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A multispecies *Lactobacillus*- and *Bifidobacterium*-containing probiotic mixture attenuates body weight gain and insulin resistance after a short-term challenge with a high-fat diet in C57/BL6J mice

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A multispecies *Lactobacillus*- and *Bifidobacterium*-containing probiotic mixture significantly reduced the adverse metabolic and inflammatory effects of a 14-week high-fat diet in wild-type C57/BL6J mice gavaged 5 days a week with the probiotic mixture or vehicle. Recent evidence indicates that the gut microbiome may play a decisive role in the onset of obesity and associated chronic metabolic diseases, such as type 2 diabetes, by modulating nutrient absorption and factors conducive to development of a persistent low-grade inflammatory state. By modifying the gut microbiome, probiotics might constitute an effective dietary strategy for managing these metabolic disorders. The tested probiotic mixture significantly attenuated the increase in body weight, serum glucose concentration and insulin resistance induced by the high-fat diet. Furthermore, it significantly reduced the up-regulation of expression of several genes encoding pro-inflammatory adipokines and leukotriene pathway enzymes (CCL-2, IL-6 and leukotriene C₄ synthase in adipose tissue, leukotriene C₄ synthase and leukotriene A₄ hydrolase in the gut). It also significantly counteracted the down-regulation of adipose tissue gene expression related to the anti-inflammatory adipokine adiponectin in mice fed the high-fat diet. These results suggest that the mechanism underlying the beneficial metabolic effects of the probiotic mixture might involve inhibition of gut and adipose tissue inflammation.

1. Introduction

Obesity, with its constellation of related metabolic diseases, such as type 2 diabetes (T2D), has become a major public health problem worldwide in view of its associated morbidity and mortality, and the increasingly large number of individuals affected. Greater consumption of energy-dense foods and an

overall shift towards more sedentary lifestyles are important factors contributing to this unprecedented rise in prevalence [1], with genetic predisposition apparently playing a relatively minor role [2,3]. More recently, the gut microbiome has elicited a surge of interest as a possible contributor to the pathogenesis of obesity and T2D. Obesity appears to be associated with reduced bacterial diversity and changes in the relative abundance of different bacterial species [3,4]. Studies have indicated differences in gut microbiota between obese and lean individuals [2], as well as between diabetic and non-diabetic individuals [5,6]. Experiments in various animal models have consistently demonstrated that the gut microbiota affects host metabolism in numerous ways. In particular, it can modify the amount of energy harvested from the diet, the integrity of the intestinal barrier, the qualitative composition and metabolism of fatty acids in adipose tissue and the liver, the secretion of gut-derived peptides, and lipopolysaccharide (LPS)-induced inflammation [7].

Abbreviations: CLAs, conjugated linoleic acids; CT, control diet; FIAF, fasting-induced adipocyte factor; GLP-1, glucagon-like peptide-1; HF, high-fat diet; HF-Pb, high-fat diet with probiotic mixture; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; IBS, inflammatory bowel syndrome; LPS, lipopolysaccharide; LTA₄H, leukotriene A₄ hydrolase; LTC₄S, leukotriene C₄ synthase; Pb, probiotic mixture; SREBP-1, sterol regulatory element-binding protein 1; T2D, type 2 diabetes; Treg cells, T regulatory cells.

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Metabolic disorders, including obesity and T2D, are associated with a state of chronic, low-grade inflammation in peripheral tissues and the circulation [8]. Expansion of adipose tissue is accompanied by enhanced secretion of pro-inflammatory adipokines, e.g. IL-6 and CCL-2, and reduced secretion of the anti-inflammatory and insulin-sensitizing adipokine, adiponectin [9]. This results in chronic, low-grade inflammation affecting multiple tissues and organs, including the liver, skeletal muscle and heart [10]. Adipose and peripheral inflammation may favour the development of debilitating behavioural symptoms, including depressive symptoms and cognitive impairment [11], as well as various chronically painful conditions, such as osteoarthritis [12]. Spinal adiponectin mRNA expression was found to be modified in obese rats, suggesting that adiponectin may also promote centrally mediated pathological changes underlying obesity-related exacerbation of pain and inflammation [13].

A logical corollary of these observations is that manipulation of the gut microbiota by qualitative and quantitative changes in the intake of specific food components, such as fatty acids [14], high-protein diets [15], probiotics [16,17] and prebiotics [18,19] could be an effective dietary strategy to manage lifestyle-related metabolic disorders such as obesity and T2D. Besides decreasing abdominal adiposity and total cholesterol, probiotics can normalize low-grade inflammation and favour mucosal integrity [20]. Recent studies have indicated that certain bacterial strains may prevent diet-induced obesity and related disorders. *Lactobacillus rhamnosus* PL60 and *L. plantarum* PL62, bacterial species capable of producing conjugated linoleic acids (CLAs) conducive to body fat reduction, have been shown to exert beneficial effects on diet-induced obesity in mice. Consumption of fermented skimmed milk containing *Lactobacillus gasseri* SBT2055 regulated adipose tissue growth in rats, possibly through inhibition of dietary fat absorption. *Lactobacillus curvatus* HY7601 and *L. plantarum* KY1032 reduced weight gain and fat accumulation in diet-induced obesity in mice by modulating pro-inflammatory genes in adipose tissue and fatty acid oxidation-related genes in the liver [16].

