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ORIGINAL ARTICLE

A vegan or vegetarian diet substantially alters the human colonic faecal microbiota

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Background/Objectives: Consisting of $\sim 10^{14}$ microbial cells, the intestinal microbiota represents the largest and the most complex microbial community inhabiting the human body. However, the influence of regular diets on the microbiota is widely unknown.
Subjects/Methods: We examined faecal samples of vegetarians ($n=144$), vegans ($n=105$) and an equal number of control subjects consuming ordinary omnivorous diet who were matched for age and gender. We used classical bacteriological isolation, identification and enumeration of the main anaerobic and aerobic bacterial genera and computed absolute and relative numbers that were compared between groups.
Results: Total counts of *Bacteroides* spp., *Bifidobacterium* spp., *Escherichia coli* and *Enterobacteriaceae* spp. were significantly lower ($P=0.001$, $P=0.002$, $P=0.006$ and $P=0.008$, respectively) in vegan samples than in controls, whereas others (*E. coli biovars*, *Klebsiella* spp., *Enterobacter* spp., other *Enterobacteriaceae*, *Enterococcus* spp., *Lactobacillus* spp., *Citrobacter* spp. and *Clostridium* spp.) were not. Subjects on a vegetarian diet ranked between vegans and controls. The total microbial count did not differ between the groups. In addition, subjects on a vegan or vegetarian diet showed significantly ($P=0.0001$) lower stool pH than did controls, and stool pH and counts of *E. coli* and *Enterobacteriaceae* were significantly correlated across all subgroups.
Conclusions: Maintaining a strict vegan or vegetarian diet results in a significant shift in the microbiota while total cell numbers remain unaltered.
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Keywords: colonic microbiota; faecal microbiota; diet; vegetarian; vegan; gender

Introduction

With an estimated 10^{14} bacterial cells, the intestinal microbiota outnumbers the human somatic and germ cells by a factor of 10. This multitude indicates its undisputed importance to host physiology: first, it forms a microbial barrier against implantation and augmentation of pathogenic or potential pathogenic organisms such as *Clostridium difficile* and *Salmonella*. This function is partly fulfilled by anaerobic species like *Bacteroides* and *Bifidobacterium*, but *Lactobacillus*, *Escherichia coli* and *Enterococcus* species also contribute to the barrier microbiota. This feature called 'colonisation resistance' (Van der Waaij, 1984) is not based on one single mechanism, but is rather described as a variety of different mechanisms complementing one another: first of all, the dominant microbiota inhibits colonisation of pathogens by occupying mucosa receptors and dense population of the superimposed mucin layer (Savage, 1977). A second strategy is based on releasing bacteriostatically or microbicidally acting substances (such as short-chain fatty acids, hydrosulphide, hydrogen peroxide, antibiotics), which additionally inhibit the growth of pathogenic germs (Hentges, 1983). Other products released by *Bifidobacterium* and *Lactobacillus* species, for instance, lactic acid or acetic acid, decrease the pH value. Considering chemical mechanisms, the oxygen partial pressure is reduced and a low redox potential of −150 mV in the terminal ileum and −250 mV in

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the colon and caecum is maintained. Finally, there is a competition for nutrients, vitamins and growth factors additionally contributing to the barrier microbiota (Gorbach, 2000).

During the 1970s, epidemiological studies indicated a link between diet and colorectal cancer. As a consequence, intensive culture-based research examined the ability of diet to alter the composition of the intestinal microbiota. Among these studies were some small-scale investigations of the importance of maintaining a vegetarian diet (Noack-Loebel *et al.*, 1983; Peltonen *et al.*, 1997). Although these attempts resulted mostly in inconsistent findings (Aries *et al.*, 1969), more recently the intestinal microflora was linked to immune (Belkaid *et al.*, 2010) and autoimmune diseases (Tjellström *et al.*, 2005), metabolic disorders (Serino *et al.*, 2009) and inflammatory (Macfarlane *et al.*, 2009) and functional gastrointestinal disorders (Kassinen *et al.*, 2007). Lately, it was pointed out that the colonic microbiome may be a contributing factor to obesity in mice (Turnbaugh *et al.*, 2006) and humans (Ley *et al.*, 2006), and other eating disorders have been proposed as well (Armougom *et al.*, 2009).

One reason for conflicting data in the literature may be the use of inappropriate control groups (CGs). CGs are usually small and have—if at all—only been poorly matched with various subjects under investigation. For example, age-related and gender differences in the quantity of certain intestinal bacteria have been demonstrated in earlier studies by us (Enck *et al.*, 2009a, b) and by others, but are widely ignored when CGs are composed.

