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Metabolism of Linoleic Acid by Human Gut Bacteria: Different Routes for Biosynthesis of Conjugated Linoleic Acid[▽]

Estelle Devillard, Freda M. McIntosh, Sylvia H. Duncan, and R. John Wallace*

Gut Health Division, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB, United Kingdom

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A survey of 30 representative strains of human gram-positive intestinal bacteria indicated that *Roseburia* species were among the most active in metabolizing linoleic acid (*cis*-9,*cis*-12-18:2). Different *Roseburia* spp. formed either vaccenic acid (*trans*-11-18:1) or a 10-hydroxy-18:1; these compounds are precursors of the health-promoting conjugated linoleic acid *cis*-9,*trans*-11-18:2 in human tissues and the intestine, respectively.

Linoleic acid (LA) (*cis*-9,*cis*-12-18:2) is metabolized in the human colon via conjugated linoleic acids (CLA) (mainly *cis*-9,*trans*-11-18:2) to vaccenic acid (VA) (*trans*-11-18:1) (both of the latter compounds are considered to be beneficial for health [5, 11, 27, 28, 31, 41]) and then to stearic acid (18:0) (17, 26). A similar pathway occurs in the rumen (12, 20), where this process, commonly known as biohydrogenation, has important implications for the fatty acid composition of meat and milk (22, 38). The microbiology of biohydrogenation in the rumen has received a great deal of attention (12, 32, 40, 42), but similar investigations have not been carried out for the human intestinal microbiota. Therefore, the aims of the present study were to identify human gut bacteria that can perform fatty acid biohydrogenation and to assess their likely importance in the mixed intestinal ecosystem. We found that *Roseburia* spp. were probably the most important organisms; some *Roseburia* species metabolized LA by the same pathway found in ruminal bacteria (20, 32), while others formed a hydration product that is a precursor of CLA in the mixed community.

Thirty bacterial strains (2 lactobacilli, 1 lactococcus, 5 propionibacteria, 3 bifidobacteria, and 19 strains of the low-G+C-content *Clostridium* cluster) isolated from or related to bacteria found in the human large intestine were studied to determine their ability to metabolize LA (Table 1). The bacteria were grown either in the liquid form of anaerobic basal M2 medium (13) or in the same medium supplemented with 50 μg/ml LA. For labeling experiments the medium was prepared using enough deuterium oxide to provide around 50% enrichment of the medium water. The linoleate isomerase activity in bacteria grown on unsupplemented M2 medium was determined by the method described by Wąsowska et al. (43). The methods used for extraction and derivatization of the total fatty acids to fatty acid methyl esters (FAME) and the identification methods were similar to the methods described by Devillard et al. (7). In order to determine the fate of the hydroxy-18:1 fatty acid (HFA) produced by some of the *Roseburia* isolates, *Roseburia intestinalis* L1-952 was grown in M2 medium containing 50 μg/ml LA, the culture was centrifuged (10,000 × *g*, 10 min, 4°C), and the supernatant was used to prepare a modified M2 medium enriched in HFA. This medium was inoculated with freshly voided human feces from two omnivorous volunteers consuming a Western diet, which had been diluted (0.2 g in 1 ml) in sterile anaerobic 0.1 M potassium phosphate buffer (pH 7.0). Duplicate aliquots of each fecal sample were removed after different times for fatty acid determination for up to 72 h.

The linoleate isomerase activity was >10 nmol CLA formed (mg protein)$^{-1}$ min^{-1} in 8 of the 30 isolates (Table 1), and *Butyrivibrio fibrisolvens* 16.4 and two strains of *Roseburia inulinivorans* (A2-194 and L1-83) exhibited the highest activity. When inoculated into medium containing LA, most of the 30 strains showed a lag time before they started to grow (Table 1). The same eight isolates that had high levels of isomerase activity also metabolized LA most extensively (Table 1). The final products for these strains were VA for the *Roseburia* and *B. fibrisolvens* isolates, a mixture of CLA (mainly *cis*-9,*trans*-11-18:2, *trans*-9,*trans*-11-18:2, and *trans*-10,*cis*-12-18:2) for *Propionibacterium freudenreichii* subsp. *shermani*, and *cis*-9,*trans*-11-18:2 and *trans*-9,*trans*-11-18:2 for *Bifidobacterium breve* (Table 1). Thus, it appears that whereas other species produced a mixture of CLA products, bacteria belonging to *Clostridium* cluster XIVa formed one product, either VA or HFA. Thirteen strains, belonging to both phylogenetic groups, metabolized LA (range, 26% to 88%) despite their low linoleate isomerase activity (Table 1). Gas chromatography traces indicated that all these strains produced the same compound, which was subsequently identified by gas chromatography-mass spectrometry (Fig. 1A). The main fragmentation in mass spectrometry led to the formation of ions at *m*/*z* 169 and 201, which are characteristic of a 10-HFA with the first 10 bonds saturated. When *R. intestinalis* L1-952 was grown in the presence of 50 μg/ml LA and deuterium oxide, the two major peaks were shifted to ions at *m*/*z* 171 and 203 (Fig. 1B). This shift corresponded to an addition of deuterium atoms at the double bond between carbons 9 and 10, suggesting that hydration occurred on this double bond (Fig. 1B). The location of the double bond could not be determined by analysis of the FAME. However, the signature masses at *m*/*z* 201 and 294 identified in 10-hydroxy-*cis*-12-18:1 by Schroepfer et al. (37) were present in the FAME spectrum. This information, together with purely biochemical considerations and comparison