In the study presented here, we investigated whether treatment with a multispecies probiotic mixture comprising five bacterial strains (*Lactobacillus acidophilus*, *L. plantarum*, *L. salivarius* and two strains of *Bifidobacterium lactis*) could attenuate experimental obesity induced in mice by a high-fat diet. Previous studies conducted by our group demonstrated the anti-inflammatory properties of this probiotic mixture [21] and also showed that it can prevent disruption of the gut epithelial barrier and up-regulate the expression of tight-junction proteins [22]. Our objective was to further explore the metabolic effects of the probiotic mixture and the potential mechanisms underlying its anti-inflammatory action.

2. Materials and methods

2.1. Probiotic mixture

The tested probiotic dietary supplement (Lactibiane Tolérance[®], PiLeJe, France) comprises a mixture of five viable lyophilized lactic acid bacterial strains (*B. lactis* LA 303, *B. lactis* LA 304, *L. acidophilus* LA 201, *L. plantarum* LA 301 and *L. salivarius* LA 302) at a total concentration of 1×10^{10} colony-forming units (CFU) per capsule. Fresh suspensions of the lyophilized probiotic mixture (Pb) were prepared daily in sterilized phosphate-buffered saline (PBS) and administered intragastrically to mice at the dose of 1×10^9 CFU/mouse (in 0.2 mL of PBS) on 5 consecutive days a week for 14 weeks.

2.2. Animals and experimental diets

The study was performed according to French government guidelines for animal experiments (protocol number 12/1048/03/

15). Twenty-seven male C57/BL6J mice aged 6 weeks at the beginning of the experiment (Charles River Laboratories, France) were housed in groups of three per cage in a controlled environment (12-h daylight cycle) with free access to food and water. After two weeks of acclimatization, the mice were randomly allocated to receive for 14 weeks one of three different experimental diets ($n = 9/\text{group}$): (1) a control (CT) diet (A04, SAFE, Villemoison-sur-Orge, France) containing (w/w) 16.1% protein, 60% carbohydrate and 3.1% fat (total energy supply: 2.9 kcal/g consumed); (2) a high-fat (HF) diet (Research Diet Inc.) containing 26% protein, 26% carbohydrates and 35% trans-type saturated fats (total energy supply: 5.2 kcal/g consumed); (3) the HF diet plus the probiotic mixture (1×10^9 CFU/mouse administered in 0.2 mL sterilized PBS by oral gavage on 5 consecutive days per week) (HF-Pb diet). The groups allocated to the CT and HF diets received the same volume of sterilized PBS alone by oral gavage on 5 consecutive days a week during 14 weeks.

At the end of the experiment, the mice were killed by cervical dislocation. The small intestine (from duodenum to ileum), colon, and subcutaneous and perigonadal adipose tissues were excised, rinsed with physiological saline solution, weighed and stored at -70°C pending further analysis.

2.3. Body weight and fat mass measurement

Body weights were recorded in all three groups of mice once a week. Body fat percentage was measured at week 13 by nuclear magnetic resonance (NMR) using an EchoNMR[™] 100H apparatus (Echo Medical Systems LLC, New York, USA). Mice were placed in a clear plastic holder for scanning, without sedation. Fat and lean masses were determined on the basis of the differences in relaxation times of the hydrogen proton spins in disparate environments. Fat mass corresponds to the mass of all fat molecules in the body and is expressed as the equivalent weight of canola oil. Lean (muscle tissue) mass corresponds to all water-containing body parts excluding fat, bone minerals, hair, claws, etc. At the end of the study (week 14), perigonadal and subcutaneous adipose tissues were excised and weighed separately.

2.4. Determination of blood glucose, insulin and homeostasis index (HOMA-IR)

Blood samples (5 μL) were taken from the tail, after a 6-h fast, at baseline (before allocation to the experimental diets) and at weeks 6 and 12. Serum glucose and insulin concentrations were determined using a glucometer and by ELISA, respectively. The glucometric assay was chosen for measurement of serum glucose concentrations as it requires small blood volumes, reducing stress on the animals. Insulin resistance was determined as HOMA-IR, calculated according to the equation $\text{HOMA-IR} = \text{fasting serum glucose concentration (mg/mL)} \times \text{fasting serum insulin concentration } (\mu\text{IU/mL})/22.5$.

2.5. Expression of genes encoding pro- or anti-inflammatory agents in adipose (perigonadal or subcutaneous) and gut (small intestine or colon) tissues

Tissues were disrupted using a tissue homogenizer (Precellys[®], Ozyme, France) in the presence of lysis buffer (QIAzol[®], Qiagen, France; 1 mL per 100 mg of tissue). Total mRNA was isolated from adipose tissues and gut using a solid-phase RNA purification kit (GenJET[®], Fermentas, France) and by classical liquid/liquid (phenol/chloroform) extraction, respectively. Reverse transcription was then performed on 1 μg samples of mRNA from each tissue using a Superscript II kit (Invitrogen, France), according to the manufacturer's protocol. Samples were prepared for high-throughput

real-time qPCR on a BioMark 96.96 Dynamic Array (Fluidigm, France) according to the manufacturer's protocol. Real-time qPCR was chosen for this analysis as being the most appropriate means of assessing several genes in a single real-time experiment.