We analysed the faecal flora of a large group of healthy volunteers on a strict vegetarian or vegan diet with classical microbiological culture techniques. We collected these data from volunteers attending the World Vegetarian and Vegan Congress 2008 and compared them with an equal number of subjects—matched for age and gender—on an omnivorous diet.

Materials and methods

Collection of stool samples for microbiological analysis

Our test group consisted of volunteers on a strict vegetarian or vegan diet who were approached during the 38th World Vegetarian and Vegan Congress in Dresden, Germany, between 27 July and 3 August 2008.

Subjects were contacted by booth staff present during the conference (JZ and BL) and asked for participation. After oral information, subjects were given an envelope with a questionnaire (see additional data), a consent form and a stool sampling kit to be returned. The completed forms and stool samples were usually returned the subsequent morning, labelled with barcodes and sent to the company that routinely conducts such microbiological analyses at a commercial setting (Institute of Microecology, Herborn, Germany) on the very same day (see below). In general, samples reached the laboratory within 24 h day and were processed immediately. Samples were not frozen and thawed before analysis.

Subjects who acknowledged being on an omnivorous diet, using antibiotics currently or during the preceding month, with regular intake of drugs, and chronic diseases, specifically inflammatory bowel diseases were excluded from further analysis. A vegetarian's diet was assumed when subjects acknowledged not to consume meat in any form but to eat animal products such as milk, cheese and eggs. A vegan diet would also exclude such animal products. Both groups were required to be on their diet for at least 4 weeks.

Complete data were available from 144 subjects on a vegetarian diet (49 males and 95 females) and from 105 subjects (45 males, 60 females) on a strict vegan diet.

With regard to control subjects, we obtained two samples

Control group 1. Faecal samples analysed routinely by the Institute of Microecology, during 1 year (2006) were made available to the investigators. From the total of 35 000, two random samples of 144 and 105 subjects were drawn using age and gender of the respective test samples (vegans, vegetarians) as key variables. Samples drawn were matched to the vegan and vegetarian groups (comparison 1, see Figure 1).

As we have shown previously (Enck *et al.*, 2009a, b), large samples allow to estimate the 'normal' flora despite the fact that it derived from patients, as disease-specific alterations

