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

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Running title: Diet effects on human colonic microbiome

Word count: Total: 3858, Abstract: 219, 3 Tables, 3 Figures, 28 references

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Abstract

Background/Objectives: Consisting of ~ 10^{14} microbial cells, the intestinal microbiota represents the largest and 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.*, *E. coli,* and *Enterobacteriaceae spp*. were significantly lower (p=.001, p=.002, p=.006 and p=.008, resp) in the vegan samples compared to controls while 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=.0001) lower stool pH than controls, and stool pH and counts of *E. coli* and *Enterobacteriaceae* were significantly correlated across all subgroups. Conclusion: Maintaining a strict vegan or vegetarian diets results in a significant shift in the microbiota while the total cell numbers remains unaltered.

Keywords: colonic microbiota, faecal microbiota, diet, vegetarian, vegan, nutrition

Acknowledgements:

We would like to express our gratitude to study volunteers of the 38th International Vegetarian Union (IVU) World Congress in Dresden, Germany who helped us to conduct this study, and to Mahi Klosterhalfen (Albert Schweitzer Stiftung für unsere Umwelt, Berlin, Germany) and Thomas Schoenberger (Vegetarier Bund, Hannover, Germany) for their logistic support.

Supported by a research grant from the interfaculty Centre of Nutritional Medicine of the Universities of Tübingen and Stuttgart-Hohenheim, Germany.

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

Introduction

With an estimated 10^{14} bacterial cells, the intestinal microbiota outnumbers the human somatic and germ cells by a factor of ten. 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 *C. difficile* and *Salmonella.* This function is partly fulfilled by anaerobic species like *Bacteroides* and *Bifidobacterium*, but also *Lactobacillus*, *E. coli* and *Enterococcus* species contribute to the barrier microbiota. This feature called "colonisation resistance“ (Van der Waaij, 1984) is not based on one single mechanism, but rather describes as a variety of different mechanisms complementing one another: first of all, the dominant microbiota inhibits the 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 (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, lower 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 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 micribiota. 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). While 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 men (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. Control groups are usually small and have – if at all – only been poorly matched with the various subjects under investigation. E.g. 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 others but are widely ignored when control groups 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 to 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 July 27th and August 3rd 2008.

Subjects were contacted by booth staff present during the conference (JZ, BL) and ask for participation. After oral information, the 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 were and the stool sample were usually returned the subsequent morning, were labeled with barcodes, and were sent to the company that routinely conducts such microbiological analyses at a commercial setting (Institute of Microecology, Herborn, Germany) the very same day (see below). In general, samples reached the laboratory within 24 hours day and were processed immediately. Samples were not frozen and thawed prior to 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 vegetarians diet was assumed when subjects acknowledged to not consume meat in any form but would 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.

Regarding control subjects, we obtained two samples:

Control Group 1 (CG1):

Fecal samples analysed routinely by the Institute of Microecology in Herborn, Germany during one year (2006) were made available to the investigators. From the total of 35.000, two random samples of 144 and 105 resp. 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

group (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 patient since disease specific alteration are averaged out across different diseases and clinical conditions. Any random selection from the total sample should therefore mirrors the distribution of the total sample, however with larger standard deviations. To further test this hypothesis we collected data from a second and truly healthy control group.

Control Group 2 (CG2):

CG2 consisted of volunteering attendants of the 64th Congress of German Gastroenterology Society (DGVS) meeting in Hamburg, Germany October 30th and September 3rd, 2009. Subjects were contacted by booth staff present during the conference (JZ, BL, HS) and ask for participation. After oral information, the 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 were and the stool sample were usually returned the subsequent morning, were labeled with barcodes, and were sent to the company that routinely conducts such microbiological analyses at a commercial setting (Institute of Microecology, Herborn, Germany) the very same day (see below).

After exclusion of individuals on vegetarian and vegan diet, persons that 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 to the vegan and vegetarian groups, two random sample of 46 vegans and 46 vegetarians were drawn from all test subjects and matched for age and gender to controls (Comparison 2, Figure 1)

***** 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 Herborn, Germany. In general, samples reached the laboratory within one day and were processed immediately.