2.6. Statistical evaluation

Data are expressed as means \pm standard deviation (SD). CT, HF and HF-Pb treatment groups were compared using one-way ANOVA for multiple comparisons with Tukey's post hoc test. Repeated measures ANOVA was used to determine the statistical significance of intergroup differences in body weight at successive time points. The 95% confidence interval was calculated and statistical significance was concluded at $p < 0.05$.

3. Results

3.1. Anti-obesity effect of the probiotic mixture

As expected, body weight increased to a greater extent in mice fed the high-fat diet than in those consuming the control diet, the difference between these two groups being significant from 6 weeks onwards. However, the body weight increase induced by the high-fat diet was reduced by concomitant administration of the probiotic mixture, the difference between the HF and HF-Pb groups being statistically significant from week 10 to the end of the study at week 14 (Fig. 1). The mean body weight gains during the total 14 weeks of experimental diet consumption were 6.23 ± 1.67 g, 18.71 ± 1.53 g and 15.20 ± 0.65 g in the CT, HF and HF-Pb groups, respectively, the difference between the HF and HF-Pb groups being statistically significant. The mean total weight gain determined at the end of the study (week 14) was $41.9 \pm 3.1\%$ higher in the HF group and $28.3 \pm 4.4\%$ higher in the HF-Pb group than in the CT group. The HF-Pb group showed a lower proportion of fat mass (30.7 versus 36.1%) and a higher proportion of lean mass (54.4 versus 49.1%), compared to the HF group (Fig. 2, panels A and B), but the differences between the two groups were not significant ($p > 0.05$). Perigonadal and subcutaneous adipose tissue weights were significantly decreased, by 22.1% and 21.2% respectively, in HF-Pb mice compared to HF mice (Fig. 2, panels C and D).

3.2. Serological analyses

Mean serum glucose and insulin concentrations determined at week 12 were significantly higher in the HF and HF-Pb groups than in the CT group, but significantly lower (by 17 and 37%, respectively) in the HF-Pb group compared to the HF group

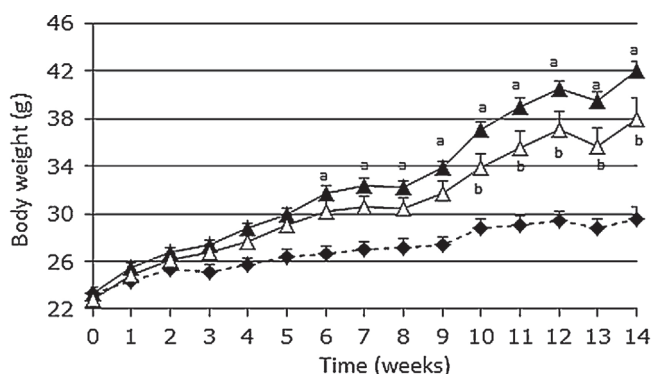


Fig. 1. High-fat diet and probiotic effect on body weight (g). The mice fed the control diet (CT) (\blacklozenge), the high-fat diet (HF) (\blacktriangle), and the high-fat diet plus the probiotic mixture (HF-Pb) (\triangle) were weighed once a week and the results averaged. Data are presented as the mean \pm SD. ^a $p < 0.05$ HF versus CT; ^b $p < 0.05$ HF-Pb versus HF.

(Table 1). Similarly, mean HOMA-IR, a representative index of insulin resistance, was significantly higher in both the HF and HF-Pb groups than in the CT group, but significantly reduced by 47% in the HF-Pb group compared to the HF group (Table 1).

3.3. Expression of genes encoding pro- or anti-inflammatory agents in adipose and gut tissues (high-throughput real-time qPCR analysis)

3.3.1. Effect of the probiotic mixture on adipokine gene expression in adipose tissue

Compared to the CT group, the HF group showed 12.6-fold and 7.2-fold higher expression levels of the gene encoding the pro-inflammatory adipokine CCL-2 in the perigonadal and subcutaneous adipose tissue, respectively (Fig. 3). These elevations in CCL-2 gene expression induced by the high-fat diet were significantly reduced (2.6- and 1.7-fold, respectively) by concomitant intake of the probiotic mixture (Fig. 3). In contrast, CCL-2 gene expression in the small intestine and colon did not differ significantly between the HF and HF-Pb groups. The elevation of IL-6 gene expression induced by the HF diet in the perigonadal adipose tissue was significantly (1.6-fold) reduced by concomitant intake of the probiotic mixture. Expression of the gene encoding the anti-inflammatory adipokine, adiponectin, in this tissue was significantly (1.8-fold) increased in the HF-Pb group compared to the HF group, approaching the level observed in the control group (Fig. 4). In the perigonadal adipose tissue, no significant differences were observed between the CT and HF-Pb groups with regard to CCL-2, IL-6 and adiponectin gene expression.

3.3.2. Effect of the probiotic mixture on the expression of genes encoding enzymes of the leukotriene pathway

The mRNA expression of leukotriene A_4 hydrolase (LTA₄H), implicated in the production of LTB₄, and that of leukotriene C₄ synthase (LTC₄S), an enzyme involved in the synthesis of the cysteinyl leukotrienes C, D and E, were also evaluated (Fig. 5, panels A and B respectively). Statistically significant inhibition of LTA₄H gene expression in adipose and colon tissues was observed in the HF-Pb group compared to the HF group. LTC₄S gene expression was also significantly inhibited in the HF-Pb group compared to the HF group with regard to gut (small intestine and colon) tissues. The level of LTC₄S gene expression in the perigonadal and subcutaneous adipose tissues did not differ significantly between the HF and HF-Pb groups. Administration of the probiotic mixture in conjunction with the high-fat diet also led to significantly (1.5-fold) up-regulated expression of the gene encoding the LXA₄ receptor Fpr2 in the colon, compared to that observed in mice receiving the high-fat diet alone (Table 2).

