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Changes in the intestinal microbiota after a short period of dietary over-indulgence, representative of a holiday or festival season

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Abstract

The effects on the intestinal microbiota of a short period of marginal over-eating, characteristic of holiday or festival periods, were investigated in a pilot study. Fourteen healthy male subjects consumed a diet rich in animal protein and fat for seven days. During this period, the subjects significantly increased their dietary energy, protein, carbohydrate and fat intakes by 56, 59, 53 and 58%, respectively (all $P < 0.05$). The mean weight gain of 0.27 kg was less than the expected 1 kg, but this was consistent with a degree of under-reporting on the baseline diet. Fluorescence *in situ* hybridisation analysis confirmed the relative stability of each individual's faecal microbiota but showed considerable variations between them. The diet was associated with a significant increase in numbers of total faecal bacteria and the bacteroides group, as detected by the universal bacterial probe (DAPI) and *Bacteroides* probe (Bac 303), respectively. Overall, there was a decrease in numbers of the *Lactobacillus/Enterococcus* group (Lab 158 probe; 2.8 ± 3.0% to 1.8 ± 1.8%) and the *Bifidobacterium* group (Bif 164 probe; 3.0 ± 3.7% to 1.7 ± 1.2%), although there was considerable inter-individual variation. Analysis of the relative proportions of each bacterial group as a percentage of the subject's total bacteria showed a trend for a change in the intestinal microbiota that might be considered potentially unhealthy.

Keywords: intestinal microbiota, effect of diet, obesity, FISH

1. Introduction

1.1 The association between diet, the intestinal microbiota and health

As many as 1000 species of bacteria (Suau *et al*. 1999; Hughes *et al*. 2001; Xu and Gordon 2003; Nicholson *et al*. 2005) colonise the human gastrointestinal tract. This complex ecosystem has a significant effect on the processes of the body and its overall health, with its influence reaching beyond the gut.

Epidemiological and other studies have shown that diet is another important influence on health. For example, high rates of colorectal cancer are found for populations consuming diets high in meat and fat and low in starch, non-starch polysaccharides, fibre and vegetables (Yoon *et al*. 2000; Hughes *et al*. 2001; Sandhu *et al*. 2001; Norat *et al*. 2002; World Cancer Research Fund/American Institute for Cancer Research 2007).

Early ecological studies showed that the faecal microbiota could be influenced by different diets associated with certain health risks. These studies confirmed that protein

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and fat, as well as type of carbohydrate (simple sugars *vs.* complex carbohydrates) were dietary factors that particularly affected the composition and activity of this microbiota (Hill *et al.* 1971; Finegold *et al.* 1974; Goldin *et al.* 1978; Hill 1981, 1998).

1.2 The impact on health of the metabolic activities of the intestinal microbiota

The nature and extent of this fermentation depends upon the characteristics of the bacterial microbiota, colonic transit time and the availability of nutrients. The products of carbohydrate metabolism are thought to benefit the host, particularly when compared with the toxic and potentially carcinogenic end products of protein metabolism (Clinton *et al.* 1988; Allison and MacFarlane 1989; MacFarlane and Cummings 1991; Roberfroid *et al.* 1995; Smith and MacFarlane 1996; Tricker 1997). Both bifidobacteria and lactobacilli belong to saccharolytic microbiota and have a role in controlling the pH of the large intestine through the production of lactic and/or acetic acids. A low pH is thought to help inhibit the growth of certain potential pathogens and putrefactive bacteria (Modler *et al.* 1990; Gibson and Wang 1994), and also depress the formation of secondary bile acids from primary ones, enhancing the precipitation of bile acids (Rafter *et al.* 1986; Nagengast *et al.* 1988; Hofman and Mysels 1992; Van Munster *et al.* 1994). Higher numbers of bifidobacteria have also been related to increased butyrate levels, which is an important energy source for the colonocytes and have possible cancer protective effects (MacFarlane and Cummings 1991; Smith *et al.* 1998).

The large intestine is also a site of intense protein turnover, with the amount of dietary protein rather than its source determining how much reaches the colon (Macfarlane *et al.* 1986; Silvester and Cummings 1995; Macfarlane and Macfarlane 2003). Numerically important proteolytic species identified in the colon include species of *Bacteroides, Propionibacterium, Clostridium, Fusobacterium* and *Streptococcus* (MacFarlane and Cummings 1991). Animal studies have implicated cooked meat containing high levels of heterocyclic amines in the development of colorectal cancer (Layton *et al.* 1995; Skog *et al.* 1995; Pence *et al.* 1998).

