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Effect of overweight on gastrointestinal microbiology and immunology: correlation with blood biomarkers

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A cross-sectional study was carried out in order to compare intestinal microbiological and immunological biomarkers with blood glucose and lipids, satiety-related hormones and inflammatory biomarkers characterising differences between obese and normal weight subjects. Faecal and blood samples were obtained from twenty obese subjects with an average BMI of 32·9 kg/m^2 and twenty normal weight subjects with an average BMI of 23·3 kg/m^2. Blood insulin, TAG and leptin were significantly elevated, whereas concentrations of HDL and ghrelin were significantly decreased in the obese subjects. Inflammatory status in the obese subjects was characterised by a trend for elevated blood C-reactive protein (CRP; $P=0·06$) and IL-6 ($P=0·02$). The faecal microbial composition differed between the groups; less sulphate-reducing bacteria ($P=0·05$) and a trend for less *Bacteroides* ($P=0·07$) were measured for overweight subjects. Furthermore, an inverse correlation was demonstrated between faecal *Bacteroides* levels and waist circumference ($P=0·05$). The faecal microbial metabolites differed between the groups; increased concentrations of branched-chain fatty acids, phenolics, valeric acid, di- and hydroxy acids were described in the obese subjects. No differences between the measured intestinal inflammatory biomarkers were detected. However, systemic inflammation (CRP and IL-6) was correlated with the faecal concentrations of phenolics and lactic acid ($P<0·05$ and 0·05, and $P<0·01$ and 0·05, respectively). In summary, weight-related differences were observed both in the intestinal microbial composition and its activity. The role of intestinal signals, such as phenolics and lactic acid in the development of weight-related problems, needs to be studied further.

Intestinal microbiota: Inflammation: Overweight

Overweightness and obesity have become globally common conditions that can significantly reduce quality of life and life expectancy[1]. Alongside overweight, the prevalence of metabolic syndrome is increasing[2]. Increased concentrations of blood lipids and glucose in overweight subjects are risk factors for the development of CVD and diabetes[3]. It is well documented that obesity is a systemic inflammatory condition, characterised by increased plasma concentrations of C-reactive protein (CRP) and inflammatory cytokines[4,5]. Furthermore, it has been demonstrated that there is a correlation between weight, or waist circumference, adiposity and the blood-inflammatory status[6,7].

Changes in gastrointestinal (GI) functions in overweight subjects have not been studied extensively. Intestinal glucose and amino acid transporters are typically adapted to the luminal nutrient concentrations[8]. Excessive luminal nutrient load may exceed the absorptive capacity of the transporters[9], allowing increased availability of nutrients for colonic microbes. Thus, nutrients not available in the colon of healthy normal weight subjects may have an impact on the composition of the microbial community and the amount of energy harvested from the diet. Recently, a new role for intestinal microbes and the production of SCFA[10,11] in the development of obesity has been suggested. Specifically, body weight-based differences between the balance of the relative proportions of the two major intestinal bacterial phyla, *Firmicutes* and *Bacteroidetes*, have been described[10].

The aim of the present study was to characterise the intestinal immunological and microbiological environment in obese and normal weight subjects in more detail, and furthermore, to correlate intestinal biomarkers with well-described blood biomarkers for obesity.

Materials and methods

Study subjects

In total, forty subjects were recruited to take part in the present study in the area of Kuopio, Eastern Finland. The main inclusion criteria of the study subjects were for them to be aged between 20 and 55 years and to have a BMI of 20–25 kg/m^2 for the normal weight group and a BMI of 30–37 kg/m^2 for the obese group. In addition, the subjects had to be accustomed to consuming a mixed diet with moderately low-fibre content (a typical fibre intake of less than 19 g/d for men and 17 g/d for women). The exclusion criteria were critical illness, inflammatory bowel disease, celiac disease or malignancy in the GI tract. Use of anti-obesity drugs, laxatives

Abbreviations: BCFA, branched-chain fatty acids; CRP, C-reactive protein; GI, gastrointestinal; PYY, peptide tyrosine–tyrosine.

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and weight control products was prohibited. Furthermore, regular (daily) use of probiotics, fibre supplements or bran as well as the regular and/or abundant use of non-steroidal anti-inflammatory drugs was not allowed. The use of antibiotics was prohibited for 3 months before and also during the study. Subjects with a tendency towards constipation (defecation frequency <3/week) and subjects with recent (within 1 month) severe acute diarrhoea were excluded. In addition, alcohol or drug abuses were exclusion criteria. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Research Ethics Committee, Hospital District of Northern Savo. The purpose of the study was explained to the participants, and written informed consent was obtained from all subjects.

Study design and background information

In telephone screening, potential study subjects were interviewed on their consumption of fibre containing food items using an internet-based questionnaire (www.leipatiedotus.fi)[12]. The questionnaire calculated the daily fibre intake in grams per day. The cross-sectional study consisted of two study visits. On the first visit, the demographic data and other background information on the study subjects were collected on a structured form (background demographics and diet in Table 1). Furthermore, anthropometric measurements were taken and subjects were instructed how to collect the faecal samples. Body weight was measured twice on the first study visit on a digital scale (Scale Seca 707, Vogel & Falke GmpH & Co., Hamburg, Germany) and the mean was used in the results. Body height was measured with a Seca telescoping measuring rod, type 221, to the nearest crossed millimetre. The waist circumference was measured at a level midway between the superior aspect of the iliac crest and the lower lateral margin of the ribs. The circumference was measured twice and the second measurement was used as the final result. Typically, study subjects are more relaxed during the second measurement and thus the last measurement is considered more reliable. No overnight fasting was requested before the anthropometric measurements. During the 1-week study period, subjects recorded in a diary all changes in their medication and health status as well as their bowel function. On the second study visit, subjects returned the faecal samples and blood samples were taken.

Sample collection

All blood samples were extracted after a 10- to 12-h overnight fast during the second study visit. The samples were collected to serum tubes for S-insulin, S-hs-CRP, S-leptin and S-adiponectin analysis; to citrate-containing tubes for B-glucose analysis; to gel tubes for total cholesterol, fS-LDL and fS-HDL and fS-TAG analysis; and to EDTA-containing tubes for P-IL-6, P-TNF-α, P-ghrelin, P-peptide tyrosine–tyrosine (PYY) and P-orexin analysis. The samples were analysed as fresh (B-glucose, S-insulin, total cholesterol, LDL- and HDL-cholesterol and TAG) or stored at -70°C (S-leptin, S-adiponectin, S-hs-CRP, P-IL-6, P-TNF-α, P-ghrelin, P-PYY and P-orexin) until analysis. Three spot samples of faeces were collected in a 60 ml specimen tube with an applicator. All faecal samples were placed immediately after collection into a home freezer or were freshly transferred, with a maximum of 1·5 h delay, for freezing in the study unit. Both frozen and fresh samples were transferred to the study unit in a polystyrene box with frozen cool bags. Thereafter, the samples were stored at -20°C until analysed.

