Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues

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ABSTRACT

Background: Recent reports suggest that the metabolic activity of the gut microbiota may contribute to the pathogenesis of obesity and hepatic steatosis.

Objective: The objective was to determine whether the fat composition of host tissues might be influenced by oral administration of commensal bifidobacteria previously shown by us to produce bioactive isomers of conjugated linoleic acid (CLA).

Design: Murine trials were conducted in which linoleic acid–supplemented diets were fed with or without Bifidobacterium breve NCIMB 702258 (daily dose of 10^9 microorganisms) to healthy BALB/c mice and to severe combined immunodeficient mice for 8–10 wk. To ensure that the observations were not peculiar to mice, a similar trial was conducted in weanling pigs over 21 d. Tissue fatty acid composition was assessed by gas-liquid chromatography.

Results: In comparison with controls, there was an increase in cis-9, trans-11 CLA in the livers of the mice and pigs after feeding with linoleic acid in combination with B. breve NCIMB 702258 (P < 0.05). In addition, an altered profile of polyunsaturated fatty acid composition was observed, including higher concentrations of the omega-3 (n–3) fatty acids eicosapentaenoic acid and docosahexaenoic acid in adipose tissue (P < 0.05). These changes were associated with reductions in the proinflammatory cytokines tumor necrosis factor-α and interferon-γ (P < 0.05).

Conclusions: These results are consistent with the concept that the metabolome is a composite of host and microbe metabolic activity and that the influence of the microbiota on host fatty acid composition can be manipulated by oral administration of CLA-producing microorganisms.


INTRODUCTION

The mammalian gut is one of the most densely populated ecosystems on the planet. Long neglected and considered by some to be impenetrable, the gut microbiota is attracting increasing scientific interest because of the unfolding role of host-microbe interactions in health and disease. With a bacterial cell load outnumbering host cells 10^10-fold and a microbial genetic content (microbiome) well in excess of that of the host, the gut microbiota has a metabolic capacity and diversity tantamount to that of a virtual, or hidden, inner organ (1). Furthermore, the concept of the “superorganism” has emerged to reflect the physiologic importance of mutually advantageous bidirectional host-microbe interactions in the gut, with the metabolome being the composite product of both the host genome and the microbiome (2, 3). From comparative studies of germ-free and conventionally colonized animals, it can be inferred that the gut microbiota must be a critical source of regulatory signals influencing the structural and functional development of the gut and maintaining mucosal homeostasis (1, 4). The identity of some of these microbiologically derived signals is becoming apparent; they exhibit a wide versatility and diversity ranging from immunomodulatory polysaccharides (5) to cytoprotective proteins and other bioactive bacterial products (6, 7). More recently, the influence of the enteric microbiota on disease processes outside the gut has become apparent and includes a bearing on common disorders in developed societies, such as obesity and its complications (8). The microbiota is a regulator of fat storage (9) and a contributor to a fatty liver phenotype in insulin-resistant mice (10). Note that the composition of the microbiota may be responsive to dietary modification from which an alteration in host metabolism, fat storage, and body mass may ensue (8).

Because the influence of diet and the microbiota on fat storage may be qualitative as well as quantitative, the question arises as to whether manipulation of the microbiota might influence the fatty acid composition of host tissues. We explored this by feeding different host species a metabolically active commensal microorganism, previously shown by us to synthesize bioactive isomers of conjugated linoleic acid (CLA) from free linoleic acid (11). CLAs are a family of positional (eg, 9,11; 10,12; 11,13) and geometric (cis or trans) isomers of linoleic acid [C18:2 cis-9 (c9), cis-12 (c12) octadecadienoic acid] with conjugated double bonds (12). CLA is a natural component of bovine milk fat as a result of the action of the ruminal microbiota on dietary linoleic acid.

1 From the Alimentary Pharmabiotic Centre, Cork, Ireland (RW, RPR, FS, LO, CO, EMQ, BK, GFF, and CS); Teagasc Moorepark Food Research Centre, Fermoy, County Cork, Ireland (RW, RPR, MC, OH, PL, and CS); and the Department of Microbiology, University College Cork, National University of Ireland, Cork, Ireland (RW and GFF).