The present preliminary study was conducted with human volunteers to investigate the effects of short-term dietary overindulgence with consumption of high levels of animal protein and fat. Molecular methods were used to analyse relevant bacterial groups in the faecal microbiota, as has been done in other studies (Harmsen *et al.* 2002; Lay *et al.* 2005). Recent work has indicated that there are no significant differences in the microbiota from colonic biopsies and faeces, as revealed by fluorescence *in situ* hybridisation (FISH) microscopy using 16S rRNA-targeted probes (Van der Waaij *et al.* 2005).

2. Materials and methods

2.1 Aim of the study

Diets consumed during holiday and festive periods are often high in energy, protein and fat. Overeating, often common at these times, is consistent with a behavioural tendency to follow positive social signals to eat, rather than internal signals to stop. However, the gut has a limited capacity of digestive and absorptive function; if overloaded, more protein, fat and carbohydrate may pass through the small intestine to the colon. Such short periods of dietary indulgence can also be associated with small rises of body weight in the population (no more than about 0.4 kg) and short-term rises of blood cholesterol (Andersson and Rössner 1992). In all published reports, these changes revert to 'normal' quickly after the holiday period. Minor digestive health problems (indigestion, heartburn, diarrhoea, bloating and constipation), however, may also be common at these times. One survey, for instance, found that 30% of patients consulted their general practitioner during the Christmas-New Year season with a range of problems attributed to seasonal over-eating and drinking (Medix Intelligent Information 2005). Such problems may be linked to fermentation of food residues in the colon.

There are no published studies on the effect of such a holiday diet on the intestinal microbiota. The present small, pilot study was designed to investigate this by mimicking an 'overload' of the digestive system, and to assess whether this might have any potential health impacts relating to changes in the intestinal microbiota.

2.2 Study design

Observations were made for 14 days: seven days 'run-in' followed by a seven day holiday diet period.

During the 'run-in', subjects were asked to make a weighed diet record and to keep a 'bowel-function' diary. At the beginning of the 'run-in', body weight and height were measured and these were repeated at the end of the holiday diet period.

On day one of the seven day diet period, subjects attended the metabolic unit and were asked to eat their usual breakfast. Body weight was again measured. The first faecal sample passed after this meal was sampled and processed for microbiological analysis. Throughout the seven day diet period, subjects consumed their main and evening meal in the metabolic unit (pre-packed breakfasts were provided for consumption at home). The food consumed was a rotating menu of holiday and festive season

food dishes, each of which was weighed so that a full weighed dietary intake could be recorded.

On the day after the seventh day of the diet, faecal samples were collected for microbiological studies. Subjects were asked to consume their usual alcohol quantities and types but to record the consumption in the diary.

2.2.1 Subjects

Potential subjects were screened by questioning, to exclude any who had medical conditions, were using medication, or were unable to eat all meats. Fourteen healthy male subjects aged 22–65 were recruited from among staff and students of King's College London. They had no history of gastrointestinal disorders or of eating or weight problems, nor were they taking any regular medication. The study was approved by the College Research Ethics Committee of King's College London.

2.2.2 Diet characteristics

The menu included main meals based on traditional Christmas foods with a rotating menu of chicken, turkey, ham, lamb, beef and pork, with traditional vegetables (potatoes, carrots, peas, parsnips, etc.) followed by dessert courses ranging from Christmas pudding with cream, trifle, a variety of fruit pies, followed by mince pies. The diet was consumed *ad libitum* though a convivial ambience was created to enhance social cues to consume as if in holiday mood, which would encourage marginal overeating.