Blood measurements

Blood lipids, sugar and insulin. Serum total TAG, total-, LDL- and HDL-cholesterol were analysed by enzymatic photometric assay (Konelab 60i Clinical Chemistry Analyzer, Thermo Electron Corp., Vantaa, Finland) using commercial reagents (Konelab cholesterol, LDL-cholesterol, HDL-cholesterol and TAG, Thermo Electron Corp.) and plasma glucose by using commercial reagents (Konelab glucose, Thermo Electron Corp.). Also serum insulin was analysed by TR-FIA using commercial kits (AutoDELFIA^{TMo} insulin kit, Perkin-Elmer, Boston, MA, USA).

Table 1. Sex, age, life style, dietary restrictions, intake of dietary fibre, bowel function and use of food supplements of the study subjects (*n* 40)
(Mean values with their standard errors)

	Normal weight group (*n* 20)				Obese group (*n* 20)			
	Mean	SEM	*n*	%	Mean	SEM	*n*	%
Men/women (number)			8/12				8/12	
Age (years)	45·0	7·2			46·2	5·8		
BMI (kg/m^2)	23·3	1·6			32·9	2·3		
Waist circumference (cm)	82·2	7·5			107·5	7·4		
Number of subjects doing regular physical exercise*			17	85			13	65
Number of smokers			3	15			4	20
Number of alcohol users			17	85			15	75
Number of subjects having dietary restrictions (lactose etc.)			5	25			6	30
Dietary intake of fibre (g/24 h)	16·1	0·5			14·9	0·9		
Number of subjects using food supplements								
Vitamins, minerals			6	30			7	35
Other food supplements (*n*-fatty acids etc.)			6	30			8	40
Defecation frequency as reported in the interview (times/d)	1·3	0·9			1·3	0·7		

* At least half an hour, two to three times a week.

Satiety-related peptides. Serum leptin and ghrelin were analysed by RIA (Multigamma 1261-001, Perkin-Elmer/Wallac Oy, Turku, Finland) using commercial kits (Human Leptin RIA Kit, cat. no HL-81K, Linco Research Inc., St Charles, MO, USA and Ghrelin (Total) RIA Kit, cat. no GHRT-89HK, Linco Research Inc., respectively). Serum adiponectin was analysed by an immunoenzymometric assay using commercial kits (Human Adiponectin/Acrp30 Immunoassay, R&D Systems Inc., Minneapolis, MN, USA). PYY was analysed by RIA (Multigamma 1261-001, Perkin-Elmer/Wallac Oy) using commercial kits (PYY (Total) RIA Kit, cat. no. PYYT-66HK, Linco Research Inc.). Plasma orexin-A concentrations were analysed with RIA using commercial kits (Orexin-A (Human, Rat, Mouse) RIA Kit; Cat no RK-003-30, Phoenix Pharmaceuticals, Inc., Belmont, CA, USA).

Inflammatory biomarkers. IL-6 and TNF-α were analysed with solid phase ELISA method using commercial kits (Quantikine® HS/Human IL-6 immunoassay and Quantikine® HS/Human TNF-α Immunoassay, R&D Systems Inc., Minneapolis, MN, USA). Hypersensitive CRP was analysed with turbidimetry (Hitachi 912, Roche, GmbH, Germany) using Tina-quant CRP (latex) high sensitive assay (Product number 1972855, Roche Diagnostics).

Faecal measurements

DM, pH, ions and energy. Immediately after melting, the frozen faecal samples were weighed for various physico-chemical analyses. For DM determination, approximately 1 g faecal sample was weighed, dried at 105°C for 16 h, cooled down in a desiccator to room temperature, reweighed and the DM content (%) calculated. For pH measurements, 1 g faecal sample was mixed with 1 ml distilled water and the pH measured immediately (SevenEasy pH, Mettler Toledo GmbH, 8603 Schwerzenbach, Switzerland). For Na^+ and K^+ analyses, 1 g faecal sample was washed and then dissolved in HCl. The solution was diluted with water, and the K content of the solution was determined by an inductively coupled plasma emission spectrometer at wavelength 766·4 nm and the Na content at wavelength 589·5 nm. The energy contents of the faecal samples were determined by an adiabatic bomb calorimeter.

Protein and fat. The faecal protein measurement was based on total n ($N \times 6{\cdot}25 =$ protein) as analysed by the Kjeldahl method. Determination of the total faecal fat was modified from the American Organization of Analytical Chemists 963.15 method (1973; Fat in Cacao Products, Soxthlet Extraction Method). In short, the total amount of petrol diethyl ether-soluble fat was determined after hydrolysis gravimetrically. Fatty acids were analysed as their methyl esters (fatty acid methyl esters) by GC[13].

Phenolic and acidic compounds. The method of analysis for phenolic and acidic compounds in human faeces was based on the method by Knust *et al.* [14]. Briefly, a faecal matrix was initially extracted with a phosphate buffer, and the extract was then acidified and re-extracted with a diethyl ether. Individual phenolic and acidic compounds were identified as their silyl derivatives by GC–MS and semi-quantitated using an internal surrogate standard (salicylic acid). Of the forty-six identified compounds, twenty belonged to di- and hydroxy acids, eleven belonged to phenolics, ten belonged to fatty acids and four belonged to sterols. The results were expressed as a sum of the compounds belonging to each subgroup. Moreover, the individual compounds (succinic acid, lactic acid, 3-OH-benzenepropanoic acid and cholesterol) with the highest concentrations in the subgroups were selected for more detailed statistical analysis.

