Differences between human subjects in the composition of the faecal bacterial community and faecal metabolism of linoleic acid

Estelle Devillard,^{1†} Freda M. McIntosh,¹ Delphine Paillard,¹ Nadine A. Thomas,¹ Kevin J. Shingfield² and R. John Wallace¹

Correspondence

R. John Wallace
John.Wallace@rowett.ac.uk

¹Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

²Animal Production Research, MTT Agrifood Research Finland, 31600, Jokioinen, Finland

Conjugated linoleic acid (CLA) is formed from linoleic acid (LA; *cis*-9,*cis*-12-18:2) by intestinal bacteria. Different CLA isomers have different implications for human health. The aim of this study was to investigate LA metabolism and the CLA isomers formed in two individuals (V1 and V2) with different faecal metabolic characteristics, and to compare fatty acid metabolism with the microbial community composition. LA incubated with faecal samples was metabolized at similar rates with both subjects, but the products were different. LA was metabolized extensively to stearic acid (SA; 18:0) in V1, with minor accumulation of CLA and more rapid accumulation of vaccenic acid (VA; *trans*-11-18:1). CLA accumulation at 4 h was almost tenfold higher with V2, and little SA was formed. At least 12 different isomers of CLA were produced from LA by the colonic bacteria from the two individuals. The predominant (>75%) CLA isomer in V1 was rumenic acid (RA; *cis*-9,*trans*-11-18:2), whereas the concentrations of RA and *trans*-10,*cis*-12-18:2 were similar with V2. Propionate and butyrate proportions in short-chain fatty acids were higher in V1. A 16S rRNA clone library from V1 contained mainly *Bacteroidetes* (54% of clones), whereas *Firmicutes* (66% of clones) predominated in V2. Both samples were devoid of bacteria related to *Clostridium proteoclasticum*, the only gut bacterium known to metabolize VA to SA. Thus, the CLA formed in the intestine of different individuals may differ according to their resident microbiota, with possibly important implications with respect to gut health.

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INTRODUCTION

Conjugated linoleic acid (CLA) is a collective term used to describe positional and geometric isomers of linoleic acid (LA; *cis*-9,*cis*-12-18:2) containing a conjugated double bond. Evidence derived from studies in animal models and clinical trials indicates that CLA could have a number of beneficial effects on human health, including decreasing carcinogenesis and atherosclerosis, controlling body fat gain and enhancing the immune response as well as decreasing inflammation and other adverse effects typically associated with immune enhancement (McLeod *et al.*, 2004; O'Shea *et al.*, 2004; Wang & Jones, 2004; Lee *et al.*, 2005; Tricon *et al.*, 2005).

The main source of CLA in man is ruminant-derived foods (Lawson *et al.*, 2001). CLA may also be formed during metabolism of LA by intestinal bacteria, as demonstrated in rats (Eyssen & Parmentier, 1974; Chin *et al.*, 1994; Ewaschuk *et al.*, 2006) and man (Howard & Henderson, 1999; Devillard *et al.*, 2006; Kamlage *et al.*, 1999, 2000). CLA shows anti-inflammatory properties with colonocytes (Hontecillas *et al.*, 2002; Bassaganya-Riera *et al.*, 2004; Bassaganya-Riera & Hontecillas, 2006), and therefore could be therapeutic for inflammatory bowel diseases, including ulcerative colitis and Crohn's disease. It has also been suggested that CLA inhibits the development of colorectal cancer (Cho *et al.*, 2005; Lampen *et al.*, 2005; Lee *et al.*, 2005; Beppu *et al.*, 2006). Positional and geometric isomers of CLA may have different physiological effects in humans (Tricon *et al.*, 2005). It is therefore important to understand the formation of specific isomers of CLA during LA metabolism in relation to gut health.

Pure-culture studies indicated that the most active LA-metabolizing bacteria in the human intestine were *Roseburia* spp. and *Butyrivibrio fibrisolvens* (Devillard *et al.*, 2007). *Roseburia inulinivorans* and *Roseburia hominis*

[†]Present address: ADISSEO France SAS, Route de Chamblet, 03600 Commeny, France.