3.3.3. Effect of the probiotic mixture on T regulatory gene expression

The mRNA expression of three T regulatory genes encoding agents modulating inflammation, namely Foxp3, CD25 and IL-10 in colon tissue was up-regulated in the group consuming the high-fat diet compared to their expression in the control group. Concomitant administration of the probiotic mixture significantly increased the expression of these T regulatory genes in colon tissue by 1.5–2.3-fold compared to the HF group (Table 2). A trend towards enhanced (1.6-fold) expression of CD25 in perigonadal adipose tissue and small intestine tissue was observed in the HF-Pb group compared to the HF group.

4. Discussion

The role of the gut microbiota in the pathogenesis of obesity-related disorders is increasingly recognized and may involve the provision of additional energy by the conversion of dietary fibres to

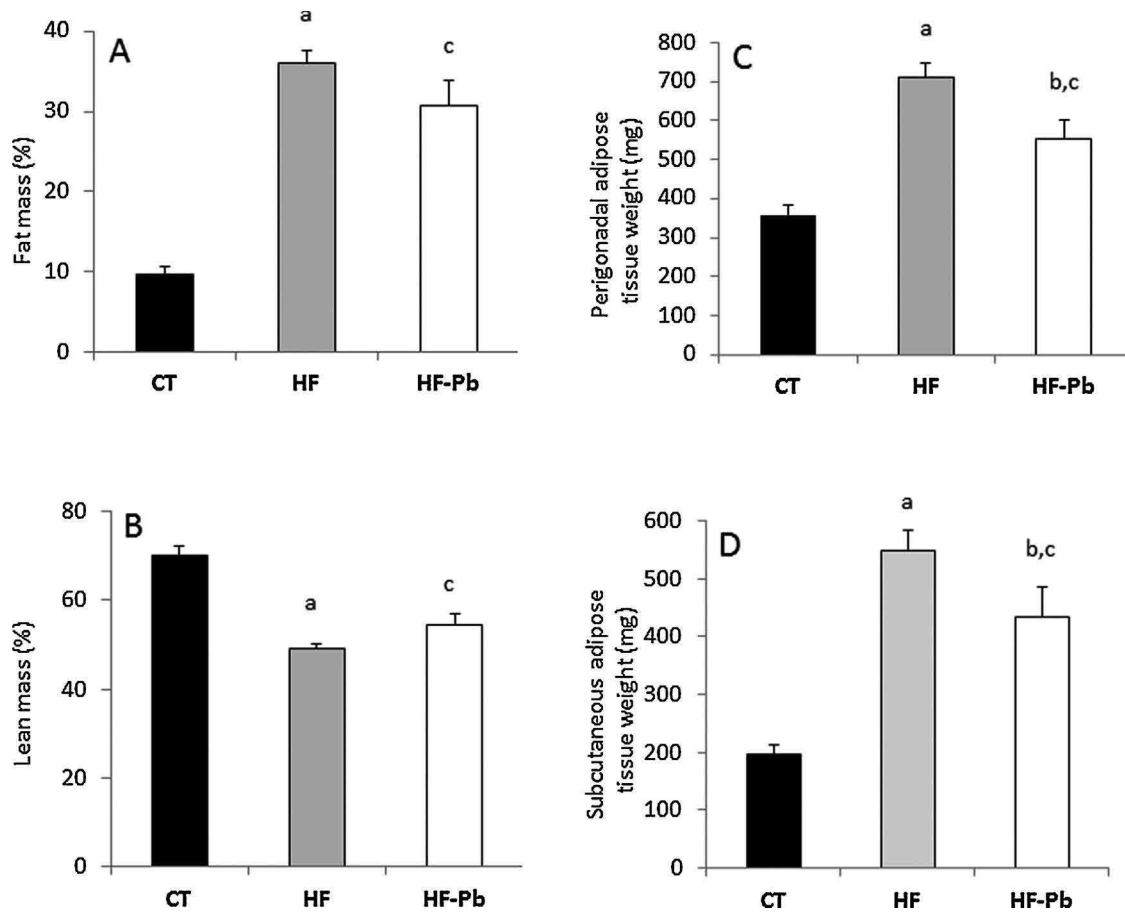


Fig. 2. High-fat diet and probiotic effects on fat mass (A), lean mass (B), perigonadal adipose tissue (C) and subcutaneous adipose tissue (D) weights. ^a $p < 0.05$ HF versus CT; ^b $p < 0.05$ HF-Pb versus HF; ^c $p < 0.05$ HF-Pb versus CT.

short-chain fatty acids, effects on gut-hormone production, and increased intestinal permeability leading to elevated systemic lipopolysaccharide (LPS) levels [23]. The ability of probiotics and prebiotics to reduce intestinal permeability has been tested in various animal models of metabolic disorders [24]. The aim of our study was to investigate the effects of concomitant administration of a probiotic mixture to mice fed a high-fat diet on body weight, glucose metabolism and the expression of genes encoding agents modulating inflammatory processes, as obesity is known to be associated with a state of chronic low-grade inflammation [8]. Previous studies have demonstrated the anti-inflammatory properties of the tested probiotic mixture [21] as well as its capacity to restore epithelial barrier disruptions induced by LPS, stress or soluble factors associated with irritable bowel syndrome (IBS) and to down-regulate the response mediated by the inflammatory cytokine TLR-4 in vitro and in vivo [22]. In this study, administration of this probiotic mixture for 14 weeks to mice fed a high-fat diet diminished the increase in body weight

Table 1
Serum glucose and insulin concentrations and HOMA-IR determined 12 weeks after the start of experimental diet consumption.