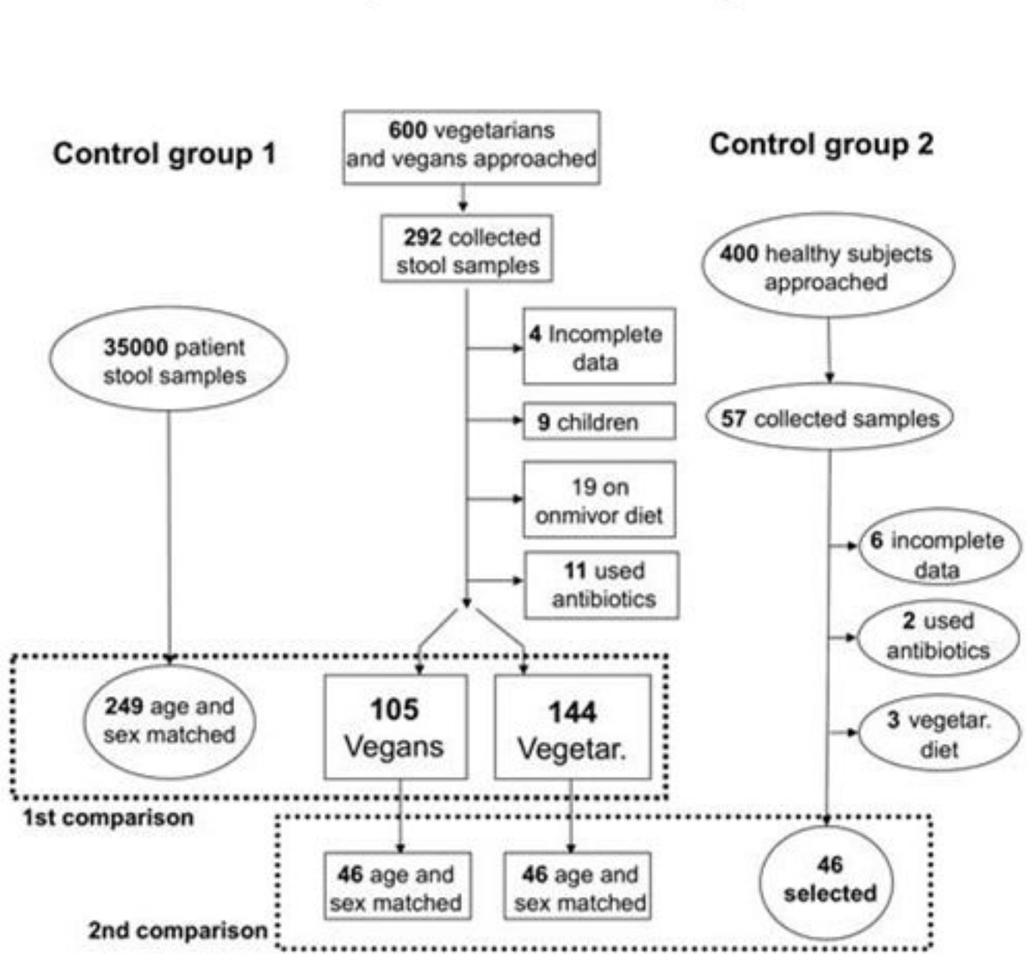

Figure 1 Recruitment of study samples from different cohorts: Control group 1 (CG1) was drawn from 35 000 adult patient stool samples from the database of the company that analysed the samples for commensal bacteria (Institute of Microecology), control group 2 (CG2) was collected during a gastroenterology meeting in Hamburg, Germany. Vegan and vegetarian subjects were recruited during the 38th World Vegetarian Congress in Dresden, Germany 2008.

are averaged out across different diseases and clinical conditions. Therefore, any random selection from the total sample should mirror the distribution of the total sample, however, with larger s.d. To further test this hypothesis, we collected data from a second and truly healthy CG.

Control group 2. Now, CG2 consisted of volunteering attendants of the 64th Congress of German Gastroenterology Society (DGVS) meeting in Hamburg, Germany, between 30 October and 3 September 2009. Subjects were contacted by booth staff present during the conference (JZ, BL and HS) and asked for participation. After oral information, subjects were given an envelope with a questionnaire (see additional data), a consent form and a stool sampling kit to be returned. The completed forms and stool samples were usually returned the subsequent morning, labelled with barcodes and sent to the company that routinely conducts such microbiological analyses at a commercial setting (Institute of Microecology) on the very same day (see below).

After exclusion of individuals on vegetarian and vegan diet, individuals who were on antibiotics during the preceding month, used drugs regularly, or had chronic diseases, specifically inflammatory bowel diseases; complete data were available from 46 subjects (28 males and 18 females).

To account for the different age and gender composition of CG2 in comparison with the vegan and vegetarian groups, two random samples of 46 vegans and 46 vegetarians were drawn from all test subjects and matched for age and gender to controls (comparison 2, Figure 1)

Identification and enumeration of microorganisms

All faecal samples were sent for routine microbiological analysis of non-pathogen faecal bacterial flora (KyberStatus) to the Institute of Microecology. In general, samples reached the laboratory within 1 day and were processed immediately.

To ensure that the transport did not have any effects on the cultured species, a storage study was performed with 20 fresh samples. In short, 0.2 g of faeces was serially diluted in 1 ml phosphate-buffered saline (pH 7.2). The solution was vortexed for 5 s and serially diluted (to 109) in phosphate-buffered saline, pH 7.2. In all, 1 ml of each dilution was plated onto enrichment or selective agar media. The remaining faeces were stored for 3 days at a temperature of 25 °C, which represents the average temperature during shipment. After the incubation period, samples were processed as described and results compared. No significant discrepancy in cell counts of the investigated microbiota could be detected within 2 days. Thus, it was concluded that a shipment of <2 days will have no effect on the composition of the culturable microbiota (Enck *et al.*, 2009a, b).

Viable bacterial cell counts in faeces were enumerated on the following selective media: Columbia blood agar (total cell count; BioMerieux, Nürtingen, Germany), U3G agar (enterobacteriacae, enterococci; Heipha, Heidelberg, Germany), Rogosa agar (lactobacilli; Heipha), DIC agar (bifidobacteria; Heipha), Schaedler agar (*Bacteroides*; Heipha) and SPM agar (clostridia; Heipha). Faecal samples were serially diluted in 1 ml phosphate-buffered saline (pH 7.2) and subsequently plated on selective agar plates using a fully automated spiral plater capable of plating 12 agar plates simultaneously. Subsequently, the plates were incubated under either aerobic or anoxic conditions at 37 °C for at least 2 days. Bacteria were first identified by Gram staining and colony morphologies. In addition, identifications were performed by the API and VITEK systems (BioMerieux). All counts were recorded as the numbers of log 10 colony-forming units per ml of sample.

The following bacteria were routinely analysed: *Clostridium* sp., *Bifidobacteria*, *Bacteroides* sp., subdominant (*E. coli*, *Enterococcus* sp., *Lactobacillus* sp.) and other bacteria (*Pseudomonas* sp., *Klebsiella* sp., *Proteus* sp., *Citrobacter* sp., aerobic bacteria). Only bacteria that were identified in at least 50% of the respective samples were included in further analysis.

