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ORIGINAL ARTICLE

Impact of Administered *Bifidobacterium* on Murine Host Fatty Acid Composition

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Abstract Recently, we reported that administration of *Bifidobacteria* resulted in increased concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in murine adipose tissue [1]. The objective of this study was to assess the impact of co-administration of *Bifidobacterium breve* NCIMB 702258 and the substrate for EPA, α-linolenic acid, on host fatty acid composition. α-Linolenic acid-supplemented diets (1%, wt/wt) were fed to mice ($n = 8$), with or without *B. breve* NCIMB 702258 (daily dose of 10^9 microorganisms) for 8 weeks. Two further groups received either supplement of *B. breve* alone or unsupplemented diet. Tissue fatty acid composition was assessed by gas liquid chromatography. Dietary supplementation of α-linolenic acid resulted in higher ($P < 0.05$) α-linolenic acid and EPA concentrations in liver and adipose tissue and lower ($P < 0.05$) arachidonic acid in liver, adipose tissue and brain compared with mice that did not receive α-linolenic acid. Supplementation with *B. breve* NCIMB 702258 in combination with α-linolenic acid resulted in elevated ($P < 0.05$) liver EPA concentrations compared with α-linolenic acid supplementation alone. Furthermore, the former group had higher ($P < 0.05$) DHA in brain compared with the latter group. These results suggest a role for interactions between fatty acids and commensals in the gastrointestinal tract. This interaction between administered microbes and fatty acids could result in a highly effective nutritional approach to the therapy of a variety of inflammatory and neurodegenerative conditions.

Keywords Omega-3 fatty acids · Eicosapentaenoic acid · Docosahexaenoic acid · *Bifidobacteria* · Microbiota · Probiotics

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Abbreviations

ANOVA	Analysis of variance
CFU	Colony forming units
CLA	Conjugated linoleic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
IBD	Inflammatory bowel disease
IFN-γ	Interferon-γ
MTP	Microsomal triglyceride transfer protein
MRS	de Man, Rogosa and Sharpe
PBS	Phosphate buffered saline
PUFA	Polyunsaturated fatty acids
PFGE	Pulse-field gel electrophoresis
SDS	Special diets services
SEM	Standard error mean
TNF-α	Tumor necrosis factor-α

Introduction

Mammals can produce all but two of the fatty acids they require; thus linoleic acid (C18:2n-6, precursor of n-6

series of fatty acids) and α-linolenic acid (C18:3n-3, precursor of n-3 series of fatty acids) are essential dietary fatty acids. Although mammalian cells cannot synthesize these fatty acids, they can metabolize them into more physiologically active compounds through a series of elongation and desaturation reactions, in which linoleic acid is converted to arachidonic acid (C20:4n-6) and α-linolenic acid is metabolized to eicosapentaenoic acid (EPA) (C20:5n-3) via the action of the enzymes Δ^6 desaturase, Δ^5 desaturase and elongase [2]. The resulting highly unsaturated fatty acid metabolites play essential roles in cell membrane function, brain and nervous system development and function, and through the production of eicosanoids (thromboxanes, leukotrienes and prostaglandins) in the inflammatory process [2]. Eicosanoids derived from arachidonic acid, such as the 2-series prostaglandins and the 4-series leukotrienes are in general, regarded as being proinflammatory in nature [3, 4], whereas the eicosanoids derived from EPA, such as the 3-series prostaglandins and the 5-series leukotrienes are considered less inflammatory or even anti-inflammatory in nature [3–5]. Thus, by increasing the ratio of n-3 to n-6 fatty acids in the diet, and consequently favouring the production of EPA, the balance of eicosanoids can be shifted in a less inflammatory direction. EPA can be further metabolized to docosahexaenoic acid (DHA, C22:6n-3), which is one of the major n-3 polyunsaturated fatty acids (PUFA) in the brain. DHA is required for fetal brain development and is held to be critical in the newborn for appropriate development and intelligence [6]. Studies have also shown that DHA provides support to learning and memory events in animal models of Alzheimer's disease [7] and brain injury [8].