* Corresponding author. Mailing address: Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, United Kingdom. Phone: 44(0)1224-716656. Fax: 44(0)1224-716687. E-mail: john.wallace@rowett.ac.uk.

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TABLE 1. Metabolism of linoleic acid by different bacterial species

Strain	Group	Source (reference)	Linoleate isomerase activity (nmol CLA mg protein^{-1} min^{-1})	Lag time (h)	% LA metabolized[a]	Main products formed[a]
Lactobacillus reuteri DSM 20016^T	*Firmicutes*	Human adult intestine (18)	0.7 ± 0.1	0	28 ± 3	HFA
Lactobacillus delbrueckii subsp. *bulgaricus* DSM 20081^T	*Firmicutes*	Bulgarian yoghurt (39)	0.6 ± 0.1	72	14 ± 5	None
Lactococcus lactis subsp. *lactis* DSM 20729	*Firmicutes*	Swiss cheese (35)	0.0 ± 0.0	0	44 ± 6	HFA
Propionibacterium freudenreichii DSM 20271^T	*Actinobacteria*	Swiss cheese (16)	1.2 ± 0.1	72	11 ± 1	None
Propionibacterium freudenreichii subsp. *shermani* DMS 20270	*Actinobacteria*	Cheese (36)	14.3 ± 1.1	24	96 ± 9	CLA (c9t11, t9t11, t10c12)
Propionibacterium freudenreichii subsp. *shermani* DMS 4902^T	*Actinobacteria*	Unknown (16)	17.0 ± 1.7	72	85 ± 7	CLA (c9t11, t9t11, t10c12)
Propioniobacterium jensenii DSM 20274	*Actinobacteria*	Silage (36)	2.4 ± 0.1	0	12 ± 1	None
Propionibacterium thoenii DSM 20276^T	*Actinobacteria*	Emmental cheese (36)	3.5 ± 0.2	48	31 ± 9	HFA
Bifidobacterium adolescentis NCFB 2204	*Actinobacteria*	Human adult intestine (6)	0.7 ± 0.0	24	68 ± 6	HFA
Bifidobacterium breve NCFB 2258	*Actinobacteria*	Human infant intestine (6)	15.4 ± 0.9	0	95 ± 8	CLA (c9t11, t9t11)
Bifidobacterium infantis NCFB 2256	*Actinobacteria*	Human infant intestine (6)	3.6 ± 0.2	24	79 ± 4	HFA
Faecalibacterium prausnitzii L2-6	Cluster IV	Human feces (3)	0.5 ± 0.0	48	32 ± 9	HFA
Eubacterium siraeum DSM 15702^T	Cluster IV	Human feces (29)	2.7 ± 0.0	24	26 ± 5	HFA
Anaerostipes caccae L1-92^T	Cluster XIVa	Human feces (24)	0.0 ± 0.0	0	15 ± 4	None
Eubacterium hallii L2-7	Cluster XIVa	Human feces (24)	3.5 ± 0.5	24	10 ± 2	None
Eubacterium ventriosum L2-12	Cluster XIVa	Human feces (3)	0.0 ± 0.0	0	14 ± 5	None
Eubacterium ruminantium L2-50	Cluster XIVa	Human feces (24)	0.0 ± 0.0	24	38 ± 3	HFA
Eubacterium rectale T1-815	Cluster XIVa	Human feces (24)	1.3 ± 0.0	24	11 ± 2	None
Eubacterium rectale A1-86	Cluster XIVa	Human feces (24)	3.5 ± 0.2	24	15 ± 1	None
Eubacterium rectale M104/1	Cluster XIVa	Human feces (3)	2.4 ± 0.2	24	10 ± 2	None
Roseburia inulinivorans A2-194^T	Cluster XIVa	Human feces (8)	23.3 ± 1.6	24	100 ± 6	VA
Roseburia inulinivorans L1-83	Cluster XIVa	Human feces (8)	30.0 ± 2.3	24	100 ± 4	VA
Roseburia hominis A2-183^T	Cluster XIVa	Human feces (8)	10.6 ± 0.8	0	100 ± 6	VA
Roseburia hominis A2-181	Cluster XIVa	Human feces (8)	11.6 ± 0.6	0	100 ± 8	VA
Roseburia intestinalis L1-82^T	Cluster XIVa	Human feces (8)	0.4 ± 0.0	24	84 ± 11	HFA
Roseburia intestinalis L1-952	Cluster XIVa	Human feces (8)	0.5 ± 0.1	24	85 ± 13	HFA
Roseburia faecis M6/1	Cluster XIVa	Human feces (8)	0.9 ± 0.1	24	86 ± 9	HFA
Roseburia faecis M88/1	Cluster XIVa	Human feces (8)	0.9 ± 0.0	24	76 ± 8	HFA
Roseburia faecis M72/1^T	Cluster XIVa	Human feces (8)	1.0 ± 0.0	24	88 ± 12	HFA
Butyrivibrio fibrisolvens 16.4	Cluster XIVa	Human feces (24)	45.3 ± 3.1	24	100 ± 4	VA

