in females in our

study may actually be the consequence of higher pH of their stool [19]. As the *Enterobacteriaceae* abundancy (*16S rDNA* copies/g and %) was negatively correlated with body mass and height ($p < 0.05$ and $p < 0.01$), too (Table 5), it may be related to gender-specificity.

DGGE bands identified by sequencing (Supplemental Table 4) were derived from bacteria identified as members of four phyla, *Bacteroidetes* (1/26), *Firmicutes* (13/26), *Proteobacteria* (2/26) and *Actinobacteria* (10/26) which coincides with the phyla determined as dominant in majority of studies, for example in a study on 17 human fecal DNA samples based on metagenomic 16S rRNA sequencing [5]. The more detailed taxonomic assignment of the bands (*Bacteroides*, *Eubacterium*, *Faecalibacterium*, *Ruminococcaceae*, *Bifidobacteria*, *Lachnospiraceae*) also coincided with the prevalent or dominant genera/families [5]. In another recent study on 200 individuals, the OTUs in the stool belonging to *Lachnospiraceae*, *Ruminococcaceae* and *Bacteroidaceae* families were proposed as the members of the so-called core microbiome. Among them, *Faecalibacterium*- and *Bacteroides*-specific OTUs were commonly found, like in our study [3]. *Faecalibacterium*, *Bifidobacterium*, *Ruminococcus*, *Bacteroides* and *Eubacterium* were also found among the most abundant genera in the fecal samples of 22 children (11–18 years old) and 10 adults (22–66 years old) [49]. In a study of fecal microbiota of 15 healthy Finnish subjects examined using the HITChip microarray analysis, representatives from *Ruminococcaceae* (*Ruminococcus*), *Faecalibacterium*, *Lachnospiraceae* (*Lachnospira*), *Streptococcus* and *Colinsella* were identified as the members of the core microbiota [50]. The comparison of the DGGE results from our study with results from the above-mentioned studies indicates that although much less powerful from the modern massive sequencing and microarray approaches, the PCR–DGGE analysis combined with band sequencing makes possible the identification of the common representatives of fecal microbiome.

Very high individual variability was reported in different previous studies of fecal microbiota. Tap et al. [5] reported 78.6 % of OTUs to be individual specific. In their study on 9 omnivorous and 8 vegetarian volunteers, only about 5 % of the variability of fecal microbiota was explained by the dietary type (vegetarian vs. omnivorous), which is in agreement with our observations made by the DGGE.

The absence of clear association of particular foods consumption and real-time PCR quantification of selected bacterial groups was not surprising. It may be partially explained by non-quantitative dietary intake assessment approach based only on the inclusion/exclusion criterion. Of course, the detailed analysis of the nutritional composition of individual subject's diets in the omnivore and vegetarian groups would only enable detailed analyses of particular effects. However, this was outside the scope of this study.

Conclusions

Our results of the analyses of fecal microbiota of 60 healthy subjects showed shifts in the quantity and diversity of their fecal microbiota in association with a single parameter—omitted or restricted ingestion of food of animal origin, occurring regardless of the individual's age, gender, body mass or social status. It would be too speculative to conclude that gut microbiota was clearly enriched in what may be described as beneficial bacteria; however, the study provided some indications such as the increased abundance of *F. prausnitzii*- and *Bacteroides–Prevotella* group-specific DNA in vegetarian group.

Although fecal microbiota DGGE profiles did not cluster in accordance to the dietary types (vegetarian or omnivorous), specific bands were more abundant among omnivores (*Bifidobacterium*, *Streptococus*, *Collinsella* and *Lachnospiraceae* representatives) or vegetarians (*Subdoligranuluim*), suggesting the association between dietary type and fecal microbial community. Due to high individual variability also observed in previous studies, only about 4 % of the explained variance in microbial community analyzed by DGGE was not surprising.

Consumption of foods of animal origin (eggs, red meat, white meat, milk, yoghurt, other dairy products, fish and seafood) and vegetarian type of diet, however, explained the largest share of the variance in microbial community structure.

The DGGE bands identified by sequencing (*Bacteroides*, *Eubacterium*, *Faecalibacterium*, *Ruminococcaceae*, *Bifidobacteria*, *Lachnospiraceae*) coincided with the dominant taxonomic groups identified in previous studies, showing the remaining usefulness of the DGGE method. Among the associations between demographic characteristics and fecal microbiota in our study group, it is worth to mention that bifidobacteria (*16S rDNA* copies/g and %) decreased with age and, interestingly, *F. prausnitzii* fecal concentration increased with height and was higher in males. The association of particular foods consumption and PCR quantification of selected bacterial groups was not found.

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Conflict of interest The authors declare that they have no conflict of interest.

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