Abbreviations: CLA, conjugated linoleic acid; DMOX, 4,4-dimethyloxazoline fatty acid derivatives; FAME, fatty acid methyl ester; LA, linoleic acid (*cis*-9,*cis*-12-18:2); MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; RA, rumenic acid (*cis*-9,*trans*-11-18:2); SA, stearic acid (18:0); SCFA, short-chain fatty acid; VA, vaccenic acid (*trans*-11-18:1).

formed vaccenic acid (VA; *trans*-11-18:1), as did *B. fibrisolvens*. Other *Roseburia* spp. formed a hydroxy 18:1 fatty acid, which was converted subsequently to VA. In contrast, *Propionibacterium* and *Bifidobacterium* spp. formed a mixture of CLA isomers. As the proportion of predominant bacterial species varies between individuals (Zoetendal *et al.*, 1998; Eckburg *et al.*, 2005; Duncan *et al.*, 2007; Flint *et al.*, 2007), we considered it plausible that LA metabolism might vary in a similar way, with possible implications for gut health. Preliminary experiments were carried out with faecal samples from a panel of human volunteers, from which two were selected as having different biohydrogenation characteristics. Here we report detailed analysis of faecal CLA formation and community structure in those individuals, which offers an insight into how LA metabolism and potential consequences on human health may vary according to the intestinal bacterial population.

METHODS

Donors and sample collections. Faecal samples were collected from two healthy 33-year-old females. The volunteers had no history of gastrointestinal disorders and no antibiotic treatment during the 2 months preceding the study. The diets of the two subjects were not controlled, though both consumed diets that would be considered typical of a Western diet. Freshly voided faeces were collected in sterile containers and were used in incubations with fatty acid substrates within 1 h of collection. Subsamples were stored at -20°C for later DNA extraction and short-chain fatty acid (SCFA) determinations.

Incubation of faecal samples with LA. Experiments were conducted in 12.5×1.5 cm culture tubes equipped with screw caps and butyl rubber septa (Bellco Biotechnology) to maintain anaerobic (CO_2) conditions during incubations. Fresh samples of faeces were homogenized, and subsamples (between 2 and 4 g) were transferred into sterile 50 ml tubes. Then 0.1 M potassium phosphate anaerobic buffer, pH 7.0, was added to obtain a final suspension of 0.1 g faecal sample per ml. The diluted sample was homogenized three times for 1 min on ice (Ultra Turrax, IKA-Werke) and filtered through three layers of muslin cloth to remove large particles. At time 0, 5 ml of the suspension was transferred into a tube maintained at 37°C under CO_2 and containing 100 μl of an aqueous solution of 25 mg LA ml^{-1} (Sigma) prepared by sonication. Immediately, a 1 ml aliquot was removed from the incubation mixture. To prevent further metabolic activity, the samples were heated at 100°C for 10 min. The remainder was incubated under CO_2 at 37°C , with samples being collected at 1, 4, and 8 h. Each incubation was performed in triplicate.

Fatty acid extraction and analysis. Following the addition of internal standard (C19:0), samples were saponified in 2 M NaOH for 30 min at 85°C (Devillard *et al.*, 2006). Use of pure unsaturated fatty acids standards treated or not with 2 M NaOH for 30 min at 85°C (results not shown) was used to verify that the saponification procedure liberated non-esterified fatty acids from complex lipids, without causing isomerization of long-chain unsaturated fatty acids. Fatty acids were extracted using a mixture of methanol and chloroform (Folch *et al.*, 1957), converted to fatty acid methyl esters (FAMES) using 1% (v/v) methanolic sulphuric acid as a catalyst, and analysed by GC (Devillard *et al.*, 2006; Wařowska *et al.*, 2006). Experimental data were analysed by two-way ANOVA (Genstat 8th edition, release 8.1; VSN International) using a model that included

the fixed effects of sampling time, random effects of volunteer and their interaction. Least-square means are reported and treatment effects were considered significant at $P < 0.05$.