The human gut is a diverse microbial ecosystem containing about 100 trillion microorganisms, comprised of more than 1,000 different species, whose collective genome, the microbiome, contains ~100-fold more genes than the entire human genome [9]. It has been well documented that the enteric microbiota play an important role in the health and well-being of the host, exerting effects on host lipid metabolism and acting as an environmental factor that contributes to development of obesity [10, 11]. In this respect, recent studies suggest that symbiosis between the microbiome and the host influences energy extraction from the diet. In addition, the promotion of fat deposition and influence on systemic inflammation have been proposed as mechanisms by which the microbiome contribute to obesity [12].

Little is known regarding the interplay between members of the enteric microbiota and fatty acids. However, some interactions between PUFAs and components of the indigenous gut microbiota and some probiotics have been reported, which might affect the biological roles of both. Recent studies by our group and others have reported that intestinal bacteria of human origin can convert linoleic and linolenic acids to bioactive isomers of conjugated linoleic acid (CLA) and conjugated α-linolenic acid, respectively [13–15]. Some bacteria of marine origin are also known to synthesise EPA and DHA de novo through the actions of polyunsaturated fatty acid synthase genes, which results in EPA and DHA being abundantly present in fish and fish oil [16–19]. Furthermore, it has been shown that administration of probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* Bb12) to pregnant women had an affect on placental fatty acid composition [20]. It has also been demonstrated that administration of formula supplemented with different probiotics (*B. animalis* subsp. *lactis* Bb12 and *L. rhamnosus* GG) to infants resulted in changes in serum fatty acid composition [21].

We have recently shown that feeding different animal species a CLA-producing *Bifidobacterium* of human origin (*B. breve* NCIMB 702258), in combination with linoleic acid as substrate, resulted in modulation of the fatty acid composition of the host, including significantly elevated concentrations of *c*9, *t*11 CLA in the liver. This study also demonstrated that oral administration of *B. breve* NCIMB 702258 to mice resulted in significantly higher concentrations of EPA and DHA in adipose tissue, coupled with reductions in the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) [1]. The objective of this study was, therefore, to investigate the effects of co-administration of *B. breve* NCIMB 702258 and the substrate for EPA, α-linolenic acid, on fatty acid composition of different host tissues in mice.

Experimental Procedure

Preparation and Administration of *B. breve* NCIMB 702258

Rifampicin resistant variants of *B. breve* NCIMB 702258 were isolated by spread-plating ~10^9 colony forming units (CFU) from an overnight culture onto MRS agar (de Man, Rogosa & Sharpe; Difco Laboratories, Detroit, MI, USA) supplemented with 0.05% (wt/v) L-cysteine hydrochloride (98% pure; Sigma Chemical Co., St. Louis, MO, USA) (mMRS) containing 500 μg/ml rifampicin (Sigma Chemical Co., Poole, Dorset, UK). Following anaerobic incubation at 37 °C for 3 days, colonies were stocked in mMRS broth containing 40% (v/v) glycerol and stored at −80 °C. To confirm that the rifampicin resistant variant was identical to the parent strain, molecular fingerprinting using pulse-field gel electrophoresis (PFGE) was employed.

Prior to freeze drying, *B. breve* NCIMB 702258 was grown in mMRS by incubating overnight at 37 °C under anaerobic conditions. The culture was washed twice in

phosphate buffered saline (PBS) and then resuspended at a concentration of $\sim 1 \times 10^{10}$ cells/ml in 15% (wt/v) trehalose (Sigma) in dH_2O. One millilitre aliquots were freeze-dried using a 24 h programme (freeze temp. −40 °C, condenser set point −60, vacuum set point 600 mTorr). Each mouse that received *B. breve* consumed approximately 1×10^9 live microorganisms per day. This was achieved by resuspending appropriate quantities of freeze-dried powder in water which mice consumed ad libitum. Mice that did not receive the bacterial strain received placebo freeze-dried powder (15% wt/v trehalose in dH_2O).