[a] When the optical density at 600 nm of a culture reached 0.6, fatty acids were extracted from the culture, the percentages of linoleic acid metabolized were calculated, and the products formed during growth were identified. These analyses were conducted in triplicate.

of the elution time of the FAME with that of ricinoleic acid (12-hydroxy-*cis*-9-18:1), indicated that the product was most likely 10-hydroxy-*cis*-12-18:1.

Modified M2 medium prepared from the culture supernatant of *R. intestinalis* L1-952 initially contained 5 μg/ml LA and 25 μg/ml HFA. The main fatty acids produced during incubation with diluted feces were *cis*-9,*trans*-11-18:2, VA, and stearic acid (Fig. 2). After a few hours, LA was almost completely metabolized, but the synthesis of CLA, VA, and stearic acid continued and corresponded to the disappearance of HFA (Fig. 2). Thus, we concluded that HFA is a precursor of *cis*-9,*trans*-11-18:2 in the mixed community. None of the pure cultures examined metabolized the HFA further.

Animal studies and clinical trials have indicated that CLA may be useful in improving human health (5, 27, 31). The uptake of CLA formed in the intestine seems to be minor (17). However, local effects on gut tissue might be anticipated. It is now well established that CLA have antiproliferative and anti-inflammatory effects on colonocytes (4, 19), so provision of CLA in the intestinal lumen could be considered beneficial, particularly for inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease (10). Bacteria from other ecosystems and from food products which are also found in the human gut, including strains of *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* (1, 6, 15, 23, 33, 34), have been known for some time to possess the ability to generate CLA. For the first time, we found here that the more abundant bacterial species belonging to clostridial clusters IV and XIVa also metabolize LA at some of the highest rates of all bacteria investigated, forming products that can be precursors of CLA (Fig. 3). Given the greater abundance of *Clostridium*-like bacteria in the human intestinal microbiota (9)—the numbers of lactobacilli, propionibacteria, and bifidobacteria are low, less