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noleic and linolenic acids (13). The predominant CLA isomer found in nature is the \( \text{c}9, \text{trans}-11 \) (t11) CLA isomer, which has been shown to exert a variety of beneficial biological activities in several experimental animal models and human cell culture studies, including inhibition of cell proliferation (14), antiatherosclerotic (15), antiobesogenic, and immunomodulatory properties (16, 17) and the ability to reduce body fat (18). Commensal lactobacilli and bifidobacteria from the mammalian gut have also been shown to generate CLA, predominantly the \( \text{c}9, \text{t}11 \) isomer, from free linoleic acid (11, 19–21). Two studies have also reported the in vivo production of \( \text{t}10, \text{c}12 \) CLA by using 2 strains of human origin, \textit{Lactobacillus rhamnosus} PL60 and \textit{Lactobacillus plantarum} PL62 (22, 23). However, no studies have addressed the in vivo production of \( \text{PL62} \) (22, 23). However, no studies have addressed the origin, mensals. We hypothesized that introduction of a \textit{Bifidobacterium} producing model of inflammatory bowel disease. inflammation in a severe combined immunodeficient (SCID) mouse.

**MATERIALS AND METHODS**

All laboratory animal experimentation were performed according to the guidelines for the care and use of laboratory animals approved by the Department of Health and Children of the Irish government.

**BALB/c mice and dietary treatment**

Male BALB/c mice were purchased from Harlan Ltd (Briester, Oxon, United Kingdom) at 8 wk of age and were fed ad libitum with standard nonpurified CRM(P) diet (Special Diets Services, Witham, Essex, United Kingdom) with free access to water at all times. The diet contained the following nutrient composition: nitrogen-free extract (57.39%), crude protein (18.35%), moisture (10%), ash (6.27%), crude fiber (4.23%), and crude oil (3.36%); which consisted of the saturated fatty acids lauric acid (C12:0; 0.03%), myristic acid (C14:0; 0.14%), palmitic acid (C16:0; 0.33%), and stearic acid (C18:0, 0.06%); the monounsaturated fatty acids myristoleic acid (C14:1; 0.02%), palmitoleic acid (C16:1; 10%), and oleic acid (C18:1, 0.87%); and the polyunsaturated fatty acids linoleic acid (C18:2n−6; 0.96%), linolenic acid (C18:3n−3; 0.11%), and arachidonic acid (C20:4n−6; 0.11%)]. Mice were individually caged and exposed to a 12-h light-dark cycle, maintained at a constant temperature of 25°C.

One week after arrival, mice were randomly assigned to one of the following dietary treatments (n = 9/group). One group daily received 1% linoleic acid (triglyceride bound form; Larodan Fine Chemicals AB, Malmo, Sweden) in their diet together with \( \approx 1 \times 10^9 \) live \textit{Bifidobacterium breve} microorganisms (accession no. NCIMB 702258; NCIMB, Aberdeen, Scotland, United Kingdom). Another group received 1% linoleic acid in their diet and placebo freeze-dried powder (15% wt:vol trehalose in distilled water). For linoleic acid treatment, a powdered diet [milled standard nonpurified CRM(P) pellets] was blended with the linoleic acid to yield a dose of \( \approx 90 \) mg linoleic acid/d per mouse (based on experiments by Bassaganya-Riera et al (24) that established an optimal intake of fatty acids of 1 g/100 g).

After 8 wk on the experimental diets, the mice were killed by cervical dislocation. Tissues were removed from the carcasses, blotted dry on filter paper, weighed, and frozen in liquid nitrogen. All samples were stored at −80°C until processed.

**Pigs and dietary treatment**

Sixteen crossbred (Large White × Landrace) weaning pigs (male) were used in a 21-d experiment (day −7 to day 21). Pigs were weaned at \( \approx 26 \) d of age and housed individually 3 wk before the trial. Pigs were fed a medicated diet [experimental (nonmedicated) diet (Table 1)] supplemented with 3 g/kg zinc oxide for 2 wk (from weaning) and a nonmedicated diet (Table 1) for 1 wk (before start of experiment 7); they were blocked by weight (similar weight in each group) and randomly assigned to treatments. The pig feeding trial complied with the European Union Council Directive 91/630/EEC, which outlines minimum standards for the protection of pigs, and the European Union Council Directive 98/58/EEC, which concerns the protection of animals kept for farming purposes.