Animals and Treatment

Female BALB/c mice were purchased from Harlan Ltd. (Briester, Oxon, UK) at 8 weeks of age and were fed ad libitum with standard non-purified CRM(P) diet (Special Diets Services (SDS), Witham, Essex, UK) with free access to water at all times. The diet contained the following nutrient composition (wt/wt): nitrogen free extract (57.39%), crude protein (18.35%), moisture (10%), ash (6.27%), crude fibre (4.23%) and crude oil (3.36%), which consisted of 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%), monounsaturated fatty acids: myristoleic acid (C14:1, 0.02%), palmitoleic acid (C16:1, 10%) and oleic acid (C18:1, 0.87%), 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 maintained at four per cage and kept in a controlled environment at 25 °C under a 12-h-light/12-h-dark cycle. All laboratory animal experiments 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.

One week after arrival, the mice were divided into four groups (A–D, $n = 8$) and subjected to the following dietary treatments daily: Group A received standard nonpurified CRM(P) diet supplemented with 1% α-linolenic acid (C18:3n-3, wt/wt, triglyceride bound form, Larodan Fine Chemicals AB, Malmo, Sweden) in combination with approximately 1×10^9 live *B. breve* NCIMB 702258 per mouse, Group B received standard non-purified CRM(P) diet supplemented with 1% α-linolenic acid and placebo freeze-dried powder, Group C received standard nonpurified CRM(P) diet and $\sim 1 \times 10^9$ live *B. breve* NCIMB 702258, Group D received standard nonpurified CRM(P) diet and placebo freeze-dried powder. For α-linolenic acid treatment, a powdered diet (milled standard non-purified CRM(P) pellets) was blended with the α-linolenic acid to yield a concentration of approximately 90 mg α-linolenic acid per mouse per day (based on studies by Bassaganya-Riera et al. [22] who reported an optimal intake of fatty acids of 1 g/100 g per day). All prepared diets were stored at −20 °C and fresh diets were provided twice weekly. Following 8 weeks on experimental diets, the animals were sacrificed by cervical dislocation. Liver, adipose tissue and brain 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.

Microbial Analysis

Fresh faecal samples were taken directly from the anus of each mouse every second week for microbial analysis. Large intestinal contents were also sampled at sacrifice for enumeration of the administered *B. breve* strain. Microbial analysis of *B. breve* NCIMB 702258 was performed by pour plating onto mMRS agar supplemented with 100 μg of mupirocin (Oxoid)/ml and 100 μg rifampicin (Sigma)/ml. Agar plates were incubated anaerobically at 37 °C for 72 h. Anaerobic environments were created using CO_2 generating kits (Anaerocult A; Merck, Darmstadt, Germany) in sealed gas jars.

Lipid Extraction and Fatty Acid Analysis

Lipids were extracted according to the method of O'Fallon et al. [23]. Briefly, tissue samples were cut into 1.5-mm rectangular strips and placed into a screw-cap Pyrex culture tube together with 0.7 ml of 10 mol/l KOH in dH_2O and 5.3 ml of MeOH. The tubes were incubated in a water bath at 55 °C for 1.5 h with vigorous hand-shaking every 20 min. After cooling below room temperature, 0.58 ml of 12 mol/l of H_2SO_4 in dH_2O was added. The tubes were mixed by inversion and with precipitated K_2SO_4 present incubated again at 55 °C for 1.5 h with hand-shaking every 20 min. Fatty acid methyl esters (FAME) were recovered by addition of 3 ml hexane and vortex mixed and separated by gas liquid chromatography (Varian 3400, Varian, Walnut Creek, CA, USA fitted with a flame ionisation detector) using a Chrompack CP Sil 88 column (Chrompack, Middleton, The Netherlands, 100 m × 0.25 mm i.d., 0.20 μm film thickness) and He as the carrier gas. The column oven was initially programmed at 80 °C for 8 min, and increased at 8.5 °C/min to a final column temperature of 200 °C. The injection volume was 0.6 μl, with automatic sample injection on a SPI 1093 splitless on-column temperature programmable injector. Data were recorded and analysed on a Minichrom PC system (VG Data System, Manchester, UK). Peaks were identified with reference to retention times of fatty acids in a standard mixture. All fatty acid results are shown as means ± standard error means (SEM) g/100 g FAME.