Soluble and insoluble carbohydrates. To separate the soluble and insoluble carbohydrates from faecal samples, 1 g faeces was weighed into a centrifuge tube and 9 ml PBS was added (the PBS consisted of 8·5 g NaCl, 1·21 g K_2HPO_4, 0·34 g KH_2PO_4, 0·5 g NaN_3 (to prevent bacterial growth), 1 mM phenylmethyl sulphonium fluoride (to prevent serine proteases), 2 mM iodoacetamide (to inhibit cystein-containing enzymes), 10 mM EDTA (to inhibit metalloproteases) and 1 litre H_2O) and stored on ice while weighing other tubes. The samples were shaken vigorously for 1 h at 4°C and then centrifuged for 30 min at 4°C at 15 000 ***g***. The clear supernatants were used for soluble carbohydrate analysis, and the pellet was used for the analysis of insoluble polysaccharides. The samples were stored at −20°C until analysed.

The extraction of soluble carbohydrates from faecal supernatants was modified from the method described by Miller & Hoskins[15]. The faecal supernatants were centrifuged in a 35 ml tube for 30 min at 4°C at 15 000 ***g*** to remove any particulate matter. The clear faecal supernatant was (via filter through a 0·45 μm filter) transferred to another centrifuge tube and 25 ml ice-cold (−18°C) ethanol was added to a final percentage of 70–80 %. The samples were mixed and allowed to stand at 4°C for 1 h. After centrifugation at 4°C for 20 min at 10 000 ***g***, the supernatants were discarded and the pellets were dissolved in 1 ml water. The amount of soluble carbohydrates is the sum of hydrolysed hexoses and amino sugars. To hydrolyse hexoses in the soluble carbohydrate, a fraction of 0·1 ml of the faecal suspension or the hexose standard solution (fucose, arabinose, rhamnose, galactose, xylose, glucose and mannose) was pipetted into a 2 ml microfuge vial, and 0·1 ml 4 M trifluoroacetic acid was added. The samples were incubated at 100°C for 3 h and evaporated to dryness. The samples were dissolved in 1 ml water and filtered through a 0·45 μm membrane filter. The separation and analyses of hexoses were made using high pH ion exchange chromatography (HPLC) and a pulsed electrochemical detector. The analytical column was CarboPac PA1 (4 mm × 250 mm), and the separation was done using a gradient elution with a mobile phase that consisted of a mixture of water and 0·2 M NaOH. To hydrolyse amino sugars in a soluble carbohydrate, a fraction of 0·1 ml of the faecal suspension or the standard solution (galactose amine and glucose amine) was pipetted into a 2 ml microfuge vial, and 0·1 ml 8 M HCl was added. The samples were incubated at 100°C for 5 h and evaporated to dryness. The samples were dissolved in 2 ml water and filtered through a 0·45 μm membrane filter. The separation and analyses of glucose amine and galactose amine were made by HPLC–pulsed electrochemical detector as described earlier.

To analyse the insoluble carbohydrates in the faecal samples, 2 ml of 60 % ice-cold H_2SO_4 was added to the solid pellet obtained after separation of the soluble and insoluble faecal fractions (see above). A glass rod was used to disperse the pellet thoroughly. The samples were allowed to stand at room temperature (20–25°C) for 1 h, then 22 ml water was added to the samples and they were kept at 100°C for 5 h. The samples

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were allowed to cool and then diluted to 50 ml with water and filtered through a 0·45 μm membrane filter. The samples were further diluted 1/10 with 20 mM NaOH. The amino sugars and hexoses were analysed by HPLC–pulsed electrochemical detector as described earlier.

Ammonia, SCFA and biogenic amines. Ammonia, SCFA and branched-chain fatty acids (BCFA) concentrations in faecal samples were measured according to a method of Ouwehand *et al.* [16]. The biogenic amines in faeces were analysed using the method described by Saarinen[17].

Microbial analyses

Total microbial counts. The total bacterial cell counts in digesta samples were determined by flow cytometry (FACS-Calibur, Becton Dickinson, Franklin Lakes, NJ, USA) as previously described[18]. The bacterial fractions were recovered by suspending faecal samples in a 50 mM sodium phosphate buffer (pH 9·0), followed by centrifugation (48 000 ***g***, 30 min, 22°C) and washing. The cell samples were diluted, fixed and stained with the fluorescent nucleic acid-binding dye SYTO 24 (Molecular Probes, Leiden, The Netherlands). The results were expressed as the quantity of bacteria/g fresh digesta weight.

DNA extraction and PCR. DNA was extracted from the washed bacteria using the method described by Apajalahti *et al.* [19], whereby bacteria were subjected to five freeze-thaw cycles and subsequently treated with lysozyme (17·5 mg/ml, 4 h, 37°C) and proteinase K (0·1 mg/ml, 1 h, 37°C). The recovered bacterial DNA was used to quantify total bifidobacteria, lactobacilli, *Clostridium* group XIVab, *Clostridium perfringens*, *Bacteroides* and sulphate-reducing bacteria using primers and probes as described in Table 2.

Quantitative real-time PCR was performed using 1 μg isolated bacterial genomic DNA. A 25-μl amplification reaction consisted of 1 × TaqMan Universal Master Mix (Applied Biosystems, Forster City, CA, USA) with 300 nM of both reverse and forward primers, and 200 nM TaqMan probe (Applied Biosystems). All assays were run on an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using the instrument's default settings for thermal cycling and fluorescence measurements. For standard curves, bacterial genomic DNA from *C. perfringens* (ATCC 13 124) and in-house isolated genomic DNA from *Desulfovibrio intestinalis* (DSM 11 275) were applied. Based on the genomic sizes, the weight of one copy of each of the *C. perfringens* and *D. intestinalis* genome was calculated, and the amount of chromosomes in 1 pg was estimated. Standard amplification curves were constructed by using 1, 10, 100 and 1000 pg bacterial genomic DNA as a template. The results are expressed as the quantity of bacteria/g faeces.

Immunological analyses

The concentrations of IgA, TNF-α, calprotectin and PGE_2 were measured from the soluble fraction of the faeces as previously described[16]. Briefly, the frozen samples were thawed and extracted with bovine serum albumin and stored at −20°C before analysis. Concentrations of IgA, TNF-α and PGE_2 were determined with an ELISA in accordance with the respective manufacturer's instructions (E80-102, Bethyl Laboratories, Inc., Montgomery, TX, USA; Biosource Europe S.A., Nivelles, Belgium; Cayman Chemical Company, Ltd, Ann Arbor, MI, USA), and the results were expressed as μg or pg per gram fresh digesta weight.