Additional data

Together with the faecal samples, we obtained additional data using a short questionnaire including the following: age, gender, weight, height, duration of vegetarian or vegan nutrition, intake of antibiotics during the preceding month, general intake of drugs, chronic diseases, chronic inflammatory bowel diseases, alcohol consumption, consumption of milk and dairy products, intake of dietary supplements, stool frequency and consistency.

Stool pH was measured in the laboratory by manually placing a pH-sensitive probe in the faecal sample. Stool consistency was rated between 1 = solid and 5 = liquid by the same experienced person during pH measurement.

Statistical analysis

Data were analysed using the SPSS Version 13 Statistical Package (SPSS Inc., Chicago, IL, USA). We performed an ANOVA (analysis of variance) using age and gender as covariates, and unpaired *t*-tests for group comparison. Pearson's correlation coefficients were computed to test for inter-correlations between clinical data (pH, stool consistency) and bacterial species. Data are reported as mean ± s.e.m. A 1% rather than a 5% α-level was set to indicate statistical significance in all ANOVAs, to account for multiple testing with potentially dependent variables.

Results

Table 1 lists the characteristics of the two samples and the respective control samples.

Table 1 Characteristics of the four study samples (mean ± s.e.m.)

	Vegetarian		*Vegan*		*CG1*	*CG2*
	Complete	*Reduced*	*Complete*	*Reduced*	*Complete*	*Complete*
N	144	46	105	46	249	46
Age	56.75 ± 15.07	47.80 ± 12.09	49.24 ± 14.63	46.50 ± 12.62	53.71 ± 14.85	46.50 ± 12.26
M:F	49:95	28:18	45:60	28:18	94:15	28:18
Weight (kg)	65.73 ± 11.35	68.13 ± 13.95	65.19 ± 11.00	68.29 ± 12.04	NA	72.73 ± 12.84
BMI	23.08 ± 3.97	22.73 ± 3.51	22.30 ± 3.28	22.57 ± 3.42	NA	24.14 ± 3.42
TC	$2.47^{11} \pm 4.51^{11}$	$1.79^{11} \pm 3.73^{11}$	$3.03^{11} \pm 6.37^{11}$	$3.29^{11} \pm 7.30^{11}$	$3.09^{11} \pm 5.01^{11}$	$3.39^{11} \pm 4.71^{11}$
pH[a]	6.7 ± 0.7	6.6 ± 0.8	6.3 ± 0.8	6.3 ± 0.7	6.8 ± 0.04	6.9 ± 0.8

Abbreviations: BMI, body mass index; CG, control group; F, female; M, male; NA, not available; TC, total germ count.
[a]pH represents stool pH as determined in the laboratory.