Statistical Analysis

Results in the text, tables and figures are presented as means per group ± SEM. Data were analysed using analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad InStat for Windows (GraphPad Software, La Jolla, CA, USA) in order to assess if differences between treatment groups (A–D) were significant. Probability values of $P < 0.05$ were set as a threshold for statistical significance.

Results

Microbial Analysis

The administered *B. breve* NCIMB 702258 was recovered in faeces from all mice that received the strain, within 2 weeks of feeding, confirming gastrointestinal transit and survival of the strain. Stool recovery of *B. breve* NCIMB 702258 was approximately 4×10^5 CFU/g faeces by week 8 of the trial in mice that received *B. breve* in combination with α-linolenic acid (group A) and approximately 2.2×10^6 CFU/g faeces in mice that received *B. breve* without α-linolenic acid (group C) (data not shown). The *B. breve* strain was detected in large intestinal contents at $\sim 4.6 \times 10^5$ CFU/g in mice that received *B. breve* and α-linolenic acid (group A) and $\sim 1.4 \times 10^6$ CFU/g in mice that received *B. breve* alone (group C). *B. breve* NCIMB 702258 was not isolated from any of the mice within group B (administered α-linolenic acid alone) or group D (unsupplemented).

Tissue Fatty Acid Composition

Oral administration of *B. breve* NCIMB 702258 and/or α-linolenic acid (C18:3n-3) did not significantly influence body weight throughout the trial period. Supplementation of α-linolenic acid, either in combination with *B. breve* or in the absence of the *B. breve* strain (group A and group B) resulted in tenfold higher concentrations of α-linolenic acid and EPA (C20:5n-3) in liver ($P < 0.05$; Table 1) and adipose tissue ($P < 0.05$; Table 2) compared with groups that did not receive the fatty acid supplement (group C and group D). In addition, the α-linolenic acid supplemented groups exhibited significantly higher concentrations of docosapentaenoic acid (DPA, C22:5n-3) in liver ($P < 0.05$; Table 1) and adipose tissue ($P < 0.05$; Table 2), significantly higher concentrations of DHA (C22:6n-3) in liver ($P < 0.05$; Fig. 2) and significantly lower concentrations of arachidonic acid (C20:4n-6) in liver ($P < 0.05$; Table 1), adipose tissue ($P < 0.05$, Table 2) and brain ($P < 0.05$, Table 3) compared with groups that did not receive fatty acid supplementation (group C and group D). The arachidonic acid/EPA ratios in liver and adipose tissue were approximately 30-fold and 20-fold lower, respectively, in the α-linolenic acid supplemented groups (group A and B) compared with unsupplemented controls (group D) ($P < 0.05$). In addition, the n-6/n-3 ratio was significantly lower in all tissues except the brain of animals supplemented with α-linolenic acid (group A and B) ($P < 0.05$; Table 1 and 2).

Administration of *B. breve* in combination with α-linolenic acid resulted in significant changes in the fatty acid composition of host liver and brain in comparison to animals that were administered α-linolenic acid alone. Mice that received *B. breve* in combination with α-linolenic acid (group A) exhibited on average, 23% more EPA (C20:5n-3) and 20% more dihomo-γ-linolenic acid (C20:3n-6) in the liver compared with the group that was administered α-linolenic acid alone (group B) ($P < 0.05$; Fig. 1; Table 1). Group A also exhibited a 12% higher concentration of DHA (C22:6n-3) in brain ($P < 0.05$; Fig. 2), as well as numerically, though not significantly, higher concentrations of DHA (C22:6n-3) in adipose tissue and liver (27 and 16%, respectively) compared with group B (Fig. 2). In addition, mice that received *B. breve* without α-linolenic acid (group C), exhibited numerically, though not significantly, higher concentrations of DHA (C22:6n-3) in brain tissue in comparison to unsupplemented controls (group D) (Fig. 2).