To ensure that the transport did not have any effect on the cultured species, a storage study had been performed with 20 fresh samples. In short, 0.2 g of feces was serially diluted in 1mL of phosphate-buffered saline (PBS, pH7.2). The solution was vortexed for 5 seconds and serially diluted (to 10−9) in PBS, pH 7.2. One mL of each dilution was plated onto enrichment or selective agar media. The remaining feces were stored for three days at a temperature of 25°C, which represents the average temperature during shipment. Following the incubation period, the samples were processed

as described and the results were compared. No significant discrepancy in the cell counts of the investigated microbiota could be detected within two days. Thus, it was concluded that a shipment of less than two 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). Fecal samples were serially diluted in 1mL of phosphate-buffered saline (PBS, pH 7.2) and subsequently plated on selective agar plates by 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 two days. Bacteria were first identified by Gram staining and colony morphologies. Additionally, identifications were performed by the API and VITEK systems (bioM´erieux). All counts were recorded as the numbers of log10 CFU per mL of sample.

The following bacteria were routinely analyzed: *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 into further analysis.

Additional data

Together with the faecal samples we obtained additional data via 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 into the fecal sample. Stool consistency was rated between 1 = solid and 5 = liquid by the same experienced person during pH measurement.

Statistical analysis

Data was analysed using the SPSS Version 13 Statistical Package. We performed an analysis of variance (ANOVA) 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 ± SEM. A 1% rather than a 5% alpha-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 *****

Vegetarians

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

Among the 14 bacterial species or genera, the following showed significant differences when compared to CG1: Vegetarian 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 males and 18 females subjects, 47.9 ± 1.9 [23 to 70] years). When this vegetarian sample was compared to 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 done.

Vegans

The vegan sample 1 consisted of 105 subjects (45 males, 60 females, 49.4 ± 1.0 [22 to 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 to 69] years). When this vegan sample was compared to 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, the statistical analysis regarding *Pseudomonas, Proteus, Citrobacter, Aerob A* and *B* species was not performed due to 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 summarizes the results of the statistical comparison of the two test samples (vegans, vegetarians) to the two control samples (patients, 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 to CG2.

***** Figure 2, Table 2 *****

Vegans versus vegetarians

All four bacterial species that were found to be different between vegans or vegetarians and the respective controls 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, the statistical analysis regarding *Pseudomonas, Proteus, Citrobacter, Aerob A* and *B* species was not performed due to 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 control groups (Figure 3), with females in all groups showing a significantly higher pH than males. Both the total vegan sample (pH = 6.3 ± 0.8) as well as the reduced vegan sample (pH = 6.3 ± 0.8) had a significantly ($p = 0.001$) lower stool pH-value than CG1 (pH = 6.8 ± 0.1) and CG2 (pH = 6.9 ± 0.8) with no gender differences.

***** Figure 3 *****

Stool pH and bacteria

As can be seen in Table 3, strong positive correlations exists 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.

***** Table 3 *****

Stool consistency

Analyses of the stool consistency did not show significant differences between our vegetarian and vegan test subjects and both control groups, and was not different between gender (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. We have here reported the 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 minimized and subtle dietary influences on the intestinal microbiota of both the test and the control group can be identified. We included a similar number of controls on an omnivorous diet, carefully and pair-wise 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 to 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 similar result with respect to *Bacteroides* species being elevated under the conditions of a high-meat diet. This analysis, however, 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-days 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*. 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 (A) normal diet and (B) lacto-ovo-vegetarian diet. As a result, they noted that numbers of *Bifidobacterium* species were significantly higher in the vegetarian test group. This study included 20 children in each group only, and the diet group received oral vaccine with non-pathogenic with *S. 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 one month) in 9 adult male and female subjects, while 9 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 (due to their technique used, gas liquid chromatography) to changes in specific bacterial flora. When the same diet was applied to patients with rheumatoid arthritis for one year (Peltonen *et al*., 1997), 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-months trial in arthritis patients (Kjeldsen-Kragh *et al.,* 1991). In the same patient group, a "Mediterranean diet" (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 of the bacterial microbiota after eight days, but confirmed clinical benefit (Michalsen *et al*., 2005) that may therefore be attributable to placebo responses (Enck & Klosterhalfen, 2005).