Identification of CLA isomers. Formal structural identification of CLA formed during *in vitro* incubations was based on GC-MS analysis of 4,4-dimethyloxazoline (DMOX) fatty acid derivatives. DMOX derivatives were prepared from selected samples of FAMES using 2-amino-2-methyl-1-propanol (500 μl) and heating overnight under a nitrogen atmosphere according to Fay & Richli (1991), with the exception that a temperature of 150°C was used (Shingfield *et al.*, 2006). GC-MS analysis of DMOX derivatives was performed using a gas chromatograph (6890; Hewlett Packard) equipped with a 100 m CP-SIL 88 column and selective quadrupole mass detector (model 5973N, Agilent Technologies), under an ionization voltage of 70 eV using helium as the carrier gas. Both the ion source and interface temperatures were maintained at 230°C . DMOX derivatives in a 4 μl sample were injected at a split ratio of 1:50 and separated using a temperature gradient with a total run time of 98.5 min. Following sample injection, column temperature was maintained at 70°C for 4 min, increased at a rate of $8^{\circ}\text{C min}^{-1}$ to 110°C , raised to 170°C at a rate of $5^{\circ}\text{C min}^{-1}$, held at 170°C for 10 min, and then increased at a rate of $4^{\circ}\text{C min}^{-1}$ to a final temperature of 240°C that was maintained for 50 min. Electron impact ionization spectra obtained were used to locate double bonds based on atomic mass unit distances, with an interval of 12 atomic mass units between the most intense peaks of clusters of ions containing n and $n-1$ carbon atoms being interpreted as cleavage of the double bond between carbon n and $n+1$ in the fatty acid moiety. Identification was further validated by comparison with an online reference library of DMOX electron impact ionization spectra (<http://www.lipidlibrary.co.uk>). Implied double-bond geometry of CLA isomers based on GC-MS analysis was verified by silver-ion HPLC analysis of the parent FAME used to prepare DMOX derivatives. Analysis was performed using an HPLC system (model 1090; Hewlett Packard) equipped with photodiode array detector and four silver-impregnated silica columns (ChromSpher 5 Lipids, 250×4.6 mm, 5 μm particle size; Varian) coupled in series. Methyl esters of CLA were separated under isocratic conditions at 22°C using 0.1% (v/v) acetonitrile in heptane at a flow rate of 1 ml min^{-1} (total run time 100 min) and monitoring column effluent at 233 and 210 (reference wavelength) nm (Shingfield *et al.*, 2003).

Determination of faecal SCFA concentrations. Diluted faecal samples (0.1 g ml^{-1} in 0.1 M phosphate buffer pH 7.0) were analysed in triplicate for SCFA concentrations. After homogenization, samples were centrifuged (10 000 g for 10 min) and the supernatants containing SCFAs were recovered. After conversion to *t*-butyldimethylsilyl derivatives, the SCFAs were analysed by GC according to Richardson *et al.* (1989).

Extraction of total DNA. Total DNA was extracted from 200 μl of faecal suspensions (0.1 g ml^{-1} in 0.1 M phosphate buffer pH 7.0) using the established protocol for isolating DNA from faeces for pathogen detection (QIAmp DNA stool kit; Qiagen). Concentrations of DNA were estimated at 260 nm, and DNA integrity was verified by agarose gel electrophoresis (0.8% agarose in $1 \times$ Tris/borate/EDTA buffer).