	Control diet (CT) (n=9)	High-fat diet (HF) (n=9)	High-fat diet + probiotic mix (HF-Pb) (n=9)
Glucose (mg/dl)	124 ± 7	176 ± 6 ^a	146 ± 8 ^b
Insulin (pmol/mL)	484 ± 15	1315 ± 167 ^a	835 ± 94 ^b
HOMA-IR	1.209 ± 0.076	4.705 ± 0.747 ^a	2.483 ± 0.359 ^b

Data are presented as the mean ± SD.

^a $p < 0.05$ versus the CT group.

^b $p < 0.05$ versus the HF group.

gain and body fat mass observed in mice receiving the same high-fat diet alone. It also protected mice from the insulin resistance induced by the high-fat diet as shown by the HOMA-IR values determined in the HF and HF-Pb groups.

The chemokine CCL-2, produced by adipocytes, has a pivotal role in the recruitment of macrophages into the peripheral adipose tissue and contributes to the insulin resistance and hepatic

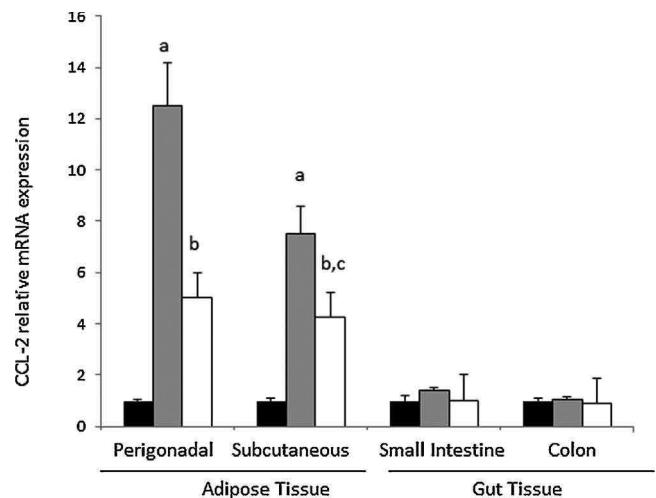


Fig. 3. Relative expression of CCL-2 mRNA in adipose and gut tissues mice fed a control diet (■), a high-fat diet alone (■) or a high-fat diet plus the probiotic mixture (□). ^a $p < 0.05$ HF versus CT; ^b $p < 0.05$ HF-Pb versus HF; ^c $p < 0.05$ HF-Pb versus CT.

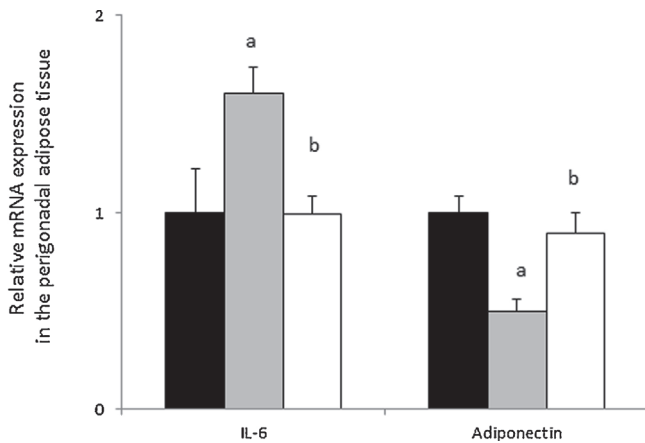


Fig. 4. Relative expression of IL-6 and adiponectin mRNA in perigonadal adipose tissue of mice fed a control diet (■), a high-fat diet alone (■) or a high-fat diet plus the probiotic mixture (□). ^a $p < 0.05$ HF versus CT; ^b $p < 0.05$ HF-Pb versus HF.

steatosis associated with obesity. Previous studies have shown enhanced expression of the CCL-2 gene in genetically obese diabetic (db/db) mice and in wild type mice with obesity induced by a high-fat diet [25], in obese mice compared to normal mice [26] and in adipose tissues of obese patients compared to lean controls [27]. In our study, the increase in CCL-2 mRNA expression in perigonadal and subcutaneous adipose tissue observed in mice consuming the high-fat diet compared to the control diet was significantly reduced by intake of the probiotic mixture. Concomitant administration of the probiotic mixture also significantly