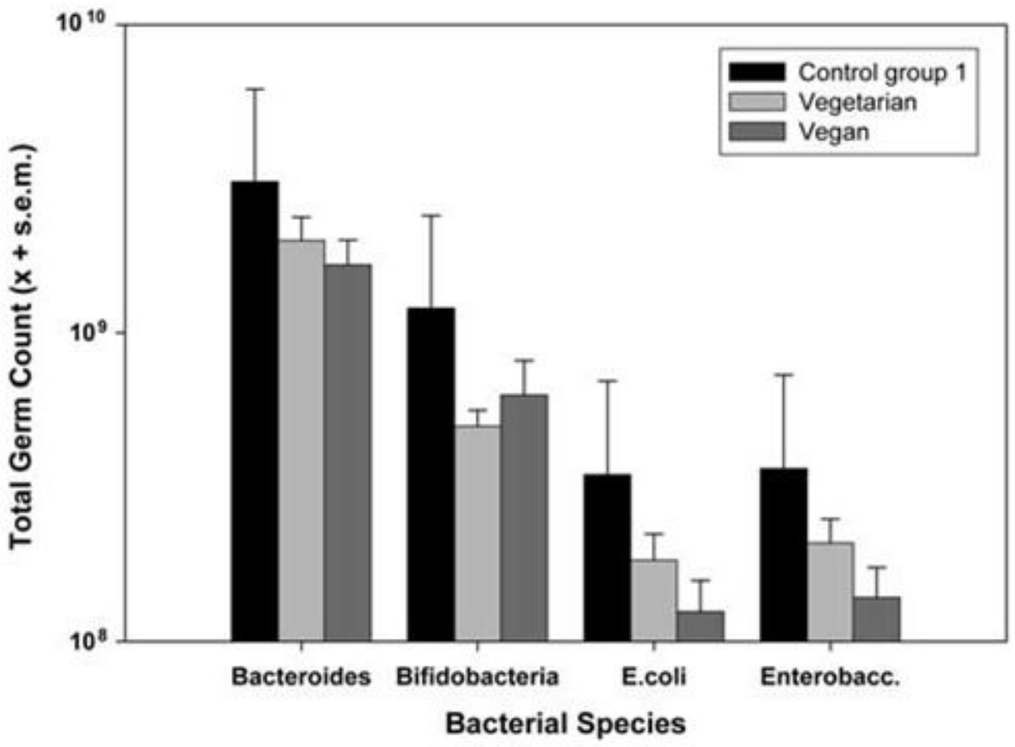

Figure 2 Total CFU (mean ± s.e.m.) of *Bacteroides* sp., *Bifidobacteria* sp., *E. coli* and *Enterobacter* sp. of the vegetarian ($n=144$) and vegan samples ($n=105$) and control group 1 ($n=249$) (see text for statistical comparison).

Vegetarians

The vegetarian sample consisted of 144 subjects (49 males and 95 females; age: 56.7 ± 0.9 (23–93) years). Analysis of the total viable count did not reveal any differences between the vegetarian group and CG1 and CG2 (Table 1).

Among the 14 bacterial species or genera, the following showed significant differences when compared with CG1: Vegetarians had significant lower microbial counts of *Bacteroides* ($P<0.001$) and *Bifidobacterium* ($P<0.001$) species (Figure 2).

The reduced vegetarian sample (for comparison 2) consisted of 46 subjects (28 male and 18 female subjects, 47.9 ± 1.9 (23–70) years). When this vegetarian sample was compared with CG2, lower microbial counts of *Bifidobacterium* ($P=0.046$), *Bacteroides* ($P=0.027$) and *E. coli* species ($P=0.053$) were detected that all did not reach significance.

With respect to *E. coli Biovare*, *Klebsiella*, *Enterobacter*, *Enterococcus*, *Enterobacteriaceae*, *Lactobacillus*, *Citrobacter* and *Clostridia* species, no significant differences were detected between the vegetarian sample and either CG1 and CG2. Microbial counts of *Pseudomonas* and *Proteus* species and aerobic bacteria were too small and therefore no comparisons were made.

Vegans

The vegan sample 1 consisted of 105 subjects (45 males, 60 females, 49.4 ± 1.0 (22–85) years). Analysis of the total viable count did not reveal any differences between the vegans and CG1 and CG2 (Table 1).

Subjects on a vegan diet had significantly lower *Bacteroides* ($P=0.001$), *Bifidobacterium* ($P=0.002$), *E. coli* ($P=0.006$) and *Enterobacteriaceae* ($P=0.008$) species than CG1 (Figure 2).

The reduced vegan sample (for comparison 2) consisted of 46 subjects (28 males and 18 females, 46.7 ± 1.9 (22–69) years). When this vegan sample was compared with CG2, a significantly lower count was found for *Bifidobacterium* species ($P=0.002$). The vegan test group also showed lower microbial counts of *Bacteroides* ($P=0.038$), *Enterobacteriaceae* ($P=0.048$) and *E. coli* species ($P=0.053$) than CG2 that did not reach significance.

With respect to *Enterobacter*, *Enterococcus*, *Clostridium*, *Klebsiella*, *Lactobacillus*, and the total viable count, no significant differences were observed. As before, statistical analysis regarding *Pseudomonas*, *Proteus*, *Citrobacter*, *Aerob A* and *B* species was not performed because of the small numbers of cases.

Figure 2 shows the mean bacterial counts for all subjects from CG1 ($n=249$) to all vegans ($n=105$) and all vegetarians ($n=144$), and Table 2 summarises the results of the statistical comparison of the two test samples (vegans and vegetarians) with the two control samples (patients and healthy controls). As is evident, the number of subjects in the reduced samples of vegans and vegetarians was too small to reach significance, except for bifidobacteria comparing vegans with CG2.

Vegans versus vegetarians

All four bacterial species that were found to be different between vegans or vegetarians and the respective controls

Table 2 Results of the comparison of the full and the reduced vegan and vegetarian samples with the respective control groups (CG1, CG2) for four different bacterial strains

	Vegan/CG1 N = *105/105*	*Vegan/CG2* N = *46/46*	*Vegetarian/CG1* N = *144/144*	*Vegetarian/CG2* N = *46/46*
Bacteroides	***P*<0.001**	*P*=0.038 (NS)	***P*<0.001**	*P*=0.027 (NS)
Bifidobacteria	***P*<0.001**	***P*=0.002**	***P*<0.001**	*P*=0.046 (NS)
Escherichia coli	***P*=0.006**	*P*=0.053 (NS)	NS	*P*=0.053 (NS)
Enterobacter	***P*=0.008**	*P*=0.048 (NS)	NS	NS

Abbreviations: CG, control group; NS, not significant.
Bold values indicate significant differences ($P<0.001$). It must be noted that *P*-values between 0.01 and 0.05 were regarded as NS because of multiple testing.