No data have been reported previously regarding our finding of significantly reduced *E.coli* and *Enterobacteriaceae* in vegetarians and vegans compared to controls, and with respect to lowered stool pH in the dieting subjects. While the correlations between bacterial counts and stool pH are low (and explain only 10 to 15% of the data variance) they are consistent across all groups and therefore indicate a diet-independent effect that has been observed also 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 to 59% in vegans) and higher fibre content (Haddad *et al*., 1999) that 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 greater than 6.5 (Adler, 1973). Therefore, vegans significantly lower stool pH via augmented metabolites 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 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 the higher stool pH values in subjects on an omnivorous diet, since 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, resp.) (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 colonization density, availability of nutrients, colonic supply, sulphate and bile acids, and bacterial adaptation may all involved in the composition and activity of colonic microflora.

This may help understanding the lower abundance of *Bifidobacteria* and *Bacteroides* species in vegans and vegetarians that 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, the disagreements in literature and the conflicting findings in the 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 men (Enck et al. 2009a,b), despite the fact that women consume – on average – similar amounts of dietary fibres than 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 gender cannot be found any longer (s. 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 reflect the overall intestinal (mucosal) microbiome along the whole gastrointestinal tract (Eckburg *et al*., 2006). The benefit in using faecal samples to investigate the intestinal microbiota is obvious: the samples are collected easily and the test subjects have not to suffer from adverse effects as they can occur following a colonoscopy. Compared to previous culture-based research our study reports data obtained from a larger sample size in comparison to most studies before. Additionally, our subjects consisted of long-term vegetarians and vegans and not of test subjects normally consuming a mixed diet, which may lead to a more marked change of the colonic microbiome.

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Table 1: Characteristics of the four study samples (mean + SEM) (n.a. = not available)

	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	n.a.	72.73 ± 12.84
BMI*	23.08 ± 3.97	22.73 ± 3.51	22.30 ± 3.28	22.57 ± 3.42	n.a.	24.14 ± 3.42
TC**	2.47E+011 ±4.51E+011	1.79E+011 ±3.73E+011	3.03E+011 ±6.37E+011	3.29E+011 ±7.30E+011	3.09E+011 ±5.01E+011	3.39E+011 ±4.71E+011
pH***	6.7 ± 0.7	6.6 ± 0.8	6.3 ± 0.8	6.3 ± 0.7	6.8 ± 0.04	6.9 ± 0.8

+: Male : Female; *: body mass index; **: Total germ count; ***: pH: stool pH as determined in the laboratory

Table 2: Results of the comparison of the full and the reduced vegan and vegetarian samples to the respective control groups (CG1, CG2) for 4 different bacterial strains. Note that p-values between 0.01 and 0.05 were regarded as not significant (n.s.) due to multiple testing.

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

Table 3: Pearson' s correlation coefficients between the pH-value and various bacterial counts (*E. coli*, *Enterobacteriacea, Bifidobacterium*, and *Bacteroides species*) in vegetarian and vegan subjects and control groups. Bold print indicates significance (p<.05)