Amplification, cloning and sequencing of 16S rRNA genes. 16S rRNA genes were amplified as described by Suau *et al.* (1999), using the same primers (S-D-Bact-0008-a-S-20 and S*-Univ-1492-b-A-21) and the same PCR conditions, which included a low number of amplification cycles (10 cycles) to avoid bias due to the amplification. After the PCR, six tubes of 50 μl each were pooled, purified and concentrated with the QIAquick spin PCR purification kit (Qiagen). The concentration and the size of the purified PCR products were

estimated by agarose electrophoresis. Purified PCR products were cloned into the pCR4-TOPO vector using the TOPO TA cloning kit (Invitrogen). Chemically competent *Escherichia coli* TOP10 cells were transformed with the ligation products by heat shock (30 s at 42 °C). Recombinant cells were selected on Luria–Bertani medium with kanamycin (50 µg ml⁻¹) and X-Gal (40 µg ml⁻¹). One hundred white colonies were transferred to 200 µl Luria–Bertani broth containing 50 µg kanamycin ml⁻¹.

Inserts were amplified directly from 1.5 µl of cultures using the primers on the pCR4-TOPO vector (T7 promoter primer and M13 reverse primer). PCR products were cleaned up using a Montage PCR96 plate (Millipore) in accordance with the recommendations of the manufacturer. Finally, purified PCR products were sequenced using the primer S-D-Bact-0008-a-S-20, on a Beckman Coulter CEQ 8000 DNA analyser.

rRNA sequence analysis. The 16S rRNA sequences from the clone libraries were compared to the public database Ribosomal Database Project (RDP, <http://rdp.cme.msu.edu/>). The classifier from RDP was used to assign 16S rRNA sequences to the taxonomical hierarchy proposed in *Bergey's Manual of Systematic Bacteriology*, release 6.0 (Cole *et al.*, 2005). Hierarchical taxa are based on a naïve Bayesian rRNA classifier. Analysis of the 16S rRNA sequences was also performed using the GenBank DNA database and the BLAST algorithm.

Detection of stearate-producing bacteria by real-time PCR. The oligonucleotides used to detect stearate-producing bacteria were designed to detect the DNA from ruminal stearate-producing bacterial strains from a group-specific region on the 16S rRNA gene (Paillard *et al.*, 2007). Dilutions of purified genomic DNA from the control strain *Clostridium proteoclasticum* P-18 were used to construct specific calibration curves. Amplification was carried out in a final volume of 25 µl containing 12.5 µl iQ Supermix (Bio-Rad), 400 and 800 nM forward and reverse primer respectively, 250 nM probe, and 2.5 µl purified genomic DNA. Amplification (10 min at 95 °C, then 40 cycles of three steps consisting of 30 s at 95 °C, 1 min at 55 °C and 30 s at 72 °C) was performed with the iCycler iQ thermal cycler (Bio-Rad). Fluorescence data were obtained at the end of the hybridization step, at excitation and emission wavelengths of 490 and 530 nm, respectively. All samples were run in triplicate. Results were analysed using the iCycler iQ detection system software (Bio-Rad).

RESULTS

Fatty acid composition of faecal samples

The fatty acid composition of the faecal samples from the two human volunteers was determined in the zero-time samples from incubations with LA (Table 1). The total

Table 1. Metabolism of LA by diluted faecal suspensions collected from two human volunteers

The initial LA concentration was 500 µg ml⁻¹ of 10 % (wet w/v) faecal suspension. Results are expressed as µg fatty acid per g of faecal sample and represent the mean of three determinations; NA, not applicable.