2.2.3 Bacterial enumeration by FISH

Differences in bacterial populations were assessed by FISH with oligonucleotide probes designed to target specific regions of 16S rRNA. These were commercially synthesized and labelled with the fluorescent dye cyanine 3 (Cy3; excitation λ 514 nm; emission λ 566 nm; fluorescent colour, orange-red, Sigma Genosys, Cambridge, UK). The probes used were Bif 164 (Langendijk *et al*. 1995), Bac 303 (Manz *et al*. 1996), Lab 158 (Harmsen *et al*. 1999), His 150 (Franks *et al*. 1998) and Erec 482 (Franks e*t al*. 1998), specific for *Bifidobacterium* spp., *Bacteroides*/*Prevotella* group, *Lactobacillus*/*Enterococcus* group, *Clostridium* clusters I and II which encompass more than 60 species (including *Clostridium perfringens*/*Clostridium histolyticum*) and *Clostridium coccoides*/*Eubacterium rectale* group (*Clostridium* cluster XIVa), respectively. For total bacterial counts the nucleic acid stain 4,6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, Cambridge, UK) was used. Faecal samples were weighed and phosphate buffered saline (PBS; 0.1M, pH 7.0) was added to make 10% (wt/vol) solution. Samples were homogenised in a stomacher for 2 min. Five millilitres were pipetted into a plastic centrifuge tube and vortexed with the help of glass beads. Samples were then centrifuged for 2 min at 13 000 rpm to remove particulate matter. Three hundred and seventy-five microlitres of supernatant were removed and added to 1125 μL of filtered 4% (wt/vol) paraformaldehyde solution in a 1.5 mL microcentrifuge tube (paraformaldehyde was added first, then supernatant). Samples were mixed and stored overnight at 4°C. The 1.5 mL of fixed cells were then centrifuged at 13 000 rpm for 5 min, the supernatant was removed and the pellet was washed twice with 1 mL of filtered PBS, resuspended in a mixture of 300 μL PBS/99% ethanol (1:1 wt/vol) and stored at −20°C for at least 1 h. Samples for *Lactobacillus*/*Enterococcus* probe after overnight fixation with 4% (wt/vol) paraformaldehyde solution were centrifuged at 13 000 rpm for 5 min, after which the supernatant was removed and the pellet was washed twice with 1 mL of filtered PBS and remaining pellet was treated with 145 μL *Lactobacillus* enzyme buffer at 37°C for 2 h. (*Lactobacillus* enzyme buffer: 25mM Tris-HCl, 585mM sucrose, 5mM $CaCl_2$, 10mM EDTA, 30 mg taurocholic acid, pH at 7.6, filtered through 0.2 μm filter and then added fresh 2 mg/mL lysozyme (50 000 Units) and 1 mg/mL lipase (100–400 Units, porcine pancreas type II)). After enzymatic treatment, 5 μL of 4% (wt/vol) paraformaldehyde were added and samples were left at 4°C for 10 min. The 150 μL of fixed and enzymatically treated cells were then centrifuged at 13 000 rpm for 5 min, the supernatant was removed and the pellet was washed twice with 1 mL of filtered PBS, resuspended in a mixture of 300 μL PBS/99% ethanol (1:1 wt/vol) and stored at −20°C for at least 1 h.

Samples were removed from the freezer and allowed to warm to room temperature before 16 μL of the cell suspension were added to 264 μL of pre-warmed (50°C) hybridisation buffer (30mM Tris-HCl, 1.36M NaCl, 1.5 mL of 10% SDS, pH at 7.2 and filtered through 0.2 μm filter). Ninety microlitres of this mixture was added to 10 μL of the appropriate probe and left overnight for hybridisation at the appropriate temperature. The samples were then washed at their respective hybridisation temperatures, in approximately 5 mL of washing buffer (20mM Tris-HCl, 0.9M NaCl, pH at 7.2 and filtered through 0.2 μm filter) for 30 min before 20 μL of DAPI were added to enable enumeration of total cell counts. The amount of sample added to the Sterilin tubes were as follows: 100 μL of lactobacilli/enterococci probe (Lab 158) and fixed cells; 20 μL of bifidobacteria probe (Bif 164) and fixed cells; 20 μL of *Bacteroides* probe (Bac 303) and fixed cells; 100 μL of clostridia subgroup *C. histolyticum*/*perfringens* (His 150 probe) and fixed cells; 20 μL *C. coccoides*/*E. rectale* group probe (Erec 482) and fixed cells; and 5 μL DAPI. The cells were counted visually with a fluorescence microscope (Nikon Eclipse, E400). The DAPI-stained cells were examined under UV light and hybridised cells viewed using a DM510 filter. For each slide, at least 15 different fields of view were counted.