Statistical analyses

The basic statistics (mean and standard error) were applied to the data. The comparison of normal weight and obese group means was carried out using the two-sample *t* test, and a *P*-value lower than 0·05 was considered as significant. For addressing associations between inflammatory blood biomarker and intestinal biomarkers, Pearson correlation coefficients were calculated by combining the data from both groups. The association between the parameters was considered significant if the *P*-value was below 0·05.

Results

A number of different biomarkers both from blood and faecal samples were measured from twenty normal weight and twenty obese subjects. The waist circumference was on average 82 and 108 cm, and the BMI was 23 and 33 kg/m^2, in the normal weight and obese subjects, respectively (Table 1).

Blood biomarkers

Concentrations of blood insulin and TAG were higher ($P<0·001$ and $P=0·026$, respectively), whereas concentrations of HDL-cholesterol were lower in the obese subjects ($P=0·006$). Blood glucose, total cholesterol and LDL-cholesterol were similar in both groups. The inflammatory biomarkers, CRP and IL-6, were higher in the obese subjects ($P=0·055$ and 0·024), whereas concentrations of TNF-α were similar in both groups. Of the measured satiety hormones,

Table 2. Primers and probes used to quantify selected members the faecal microbiota

Target	Forward	Reverse	Probe	Reference
Bifidobacterium	CCT GGT AGT CCA CGC CGT AA	CAG GCG GGA TGC TTA ACG	ATC CAG CAT CCA CCG	Makivuokko *et al.* [54]
Lactobacillus	TGG AAA CAG RTG CTA ATA CCG	GTC CAT TGT GGA AGA TTC CC		Byun *et al.* [55]
Clostridium perfringens	TTT GGA GAT ATA GAT ACT CCA TAT CAT CCT	GTG CAA AAG TCT CAA ACT TAA CAT GTC	TAA TGT TAC TGC CGT TGA T	Tiihonen *et al.* [30]
Clostridium group XIVab	GAW GAA GTA TYT CGG TAT GT	CTA CGC WCC CTT TAC AC		Song *et al.* [56]
Bacteroides	GGC GAC CGG CGC ACG GG	GRC CTT CCT CTC AGA ACC C		Nakanishi *et al.* [57]
Sulphate reducers	GGC GCT GAA ATG ACC ATG AT	GGC CGT AAC CGT CCT TGA A	TTC GTG CCC GCC CG	Tiihonen *et al.* [30]

Table 3. Blood biomarkers in the obese and normal weight subjects
(Mean values with their standard errors)

	Normal weight group			Obese group			
Variable	*n**	Mean	SEM	*n**	Mean	SEM	*P*
Biomarkers of sugar and lipid metabolism							
Total cholesterol (mmol/l)	20	4·65	0·19	20	4·49	0·19	0·568
LDL-cholesterol (mmol/l)	20	2·95	0·14	20	2·95	0·16	0·981
HDL-cholesterol (mmol/l)	20	1·57	0·10	20	1·19	0·09	0·006†
Total TAG (mmol/l)	20	0·94	0·10	20	1·54	0·23	0·026†
Fasting plasma glucose (mmol/l)	20	5·32	0·17	19	5·59	0·12	0·198
Fasting serum insulin (mU/l)	20	5·10	0·67	19	10·21	1·00	0·0001†
Inflammatory biomarkers							
Hypersensitive CRP (mg/l)	20	1·23	0·38	20	2·50	0·52	0·055
IL-6 (pg/ml)	20	0·85	0·13	19	1·77	0·36	0·024†
TNF-α (pg/ml)	20	1·00	0·24	20	1·09	0·12	0·732
Satiety-related hormones							
Adiponectin (μg/ml)	20	10·32	1·61	20	8·53	1·60	0·436
Ghrelin (pg/ml)	20	1058·7	79·8	20	840·7	58·8	0·034†
Leptin (ng/ml)	20	6·89	1·14	20	22·75	2·95	<0·0001†
PYY (pg/ml)	20	70·84	3·32	20	80·83	6·38	0·176
Orexin (pg/ml)	20	12·13	0·67	19	14·51	2·49	0·366

CRP, C-reactive protein; PYY, peptide tyrosine–tyrosine.
* The sample size used for calculating the descriptive statistics. *n* is smaller than twenty in some cases due to missing data. Two-sample *t* test was used to compare the mean values between the two groups.
† A *P*-value <0·05 was considered to indicate significant difference between the groups.

concentrations of leptin were higher and concentrations of ghrelin were lower in the obese subjects ($P<0·0001$ and $P=0·03$, respectively). Concentrations of adiponectin, PYY and orexin were similar in both groups (Table 3).

Faecal measurements

Chemical composition of the faecal samples was studied with focus on the available nutrients and energy (Table 4). The remaining measured energy in the faecal samples was similar in both groups, as well as the other measured physico-chemical properties (faecal weight, DM content, pH, and concentrations of Na^+ and K^+), with the exception of concentrations of ammonia and carbohydrates that showed a trend for higher levels in the obese subjects ($P=0·15$ and 0·14, respectively). The residual concentrations of macronutrients, proteins and fats were similar in both groups.

Table 4. Chemical composition of faeces from the normal weight and obese subjects
(Mean values with their standard errors)

	Normal weight group			Obese group			
Variable	*n**	Mean	SEM	*n**	Mean	SEM	*P*
Physico-chemical							
Faecal weight (g fw)	20	221	29	20	231	25	0·800
DM (%)	20	22·3	0·8	20	23·3	1·5	0·573
pH	20	7·13	0·10	20	7·01	0·09	0·387
Ammonia (mmol/kg fw)	20	22·6	1·6	19	26·6	2·2	0·151
Na^+ (g/kg fw)	8	0·25	0·08	8	0·32	0·08	0·525
K^+ (g/kg fw)	8	4·54	0·37	8	4·02	0·25	0·257
Energy (kJ/100 g fw)	8	415	36	8	425	29	0·829
Residual macronutrients (g/kg fw)							
Protein	20	75·0	3·8	20	76·6	5·7	0·814
Carbohydrates	20	26·6	1·4	20	30·5	2·2	0·140
Soluble carbohydrates	20	1·13	0·19	20	1·23	0·22	0·724
Insoluble carbohydrates	20	25·5	1·5	20	29·3	2·2	0·150
Fat	8	36·8	8·2	8	24·7	3·9	0·201
Sum of fatty acids	8	2·23	0·84	8	3·54	1·59	0·480
Sum of sterols†	8 (3)	0·54	0·28	8 (3)	0·25	0·13	0·350
Cholesterol‡	8 (4)	0·49	0·28	7 (4)	0·06	0·03	0·170

fw, fresh digesta weight.
* The sample size used for calculating the descriptive statistics including the number of samples below the detection limits (given in parenthesis). For calculation of the mean values, the data below detection limits were set to half of the limit value. Two-sample *t* test was used to compare the mean values between the two groups.
† Detection limit 0·01 g/kg fw.
‡ Detection limit 0·01 g/kg fw.