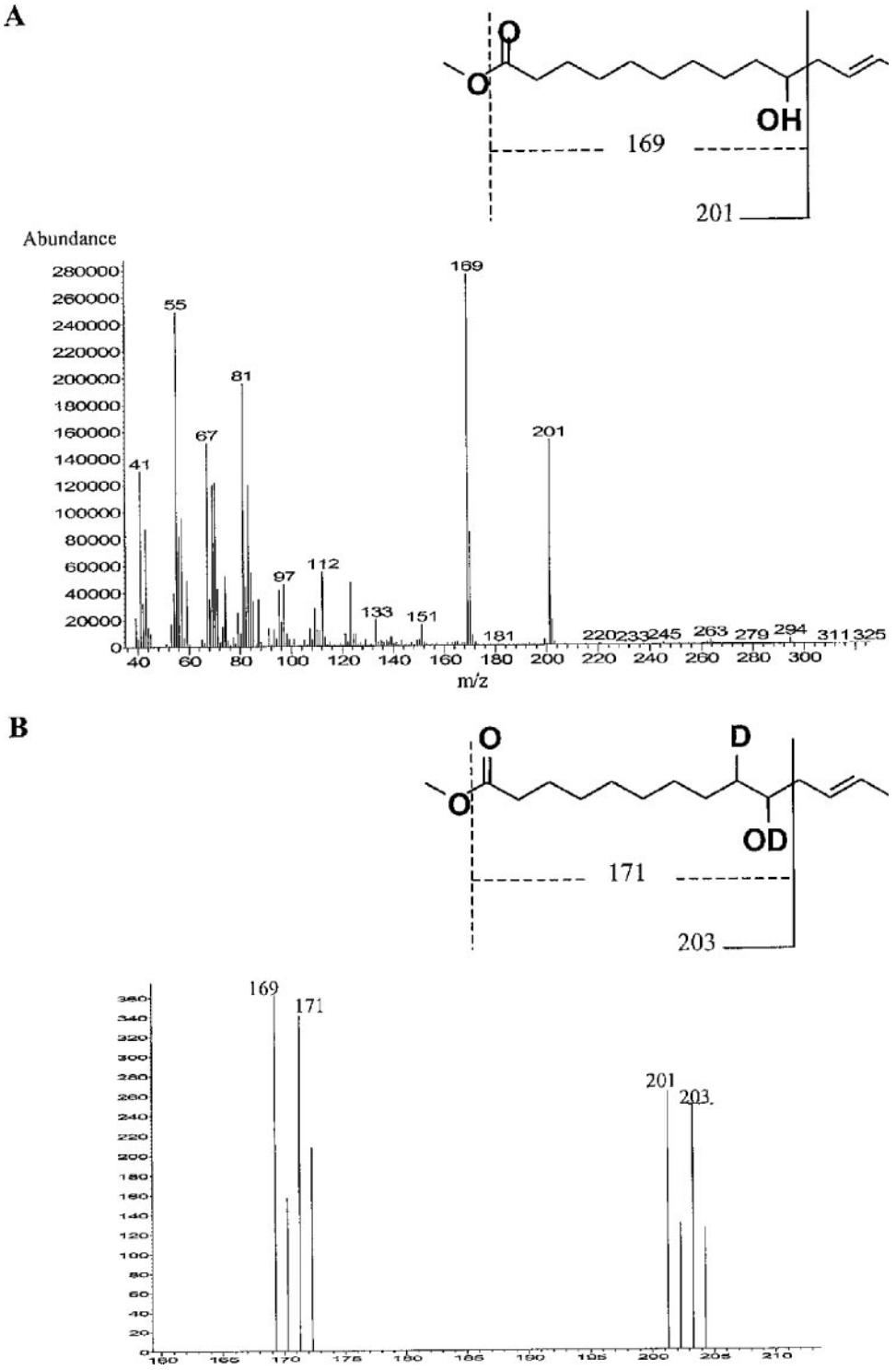

FIG. 1. Mass spectra of the methyl ester of the HFA produced by 13 of the 30 bacterial strains studied here when they were incubated with linoleic acid. (A) Mass spectrum obtained with the unlabeled FAME derivative. (B) Mass spectrum obtained with the FAME derivative obtained from cultures containing deuterium oxide, showing peaks at *m/z* 160 to 210, where the two characteristic fragments of HFA are present.

than 5% of the total microbiota (2, 21)—it may be deduced that LA metabolism by this major group is quantitatively more important than LA metabolism by the *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* groups.

The discovery that HFA is a precursor of *cis*-9,*trans*-11-18:2 in the mixed intestinal community is also new, leading to the likely scheme of CLA formation shown in Fig. 3. Similar importance of HFA was proposed for a *Lactobacillus* sp., involv-