Table 1. Dietary intake before and during the holiday diet

	Baseline	After the period of holiday diet	% change
Energy, kcal/day	1799 ± 434 (886–2310)	2812 ± 849* (1870–4067)	+56
Protein, g/day	71.1 ± 17.2 (30.2–99.4)	112.7 ± 26.7* (75.6–178.6)	+59
Carbohydrate, g/day	227.3 ± 72.7 (98–341.8)	348.1 ± 125.4* (175.2–595.1)	+53
Fat, g/day	69.3 ± 23.1 (37.9–121.9)	109.5 ± 28.9* (74.1–154.5)	+58
Alcohol, g/day	2.7 ± 9.7 (0–35.0)	3.3 ± 11.3 (0–41)	—

Values are mean ± SD and ranges within parentheses.
$n = 12$ for baseline, $n = 14$ for holiday diet.
*Significantly different from baseline $P < 0.05$.

2.2.4 Statistical analysis

The statistical significance of the effects of the differences in the dietary intakes and the effects of the holiday diet on the composition of the subject's faecal microbiota was based on two-sided paired *t*-statistics.

3. Results

3.1 Subjects

Fourteen subjects, of European and Middle-Eastern ancestry, completed the study. There were no changes of bowel habit other than an episode of transient constipation in one subject. Median weight change was zero (range: −0.9 to +2.2 kg), the mean change was +0.27 ± 0.90 kg (mean ± SD) for 13 subjects (one subject failed to return for measurement).

3.2 Dietary intake

Most subjects achieved an increase of dietary energy, protein and fat intake during the holiday diet period. The volunteers' overall total dietary energy intake rose by 56%, whereas protein, fat and carbohydrate increased by 59, 53 and 58%, respectively (Table 1). Alcohol was consumed by only one subject during the baseline period and by two in the holiday diet period. Two subjects failed to deliver their baseline dietary records despite repeated requests; thus, baseline data shown in Table 1 are for 12 subjects only. A subgroup analysis of complete and reliable data ($n = 11$) showed that the volunteers' overall total dietary energy intake rose by 39%, whereas protein, fat and carbohydrate increased by 49, 41 and 42%, respectively. The differences between baseline and holiday diet intake of energy, protein, carbohydrate and fat intakes were significantly different ($P < 0.05$).

3.3 FISH analysis

A large degree of inter- and intra-individual variability was revealed in the bacterial profile of samples measured using specific probes directed against regions of the 16S rRNA (Table 2). Numbers of total bacteria (DAPI) among the individuals ranged from 10.17 to 10.73 $\log_{10}$ cells/g of wet faeces before the holiday diet feeding period, and from 10.32 to 10.85 $\log_{10}$ cells/g of wet faeces after the holiday diet feeding period (Table 2). The highest increase in numbers of total bacteria after the feeding period was 0.40 $\log_{10}$ cells/g of wet faeces (subject K), whereas the highest decrease was 0.08 $\log_{10}$ cells/g of wet faeces (subject B). Mean numbers of total bacteria in the faecal microbiota showed a statistically significant increase after the holiday diet feeding period of 1.28×10^{10} cells/g of wet faeces (Table 3, $P = 0.003$). Interestingly, after the feeding period, numbers of total bacteria decreased only in one case (subject B) and remained the same in just one case (subject C), whereas an increase was observed in 12 of the total of 14 subjects.

After the holiday diet period, numbers of lactobacilli/enterococci decreased in 8 of 14 subjects, clostridia (clusters I and II) increased in 8 of 14 subjects, bifidobacteria decreased in 8 of 14 subjects and eubacteria (*Clostridium* cluster XIVa) decreased in 8 of 14 subjects. Bifidobacteria was below the detection limit (10^6 cells/g of wet faeces) in one subject (subject D) during the whole study.

Mean numbers of *Bacteroides*/*Prevotella* group in the faecal microbiota showed a statistically significant increase of 0.43×10^{10} cells/g of wet faeces (Table 3, $P = 0.0003$). Numbers of *Bacteroides*/*Prevotella* group among the individuals ranged from 9.07 to 10.22 $\log_{10}$ cells/g of wet faeces before the holiday diet feeding period, and from 9.30 to 10.40 $\log_{10}$ cells/g of wet faeces after the holiday diet feeding period (Table 2). Remarkably, numbers of *Bacteroides*/*Prevotella* group increased in all fourteen subjects of this pilot study after the end of the holiday diet (Table 2). The highest increase in numbers of *Bacteroides* after the feeding period was 0.46 $\log_{10}$ cells/g of wet faeces (subject L) followed by subject N (increase of 0.44 $\log_{10}$ cells/g of wet faeces). *Clostridium* clusters I and II mean numbers also increased after the holiday diet feeding period, although not statistically significant (3.64×10^7 cells/g of wet faeces, $P = 0.31$). In contrast, bifidobacteria and lactobacilli/enterococci mean numbers were lowered at the end of the holiday diet feeding period when