Fatty acid	Volunteer 1				Volunteer 2				SD*	P-values*		
	0 h	1 h	4 h	8 h	0 h	1 h	4 h	8 h		Volunteer	Time	Vol.–Time†
14:0	69	88	90	88	72	68	82	70	15	0.094	0.383	0.547
16:0	1093	1757	2183	2094	1235	1384	1419	1395	135	<0.001	<0.001	<0.001
18:0	2941	3512	4355	4260	1931	2027	2203	2129	210	<0.001	<0.001	<0.001
20:0	151	258	207	197	75	79	101	126	30	<0.001	0.022	0.023
22:0	344	350	341	242	173	147	207	163	38	<0.001	0.027	0.063
24:0	0	0	0	0	12	0	0	0	4	NA	NA	NA
Total saturated	4599	5966	7176	6881	3498	3705	4011	3883	182	<0.001	<0.001	<0.001
cis-9-16:1	34	66	51	53	85	76	97	83	13	<0.001	0.254	0.052
trans-10-18:1	41	55	53	60	39	49	36	48	12	0.072	0.183	0.702
trans-11-18:1 (VA)	337	1137	1063	952	78	124	618	1156	67	<0.001	<0.001	<0.001
cis-9-18:1	599	821	517	523	614	816	682	618	52	0.006	<0.001	0.042
18:1 others	266	378	529	765	238	104	453	545	37	<0.001	<0.001	<0.001
cis-13-22:1	196	160	340	329	0	0	0	0	30	NA	<0.001	NA
Total MUFAs	1474	2618	2554	2681	1054	1169	1885	2449	104	<0.001	<0.001	<0.001
cis-9,cis-12-18:2 (LA)	6341	5717	2595	1740	6762	5654	2156	1116	186	0.033	<0.001	<0.001
trans-9,trans-12-18:2	0	45	49	55	0	0	34	37	8	<0.001	<0.001	0.001
CLA total‡	0	301	151	39	0	644	1416	984	41	<0.001	<0.001	<0.001
18:3 n-3	120	112	197	211	65	48	44	29	18	<0.001	0.003	<0.001
20:5 n-3	0	57	0	0	0	0	0	0	8	NA	NA	NA
22:6 n-3	0	0	0	0	0	0	0	0	0	NA	NA	NA
Total PUFAs	6461	6232	2992	2045	6826	6346	3649	2166	197	0.001	<0.001	0.093

*Analysed by two-way ANOVA with a model that included the effects of volunteer, time and their interaction. Residual SD based on ANOVA output.

†Volunteer–time interaction.

‡See Table 2 for composition.

saturated fatty acid concentrations were higher in V1 than V2 (4.6 vs 3.5 mg g⁻¹), containing predominantly 16:0, 18:0 and 22:0. Oleic acid (*cis*-9-18:1) was the most abundant monounsaturated fatty acid (MUFA), at comparable concentrations in both samples. In contrast, VA concentrations in V1 were 4.3-fold higher than in V2. PUFA concentrations were similar between samples and consisted mainly of the LA that had been added to the suspensions (Table 1). CLA was not detectable.

LA metabolism by human faecal flora

LA was metabolized at similar rates in the incubations of faecal samples from the two volunteers (Table 1). CLA accumulated to a much greater extent in V2 compared to V1, whereas VA accumulation was more rapid in V1 than V2. Stearic acid (SA; 18:0) formation was also much higher in V1 than V2, where SA formation was minimal. Palmitic acid (16:0) concentration also doubled in V1, while little if any was formed with V2.

There were marked differences between the two volunteers in the profile of CLA isomers produced (Table 2). A total of 12 isomers were detected in V2, compared with six in V1. In V1, rumenic acid (RA; *cis*-9,*trans*-11-18:2) was the predominant isomer throughout, with *trans*-10,*cis*-12-18:2 being less than one-tenth of the RA concentration. *trans*-9,*trans*-11-18:2 was present at similar concentrations to *trans*-10,*cis*-12-18:2 in V1. In contrast, *trans*-10,*cis*-12-

18:2 was a much higher proportion of total CLA in V2, and even exceeded RA in concentration at 8 h.

Concentrations of SCFAs in faecal samples

Total concentrations of SCFAs in faecal samples from V1 and V2 were 86.7 mM and 68.7 mM, respectively (Table 3). The major SCFAs (acetate, butyrate and propionate) accounted for 91.6% and 89.4% of the total SCFA in V1 and V2, respectively. Other SCFAs, including isobutyrate, isovalerate, valerate, caproate and succinate, were also detected, but all samples were devoid of lactate. Proportions of propionate and butyrate in total SCFAs were higher in V1 compared with V2, with acetate/propionate/butyrate molar ratios of 58.9/17.2/15.5 and 63.5/13.9/12, respectively.