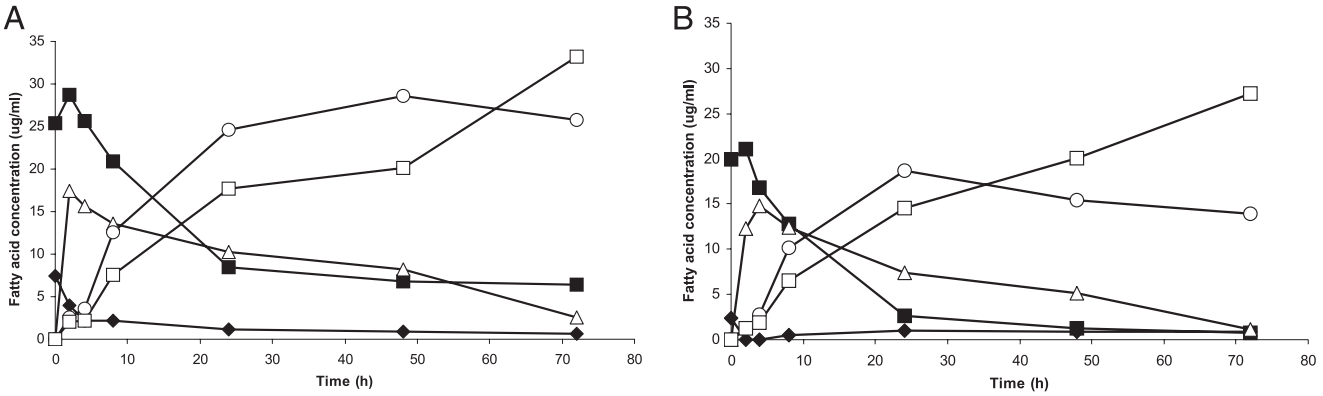

FIG. 2. Metabolism of HFA (■) and LA (◆) and formation of the products CLA (△), VA (○), and stearic acid (□) by mixed fecal flora from volunteer 1 (A) and from volunteer 2 (B). The values are the means of duplicates and are expressed in μg of fatty acid/ml of fecal suspension.

ing a hydration/dehydration process (30). No similar role for HFA has been postulated for bacteria in the rumen, where biohydrogenation is a quantitatively very important activity (12, 32).

The final product of LA metabolism by mixed fecal microbiota was shown here to be stearic acid, as shown previously by Howard and Henderson (14), yet none of the strains tested here produced stearate from LA (Fig. 3). Searching for stearate producers in the rumen has been difficult, largely because these organisms are extraordinarily sensitive to the toxic effects of unsaturated fatty acids (25, 42). The same may be true of human intestinal bacteria. Thus, stearate producers and the species that convert HFA to

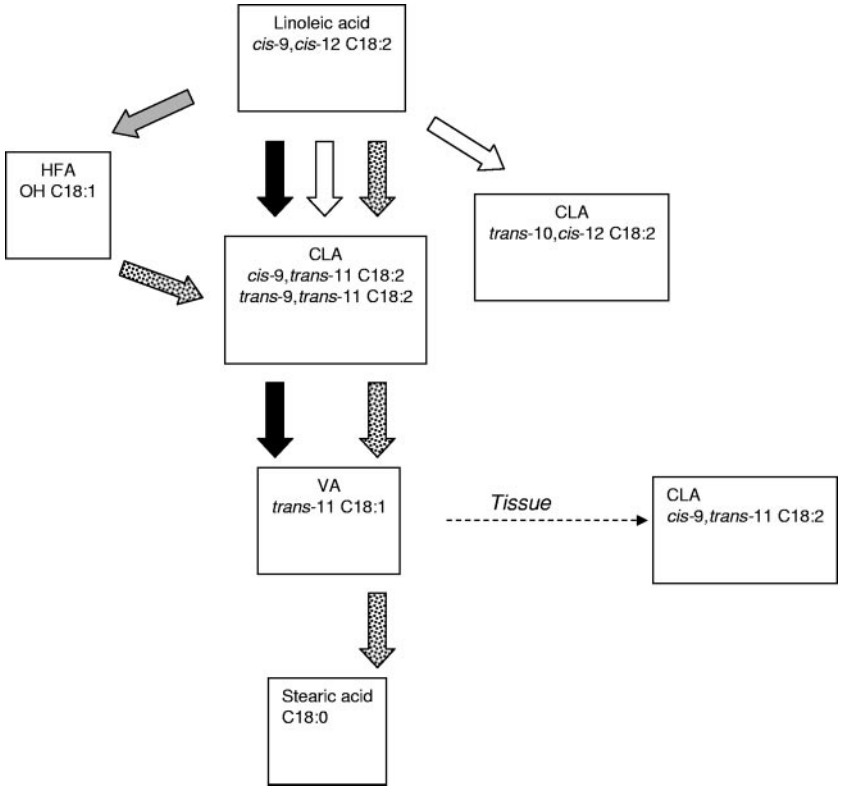

FIG. 3. Proposed pathways of LA metabolism by bacterial species isolated from the human gut. The open arrows represent the bacterial activity of *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* species leading to the formation of CLA. The shaded arrows represent the bacterial activity of some *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* species and some *Clostridium*-like bacteria belonging to clusters IV (e.g., *Eubacterium siraeum*) and XIVa (e.g., *R. intestinalis* and *Roseburia faecis*) leading to the formation of HFA. The solid arrows represent the bacterial activity of *Clostridium*-like bacteria belonging to cluster XIVa leading to the formation of VA (e.g., *Roseburia hominis* and *R. inulinivorans*). The dotted arrows represent activities observed in fecal microbiota for which the responsible bacterial species are still unknown.

CLA, both potentially very important reactions in the mixed ecosystem of the human intestine, remain to be identified.

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