Table 2. Enumeration of bacteria with DAPI and five FISH probes in the pilot study for each individual before and after the holiday-type diet

	Total cells DAPI	*Lactobacilli/Enterococci* Lab 158	*Clostridium* clusters I and II	Bifidobacteria Bif 164	*Bacteroides/ Prevotella* Bac 303	*Clostridium* cluster XIVa
A1	10.53	8.66	7.19	8.46	9.60	9.68
A2	10.63	8.28	6.97	8.28	9.68	9.09
B1	10.67	9.37	7.49	7.92	10.22	10.34
B2	10.59	9.10	7.98	7.56	10.34	9.70
C1	10.32	8.51	7.64	9.56	9.82	10.01
C2	10.32	8.43	7.77	9.04	10.01	9.28
D1	10.47	9.29	6.79	6.00*	9.85	10.09
D2	10.61	9.01	7.33	6.00*	10.09	9.56
E1	10.47	8.82	6.97	7.94	9.83	10.02
E2	10.85	9.45	7.53	8.74	10.02	9.84
F1	10.17	8.84	7.27	8.27	9.98	10.32
F2	10.55	8.93	7.09	9.03	10.32	9.80
G1	10.38	9.35	6.79	8.88	9.77	9.83
G2	10.33	9.05	6.79	8.84	9.83	9.73
H1	10.37	8.03	8.11	9.00	9.77	9.62
H2	10.52	8.44	8.45	8.90	9.80	9.87
I1	10.43	8.86	8.18	8.64	9.59	10.04
I2	10.72	8.91	8.69	8.72	9.96	10.35
J1	10.28	8.49	8.38	8.68	9.52	9.93
J2	10.53	8.29	7.92	8.85	9.91	10.04
K1	10.24	8.27	7.39	8.73	9.19	9.62
K2	10.64	8.38	7.77	8.92	9.30	10.10
L1	10.73	8.67	8.24	8.92	9.07	9.93
L2	10.75	8.56	8.21	9.15	9.53	9.91
M1	10.58	8.53	8.39	9.22	10.19	9.93
M2	10.66	8.72	7.95	8.68	10.40	10.09
N1	10.23	8.30	8.21	9.28	9.56	9.91
N2	10.59	8.19	8.55	9.07	10.00	10.01

Bacterial counts ($\log_{10}$ cells/g of wet faeces) for subjects A-N at sampling points 1–2.
Sampling point 1, at day 1 of the holiday diet feeding period; sampling point 2, at day 10 of the holiday diet feeding period.
*Detection limit of the method: 10^6 cells/g of wet faeces.

compared with the numbers at the end of the 'run in' period, although not significantly (1.65×10^8 cells/g of wet faeces, $P = 0.48$ and 0.67×10^8 cells/g of wet faeces, $P = 0.75$, respectively). Numbers of *C. coccoides/ E. rectale* group increased after the holiday diet feeding period although not significantly (1.91×10^9 cells/g of wet faeces, $P = 0.11$).

The proportions of cells that hybridised with the phylogenetic probes relative to the total bacteria are presented in Table 4. The five probes used in this study accounted for an average 50% of the total bacteria both before and after the holiday diet feeding period. *Bacteroides/Prevotella* probe (Bac 303) represented the highest proportion of the faecal bacteria before and after the holiday diet

Table 3. Bacterial numbers (cells/g of wet faeces) for total bacteria and five specific key groups expressed as the average of fourteen healthy adults, before and after the feeding period