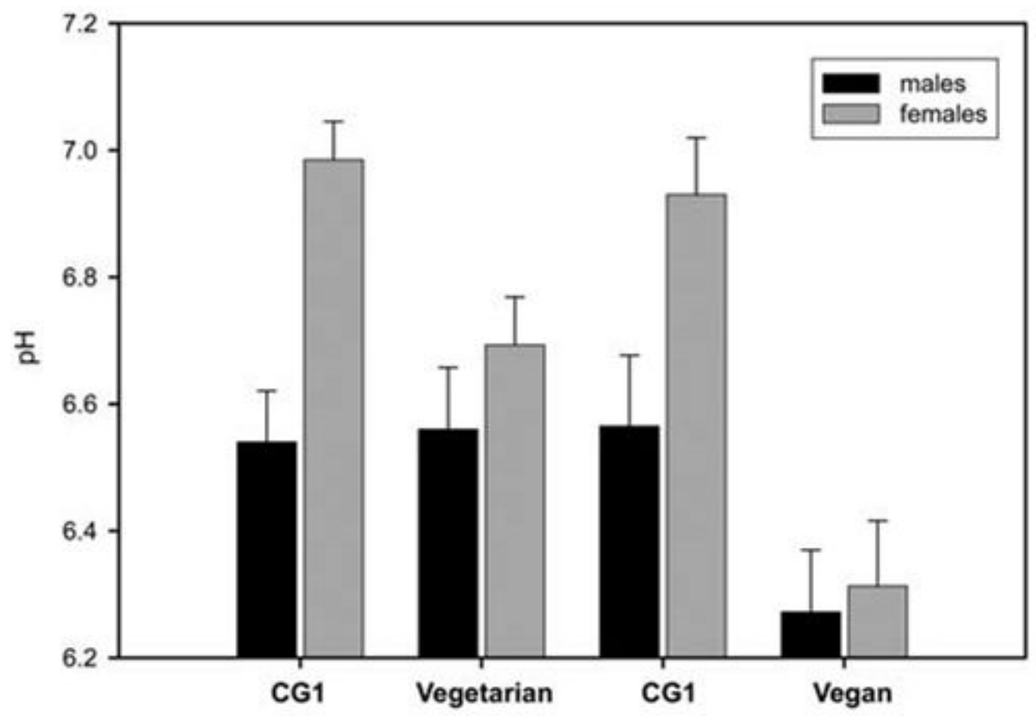

Figure 3 Stool pH in vegan ($n=105$) and vegetarian subjects ($n=144$) as compared with their respective controls from CG1, separated by gender.

samples (see Figure 2) were not significantly different between vegans and vegetarians. Neither of the other bacteria (*Enterobacter, Enterococcus, Clostridium, Klebsiella, Lactobacillus*) revealed any difference between both. As before, statistical analysis regarding *Pseudomonas, Proteus, Citrobacter, Aerob A* and *B* species was not performed because of the small numbers of cases. The total viable count also was similar between both groups (Table 1).

Clinical data

Stool pH and dietary habits. Vegetarians had similar stool pH than did both CGs (Figure 3), with females in all groups showing a significantly higher pH than males. Both the total vegan sample (pH = 6.3 ± 0.8) and the reduced vegan sample (pH = 6.3 ± 0.8) had a significantly ($P=0.001$) lower stool pH value than did CG1 (pH = 6.8 ± 0.1) and CG2 (pH = 6.9 ± 0.8) with no gender differences.

Stool pH and bacteria

As can be seen in Table 3, strong positive correlations exist between stool pH and the counts of two bacterial strains (*E. coli* and *Enterobacteriaceae*) across all samples and subsamples, indicating dependency of these strains on a specific intestinal milieu irrespective of the eating habit. Only CG1 (that was composed of a random sample of patients with various clinical diagnoses (18)) also showed a weak but negative correlation of *Bifidobacteria* and *Bacteroides* species with stool pH.

Stool consistency

Analyses of stool consistency did not show significant differences between our vegetarian and vegan test subjects and both CGs, and was not different between genders (data not shown).

Discussion

Diet is an obvious factor influencing intestinal bacteria. However, previous studies of the faecal microbiota using conventional microbiological methods in populations underlying different dietary habits revealed only moderate differences, possibly because only a limited number of individuals were included in these studies. Here, we reported data from two larger cohorts (250 subjects on a strict vegan or vegetarian diet) under the assumption that inter-individual variations in the microbiota composition may be minimised and subtle dietary influences on the intestinal microbiota of both the test and the CG can be identified. We included a similar number of controls on an omnivorous diet, carefully matched for age and gender.