Oral administration of *B. breve*, both in combination with α-linolenic acid and without α-linolenic acid supplementation (group A and C), also resulted in significantly higher concentrations of arachidonic acid (C20:4n-6) and stearic acid (C18:0) incorporated in the liver compared to mice that did not receive *B. breve* (group B and group D) ($P < 0.05$; Table 1).

Discussion

The influence of dietary PUFA on phospholipids, their eicosanoid derivatives and the transmembrane-signalling lipid rafts into which they are arranged provide multiple targets for the dietary modulation of the balance of inflammatory mediators in the human gut. In addition, gut mucosal inflammation is now recognised as being heavily influenced by the gastrointestinal microbiota [24, 25]. The delicate balance of inflammatory mediators derived from PUFA may be readjusted by members of the indigenous gut microbiota. In this study, we investigated how co-administration of *B. breve* NCIMB 702258 and the substrate for EPA, α-linolenic acid affected the EPA and DHA concentrations of different host tissues. We found that dietary supplementation of *B. breve* NCIMB 702258 in combination with α-linolenic

Table 1 Fatty acid composition (%) of liver from BALB/c mice

FAME	Liver			
	A	B	C	D
C16:0	23.11 ± 0.99^{c,d}	23.89 ± 0.71^{c,d}	27.78 ± 0.64^{a,b}	27.64 ± 0.42^{a,b}
C16:1c9	1.56 ± 0.10^{b,c,d}	2.07 ± 0.18^{a,d}	2.32 ± 0.18^{a}	2.73 ± 0.27^{a,b}
C18:0	13.83 ± 0.42^{b,c,d}	12.36 ± 0.44^{a,d}	11.50 ± 0.35^{a,d}	10.40 ± 0.35^{a,b,c}
C18:1c9	9.16 ± 0.36^{c,d}	10.16 ± 0.62^{c,d}	13.75 ± 0.57^{a,b}	15.16 ± 0.75^{a,b}
C18:2n-6	18.70 ± 0.35	18.32 ± 0.21^{d}	18.21 ± 0.32^{d}	19.38 ± 0.42^{b,c}
C18:3n-3	8.37 ± 0.91^{c,d}	9.47 ± 0.64^{c,d}	0.50 ± 0.03^{a,b}	0.58 ± 0.04^{a,b}
C18:3n-6	0.20 ± 0.02^{c,d}	0.17 ± 0.01^{c,d}	0.30 ± 0.03^{a,b}	0.32 ± 0.01^{a,b}
C18:4n-3	0.14 ± 0.01^{c,d}	0.16 ± 0.01^{c,d}	0.23 ± 0.03^{a,b}	0.22 ± 0.02^{a,b}
C20:3n-6	0.73 ± 0.05^{b}	0.61 ± 0.02^{a}	0.69 ± 0.04	0.63 ± 0.03
C20:4n-6	6.71 ± 0.29^{b,c,d}	5.57 ± 0.14^{a,c,d}	11.45 ± 0.58^{a,c,d}	9.78 ± 0.53^{a,b,c}
C22:5n-3	1.02 ± 0.06^{c,d}	0.94 ± 0.05^{c,d}	0.23 ± 0.02^{a,b}	0.26 ± 0.02^{a,b}
n-6/n-3	1.36 ± 0.10^{c,d}	1.30 ± 0.07^{c,d}	4.70 ± 0.23^{a,b}	5.07 ± 0.16^{a,b}

Results are expressed as means ± SEM g/100 g FAME ($n = 8$). Different superscript letters within a column indicate significant difference ($n = 8$, $P < 0.05$). *FAME* fatty acid methyl esters. Group A = 1% α-linolenic acid in combination with 1×10^9 live *B. breve* NCIMB 702258 per day, Group B = 1% α-linolenic acid, Group C = standard diet in combination with 1×10^9 live *B. breve* NCIMB 702258, and Group D = unsupplemented mice (standard diet). *ND* not detected