Bacterial group	Before holiday diet feeding period	After the holiday diet feeding period	Mean difference (cells/g of wet weight faeces)	SEM	*P*
Total cells (DAPI)	2.82×10^{10}	4.10×10^{10}	1.28×10^{10}	3.51×10^{09}	0.003*
Lactobacilli/Enterococci (Lab 158)	7.87×10^{8}	7.20×10^{8}	-0.67×10^{8}	4.65×10^{08}	0.75
Clostridium clusters I and II (His 150)	8.95×10^{7}	1.26×10^{8}	3.64×10^{7}	3.42×10^{07}	0.31
Bifidobacteria (Bif 164)	8.48×10^{8}	6.83×10^{8}	-1.65×10^{8}	2.27×10^{08}	0.48
Bacteroides/Prevotella (Bac 303)	6.52×10^{9}	1.08×10^{10}	0.43×10^{10}	8.89×10^{08}	0.0003*
Clostridium cluster XIVa (Erec 482)	6.25×10^{9}	8.17×10^{9}	1.91×10^{9}	1.11×10^{09}	0.11

*Level of significance: $P < 0.05$ for each bacterial group during the feeding period.

Table 4. Proportions of the *Lactobacillus*/*Enterococcus* (Lab 158), *Histolyticum*/*Perfringens* (His 150), *Bifidobacterium* (Bif 164), *Bacteroides*/*Prevotella* (Bac 303) and *C. coccoides*/*E. rectale* (Erec 482) groups in 14 healthy adults before and after a holiday diet-type feeding period, assessed with FISH microscopy

	% of total bacteria that hybridised with probe	
Probe	Before	After
Lactobacilli/Enterococci (Lab 158)	2.8 ± 3.0	1.8 ± 1.8
Clostridium clusters I and II (His 150)	0.3 ± 0.3	0.3 ± 0.4
Bifidobacteria (Bif 164)	3.0 ± 3.7	1.7 ± 1.2
Bacteroides/*Prevotella* (Bac 303)	23.1 ± 18.8	26.3 ± 19.1
Clostridium cluster XIVa (Erec 482)	22.2 ± 13.3	19.9 ± 14.6
Additivity	51.4 ± 23.5	50.0 ± 33.1

Results are presented as mean ± SD.
Total number of bacteria counted after samples stained with DAPI.

feeding period (23.1 and 26.3%, respectively). This was followed by *C. coccoides*/*E. rectale* (Erec 482) probe (22.2 and 19.9%, respectively). *Lactobacillus*/*Enterococcus* (Lab 158), *C. histolyticum*/*perfringens* (His 150) and *Bifidobacterium* (Bif 164) groups added together accounted for an average 6.1% of the total bacteria before and 3.8% after the holiday diet feeding period.

4. Discussion

Our results confirm earlier observations of large inter- and intra-individual variations for the human faecal microbiota and a high degree of variability in the intestinal microbiota composition (Suau *et al*. 1999; Barcenilla *et al*. 2000; Zoetendal *et al*. 2001; Mai *et al*. 2004). Moreover, no significant changes could be seen in most bacterial groups tested (Table 3) confirming the underlying stability of each individual's microbiota (Hill 1981; Fuller and Rowland 1989; Zoetendal *et al*. 1998, 2001). *Bacteroides* (*Bacteroides*/*Prevotella* group) and eubacteria (*Clostridium* cluster XIVa) groups were the bacterial groups present in the greatest numbers in all 14 subjects both before and after the holiday diet feeding period. This is in agreement with previous studies which showed that these bacterial groups are two of the most predominant groups in the human faecal microbiota (Gibson and Roberfroid 1995; Suau *et al*. 1999; Sghir *et al*. 2000; Mai *et al*. 2004; Lay *et al*. 2005). In the present study, numbers of total bacteria increased significantly after the short period of a diet rich in fat and animal protein. The health significance of this is not known.

A further finding from the current study was a significant increase in numbers of the *Bacteroides* group after the dietary period. *Bacteroides* (and *Clostridium*) contain numerically important proteolytic species in the colon (MacFarlane and Cummings 1991). An increase in the numbers of *Bacteroides* species has previously been associated not only with diets rich in fat and animal protein (Hill *et al*. 1971; Finegold *et al*. 1974) but also with pathological disorders such as severity of ulcerative colitis (Ishikawa *et al*. 2005). With the present study, in terms of proportional change, there was a 20 and 11% reduction of bifidobacteria and lactobacilli/enterococci respectively and a 60, 40 and 30% increase of *Bacteroides*/*Prevotella*, *Clostridium* clusters I and II and *Clostridium* cluster XIVa. These results appear to indicate that a short period of dietary overindulgence may promote the growth of potential harmful bacteria and inhibit those usually considered as being beneficial (Rafter *et al*. 1986; Nagengast *et al*. 1988; Modler *et al*. 1990; Hofman and Mysels 1992; Gibson and Wang 1994; Van Munster *et al*. 1994). However the study only lasted one week, and it is not known whether the observed changes were short-term or long-term. In addition, it is not known whether the statistically significant changes and trends seen here have any physiological significance. It is also important to note that labelling non-pathogenic commensal bacteria as either beneficial or detrimental remains speculative, and considerably more scientific investigation at the species level is needed.