Our data are only in part in agreement with the published literature. In our study, the faecal microbiota of vegetarian and vegan test subjects showed significant lower microbial counts of *Bifidobacterium* species; vegetarians and vegans also exhibit lower microbial counts of *Bacteroides* species. These observations have previously been reported when faecal samples from English people, consuming a mixed western diet, were compared with Africans from Uganda, consuming a high-carbohydrate vegetarian diet (Aries *et al.*, 1969). Others (Finegold *et al.*, 1974) found similar effects regarding *Bacteroides*, yet the differences observed were not statistically significant. Another study (Maier *et al.*, 1974) indicated a

Table 3 Pearson's correlation coefficients between the pH value and various bacterial counts (*Escherichia coli*, *Enterobacteriacea*, *Bifidobacterium* and *Bacteroides species*) in vegetarian and vegan subjects and control groups

pH	*Vegetarian*		*Vegan*		*CG1*	*CG2*
	N = *144*	N = *46*	N = *105*	N = *46*	N = *249*	N = *46*
Escherichia coli	**$R = 0.331$** **$P < 0.001$**	**$R = 0.323$** **$P = 0.045$**	**$R = 0.395$** **$P < 0.001$**	**$R = 0.374$** **$P = 0.017$**	**$R = 0.213$** **$P = 0.002$**	**$R = 0.470$** **$P = 0.002$**
Enterobacteriaceae	**$R = 0.362$** **$P < 0.001$**	**$R = 0.324$** **$P = 0.042$**	**$R = 0.392$** **$P < 0.001$**	**$R = 0.358$** **$P = 0.020$**	**$R = 0.198$** **$P = 0.004$**	**$R = 0.445$** **$P = 0.002$**
Bifidobacterium	$R = 0.050$ $P = 0.599$	$R = -0.022$ $P = 0.899$	$R = -0.026$ $P = 0.824$	$R = 0.207$ $P = 0.239$	**$R = -0.299$** **$P < 0.001$**	$R = 0.063$ $P = 0.719$
Bacteroides	$R = 0.006$ $P = 0.943$	$R = 0.000$ $P = 0.998$	$R = 0.156$ $P = 0.130$	$R = 0.187$ $P = 0.237$	**$R = -0.157$** $P = \mathbf{0.014}$	$R = 0.017$ $P = 0.914$

Abbreviation: CG, control group.
Bold values indicates significance ($P < 0.05$).

similar result with respect to *Bacteroides* species being elevated under conditions of a high-meat diet. However, this analysis was performed on only five test subjects and thus the result has to be interpreted with care.

The probably best-controlled study (van Faassen *et al.*, 1987) was conducted in 12 healthy male subjects during a 20-day cross-over trial under controlled metabolic-ward conditions. In this study, no effects of a mixed, a lacto-ovo-vegetarian and a vegan diet on anaerobic bacteria, specifically on *Bifidobacteria* and *Bacteroides* were found, but instead significantly lower counts of the aerobic strains *Lactobacilli* and *Enterococci* were found. In contrast, an earlier study (Noack-Loebel *et al.*, 1983) finally yielded opposite results regarding the *Bifidobacterium* species. They compared the composition of the faecal flora in two groups of children with (1) normal diet and (2) lacto-ovo-vegetarian diet. As a result, they noted that the numbers of *Bifidobacterium* species were significantly higher in the vegetarian test group. This study included only 20 children in each group, and the diet group received oral vaccines with non-pathogenic *Streptococcus faecalis* and *E. coli* in addition to their diet, which makes data of nutritional effects on the faecal microbiome unreliable.

The study by Peltonen *et al.* (1997) used a short-term vegan diet (for 1 month) in nine adult male and female subjects, whereas nine control subjects stayed on an omnivore diet for the same time. They found a significant decrease in bacterial cellular fatty acids with the vegan diet, but could not attribute this (because of their technique used, gas liquid chromatography) to changes in specific bacterial flora. When the same diet was applied to patients with rheumatoid arthritis for 1 year (Peltonen *et al.*, 1994), a similar effect was found, associated with an improvement in clinical symptoms. Again, no specific bacterial strains could be made responsible, but clinical effects have been observed in an earlier 3.5-month trial in arthritis patients (Kjeldsen-Kragh *et al.*, 1991). In the same patient group, a 'Mediterranean diet' (that consisted of fruit and vegetables daily, the abundant intake of whole grain bread, pasta and rice, fish and the exclusive use of olive oil) resulted in no change in the bacterial microbiota after 8 days, but confirmed clinical benefit (Michalsen *et al.*, 2005) that may therefore be attributable to placebo responses (Enck and Klosterhalfen, 2005).