The diet for the experiment was a nonmedicated diet (Table 1). Pigs were housed individually in a total of 2 rooms with 8 pigs per room, with each treatment group being housed separately to avoid cross-contamination. At day 0 the pigs received the following experimental diets once a day for 21 d; one set of pigs received 50 mL sterile reconstituted skim milk (RSM; 10% wt:vol) containing 6.2 g sunflower oil (Tribly Trading Ltd, Drogheda, Ireland) in combination with 50 mL RSM (10% wt:vol) containing \( \approx 10^{10} \) colony-forming units (CFU) \textit{B. breve} NCIMB 702258.

### Table 1

<table>
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Provided per kilogram of complete diet: 175 mg Cu, 140 mg Fe, 47 mg Mn, 120 mg Zn, 0.6 mg I, 0.3 mg Se, 6000 IU vitamin A, 1000 IU vitamin D3, 100 IU vitamin E, 4 mg vitamin K, 15 μg vitamin B-12, 2 mg riboflavin, 12 mg nicotinic acid, 10 mg pantothenic acid, 250 mg choline chloride, 2 mg vitamin B-1, 3 mg vitamin B-6, and 60 mg Endox (Kemin AgriFoods North America, Des Moines, IA).
second set of pigs (control pigs) received 100 mL RSM (10% wt:vol), without supplementation, once a day. Fatty acid analysis of the sunflower oil showed that it contained ∼63% linoleic acid. The skim milk diets were prepared daily and administered to the pigs with water available ad libitum. Blood samples were taken from all pigs on day 0 and day 19 for serum fatty acid, immunologic analysis, or both. The pigs were deprived of food for ∼18 h before blood sampling. After sampling, the serum tubes were stored at room temperature for 30 min to 2 h and centrifuged at 3315 g for 20 min. The serum was removed and stored at −20°C under nitrogen before fat extraction. Six pigs/treatment (12 in total) were killed by captive bolt on day 21. Samples of adipose tissue (back fat) and liver were collected and frozen in liquid nitrogen. All samples were stored at −80°C until processed.

**SCID mice and dietary treatment**

SCID mice were purchased from Harlan Ltd (Briester, United Kingdom) at 6 wk of age and were fed ad libitum with standard nonpurified CRM(P) diet (Special Diets Services) with free access to water at all times. Each cage contained one mouse. Mice were exposed to a 12-h light-dark cycle and maintained at a constant temperature of 25°C. One week after arrival, mice were divided into 5 groups (A–E; n = 8) for different dietary treatments. Group A was fed a linoleic acid–supplemented diet (1% wt:wt) and placebo freeze-dried powder (15% wt:vol trehalose), group B was fed linoleic acid (1% wt:wt) in combination with \( B. breve \) NCIMB 702258 (a daily dose of 10\(^8\) microorganisms), group C received the standard nonpurified CRM(P) diet in combination with \( B. breve \) NCIMB 702258 (a daily dose of 10\(^8\) microorganisms), group D received pure \( c_9, t_11 \) CLA (1% wt:wt) and placebo freeze-dried powder (15% wt:vol trehalose), and group E was fed the standard nonpurified CRM(P) diet and placebo freeze-dried powder (15% wt:vol trehalose). For linoleic acid and \( c_9, t_11 \) CLA treatments (added as triglyceride bound form; Larodan Fine Chemicals AB), a powdered diet [milled standard nonpurified CRM(P) pellets] blended with the fatty acid was administered daily for 10 wk to yield ∼90 mg fatty acid/d per mouse (24). On day 0, colitis was induced in SCID mice by adoptive transfer of the rifampicin-resistant variant was identical to the parent strain, NCIMB 702258 is an efficient CLA producer, converting ∼65% linoleic acid to \( c_9, t_11 \) CLA when grown in 0.55 mg/mL linoleic acid in vitro (11). Rifampicin-resistant variants of the \( B. breve \) strain were isolated by spread-plating ∼10\(^9\) CFU from an overnight culture onto MRS agar (de Man, Rogosa, and Sharpe; Difco Laboratories, Detroit, MI) supplemented with 0.05% (wt:vol) L-cysteine hydrochloride (mMRS, 98% pure; Sigma Chemical Co, St Louis, MO) containing 500 μg/mL rifampicin (Sigma). After anaerobic incubation at 37°C for 3 d, colonies were stocked in mMRS broth containing 40% (vol:vol) glycerol. To confirm that the rifampicin-resistant variant was comparable to the parent strain for CLA production.