However, probiotic and prebiotic research has shown that modulation of the intestinal microbiota can result in benefits for people with different states of health. The increase of toxic and carcinogenic breakdown products in the colon resulting from a high protein diet, has led to suggestions that beneficial modulation of the intestinal microbiota may reduce risk. There is some evidence for this. A double-blind, placebo-controlled human volunteer studies conducted by a group in Belgium (De Preter *et al*. 2004, 2007) found that consumption of the probiotic strain *L. casei* Shirota resulted in significant reduction of levels of ammonia and *para*-cresol in the urine, indicating a suppression of the generation and accumulation of toxic metabolites in the colon. This strain has also been linked to reduction of faecal β-glucosidase and β-glucuronidase: enzymes mainly produced by Enterobacteriaceae and clostridia, associated with carcinogen formation in the intestines (Spanhaak *et al*. 1998; De Preter *et al*. 2008). This may partly explain studies indicating possible cancer benefit for this strain (Hayatsu and Hayatsu 1993; Aso *et al*. 1995; Ishikawa *et al*. 2005).

Larger studies with the appropriate power to detect the effects of dietary interventions on the faecal microbiota composition are clearly needed to advance this field. Molecular microbiota analysis methods such as metagenomics can facilitate the determination of dietary effects

on the intestinal microbiota composition as indicated by this preliminary study. A more comprehensive microbiota analysis approach that includes more bacterial groups with the use of the appropriate molecular probes and metabolic activity assays will increase the likelihood of detecting potentially important associations between diet and either composition or activity of the gut microbiota. Establishment of such associations will be fundamental for the future design of specific dietary interventions aimed at improving human health through changes in the microbiota.

In the present study, the dietary changes were not as great as might have been achieved under more tightly controlled metabolic unit conditions. Diaz *et al.* (1992) achieved greater increases of dietary energy intake and greater increases of body weight though over a longer period of time, and under conditions which did not replicate a 'holiday diet' type of intervention. Our subjects were free living but during the 'holiday diet' ate their main meals in the metabolic unit at King's College London. Dietary data for the holiday diet period, which was mostly collected in the metabolic unit under the observation of the research team, was thought to be reliable, whereas the dietary records made before the diet were believed to be less reliable and showed evidence of under-reporting especially in one individual; moreover, two subjects failed to deliver their diet records despite repeated requests. The mean dietary energy intake on the baseline was 1799 kcal/day, which is a low value for fully grown men, again suggesting under-reporting. A sub-group analysis excluding the subject suspected of under-reporting showed a revised overall baseline energy intake of 1919 kcal/day. Any repetition of this type of study should standardise conditions by offering the baseline diet within a metabolic unit to achieve greater control. The 56% increase in dietary energy intake should have translated into a body weight gain of about 1 kg during seven days, assuming no increase of energy expenditure. The measured weight changes were variable but the average weight gain of 0.27 kg suggested that in fact the true differences in energy and macronutrient intakes were not as great as the calculated figures and may have been nearer to a more modest 25% increase rather than the 56% increase of energy intake reported above. (Exclusion of the suspected under-reporting subject reduced the increase of dietary intake to 39%.) There was relatively little alcohol consumption during this study, rendering it somewhat dissimilar to the usual pattern of holiday food and drink consumption in the United Kingdom.

In conclusion, seven days of marginal over-eating of a holiday diet, characterised by a greater than usual quantity and variety of meats and other 'festive' dishes, resulted in a significant increase in numbers of total faecal bacteria and the *Bacteroides* group, as detected by the universal bacterial probe (DAPI) and *Bacteroides* probe (Bac 303), respectively. Numbers of the lactobacilli/enterococci group and bifidobacteria decreased, despite inter-individual variation. The shift in the relative proportions of each bacterial group suggested a change in the intestinal microbiota that might be regarded as unhealthy.

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