No data have been reported previously regarding our finding of significantly reduced *E. coli* and *Enterobacteriaceae* in vegetarians and vegans compared with controls, and with respect to lowered stool pH in dieting subjects. Although correlations between bacterial counts and stool pH are low (and explain only 10–15% of the data variance), they are consistent across all groups and therefore indicate a diet-independent effect that has also been observed previously in a large cohort of both paediatric and adult patients (Enck *et al.*, 2009a, b).

Maintaining a vegan diet is associated with significantly higher consumption of carbohydrates (45% carbohydrates in omnivores compared with 59% in vegans) and higher fibre content (Haddad *et al.*, 1999), which is responsible for lower stool pH in the vegan population. The degradation of dietary fibres by exoenzymes mainly leads to greater amount of short-chain fatty acids such as acetate, propionate and butyrate that create a slightly acidic milieu with values between pH 5.5 and 6.5. This effect may have been amplified by germs that grow because of the large amount of fibres. These pH ranges do not support bacteria such as *E. coli* and *Enterobacteriacea* in their growth as they prefer pH ranges >6.5 (Adler, 1973). Therefore, the significantly lower stool pH through augmented metabolites in vegans is caused by increased fibre intake, and the dietary habit may directly be responsible for lower counts of *E. coli* and *Enterobacteriacea*. In addition, *E. coli* and *Enterobacteriacea* prefer proteins as the main source of energy that explains their higher counts in vegetarians and omnivores.

In contrast, lower abundance of *Bifidobacteria* and *Bacteroides* species in vegans and vegetarians was not associated with stool pH directly and therefore needs another explanation.

Higher consumption of animal protein is one possible explanation for higher stool pH values in subjects on an

omnivorous diet, as proteolytic putrefactive bacteria are able to increase stool pH by producing alkaline metabolites. This speculation is strengthened upon closer examination of the mean pH values. The vegetarians' mean pH value of 6.6 is between that of vegans and of omnivores (6.3 and 6.9, respectively) (see Table 1). Thus, the vegetarian eating habit represents the link between the other two forms of diet. As has been shown previously, stool pH become more alkaline with increasing age and differs significantly between gender (Enck *et al.*, 2009a, b). In addition to age, gender and diet, factors such as microbial interaction, food transit through different intestinal compartments with different bacterial colonisation density, availability of nutrients, colonic supply, sulphate and bile acids and bacterial adaptation may all be involved in the composition and activity of colonic microflora.

This may help in understanding the lower abundance of *Bifidobacteria* and *Bacteroides* species in vegans and vegetarians, which was not linked to stool pH. One other explanation may be that *Bacteroides* and *Bifidobacteria* contributed a higher percentage of the total bacteria mass in the human colon, and fluctuations in the number of cells are less relevant than in bacteria with lower abundance such as *E. coli* and *Enterobacteriacea*. Thus, the lower counts of these species are possibly independent of the acidity of the milieu. However, disagreements in the literature and the conflicting findings in several studies show that the exact mechanisms still need to be explored in future mechanistic studies.

Probably the most relevant finding of our study is the differential effects of the (vegan) diet on stool pH: in healthy omnivorous subjects and in patients (both adults and children), women exhibited higher pH values than did men (Enck *et al.*, 2009a, b), despite the fact that women consume—on average—similar amounts of dietary fibres than do men. This may be due to differences in bowel transit which is longer in females and thus may allow more time for metabolism and absorption of short-chain fatty acids. When both men and women maintain a strict vegan diet rich in fibres for prolonged periods of time, both reduce their regular stool pH, and a difference between the genders cannot be found any longer (see Figure 3). This indicates that females profit more from maintaining a strict vegan diet than do men. However, this would also need independent and experimental proof.

A number of limitations of our study need to be acknowledged. First, conventional microbiological methods assess only a fraction of the currently known intestinal microbiota (Qin *et al.*, 2010). Other limitations refer to the question whether the faecal microbiota reflects the overall intestinal (mucosal) microbiome along the whole gastrointestinal tract (Eckburg *et al.*, 2006). The benefit of using faecal samples to investigate the intestinal microbiota is obvious: the samples are collected easily and test subjects do not have to suffer from adverse effects as they can occur after a colonoscopy. Compared with previous culture-based research, our study reports data obtained from a larger sample size in comparison with most studies before. In addition, our subjects consisted of long-term vegetarians and vegans and not test subjects normally consuming a mixed diet, which may lead to a more marked change in the colonic microbiome.

Conflict of interest

PE is a consultant of the company that conducted the stool culture analysis (SymbioPharm, Herborn, Germany), and KZ, AS and KR are employees of the company. The remaining authors do not report any potential conflict of interest.

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