For use in the mice trials, \( B. breve \) was initially grown in mMRS by incubating overnight at 37°C under anaerobic conditions. The culture was washed twice in phosphate-buffered saline and resuspended at 1 × 10\(^{10}\) cells/mL in 15% (wt:vol) trehalose (Sigma). Aliquots (1 mL) were freeze-dried with the use of a 24-h program (freeze temperature: −40°C; condenser set point: −60; vacuum set point: 0.6 mm Hg). Each mouse that received \( B. breve \) consumed ∼1–2 × 10\(^8\) live microorganisms/d. This was achieved by resuspending appropriate quantities of freeze-dried powder in the water that the mice consumed ad libitum. Mice that did not receive the bacterial strain received placebo freeze-dried powder (15% wt:vol trehalose). For the pig feeding trial, a fresh culture of \( B. breve \) NCIMB 702258 was grown overnight to ∼1–2 × 10\(^8\) CFU/mL in mMRS, washed in maximum recovery diluent, and resuspended in 50 mL RSM (wt:vol) before feeding. Each pig that received \( B. breve \) consumed ∼1–2 × 10\(^8\) live microorganisms/d.

**Microbial analysis**

Fresh fecal samples were taken from BALB/c mice and pigs every week for microbial analysis and fatty acid analysis. Microbial analysis of the fecal samples involved enumeration of the \( B. breve \) strain on mMRS agar supplemented with 100 μg mupirocin (Oxoid, Basingstoke, United Kingdom)/mL, 100 μg rifampicin (Sigma)/mL, and 50 U nystatin (Sigma)/mL. Agar plates were incubated anaerobically for 72 h at 37°C. Contents from mid-large intestine and mid-small intestine were sampled at killing from 6 pigs/treatment group for enumeration of the administered \( B. breve \).

**Lipid extraction and fatty acid analysis**

Lipids were extracted with chloroform:methanol (2:1 vol:vol) according to the method by Folch et al (26). Fatty acid methyl esters (FAMEs) were prepared by first using 10 mL 0.5 N NaOH in methanol for 10 min at 90°C followed by 10 mL 14% BF\(_3\) in methanol (Sigma) for 10 min at 90°C (27). FAMEs were recovered with hexane. Before gas-liquid chromatography analysis, samples were dried over 0.5 g anhydrous sodium sulfate for 1 h and stored at −20°C. FAMEs were separated by gas-liquid chromatography (Varian 3400; Varian, Walnut Creek, CA) fitted with a flame ionization detector, using a Chrompack CP Sil 8 column (Chrompack, Middleton, Netherlands; 100 m × 0.25 mm internal diameter, 0.20 μm film thickness) and helium as carrier gas. The column oven was programmed to be held initially at 80°C for 8 min then increased 8.5°C/min to a final column temperature of 200°C. The injection volume used was 0.6 μL, with automatic sample injection on a SPI 1093 splitless on-column temperature programmable injector. Data were recorded and analyzed on a Minichrom personal computer system (VG Data System, Manchester, United Kingdom), and peaks were identified with reference to retention times of fatty acids in a standard mixture. The percentage of individual fatty acids was calculated according to the peak areas relative to the total area (total fatty acids were set at 100%). All fatty acid results are shown as mean ± SEM g/100 g FAMEs.
Immunologic analysis