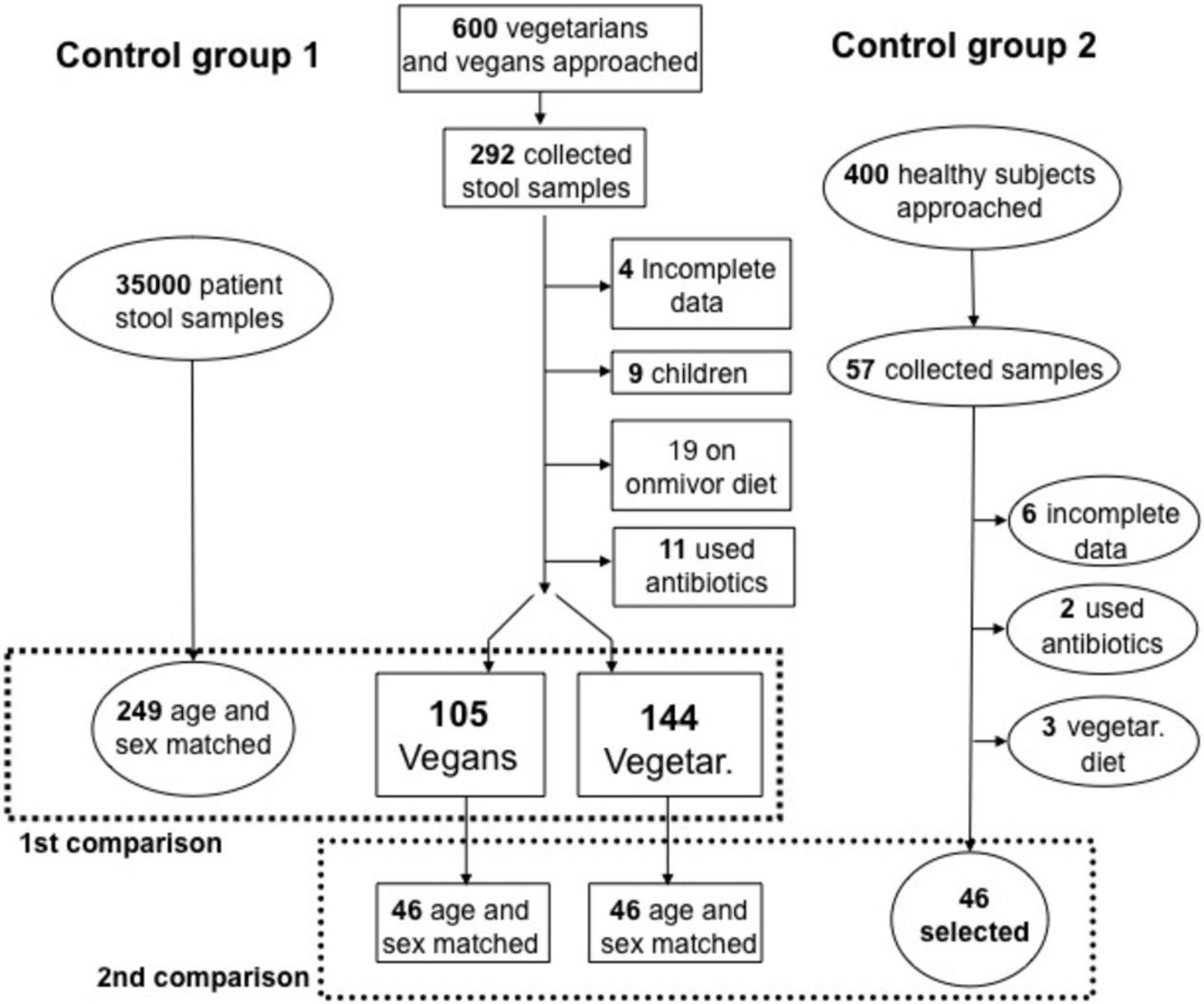
pH	**Vegetarian**		**Vegan**		**CG1**	**CG2**
	N=144	N=46	N=105	N=46	N=249	N=46
E.coli	**R = 0.331 p < 0.001**	**R = 0.323 P = 0.045**	**R = 0.395 p < 0.001**	**R = 0.374 p = 0.017**	**R = 0.213 p = 0.002**	**R = 0.470 p = 0.002**
Enterobacteriaceae	**R = 0.362 p < 0.001**	**R = 0.324 P = 0.042**	**R = 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 = 0.014**	R = 0.017 p = 0.914

Legend of Figures

Figure 1: Recruitment of study samples from different cohorts: Control Group 1 (CG1) was drawn from 35.000 adult patient stool samples from the data base of the company that analysed the samples for commensal bacteria (Institute of Microecology, Herborn, Germany), 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.