Assessment of bacterial diversity in faecal samples

Faecal samples from the two volunteers were also analysed for bacterial composition. Two hundred colonies were used for 16S rRNA gene sequencing, from which 91 sequences for each sample were analysed further. The average length of the sequences obtained was 800 bp; 650–700 bp were used for sequence analysis. All the sequences belonged to the main phyla of bacteria found in the human gut, namely *Firmicutes*, *Bacteroidetes* and *Actinobacteria* (Table 4). The

Table 2. Distribution of CLA isomers formed during the incubation of LA with diluted faecal samples collected from two human volunteers

Results are expressed as percentages of total CLA present in the samples; –, not detected

	Volunteer 1				Volunteer 2			
	0 h	1 h	4 h	8 h	0 h	1 h	4 h	8 h
<i>trans</i> -6, <i>trans</i> -8-18:2	–	–	–	–	–	–	–	–
<i>trans</i> -7, <i>cis</i> -9-18:2	–	–	–	–	–	–	–	–
<i>trans</i> -7, <i>trans</i> -9-18:2	–	–	–	–	–	–	–	–
<i>cis</i> -8, <i>cis</i> -10-18:2	–	–	–	–	–	–	–	1
<i>trans</i> -8, <i>cis</i> -10-18:2	–	–	–	–	–	–	–	–
<i>trans</i> -8, <i>trans</i> -10-18:2	–	–	–	–	–	–	–	–
<i>cis</i> -9, <i>cis</i> -11-18:2	–	–	0.9	1.4	–	2.3	9.4	1.1
<i>cis</i> -9, <i>trans</i> -11-18:2	–	100	80.3	75	–	57.4	44.7	35.7
<i>trans</i> -9, <i>cis</i> -11-18:2	–	–	–	–	–	–	–	–
<i>trans</i> -9, <i>trans</i> -11-18:2	–	–	6.6	12.4	–	7.8	13.6	8.3
<i>cis</i> -10, <i>cis</i> -12-18:2	–	–	–	0.9	–	–	–	1.5
<i>trans</i> -10, <i>cis</i> -12-18:2	–	–	7.1	6.9	–	28.4	25.7	44.2
<i>trans</i> -10, <i>trans</i> -12-18:2	–	–	5.2	3.4	–	4.2	1.3	6.9
<i>cis</i> -11, <i>cis</i> -13-18:2	–	–	–	–	–	–	–	0.8
<i>cis</i> -11, <i>trans</i> -13-18:2	–	–	–	–	–	–	–	–
<i>trans</i> -11, <i>cis</i> -13-18:2	–	–	–	–	–	–	3.2	0.6
<i>trans</i> -11, <i>trans</i> -13-18:2	–	–	–	–	–	–	0.6	–
<i>cis</i> -12, <i>trans</i> -14-18:2	–	–	–	–	–	–	1	–
<i>trans</i> -12, <i>cis</i> -14-18:2	–	–	–	–	–	–	0.7	–

Table 3. Molar proportions of SCFAs in faecal samples V1 and V2

Results reported represent the mean of three determinations and are expressed as a percentage of total SCFAs. Total concentrations of SCFAs in faecal samples from V1 and V2 were 86.7 mM and 68.7 mM, respectively.

SCFA	Percentage of total SCFAs			
	Volunteer 1		Volunteer 2	
	Mean	SD	Mean	SD
Succinate	0.0		0.5	0.1
Caproate	2.2	0.4	2.5	0.3
Valerate	2.6	0.2	1.9	0.2
Iso-valerate	1.5	0.2	1.8	0.3
Iso-butyrate	2.2	0.3	2.9	0.4
Butyrate	15.5	1.7	12.0	1.5
Propionate	17.2	1.9	13.9	1.3
Acetate	58.9	8.2	63.5	10.1

proportions of sequences assigned to these different phyla compared to the total sequences analysed were different for the two volunteers. *Bacteroidetes* predominated in V1 (53.8 % of the clones), whereas the major bacterial population in V2 belonged to the *Firmicutes* phylum (65.9 % of the clones). V2 also contained small numbers of bacteria from the phylum *Actinobacteria* (4.4 % of the total sequences), which were not present in V1. In addition, there were large variations within the two main phyla between the samples analysed. Some bacterial families were absent from the clone library of V1, such as *Eubacteriaceae* and *Rikenellaceae*, or from the clone library of V2, such as *Prevotellaceae*.