Splenocytes were isolated from spleens of SCID mice, and erythrocytes were depleted with the use of an erythrocyte lysing kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. Splenocytes were resuspended in Dulbecco’s modified Eagle’s medium (containing 10% fetal bovine serum, 1% penicillin-streptomycin; Sigma) and diluted to 1 × 10^6 cells/mL for in vitro culturing. Isolated splenocytes were cocultured with anti-CD3–anti-CD28 monoclonal antibodies and with cells/mL for in vitro culturing. Isolated splenocytes were cocultured with anti-CD3–anti-CD28 monoclonal antibodies and with the proinflammatory bacterium Salmonella typhimurium UK1 (1 × 10^8 cells/mL) at 37°C for 48 h. Cell supernatant fluids were collected and stored at −80°C. Cytokine analysis was performed on the supernatant fluids with the use of a BD Cytometric Bead Array Mouse Inflammation Kit (BD Biosciences, San Jose, CA).

Lamina propria lymphocytes (LPLs) were isolated from porcine ileum tissue samples collected at killing on day 21, as described for human LPLs (28). LPLs were stimulated with 2 µg/mL phytohemagglutinin or vehicle for 18 h. Supernatant fluids were collected for cytokine analysis by enzyme-linked immunoabsorbent assay (Quantikine kits; R&D Systems). Antibodies were used according to the manufacturer’s instructions.

Assay of myeloperoxidase activity in colon of SCID mice

Myeloperoxidase (MPO) activity in colonic tissue was measured according to the method by Krawisz et al (29). Total soluble protein concentration was measured by the method of Bradford (30), and the MPO activity of colonic tissue was expressed as units/mg protein.

Statistical analyses

Data were presented as mean per group ± SEM. Data from SCID mice were analyzed by MINITAB RELEASE 14 statistical software (Minitab Inc, State College, PA) and tested as appropriate by analysis of variance or Kruskal-Wallis tests to assess whether differences between treatment groups (A–E) were significant. Student’s t test was performed to determine whether data from BALB/c mice (linoleic acid compared with linoleic acid in combination with B. breve–supplemented diets) were significant. Data from the pig trial (sunflower oil in combination with B. breve compared with unsupplemented diets) were analyzed with the use of the general linear models procedure of SAS (version 9.1 for WINDOWS; SAS Institute Inc, Cary, NC). Results were considered significant as follows: *P < 0.05, **P < 0.01, ***P < 0.001 (asterisks correspond to symbols used in figures for this article). Correlations between c9,t11 CLA and linoleic acid in feces from BALB/c mice were assessed by Pearson’s (r) correlation test with the use of ANALYZE-IT, Microsoft Excel (2003; Microsoft, Redman, WA).

RESULTS

Survival and transit of B. breve NCIMB 702258 in mouse and pig

Quantification of the numbers of bacteria of the B. breve strain monitored in the feces of individual mice and pigs on a weekly basis confirmed gastrointestinal transit and survival of the administered strain. Stool recovery of B. breve was ≈3 × 10^6 CFU/g feces in mice and ≈1 × 10^6 CFU/g feces in pigs at the end of the trials. Furthermore, B. breve was detected in the small intestine at ≈10^5 CFU/g and in the large intestine at ≈10^7 CFU/g at killing of the pigs administered B. breve.

Oral administration of B. breve NCIMB 702258 positively affects the fatty acid composition of mammalian tissues

After 8 wk of dietary treatment, the mean c9,t11 CLA content of the liver was 2.4-fold higher in the BALB/c mice fed B. breve in combination with linoleic acid substrate than in the control mice that were fed linoleic acid alone (P < 0.001; Figure 1A). Oral administration of B. breve also led to a 2-fold higher c9,t11 CLA content in the large intestine (P < 0.001; Table 2). Higher amounts of c9,t11 CLA were also observed in the small intestine of the mice receiving B. breve in combination with linoleic acid than of the control mice (P < 0.05), as well as numerically higher amounts of c9,t11 CLA in the cecal contents of the former group compared with the latter group (Table 2). The BALB/c mice receiving B. breve in combination with linoleic acid had also a 2.4-fold higher amount of c9,t11 CLA in feces harvested after 8 wk than did the control mice (P < 0.001), which correlated with a lower amount of linoleic acid in feces (P < 0.001, r = −0.863; Figure 2). Furthermore, in comparison with the control group that received linoleic acid alone, oral administration of the combination of B. breve and linoleic acid resulted in numerically lower amounts of linoleic acid, however not significant, in the liver, large intestine, and small intestine.
indicating that it has been further bioprocessed (Table 2). In addition to an overall higher content of e9,t11 CLA and a lower content of linoleic acid in BALB/c mice receiving B. breve, these mice had higher amounts of eicosapentaenoic acid (EPA, 20:5n–3; 0 < P ≤ 0.05) and docosahexaenoic acid (DHA, 22:6n–3; 0 < P ≤ 0.01) in the large intestine (Table 2).