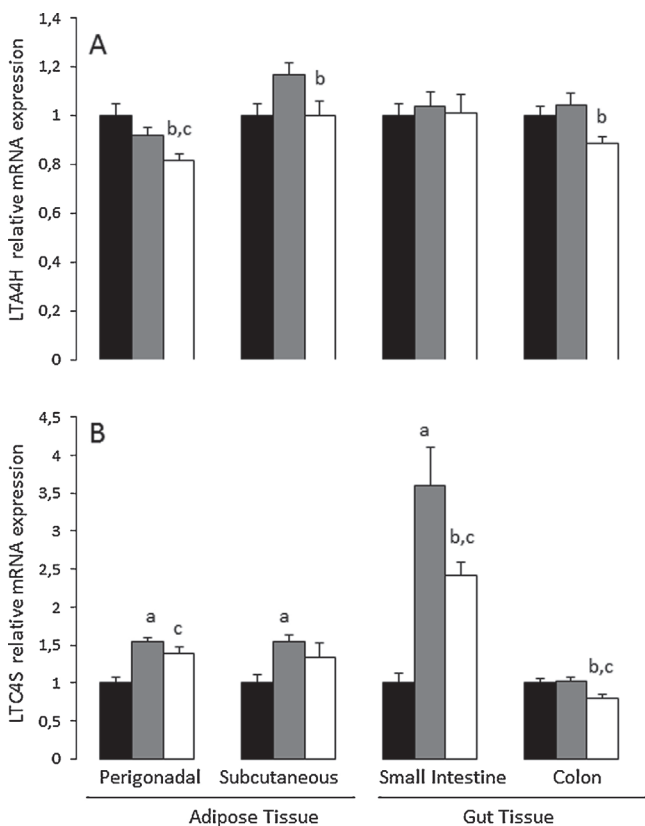


Fig. 5. Relative expression of LTA₄H (A) and LTC₄S (B) mRNA in adipose and gut tissues of male C57/BL6J mice fed a control diet (■), a high-fat diet alone (■) or a high-fat diet plus the probiotic mixture (□). ^a $p < 0.05$ HF versus CT; ^b $p < 0.05$ HF-Pb versus HF; ^c $p < 0.05$ HF-Pb versus CT.

Table 2

Relative LXA₄ receptor (Fpr2) and T regulatory gene mRNA expression in colon tissue.

	Control diet (CT) (n=9)	High-fat diet (HF) (n=9)	High-fat diet + probiotic mixture (HF-Pb) (n=9)
Fpr2	1.00 ± 0.09	2.01 ± 0.27 ^a	2.98 ± 0.29 ^b
Foxp3	1.00 ± 0.19	2.88 ± 0.36 ^a	4.46 ± 0.60 ^b
CD25	1.00 ± 0.05	1.86 ± 0.20 ^a	2.97 ± 0.28 ^b
IL-10	1.00 ± 0.12	1.16 ± 0.12	2.64 ± 0.44 ^{b,c}

Data are presented as the mean ± SD.

^a $p < 0.05$ versus the CT group.

^b $p < 0.05$ versus the HF group.

^c $p < 0.05$ versus the CT group.

inhibited the up-regulation of IL-6 and down-regulation of adiponectin in perigonadal adipose tissue observed in mice fed a high-fat diet.

Inflammation of the intestine, which is closely connected to the visceral adipose tissue via blood vessels has also been observed in the early phase of developing obesity [28]. In this study, the expression of genes implicated in inflammatory processes in the gut was explored using microfluidic tools. Real-time PCR analysis revealed a significant impact of the tested probiotic mixture on the expression of genes encoding enzymes of the leukotriene pathway and Treg genes in colon tissue from mice fed a high-fat diet, demonstrating its effect on inflammatory processes. The leukotriene precursor arachidonic acid 5-hydroperoxide (5-hydroperoxyeicosatetraenoic acid; 5-HPETE) may be converted into either LTB₄, via the activation of LTA₄H, or into LXA₄, via inhibition of this enzyme, LTC₄, LTD₄ and/or LTE₄ being produced via activation of LTC₄S. Our study showed a decrease in LTA₄H and LTC₄S mRNA expression in mice receiving the probiotic mixture in addition to the high-fat diet. This suggests that inhibition of both enzymes might lead to enhanced LXA₄ production in gut tissue. The hypothesis that probiotics affect the expression of genes involved in immunosuppression and resolution of inflammation is supported by the increase in expression of Fpr2 (LXA₄ receptor) mRNA observed in mice receiving the tested probiotic mixture in addition to the high-fat diet.

Chen et al. recently investigated the effects of LTB₄ on the differentiation of immunosuppressive CD4⁺CD25⁺Foxp3⁺Treg cells in vitro and found that LTB₄ dose-dependently decreased the percentage of Treg cells and the mRNA expression of Foxp3 [29]. Foxp3⁺Treg cells, defined by expression of the forkhead family transcription factor p3 (Foxp3) and high levels of the IL-2 receptor- α chain (CD25), constitute a subset of CD4⁺Treg cells with the function of suppressing immune responses and maintaining self-tolerance [30]. The proportion of circulating CD25⁺CD127⁻Foxp3⁺Treg cells in the total CD4⁺ cell population is inversely correlated with indices of adiposity such as body weight, body mass index and circulating leptin levels, particularly in obese subjects. Moreover, significantly fewer Treg cells are seen in individuals exhibiting elevated markers of systemic inflammation (hsCRP) or impaired glucose tolerance (HbA1c) [31]. A link between the leukotriene pathway enzymes and Treg cells was observed by Börgeson et al. [32] who showed that LXA₄ increased expression of the anti-inflammatory cytokine IL-10 in adipose tissue explants from perigonadal fat depots of mice. In our study, we demonstrated that probiotic supplementation not only modified the expression of genes encoding enzymes implicated in the leukotriene pathway in mice fed a high-fat diet but also significantly increased Foxp3, CD25, and IL-10 mRNA expression in colon tissue.