A real-time PCR approach was used to estimate the population of *C. proteoclasticum* in faecal samples. The design of the method to target this specific bacterial

population was based on sequences from ruminal bacteria. No bacteria corresponding to this specific group of SA producers were detected in the DNA extracted from either faecal sample.

DISCUSSION

This study was undertaken against a background of exploiting potential health benefits from enhancing CLA formation from LA in the human colon, either as therapy for inflammatory bowel disease or as a prophylactic with respect to colonic cancer. The aim was to assess how different compositions of the indigenous bacterial community of the human intestine could affect the formation of different isomers of CLA. There is an increasing body of evidence indicating that positional and geometric isomers of CLA exert different physiological effects in humans (Tricon *et al.*, 2005). The present study reports, we believe for the first time, that at least 12 different isomers of CLA can be produced from LA by the colonic microflora, and that CLA production varies between individuals. In particular, *trans*-10,*cis*-12-18:2, which has possible detrimental effects on health (Clement *et al.*, 2002; Wahle *et al.*, 2004), may become the predominant isomer formed depending on the bacterial population colonizing the intestine. The physiological effects of most other CLA isomers are unknown. Production of VA may elicit similar benefits to human gut health to RA, since VA can be converted to RA in mammalian tissues, including intestinal cells (Miller *et al.*, 2003). Thus, the accumulation of VA in V1 could be considered positive with regard to human health, whereas the mixture of CLA isomers accumulating in V2 would be much less clearly positive.

The pattern of LA metabolism observed here for V1 was consistent with previous studies reporting that the main route of LA in intestinal bacteria involves the isomerization of LA to RA, followed by sequential reduction of RA to VA

Table 4. Identity of clones retrieved from 16S rDNA libraries made from faecal samples V1 and V2

	Percentage of clones		Related genera
	V1	V2	
Phylum Firmicutes	46.2	65.9	
Family Clostridiaceae	8.8	23.1	<i>Faecalibacterium</i> , <i>Clostridium</i>
Family Lachnospiraceae	24.2	19.8	<i>Lachnospira</i> , <i>Roseburia</i> , <i>Butyrivibrio</i>
Family Acidaminococcaceae	8.8	4.4	<i>Dialister</i>
Family Eubacteriaceae	0	2.2	<i>Eubacterium</i>
Unclassified	4.4	16.5	
Phylum Bacteroidetes	53.8	29.7	
Family Prevotellaceae	29.7	0	<i>Prevotella</i>
Family Bacteroidaceae	14.3	11.0	<i>Bacteroides</i>
Family Rikenellaceae	0	2.2	<i>Alistipes</i>
Unclassified	9.9	16.5	
Phylum Actinobacteria	0	4.4	<i>Bifidobacterium</i> , <i>Propionibacterium</i>