After 3 wk of dietary treatment, the e9,t11 CLA content of porcine livers was 1.5-fold higher in animals administered B. breve in combination with linoleic acid–containing sunflower oil compared than in unsupplemented controls (P < 0.05; Figure 1B). Although oral administration of B. breve also led to a numerically higher e9,t11 CLA in porcine adipose tissue than in controls (0.032 ± 0.01 and 0.025 ± 0.01 g/100 g FAME, respectively) and in blood (0.25 ± 0.03 and 0.21 ± 0.04 g/100 g FAME, respectively), these were not significantly different.

After 10 wk of dietary treatment, the e9,t11 CLA content of SCID mouse liver was 4-fold higher in the group that received B. breve in combination with linoleic acid (group B) than in the group receiving linoleic acid (group A) (Figure 1A). Furthermore, the e9,t11 CLA content of SCID mouse large intestine and cecal contents were 3.0- and 2.0-fold higher, respectively, in the group that received B. breve in combination with linoleic acid compared than in the group that received linoleic acid alone (0.03 ± 0.01 and 0.16 ± 0.03 g/100 g FAME compared with 0.01 ± 0.01 and 0.08 ± 0.02 g/100 g FAME, respectively). The mice fed pure e9,t11 CLA (group D) had higher amounts of e9,t11 CLA in all tissues than did all other groups (P < 0.05). This group also exhibited decreased arachidonic acid (20:4n–6) content of liver than did the unsupplemented controls (8.81 ± 1.09 compared with 12.45 ± 0.90 g/100 g FAME; 0 < P ≤ 0.05). The mice receiving B. breve only (without linoleic acid supplementation; group C)
exhibited a 3-fold higher ($P < 0.001$) adipose tissue concentration of EPA than did the unsupplemented mice (group E) (Figure 3A). Supplementation with 9,11 CLA also resulted in higher amounts of EPA in adipose tissue than that found in unsupplemented mice ($P < 0.05$), whereas supplementation with linoleic acid (without *B. breve*) resulted in lower amounts of EPA in adipose tissue ($P < 0.05$; Figure 3A). The mice receiving *B. breve* (without linoleic acid supplementation; group C) also exhibited higher amounts of DHA in adipose tissue than did the unsupplemented mice (group E) ($P < 0.001$; Figure 3B). In contrast to fatty acid compositional changes, oral administration of *B. breve* did not significantly influence body weight gain in either the mice or pigs throughout the feeding trials (data not shown).

**Oral administration of *B. breve* NCIMB 702258 and 9,11 CLA reduces proinflammatory cytokine release in SCID mice and pigs**

The proinflammatory cytokines interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were reduced from splenocytes of SCID mice fed 9,11 CLA compared with unsupplemented controls, after stimulation with anti-CD3–anti-CD28 ($P < 0.05$; Figure 4A). Moreover, TNF-α production from splenocytes of the mice fed *B. breve* was reduced after stimulation with *S. typhimurium* UK1 compared with controls ($P < 0.05$, Figure 4B). In addition, TNF-α and IFN-γ production from splenocytes of the mice fed *B. breve* was reduced after stimulation with *S. typhimurium* UK1 compared with controls ($P < 0.05$, Figure 4B). Compared with unsupplemented control mice, there were no reductions in proinflammatory cytokine release from splenocytes of mice fed either linoleic acid or the combination of *B. breve* and linoleic acid after stimulation with anti-CD3–anti-CD28 or *S. typhimurium* UK1.