In conclusion, probiotic supplementation significantly attenuated body weight gain and protected mice from glucose intolerance and insulin resistance induced by a high-fat diet. Gene expression analysis indicated that these effects are at least in part

due to the anti-inflammatory actions of this probiotic mixture. In adipose tissue, probiotic treatment reduced expression of the gene encoding CCL-2, an important chemokine for macrophage infiltration of adipose tissue. In colon tissue, this treatment increased the expression of genes involved in the immunosuppression and resolution of inflammation. However, full-scale clinical trials would be required to assess the value of the tested probiotic with regard to the development of obesity and associated inflammatory processes in humans. Further studies would also be needed to elucidate the exact mechanisms underlying the effects of the tested probiotic mixture with regard to its anti-inflammatory impact on the gut immune system, its modification of gut microbiota, its ability to reduce overall energy intake and its modulation of gut hormone expression. The gut microbiota may participate in the regulation of energy metabolism, i.e. the energy harvested from the diet, as well as the regulation of fat storage (expression of FIAF), fat lipogenesis (acetyl-CoA carboxylase, fatty acid synthase, SREBP-1), and fatty acid oxidation (AMPK activity) [33]. Modulation of gut peptides involved in appetite regulation, such as GLP-1 and PYY, could be another mechanism by which the gut microbiota might control energy and glucose homeostasis. Data obtained in experimental models and in clinical studies have already shown that changing the gut microbiota by means of prebiotics (such as fructans) [18,34] or probiotics (such as *Lactobacillus casei* W8) [35] may contribute to regulating gut peptide synthesis and controlling food intake. So it might be interesting to complete this study by investigating whether the beneficial effect of our probiotic mixture on body weight gain could be explained by reduced food intake and/or increased energy expenditure, and to investigate more precisely the underlying mechanisms of action.

Layperson's summary

The nature of the microorganism population in the gut was recently identified as a potential contributor to the increased prevalence of obesity and type 2 diabetes. In this study, we investigated the effects of a probiotic mixture in mice fed a high-fat diet conducive to the development of obesity. The weight gain and increases in blood glucose level and insulin resistance induced by this diet were significantly reduced by probiotic supplementation. Improvement in the inflammatory state of the gut and adipose tissue might be a possible mechanism underlying the anti-obesity effect of the probiotic mix studied.

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References

[1] T. Temelkova-Kurktschiev, T. Stefanov, Lifestyle and genetics in obesity and type 2 diabetes, *Exp. Clin. Endocrinol. Diabetes* 120 (1) (2012) 1–6.
 [2] S. Devaraj, P. Hemarajata, J. Versalovic, The human gut microbiome and body metabolism: implications for obesity and diabetes, *Clin. Chem.* 59 (4) (2013) 617–628.