and VA to SA (Howard & Henderson, 1999; Devillard *et al.*, 2006; Kamlage *et al.*, 1999, 2000), in a manner similar to that found in ruminal bacteria (Harfoot & Hazlewood, 1997). The differences in metabolism between V1 and V2 tie in reasonably well with the 16S rRNA clonal analysis according to our limited understanding of intestinal biohydrogenation (Devillard *et al.*, 2007). Pure-culture studies have shown that of the bacteria studied, *Roseburia* spp. and *Butyrivibrio fibrisolvens* metabolize LA to VA most rapidly (Devillard *et al.*, 2007). Thus, the higher *Lachnospiraceae* numbers in V1 are consistent with greater biohydrogenating activity in V1. Other *Firmicutes* were much less active (Devillard *et al.*, 2007), so the larger numbers of *Clostridiaceae* in V2 would have little impact on LA metabolism. The higher abundance of CLA isomers in V2 than V1 may reflect the higher numbers of *Actinobacteria* and *Bacteroidetes* in V1. While all *Firmicutes* capable of LA metabolism to CLA were found to form VA and RA as intermediates (Devillard *et al.*, 2007), *Propionibacterium* and *Bifidobacterium* spp. (*Actinobacteria*) are known to produce a mixture of CLA isomers (Coakley *et al.*, 2003; Devillard *et al.*, 2007). *Actinobacteria* were not among the most numerous clones in V1, but were present in V2. Gram-negative intestinal bacteria do not metabolize LA (E. Devillard, unpublished observations), with the implication that the large difference between V1 and V2 in *Bacteroidetes* would have little influence on fatty acid metabolism, although it is possible that they metabolize CLA or 18:1 acids formed from LA by other species. Lactobacilli were not present in the clone libraries. Thus, although human gut-derived *Lactobacillus* spp. have been reported to produce CLA (Alonso *et al.*, 2003), their low abundance and low activity suggest that their role in CLA synthesis in the mixed indigenous community is relatively minor.

The metabolic data indicate that the bacterial community in V1 contained specific bacteria capable of converting VA to SA, which were missing or inactive in V2. The bacteria capable of this step of LA metabolism are unknown. In the rumen, the only species yet isolated that forms SA as an end product of LA metabolism is *C. proteoclasticum* (Wallace *et al.*, 2006). No *C. proteoclasticum* were present in either V1 or V2. Thus, there remains an important unknown in the microbial ecology of biohydrogenation of unsaturated fatty acids in the human colon – which species form SA? If, by analogy with the ruminal *C. proteoclasticum*, these bacteria carry out biohydrogenation because of an extraordinary sensitivity to growth inhibition by unsaturated fatty acids, they could prove elusive to isolate.

Such large differences in the main constituent bacteria might also have been expected to affect the main fermentation pathways in the two samples. Here, the effects were relatively minor, with the main difference apparently a lower acetate production in V1. The greater proportion of *Firmicutes* in V2 did not, as might have been expected based on evidence from other studies (Barcenilla *et al.*, 2000), result in a higher proportion of butyric acid.

The measurements made in the present study were from faecal samples, from which we extrapolate to conditions prevailing in the intestine itself. The microbial community composition changes during passage of digesta through the intestine (Hayashi *et al.*, 2005). For example, facultative anaerobes are much more abundant in caecal versus faecal samples (Marteau *et al.*, 2001). However, we know of no study that has compared biohydrogenating activity or fatty acid-metabolizing bacteria in different sections of the gut. Furthermore, there is little indication from the study of Eckburg *et al.* (2005) that there are consistent changes in the *Firmicutes* (of which the main human biohydrogenators are members) throughout the intestine, although diversity seemed to be higher in faeces than elsewhere.

This study of faecal microbial metabolism of fatty acids from two volunteers reveals that very important differences can exist between individuals, leading to the formation of different concentrations and isomers of CLA. Such differences would be expected to have an impact upon human health, especially gut health. The systemic impact of CLA produced by the gut flora is considered to be minor, because of low absorption via the colonic epithelium (Chin *et al.*, 1994; Kamlage *et al.*, 1999, 2000). CLA taken up by the colonocytes may exert a local effect, however (Bassaganya-Riera *et al.*, 2004; Miller *et al.*, 2003). Quantities of LA passing to the intestine may be significant (Ewaschuk *et al.*, 2006), especially in people taking dietary supplements that decrease fat absorption (Hauptman *et al.*, 2000). A much better understanding of the microbial ecology of fatty acid metabolism will be required to use this information in a predictive manner. In particular, the bacteria responsible for SA formation must be identified. Ideally, a molecular genetic approach would offer the most promising means to search for these bacteria. However, as none of the isomerase or reductase genes has yet been identified, an approach in which the gene(s) form the target of the search is not practicable at this time.

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