Table 2 Fatty acid composition (%) of adipose tissue from BALB/c mice

FAME	Adipose tissue			
	A	B	C	D
C16:0	28.84 ± 2.83^{c}	28.73 ± 2.16^{c}	30.92 ± 0.82^{a,b,d}	29.87 ± 0.83^{c}
C16:1c9	5.39 ± 0.73	6.07 ± 2.01	6.69 ± 1.95	6.54 ± 2.31
C18:0	8.76 ± 1.00	8.00 ± 2.33	7.71 ± 1.99	7.31 ± 2.87
C18:1c9	14.41 ± 1.29	14.83 ± 3.01	16.92 ± 3.07	18.35 ± 4.84
C18:2n-6	18.22 ± 0.25^{c}	18.80 ± 0.81^{c}	16.56 ± 0.77^{a,b}	17.96 ± 3.39
C18:3n-3	6.56 ± 1.00^{c,d}	7.35 ± 2.15^{c,d}	0.66 ± 0.10^{a,b}	0.79 ± 0.28^{a,b}
C18:3n-6	0.15 ± 0.02	0.13 ± 0.03	0.15 ± 0.03	0.14 ± 0.04
C18:4n-3	0.16 ± 0.02	0.12 ± 0.03	0.14 ± 0.04	0.14 ± 0.03
C20:3n-6	0.37 ± 0.04	0.30 ± 0.09	0.32 ± 0.09	0.29 ± 0.16
C20:4n-6	6.20 ± 0.97^{c}	5.27 ± 2.04^{c,d}	10.57 ± 3.45^{a,b}	9.29 ± 5.27^{b}
C22:5n-3	0.67 ± 0.06^{c,d}	0.55 ± 0.14^{c,d}	0.27 ± 0.03^{a,b}	0.27 ± 0.16^{a,b}
n-6/n-3	2.20 ± 0.21^{c,d}	2.15 ± 0.15^{c,d}	10.80 ± 0.28^{a,b}	11.56 ± 0.92^{a,b}

Results are expressed as means ± SEM g/100 g FAME ($n = 8$). Different superscript letters within a column indicate significant difference ($n = 8$, $P < 0.05$). *FAME* fatty acid methyl esters. Group A = 1% α-linolenic acid in combination with 1×10^9 live *B. breve* NCIMB 702258 per day, Group B = 1% α-linolenic acid, Group C = standard diet in combination with 1×10^9 live *B. breve* NCIMB 702258, and Group D = unsupplemented mice (standard diet). *ND* not detected

acid resulted in the modulation of host fatty acid composition, and, specifically, resulted in significantly higher EPA and dihomo-γ-linolenic acid concentrations in liver and higher DHA in brain compared to mice that received α-linolenic acid without microbial supplementation. Some recent studies have shown that the gut microbiota modifies a number of lipid species in serum, adipose tissue and liver [26] as well as the eye lipidome of mice [27] when compared to germ-free mice, suggesting that interactions between intestinal bacteria and fatty acids occur.

Supplementation of α-linolenic acid, both in combination with *B. breve* and in the absence of the *B. breve* strain, resulted in significant increases in EPA and DHA in the liver and adipose tissue, at the expense of arachidonic acid. Since EPA replaces arachidonic acid as an eicosanoid

Table 3 Fatty acid composition (%) of brain from BALB/c mice

FAME	Brain			
	A	B	C	D
C16:0	30.69 ± 0.65	32.38 ± 0.44^{c}	28.88 ± 0.90^{b,d}	31.47 ± 0.51^{c}
C16:1c9	0.81 ± 0.02^{b,c}	0.94 ± 0.03^{a,c,d}	0.73 ± 0.03^{a,d}	0.84 ± 0.03^{b,c}
C18:0	20.22 ± 0.07^{b}	19.87 ± 0.14^{a}	20.11 ± 0.20	19.85 ± 0.28
C18:1c9	17.58 ± 0.19	17.61 ± 0.24	17.14 ± 0.29	17.23 ± 0.33
C18:2n-6	1.18 ± 0.04^{b,c}	1.54 ± 0.10^{a,c,d}	1.00 ± 0.05^{a,b}	1.14 ± 0.03^{b}
C18:3n-3	0.10 ± 0.01^{b,c,d}	0.18 ± 0.02^{a,c,d}	ND	ND
C18:3n-6	ND	ND	ND	ND
C18:4n-3	ND	ND	ND	ND
C20:3n-6	ND	ND	ND	ND
C20:4n-6	7.06 ± 0.07^{c}	6.88 ± 0.12^{c,d}	7.84 ± 0.23^{a,b}	7.37 ± 0.17^{b}
C22:5n-3	0.25 ± 0.01^{c,d}	0.24 ± 0.01^{c}	0.11 ± 0.01^{a,b}	0.17 ± 0.04^{a}
n-6/n-3	0.79 ± 0.03^{b,d}	0.89 ± 0.03^{a}	0.87 ± 0.04	0.97 ± 0.04^{a}