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

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


Control group 1
600 vegetarians and vegans approached
Control group 2
292 collected stool samples
400 healthy subjects approached
35000 patient stool samples
4 Incomplete data
9 children
19 on onmivor diet
11 used antibiotics
57 collected samples
6 incomplete data
2 used antibiotics
3 vegetar. diet
249 age and sex matched
105 Vegans
144 Vegetar.
1st comparison
46 age and sex matched
46 age and sex matched
46 selected
2nd comparison

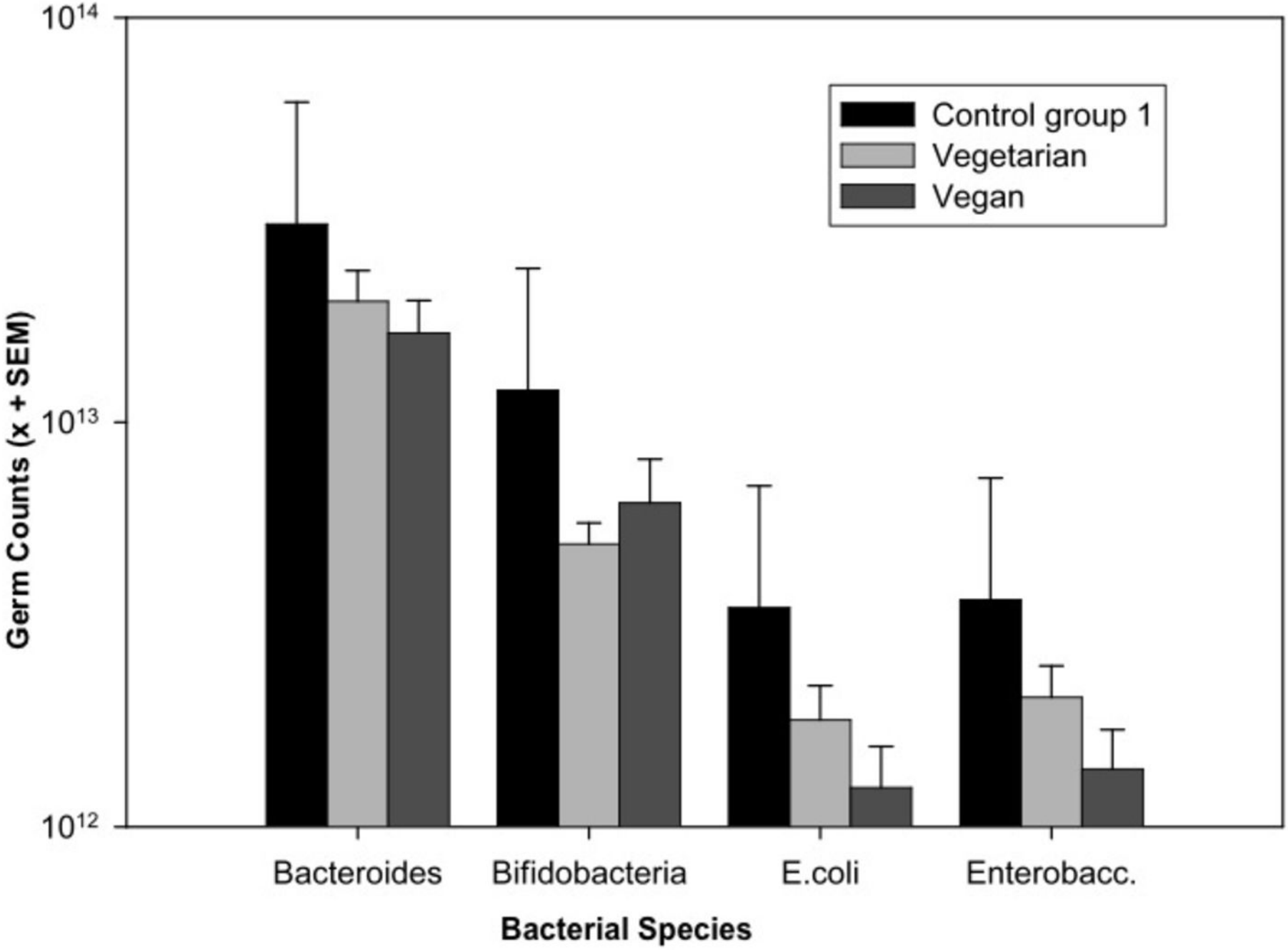

10^{14}
10^{13}
10^{12}
Germ Counts (x + SEM)
Control group 1
Vegetarian
Vegan
Bacteroides
Bifidobacteria
E.coli
Enterobacc.
Bacterial Species

Figure 3