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While the total concentration of bacteria was similar, the microbial composition was different in the obese and normal weight subjects. The level of sulphate-reducing bacteria ($P=0{\cdot}05$) and *Bacteroides* ($P=0{\cdot}07$) appeared higher in the normal weight subjects than in the obese subjects (Table 5). Of the microbial metabolites, the sum of SCFA was similar in both groups. However, the concentrations of lactic acid and valeric acid were different ($P=0{\cdot}08$ and $0{\cdot}04$) between the study groups (Table 5). The sum of BCFA ($P=0{\cdot}03$) was higher in the obese subjects, as were the two main components, 2-methylbutyric acid and isovaleric acid ($P=0{\cdot}02$ and $0{\cdot}03$, respectively). However, neither the sum of biogenic amines nor any of the single biogenic amines differed significantly between the groups. The sum of phenolic concentrations was significantly higher in the obese subjects ($P=0{\cdot}02$). In more detail, the 3-OH-benzenepropanoic acid concentrations were elevated in the obese subjects ($P=0{\cdot}06$).

No differences were detected in the concentrations of faecal immune markers, IgA, TNF-α, calprotectin and PGE_2 (Table 5).

Correlations between blood inflammatory biomarkers and intestinal biomarkers

It was of special interest to study the correlations between inflammatory blood biomarkers and the intestinal biomarkers. Serum CRP and IL-6 appeared to be correlated, especially

Table 5. Faecal microbes and microbial metabolites in the normal and obese subjects
(Mean values with their standard errors)

	Normal weight group			Obese group			
Variable	*n**	Mean	SEM	*n**	Mean	SEM	*P*
Microbes (log10 cells/g fw)							
Total counts	20	$1{\cdot}84\times10^{11}$	$2{\cdot}05\times10^{10}$	20	$2{\cdot}03\times10^{11}$	$2{\cdot}08\times10^{10}$	0·523
Clostridium group XIVab	20	$3{\cdot}77\times10^{10}$	$4{\cdot}12\times10^{9}$	20	$3{\cdot}33\times10^{10}$	$3{\cdot}29\times10^{9}$	0·403
Bacteroidetes	20	$5{\cdot}33\times10^{9}$	$1{\cdot}06\times10^{9}$	20	$2{\cdot}72\times10^{9}$	$9{\cdot}41\times10^{8}$	0·074
Bifidobacterium	20	$4{\cdot}33\times10^{9}$	$1{\cdot}18\times10^{9}$	20	$3{\cdot}45\times10^{9}$	$6{\cdot}68\times10^{8}$	0·523
Lactobacillus	20	$3{\cdot}64\times10^{8}$	$1{\cdot}53\times10^{8}$	20	$4{\cdot}26\times10^{8}$	$2{\cdot}04\times10^{8}$	0·810
Sulphate reducers†	20 (12)	$3{\cdot}62\times10^{7}$	$1{\cdot}7\times10^{7}$	20 (12)	$1{\cdot}36\times10^{6}$	$1{\cdot}28\times10^{6}$	0·055
Clostridium perfringens‡	20 (8)	$1{\cdot}43\times10^{5}$	$8{\cdot}81\times10^{4}$	20 (6)	$2{\cdot}54\times10^{3}$	$1{\cdot}19\times10^{3}$	0·128
Sum of SCFA (mmol/kg fw)	20	67·31	6·22	20	78·79	6·19	0·199
Acetic acid	20	42·13	3·84	20	47·15	3·80	0·359
Propionic acid	20	11·50	1·19	20	13·64	1·34	0·241
Butyric acid	20	11·28	1·42	20	14·73	1·47	0·100
Valeric acid	20	1·84	0·11	20	2·45	0·25	0·036§
Capronic acid‖	20	0·525	0·132	20	0·780	0·224	0·335
Sum of BCFA (mmol/kg fw)	8	2·98	0·24	20	3·89	0·33	0·034§
2-Methylbutyric acid	20	0·747	0·064	20	0·101	0·088	0·023§
Isovaleric acid	20	0·945	0·073	20	1·255	0·108	0·026§
Isobutyric acid	20	1·29	0·11	20	1·61	0·14	0·088
Sum of biogenic amines (nmol/kg fw)	19	2829	727	20	2175	360	0·428
Methylamine	19	419·17	42·37	20	409·12	49·12	0·878
Phenylethylamine¶	19 (7)	72·81	17·21	20 (4)	88·93	19·23	0·537
Putrescine¶	19 (2)	898·77	490·01	20 (2)	477·57	122·94	0·414
Cadaverine¶	19 (3)	697·68	315·72	19 (1)	560·38	268·68	0·742
Histamine**	19 (13)	17·18	4·31	20 (14)	35·77	14·03	0·218
Tyramine**	19 (11)	28·46	7·29	20 (10)	35·61	10·66	0·587
Spermidine	19	633·77	53·29	20	549·62	51·89	0·265
Spermine	19	42·06	6·33	20	31·64	3·28	0·155
Sum of di- and hydroxy acids (mg/kg fw)	8	426·5	65·5	8	765·5	127·4	0·033§
Lactic acid††	8 (2)	67·13	19·16	8 (1)	249	88·80	0·082
Succinic acid††	8 (1)	103·9	39·4	8 (1)	173	40·8	0·243
Sum of phenolic acids (mg/kg fw)	8 (3)	305·6	61·6	8 (2)	723·3	137·9	0·021§
3-OH-Benzene propanoic acid††	8	163·1	59·9	8	519	151·6	0·056
Immunological biomarkers							
IgA (μg/g fw)‡‡	20 (2)	129·3	36·2	20	189·3	35·1	0·241
PGE_2 (pg/g fw)	20	498·2	56·5	20	486·1	39·0	0·860
TNF-α (pg/g fw)§§	20 (1)	9·45	2·74	20	15·53	6·36	0·388
Calprotectin (μg/g fw)	20	34·23	17·09	20 (3)	26·44	4·89	0·666

fw, fresh digesta weight; BCFA, branched-chain fatty acid.
* The sample size used for calculating the descriptive statistics including the number of samples below the detection limits (given in parenthesis). For calculation of the mean values, the data below detection limits were set to half of the limit value. Two-sample *t* test was used to compare the mean values between the two groups.
† Detection limits: $1{\cdot}2\times10^{2}$ cells/g.
‡ Detection limits: $5{\cdot}1\times10^{1}$ cells/g.
§ A *P*-value $<0{\cdot}05$ was considered to indicate significant difference between the groups.
‖ Detection limits: 0·1 mmol/kg.
¶ Detection limits: 30 nmol/kg.
** Detection limits: 15 nmol g/kg.
†† Detection limits: 10 mg/kg.
‡‡ Detection limits: 0·4 μg/kg.
§§ Detection limits: 0 pg/g.