Results are expressed as means ± SEM g/100 g FAME ($n = 8$). Different superscript letters within a column indicate significant difference ($n = 8$, $P < 0.05$). *FAME* fatty acid methyl esters. Group A = 1% α-linolenic acid in combination with 1×10^9 live *B. breve* NCIMB 702258 per day, Group B = 1% α-linolenic acid, Group C = standard diet in combination with 1×10^9 live *B. breve* NCIMB 702258, and Group D = unsupplemented mice (standard diet). *ND* not detected

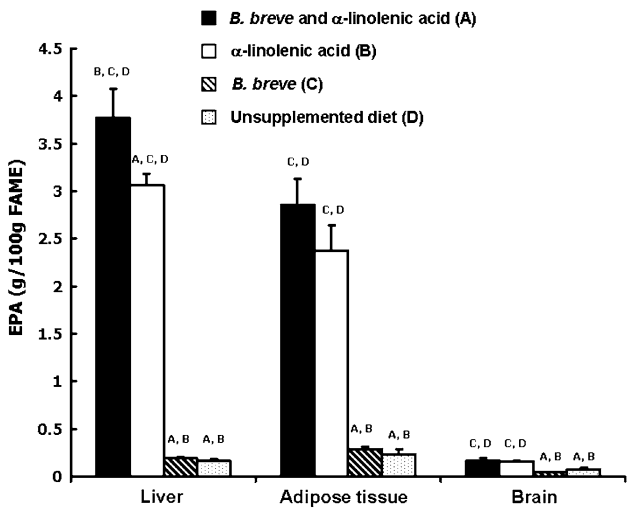

Fig. 1 Eicosapentaenoic acid (EPA) concentrations in murine liver, adipose tissue and brain. Different superscript letters within a column indicate significant differences ($n = 8$, $P < 0.05$). EPA is expressed as Mean ± SEM g/100 g fatty acid methyl esters (FAME)

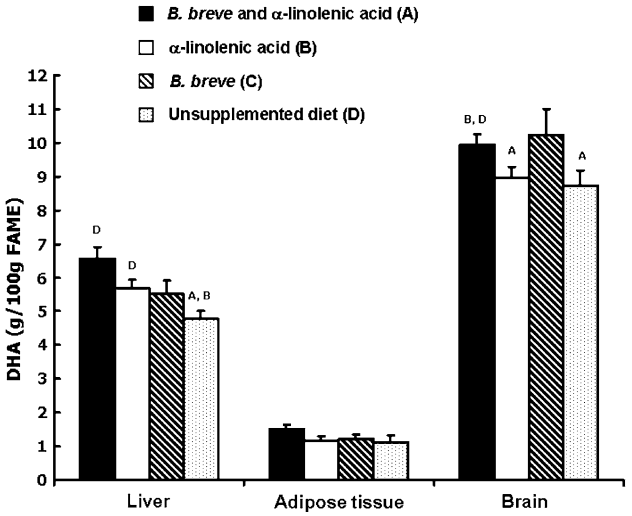

Fig. 2 Docosahexaenoic acid (DHA) concentrations in murine liver, adipose tissue and brain. Different superscript letters within a column indicate significant differences ($n = 8$, $P < 0.05$). DHA is expressed as mean ± SEM g/100 g fatty acid methyl esters (FAME)