**DISCUSSION**

This study shows that fatty acid composition of host tissue is positively influenced by oral administration of a metabolically active commensal acting on a dietary substrate. We showed that dietary supplementation with *B. breve* NCIMB 702258 in combination with linoleic acid significantly increased liver 9,11 CLA content in both mice and pigs. This result is consistent with previous evidence for synthesis of the bioactive isomer 9,11 CLA from linoleic acid by this commensal in vitro (11), and it indicates that the strain is metabolically active in the gastrointestinal tract. Oral administration of *B. breve* NCIMB 702258 also resulted in significantly higher concentrations of EPA and DHA in adipose tissue compared with concentrations in unsupplemented mice. Moreover, the ratios of arachidonic acid to EPA in liver, adipose tissue, and cecal contents were lower in the *B. breve*–fed SCID mice than in the control mice. Higher concentrations of EPA and DHA were also obtained in the large intestine of BALB/c mice that received *B. breve*.

These results have considerable clinical implications. Because CLA has been shown to alleviate nonalcoholic fatty liver disease (NAFLD) (31), a condition accompanying obesity, elevation of 9,11 CLA in the liver, as produced by *B. breve* NCIMB 702258, is of therapeutic relevance. Indeed, oral administration of the probiotic mixture VSL#3 was reported to improve NAFLD in *ob/ob* mice and to improve characteristics of liver dysfunction in patients with NAFLD (32, 33). Note that this probiotic mixture was shown to produce CLA in vitro, mainly the 9,11 CLA isomer (34).

EPA and DHA exert antiinflammatory properties (35), and the ability of these fatty acids to alter cytokine production from several cell types and to reduce proinflammatory cytokines has been reported (36, 37). The antiinflammatory actions of these fatty acids are mainly due to the replacement of arachidonic acid.
in cell membranes, which results in decreased production of arachidonic acid–derived proinflammatory eicosanoids such as prostaglandin E2 and leukotriene B4. An antiobesity effect of EPA in vivo has also been proposed (39, 40), which might, in part, be mediated by inflammatory changes.

The alteration in EPA and DHA observed in the present study suggests that feeding a metabolically active strain, such as _B. breve_ NCIMB 702258, can influence the fatty acid composition of host tissues which could, in turn, be linked with additional changes such as alterations in cytokine production. Indeed, the current study showed an antiinflammatory effect of the administered _B. breve_ NCIMB 702258, with reduced production of IFN-γ, TNF-α, and IL-6 from splenocytes of SCID mice with colitis and reduced production of TNF-α, IL-1β, IL-8, and IL-12, coupled with an increase in the regulatory cytokine, IL-10, from LPLs of pigs. This antiinflammatory effect is relevant to the proinflammatory cytokine profile in obesity, which is associated with elevated concentrations of TNF-α and IL-6 (41–43). Although _c9,t11_ CLA was produced in the SCID mice that were fed the combination of _B. breve_ and linoleic acid, these mice did not exhibit any attenuated production of IFN-γ and TNF-α. This may be due to the relatively large amount of linoleic acid administered to these mice which may have negated the antiinflammatory effect mediated by the _B. breve_ strain or _c9,t11_ CLA produced from _B. breve_ or both because linoleic acid has been shown to exhibit proinflammatory properties (44, 45). Linoleic acid is the metabolic precursor to arachidonic acid, and the bioactive eicosanoids derived from arachidonic acid such as leukotriene B4 are linked to inflammation (46). However, the SCID mice that received dietary _c9,t11_ CLA supplementation showed a positive effect on mucosal inflammation, reflected by a reduction in TNF-α, IFN-γ, and IL-6, which is consistent with previous observations in poultry (47), rats (48), and mice (49). The mechanisms underlying this antiinflammatory effects of CLA include both a reduction of the arachidonate pool with reduced downstream production of eicosanoids and subsequent cytokine modulation, or a complex series of events following agonist effects on peroxisome proliferator-activated receptors (α, β, δ, and γ) (50). Because obesity is associated with a proinflammatory state (41–43) and the gut microbiota has been proposed to play a role in its development (9), manipulation of the gut microbiota with a metabolically active _Bifidobacterium_ strain could therefore represent a therapeutic strategy for obesity and other inflammatory settings. Indeed, a recent study showed that dietary modulation of gut microbiota by increasing the numbers of bifidobacteria improved glucose tolerance and insulin secretion as well as inflammation in mice fed a high-fat diet (51).