Table 6. Pearson correlation coefficients (*r*) and *P*-values for testing whether the correlation is zero or not for faecal and clinical measurements combining data from both the groups

Faecal parameters...	Concentrations of di- and hydroxy acids			Concentrations of phenolics			Concentrations of sterols			Total microbial counts			Numbers of *Bacteroides*		
Clinical parameters	*r*	*P**	*n*†	*r*	*P**	*n*†	*r*	*P**	*n*†	*r*	*P**	*n*†	*r*	*P**	*n*†
CRP	0·51	0·04	16	0·53	0·04	16		NS		−0·51	0·04	16		NS	
Concentrations of IL-6	0·51	0·04	16	0·62	0·01	16		NS			NS			NS	
Concentrations of TNF-α		NS			NS		0·77	0·0002	16	0·42	0·006	40		NS	
Waist circumference	0·52	0·04	16		NS			NS			NS		−0·31	0·05	40
Weight	0·56	0·02	16	0·60	0·01	16		NS			NS		−0·26	0·09	40

CRP, C-reactive protein.
* A *P*-value $<0·05$ indicates significant association.
† Values refer to the number of observations for which measurements on both parameters are available.

with some of the microbial metabolites, i.e. concentrations of phenolics ($P=0·04$ and 0·01) and di- and hydroxy acids ($P=0·04$ and 0·04), whereas serum TNF-α was associated with the total number of bacteria and the faecal concentrations of sterols ($P=0·006$ and $P<0·001$). An inverse correlation between the numbers of faecal *Bacteroides* and waist circumference ($P=0·05$) as well as weight ($P=0·09$) was noted. In addition, an inverse correlation between the total faecal microbial counts and serum CRP ($P=0·04$) was found (Table 6).

Discussion

The obese subjects included in the present study were found to represent typical characteristics regarding their fasting satiety-regulating hormones, metabolic disturbances and inflammatory biomarkers. In accordance with previous findings, the plasma TAG concentrations were higher and HDL-cholesterol was lower in obese subjects than in normal weight subjects[20]. Moreover, the increased insulin concentrations in the study subjects may indicate insulin resistance. The elevated plasma leptin levels in the present study are also in line with previous findings[21] indicating leptin resistance in obese subjects[21]. Circulating ghrelin, which has the opposite effect to leptin, is, however, decreased as reported also by Tschop *et al.* [22]. Adipose tissue has been described as an origin for pro-inflammatory cytokines such as IL-6 and TNF-α[23]. It has been proposed that hypoxia caused by enlarged adipocyte size may underlie an inflammatory response, increasing IL-6 and TNF-α production and decreasing secretion of anti-inflammatory adiponectin[24]. Increased serum IL-6 concentrations are reported to be associated with visceral adiposity, whereas serum TNF-α showed more associations with total body fatness[20,25]. The present study also indicated a correlation between serum IL-6 and waist circumference, while serum TNF-α correlated with faecal sterol concentrations. The latter phenomenon possibly indicating of a feedback signalling from the stressed adipocytes, indicated by secretion of TNF-α, to the intestinal tissue to reduce absorption of sterols forms the diet, thus perhaps resulting in increased concentrations present in the faeces. Alternatively, in rodent model, inflammation has shown to impair reverse cholesterol transport to faeces[26]. High-fat feeding has shown to increase gut permeability of bacterial lipopolysaccharides to plasma, which in turn triggers low-grade inflammation and obesity-associated disorders[27,28]. Anti-inflammatory adiponectin concentrations are typically decreased in obesity[29]; however, in the present study, the decrease was not statistically significant. It is of interest that, despite the systemic inflammatory status, the measured faecal immunological biomarkers were not affected by obesity. Previously, it has been described that concentrations of faecal PGE_2 may be affected by age[30], and furthermore, that faecal IgA concentrations may be elevated during the allergy season in subjects allergic to birch pollen[31]. Both calprotectin and TNF-α are used to monitor the state of intestinal inflammation, and antibodies specific to TNF-α are also used as treatment to suppress the intestinal inflammation, in patients with inflammatory bowel disease and ulcerative colitis[32–34].

Only relatively small changes were detected in the faecal microbial composition between the obese and normal weight groups. However, the fermentation pattern in obese subjects is more pronounced in protein than carbohydrate fermentation as suggested by increased concentrations of BCFA, phenolics and a tendency for increased ammonium concentrations[35]. Surprisingly, in spite of the indirect indications of elevated amounts of protein fermentation, less sulphate-reducing bacteria were detected in the obese subjects. In accordance with previous observations, the levels of *Bacteroides* were decreased in the obese subjects[10]. Interestingly, the waist circumference was inversely correlated with the numbers of *Bacteroides*, underlining the possible role of *Bacteroides* in the absence of adiposity. Increased numbers of faecal *Bacteroidetes* have also been detected in elderly over 70 years of age than in young adults[36]. In a recently published retrospective study on children at risk of developing allergy, less bifidobacteria and more *Staphylococcus aureus* were found in the faeces of children that later became overweight[37]. In the present study, the levels of *S. aureus* were not determined, but the levels of bifidobacteria were similar in both the normal weight and obese subjects. Furthermore, other associations between chronic conditions such as irritable bowel syndrome and intestinal microbiota have also been reported[38].