precursor in cell membranes of platelets, erythrocytes, neutrophils, monocytes and hepatocytes [28], this results in a reduced synthesis of inflammatory eicosanoids from arachidonic acid and, subsequently, elevated production of anti-inflammatory eicosanoids from EPA. This alteration towards a more anti-inflammatory profile could be of importance in a variety of chronic inflammatory settings that are of high prevalence in Western societies such as inflammatory bowel disease (IBD), rheumatoid arthritis, cardiovascular disease, obesity, Alzheimer's disease and certain psychiatric diseases such as depression, which are characterised by an excessive production of arachidonic acid-derived eicosanoids [29–33]. Moreover, since excessive intake of n-6 PUFA, characteristic of modern Western diets, could potentiate inflammatory processes and so could predispose to, or exacerbate associated diseases, increasing the intake of α-linolenic acid and/or EPA may have a protective effect. A recent study using IL-10 knock-out mice (mice that spontaneously develop colitis) demonstrated significantly reduced colonic inflammation of mice that were fed fish oil (enriched in EPA and DHA) compared with mice that were fed n-6 PUFA-rich corn oil [34].

Oral administration of *B. breve*, both in combination with α-linolenic acid and without α-linolenic acid supplementation, resulted in significantly higher amounts of arachidonic acid incorporated in liver compared to mice that did not receive *B. breve*. Given that the administration of *B. breve* resulted in significantly higher concentrations of long-chain PUFA such as EPA, DHA and arachidonic acid, administration of this strain resulted in an increase in the levels of unsaturation within fatty acids. Interestingly, it was previously shown that a mixture of probiotics (*Bacillus subtilis, B. natto, B. megaterium, B. thermophilus, Lactobacillus acidophilus, L. plantarum, L. brevis, L. casei, Streptococcus faecalis, S. lactis, S. thermophilus, Clostridium butyricum, Saccharomyces cerevisiae* and *Candida utilis*) increased the activity of liver Δ6-desaturase in rats, which resulted in increased amounts of arachidonic acid derived from linoleic acid [35]. Consequently, in our study, the increased levels of long-chain PUFA in *B. breve* supplemented groups may have arisen from the reported properties of probiotics in regulating desaturase activity involved in the metabolism of fatty acids to their longer-chain unsaturated derivatives. *B. breve* NCIMB 702258 may also have influenced the mechanisms of PUFA uptake to the intestinal epithelium in the present study. It was recently demonstrated that exposure of *L. plantarum* WCFSI to human intestinal mucosa induced an upregulation of genes involved in fatty acid uptake, i.e. CD36 and microsomal triglyceride transfer protein (MTP) [36].

Quantification of the numbers of bacteria of the *B. breve* strain monitored in the feces of individual mice confirmed gastrointestinal transit and survival of *B. breve* NCIMB 702258. Faecal recovery was approximately 2×10^6 CFU/g feces in mice that received *B. breve* without α-linolenic acid and approximately 4×10^5 CFU/g feces in mice that received *B. breve* in combination with α-linolenic acid. The fecal recovery of *B. breve* NCIMB 702258 in mice that received *B. breve* without α-linolenic acid is consistent with our previous study [1], however the fecal recovery of *B. breve* was reduced in the presence of α-linolenic acid. Since free PUFA have been shown to be antibacterial and to inhibit the growth of bacteria [37–39] and adhesion of bacteria to intestinal surfaces [40], this might explain the lower numbers of *B. breve* obtained from mice that received supplementation of α-linolenic acid compared to mice that did not receive fatty acid supplementation.

Since the effect of the combined *B. breve* and α-linolenic acid intervention on EPA- and DHA concentrations was greater than that of α-linolenic acid intervention alone, this effect could be attributed to *B. breve* NCIMB 702258 and suggests that feeding a metabolically active strain can influence the fatty acid composition of host tissues. In conclusion, the present study shows that the administration of *B. breve* NCIMB 702258 is associated with alterations in the fatty acid composition of host liver and brain, including elevated concentrations of EPA and DHA. This interaction between administered microbes and n-3 fatty acids could result in more efficient probiotic preparations, which may be beneficial for a range of immunoinflammatory disorders as well as having significance for the promotion of neurological development in infants.

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