In the present study, bioformation of _c9,t11_ CLA from linoleic acid proved to be greater in the large intestine than in the small intestine. Bioformation of CLA in the proximal small intestine is also less likely to occur because of the lower intestinal load relative to the ileum and large intestine. The amount of linoleic acid available for CLA production in the large intestine varies and depends on the amount ingested and the efficacy of absorption in the small intestine. Edlonwee and Kies (52) showed that humans generally excrete about 20 mg of linoleic acid/d, indicating that the substrate is available for microbial production of CLA in the large intestine. In contrast to dietary CLA, which is primarily absorbed from the small intestine, the microbiota may be the primary source of CLA for the large intestine. Given that CLA exhibits antiinflammatory and antiangiogenic effects on colonocytes (24, 53), microbially derived CLA may be an important contributor to colonic mucosal homeostasis.

The mechanism by which _B. breve_ NCIMB 702258 administration mediated the changes in host n-3 fatty acid composition in the present study is uncertain. _B. breve_ administration might have influenced fatty acid metabolism by using or assimilating certain polyunsaturated fatty acids, such as ω-linolenic acid, or _B. breve_ might influence dietary fatty acid uptake in the intestine. The observed differences could also result from the properties of commensals in regulating desaturase activity involved in the metabolism of fatty acids to their longer-chain derivatives. Indeed, it has been shown that a variety of commensals increased the activity of rat liver Δ⁶-desaturase, which

**FIGURE 4.** Cytokine production by stimulated splenocytes obtained from severe combined immunodeficient mice with colitis. (A) Stimulation with anti-CD3–anti-CD28; (B) stimulation with *Salmonella typhimurium* UK1. Results are expressed as mean cytokine concentrations ± SEMs (n = 8). *Significantly different from un-supplemented mice, P < 0.05. _B. breve_, *Bifidobacterium breve*; _c9,t11_ CLA, _cis-9,trans-11_ conjugated linoleic acid; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6.
resulted in increased amounts of arachidonic acid derived from linoleic acid (54). Although we cannot definitively discount the contribution of coprophagy to the observed changes in host CLA liver status, it seems unlikely that this contributed in any significant way. Given that CLA was increased in both the small and the large intestine of mice receiving the combination of \textit{B. breve} and linoleic acid than of mice fed linoleic acid alone, this proves that bioformation of CLA occurred in vivo at the sites where absorption of fatty acids occurs.

In conclusion, the present study shows that feeding different animal species a CLA-producing \textit{Bifidobacterium} of human origin (\textit{B. breve} NCIMB 702258), in combination with linoleic acid as substrate, results in modulation of the fatty acid composition of the host, including significantly elevated concentrations of \(c_9,t_{11}\) CLA in the liver. The same strain is also associated with an apparent anti-inflammatory effect on cytokine production that is pertinent to the pro-inflammatory cytokine profile that characterizes obesity. The results confirm that the metabolic activity of the enteric microbiota and the dietary manipulation thereof represent a realistic target for modification of the fatty acid composition of host tissues.

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FIGURE 5. Cytokine production by phytohemagglutinin-stimulated lamina propria lymphocytes isolated from ileum tissue of pigs. Results are expressed as mean cytokine concentrations ± SEMs \((n = 6)\). * **Significantly different from unsupplemented pigs; \(\ast \ P < 0.05, \ast \ast \ P < 0.01\). IFN-\(\gamma\), interferon-\(\gamma\); \textit{B. breve}, \textit{Bifidobacterium breve}; LA, linoleic acid; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); IL-1\(\beta\), interleukin-1\(\beta\); IL-8, interleukin-8; IL-12, interleukin-12; IL-10, interleukin-10.
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