The elevated concentrations of many residual microbial metabolites, both originating from carbohydrate and protein fermentation, in the faeces suggest a different fermentation profile in the colon of the obese subjects. Previously, it has been described that faecal SCFA are found in higher levels in obese subjects than in lean subjects[39], while, in the present study, the difference was statistically significant only in the faecal concentrations of BCFA. Recently, Samuel *et al.* [40] have indicated SCFA as signalling molecules for enteroendocrine cells to produce PYY and thus decreasing gut motility and further

increasing SCFA absorption and energy extraction from the gut lumen. Microbial metabolites may also regulate food intake differently in obese and in lean subjects, as has been indicated by rat studies[41]. Many metabolites resulting from putrefaction, such as phenols[42], have been linked with health risks, such as colon cancer[43], whereas metabolites, such as butyrate, may be chemopreventive in colon carcinogenesis[44]. Obesity has been identified as a risk factor for the development of colon cancer[45]; the higher faecal levels of BCFA and phenols could potentially be a partial explanation for these epidemiological observations. Concentrations of faecal butyrate were not found to be decreased in the obese subjects in the present study. In summary, the changed microbial metabolite profile in the faeces of obese subjects may reflect the adaptation of the microbial community to the different nutrients available.

In the present study, faecal phenolics and lactic acid were found to be associated with the blood inflammatory biomarkers, CRP and IL-6. It remains to be determined whether or not these coinciding changes are somehow causally linked to each other. L-lactate is generally a signal of anaerobic metabolism in tissues and thus may induce inflammatory response in adipose tissues. Lactic acid is also produced by many different GI microbes and is typically utilised by other intestinal microbes[46] and does not accumulate in the faeces of healthy subjects. If, however, lactate is accumulated in the lower GI tract due to an imbalance in the lactate-producing and lactate-utilising bacteria, it can be absorbed into the blood circulation either by passive diffusion (D-lactate) or via the monocarboxylate transporter (L-lactate)[47]. Therefore, lactic acid originating from intestinal fermentation can have also systemic effects, e.g. with muscle cells[48]. Unfortunately, plasma lactate levels were not analysed in the present study. In obesity, elevated blood lactate concentrations are suggested to originate from increased lactate production by adipocytes[49]. However, the postprandial rise in circulating lactate may be partly explained by the intestinal lactate production. Chronic hyperlactaemia has been suggested to have a role in the development of insulin resistance in muscle cells[48]. Excessive amounts of lactic acid in the blood circulation have been shown to cause neurotoxicity and cardiac arrhythmia[50,51]. Also dietary or microbe-derived phenolics can be absorbed from the GI tract, but their uptake can be incomplete[52]. In addition to microbial metabolites, structural microbe-derived products, such as lipopolysaccharides, have been recently suggested to affect the development of metabolic diseases in animal models[53].

Overall, the changes in the microbial fermentation patterns, including increased faecal phenolics and lactic acid concentrations, described in the obese subjects most probably have an impact on host physiology including the systemic inflammatory condition. Future studies should focus on studying whether these obesity-associated intestinal parameters could be a potential therapeutic target for decreasing obesity-associated systemic effects.

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References

1. Peeters A, Barendregt JJ, Willekens F, *et al.* (2003) Obesity in adulthood and its consequences for life expectancy: a life-table analysis. *Ann Intern Med* **138**, 24–32.
2. Mikkola I, Keinanen-Kiukaanniemi S, Laakso M, *et al.* (2007) Metabolic syndrome in connection with BMI in young Finnish male adults. *Diabetes Res Clin Pract* **76**, 404–409.
3. Esteghamati A, Khalilzadeh O, Anvari M, *et al.* (2008) Metabolic syndrome and insulin resistance significantly correlate with body mass index. *Arch Med Res* **39**, 803–808.
4. Yudkin JS, Stehouwer CDA, Emeis JJ, *et al.* (1999) C-reactive protein in wealthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction – a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* **19**, 972–978.
5. Dandona P, Aljada A & Bandyopadhyay A (2004) Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* **25**, 4–7.
6. Bahceci M, Gokalp D, Bahceci S, *et al.* (2007) The correlation between adiposity and adiponectin tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size asssociated with inflammation in adults? *J Endocrinol Invest* **30**, 210–214.
7. Festa A, D'Agostino R, Williams K, *et al.* (2001) The relation of body fat mass and distribution to markers of chronic inflammation. *Int J Obes* **25**, 1407–1415.
8. Diamond JM & Karasov WH (1987) Adaptive regulation of intestinal nutrient transporters. *Proc Natl Acad Sci USA* **84**, 2242–2245.
9. Diamond J (1991) Evolutionary design of nutrient absorption: enough but not too much. *News Physiol Sci* **6**, 92–96.
10. Ley RE, Turnbaugh PJ, Klein S, *et al.* (2006) Microbial ecology – human gut microbes associated with obesity. *Nature* **444**, 1022–1023.
11. Turnbaugh PJ, Ley RE, Mahowald MA, *et al.* (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031.
12. Lyly M, Soini E, Rauramo U, *et al.* (2004) Perceived role of fibre in a healthy diet among Finnish consumers. *J Hum Nutr Diet* **17**, 231–239.
13. Cristie WW (1989) *Gas Chromatography and Lipids, A Practical Guide*. Glasgow: The Oily Press Ltd.
14. Knust U, Erben G, Spiegelhalder B, *et al.* (2006) Identification and quantitation of phenolic compounds in faecal matrix by capillary gas chromatography and nano-electrospray mass spectrometry. *Rapid Commun Mass Spectrom* **20**, 3119–3129.
15. Miller RS & Hoskins LC (1981) Mucin degradation in human colon ecosystems. Fecal population densities of mucin-degrading bacteria estimated by a 'most probable number' method. *Gastroenterology* **81**, 759–765.

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16. Ouwehand AC, Tiihonen K, Saarinen M, *et al.* (2009) Influence of a combination of *Lactobacillus acidophilus* NCFM and lactitol on healthy elderly: intestinal and immune parameters. *Br J Nutr* **101**, 367–375.
17. Saarinen M (2002) Determination of biogenic amines as dansyl derivatives in intestinal digesta and feces by reversed phase HPLC. *Chromatographia* **55**, 297–300.
18. Apajalahti JH, Kettunen H, Kettunen A, *et al.* (2002) Culture-independent microbial community analysis reveals that inulin in the diet primarily affects previously unknown bacteria in the mouse cecum. *Appl Environ Microbiol* **68**, 4986–4995.
19. Apajalahti JH, Sarkilahti LK, Maki BR, *et al.* (1998) Effective recovery of bacterial DNA and percent-guanine-plus-cytosine-based analysis of community structure in the gastrointestinal tract of broiler chickens. *Appl Environ Microbiol* **64**, 4084–4088.
20. Couillard C, Bergeron N, Prud'homme D, *et al.* (1998) Postprandial triglyceride response in visceral obesity in men. *Diabetes* **47**, 953–960.
21. Considine RV (1997) Leptin and obesity in humans. *Eat Weight Disord* **2**, 61–66.
22. Tschop M, Weyer C, Tataranni PA, *et al.* (2001) Circulating ghrelin levels are decreased in human obesity. *Diabetes* **50**, 707–709.
23. Compher C & Badellino KO (2008) Obesity and inflammation: lessons from bariatric surgery. *JPEN J Parenter Enteral Nutr* **32**, 645–647.
24. Trayhurn P, Wang B & Wood IS (2008) Hypoxia and the endocrine and signalling role of white adipose tissue. *Arch Physiol Biochem* **114**, 267–276.
25. Cartier A, Lemieux I, Almeras N, *et al.* (2008) Visceral obesity and plasma glucose-insulin homeostasis: contributions of interleukin-6 and tumor necrosis factor-alpha in men. *J Clin Endocrinol Metab* **93**, 1931–1938.
26. McGillicuddy FC, de la Llera MM, Hinkle CC, *et al.* (2009) Inflammation impairs reverse cholesterol transport *in vivo*. *Circulation* **119**, 1135–1145.
27. Cani PD, Amar J, Iglesias MA, *et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772.
28. Cani PD, Bibiloni R, Knauf C, *et al.* (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* **57**, 1470–1481.
29. Weyer C, Funahashi T, Tanaka S, *et al.* (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* **86**, 1930–1935.
30. Tiihonen K, Tynkkynen S, Ouwehand A, *et al.* (2008) The effect of ageing with and without non-steroidal anti-inflammatory drugs on gastrointestinal microbiology and immunology. *Br J Nutr* **100**, 130–137.
31. Ouwehand AC, Nermes M, Collado MC, *et al.* (2009) Specific probiotics alleviate allergic rhinitis during the birch pollen season. *World J Gastroenterol* **15**, 3261–3268.
32. Foell D, Wittkowski H & Roth J (2009) Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. *Gut* **58**, 859–868.
33. Tsianos EV & Katsanos K (2009) Do we really understand what the immunological disturbances in inflammatory bowel disease mean? *World J Gastroenterol* **15**, 521–525.
34. Yadav PK & Liu Z (2009) Current strategies for the treatment of ulcerative colitis. *Recent Pat Inflamm Allergy Drug Discov* **3**, 65–72.
35. Cummings JH & Macfarlane GT (1991) The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* **70**, 443–459.
36. Makivuokko H, Tiihonen K, Tynkkynen S, *et al.* (2009) The effect of age and non-steroidal anti-inflammatory drugs on human intestinal microbiota composition. *Br J Nutr* (Epublication ahead of print version 25 August 2009).
37. Kalliomaki M, Collado MC, Salminen S, *et al.* (2008) Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* **87**, 534–538.
38. Malinen E, Rinttila T, Kajander K, *et al.* (2005) Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* **100**, 373–382.
39. Schwiertz A, Taras D, Schafer K, *et al.* (2009) Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* (Epublication ahead of print version 4 June 2009).
40. Samuel BS, Shaito A, Motoike T, *et al.* (2008) Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci USA* **105**, 16767–16772.
41. Bray GA (2000) Afferent signals regulating food intake. *Proc Nutr Soc* **59**, 373–384.
42. Hughes R & Rowland IR (2000) Metabolic activities of the gut microflora in relation to cancer. *Microb Ecol Health Dis* **11**, 179–185.
43. Rafter JJ (2002) Scientific basis of biomarkers and benefits of functional foods for the reduction of disease risk: cancer. *Br J Nutr* **88**, S219–S224.
44. Scheppach W & Weiler F (2004) The butyrate story: old wine in new bottles. *Curr Opin Clin Nutr Metab Care* **7**, 563–567.
45. Larsson SC & Wolk A (2007) Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. *Am J Clin Nutr* **86**, 556–565.
46. Duncan SH, Louis P & Flint HJ (2004) Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol* **70**, 5810–5817.
47. Ritzhaupt A, Wood IS, Ellis A, *et al.* (1998) Identification and characterization of a monocarboxylate transporter (MCT1) in pig and human colon: its potential to transport L-lactate as well as butyrate. *J Physiol* (Pt 3), **513**, 719–732.
48. Lombardi AM, Fabris R, Bassetto F, *et al.* (1999) Hyperlactatemia reduces muscle glucose uptake and GLUT-4 mRNA while increasing (E1alpha)PDH gene expression in rat. *Am J Physiol* **276**, E922–E929.
49. DiGirolamo M, Newby FD & Lovejoy J (1992) Lactate production in adipose tissue: a regulated function with extra-adipose implications. *FASEB J* **6**, 2405–2412.
50. Chan L, Slater J, Hasbargen J, *et al.* (1994) Neurocardiac toxicity of racemic D,L-lactate fluids. *Integr Physiol Behav Sci* **29**, 383–394.
51. Vella A & Farrugia G (1998) D-Lactic acidosis: pathologic consequence of saprophytism. *Mayo Clin Proc* **73**, 451–456.
52. Jenner AM, Rafter J & Halliwell B (2005) Human fecal water content of phenolics: the extent of colonic exposure to aromatic compounds. *Free Radic Biol Med* **38**, 763–772.
53. Reigstad CS, Lunden GO, Felin J, *et al.* (2009) Regulation of serum amyloid A3 (SAA3) in mouse colonic epithelium and adipose tissue by the intestinal microbiota. *PLoS One* **4**, e5842.
54. Makivuokko H, Nurmi J, Nurminen P, *et al.* (2005) *In vitro* effects on polydextrose by colonic bacteria and caco-2 cell cyclooxygenase gene expression. *Nutr Cancer* **52**, 94–104.
55. Byun R, Nadkarni MA, Chhour KL, *et al.* (2004) Quantitative analysis of diverse *Lactobacillus* species present in advanced dental caries. *J Clin Microbiol* **42**, 3128–3136.
56. Song Y, Liu C & Finegold SM (2004) Real-time PCR quantitation of clostridia in feces of autistic children. *Appl Environ Microbiol* **70**, 6459–6465.
57. Nakanishi Y, Murashima K, Ohara H, *et al.* (2006) Increase in terminal restriction fragments of Bacteroidetes-derived 16S rRNA genes after administration of short-chain fructooligosaccharides. *Appl Environ Microbiol* **72**, 6271–6276.

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