[3] E. Le Chatelier, T. Nielsen, J. Qin, E. Prifti, F. Hildebrand, G. Falony, et al., Richness of human gut microbiome correlates with metabolic markers, *Nature* 500 (7464) (2013) 541–546.
 [4] A. Tagliabue, M. Elli, The role of gut microbiota in human obesity: recent findings and future perspectives, *Nutr. Metab. Cardiovasc. Dis.* 23 (3) (2013) 160–168.
 [5] N. Larsen, F.K. Vogensen, F.W. van den Berg, D.S. Nielsen, A.S. Andreasen, B.K. Pedersen, et al., Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults, *PLoS ONE* 5 (2) (2010) e9085.
 [6] J. Qin, Y. Li, Z. Cai, S. Li, J. Zhu, F. Zhang, et al., A metagenome-wide association study of gut microbiota in type 2 diabetes, *Nature* 490 (7418) (2012) 55–60.
 [7] G. Musso, R. Gambino, M. Cassader, Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes, *Annu. Rev. Med.* 62 (2011) 361–380.
 [8] M.F. Gregor, G.S. Hotamisligil, Inflammatory mechanisms in obesity, *Annu. Rev. Immunol.* 29 (2011) 415–445.
 [9] A.A. Bremer, I. Jialal, Adipose tissue dysfunction in nascent metabolic syndrome, *J. Obes.* 2013 (2013) 393192.
 [10] U.J. Jung, M.S. Choi, Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease, *Int. J. Mol. Sci.* 15 (4) (2014) 6184–6223.
 [11] J. Lasselin, L. Capuron, Chronic low-grade inflammation in metabolic disorders: relevance for behavioral symptoms, *Neuroimmunomodulation* 21 (2–3) (2014) 95–101.
 [12] L.I. Arranz, M. Rafecas, C. Alegre, Effects of obesity on function and quality of life in chronic pain conditions, *Curr. Rheumatol. Rep.* 16 (1) (2014) 390.
 [13] T. Iannitti, A. Graham, S. Dolan, Increased central and peripheral inflammation and inflammatory hyperalgesia in Zucker rat model of leptin receptor deficiency and genetic obesity, *Exp. Physiol.* 97 (11) (2012) 1236–1245.
 [14] W. Shen, H.R. Gaskins, M.K. McIntosh, Influence of dietary fat on intestinal microbes, inflammation, barrier function and metabolic outcomes, *J. Nutr. Biochem.* 25 (3) (2014) 270–280.
 [15] T. Iannitti, B. Palmieri, The obese patient: clinical effectiveness of a high-protein low-calorie diet and its usefulness in the field of surgery, *Minerva Gastroenterol. Dietol.* 56 (2 Suppl. 1) (2010) 1–65.
 [16] T. Arora, S. Singh, R.K. Sharma, Probiotics: interaction with gut microbiome and antiobesity potential, *Nutrition* 29 (4) (2013) 591–596.
 [17] C.L. Karlsson, G. Molin, F. Fåk, M.L. Johansson Hagslatt, M. Jaksevic, A. Hakansson, et al., Effects on weight gain and gut microbiota in rats given bacterial supplements and a high-energy-dense diet from fetal life through to 6 months of age, *Br. J. Nutr.* 106 (6) (2011) 887–895.
 [18] N.M. Delzenne, P.D. Cani, A.M. Neyrinck, Modulation of glucagon-like peptide 1 and energy metabolism by insulin and oligofructose: experimental data, *J. Nutr.* 137 (11 Suppl.) (2007) 2547S–2551S.
 [19] N.M. Delzenne, A.M. Neyrinck, P.D. Cani, Modulation of the gut microbiota by nutrients with prebiotic properties: consequences for host health in the context of obesity and metabolic syndrome, *Microb. Cell Fact.* 10 (Suppl. 1) (2011) S10.
 [20] R.H. Mallappa, N. Rokana, R.K. Duary, H. Panwar, V.K. Batish, S. Grover, Management of metabolic syndrome through probiotic and prebiotic interventions, *Indian J. Endocrinol. Metab.* 16 (2012) 20–27.
 [21] S. Drouault-Holowacz, B. Foligne, V. Dennin, D. Goudercourt, K. Terpend, A. Burckel, B. Pot, Anti-inflammatory potential of the probiotic dietary supplement Lactibiane Tolerance: in vitro and in vivo considerations, *Clin. Nutr.* 25 (6) (2006) 994–1003.
 [22] M. Nèbot-Vivinus, C. Harkat, H. Bziouche, C. Cartier, R. Plichon-Dainese, L. Moussa, et al., A multispecies probiotic protects gut barrier function in experimental models, *World J. Gastroenterol.* 20 (2014) 6832–6843.
 [23] M. Blaut, S. Klaus, Intestinal microbiota and obesity, in: H.-G. Joost (Ed.), *Appetite Control. Handbook of Experimental Pharmacology*, vol. 209, Springer, Heidelberg, 2012, pp. 251–273.
 [24] S. Tavares da Silva, C. Araújo dos Santos, J. Bressan, Intestinal microbiota; relevance to obesity and modulation by prebiotics and probiotics, *Nutr. Hosp.* 28 (2013) 1039–1048.
 [25] H. Kanda, S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, et al., MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity, *J. Clin. Invest.* 116 (2006) 1494–1505.
 [26] H. Takahashi, Y. Tsuda, M. Kobayashi, D.N. Herndon, F. Suzuki, CCL2 as a trigger of manifestations of compensatory anti-inflammatory response syndrome in mice with severe systemic inflammatory response syndrome, *J. Leukoc. Biol.* 79 (4) (2006) 789–796.
 [27] J. Panee, Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes, *Cytokine* 60 (1) (2012) 1–12.
 [28] C. Bleau, A.D. Karelis, D.H. St-Pierre, L. Lamontagne, Crosstalk between intestinal microbiota, adipose tissue and skeletal muscle as an early event in systemic low-grade inflammation and the development of obesity and diabetes, *Diabetes Metab. Res. Rev.* (2014) (in press).
 [29] H. Chen, J. Qin, P. Wei, J. Zhang, Q. Li, L. Fu, S. Li, C. Ma, B. Cong, Effects of leukotriene B4 and prostaglandin E2 on the differentiation of murine Foxp3+ T regulatory cells and Th17 cells, *Prostaglandins Leukot. Essent. Fatty Acids* 80 (4) (2009) 195–200.
 [30] R.A. Peterson, Regulatory T-cells: diverse phenotypes integral to immune homeostasis and suppression, *Toxicol. Pathol.* 40 (2) (2012) 186–204.
 [31] N.M. Wagner, G. Brandhorst, F. Czepluch, M. Lankeit, S. Eberle, S. Herzberg, V. Faustin, J. Riggert, M. Oellerich, G. Hasenfuss, S. Konstantinides, K. Schafer, Circulating regulatory T cells are reduced in obesity and may identify subjects

- at increased metabolic and cardiovascular risk, *Obesity* (Silver Spring) 21 (3) (2013) 461–468.
- [32] E. Börgeson, F.C. McGillicuddy, K.A. Harford, N. Corrigan, D.F. Higgins, P. Maderna, H.M. Roche, C. Godson, Lipoxin A4 attenuates adipose inflammation, *FASEB J.* 26 (10) (2012) 4287–4294.
- [33] P.D. Cani, N.M. Delzenne, The role of the gut microbiota in energy metabolism and metabolic disease, *Curr. Pharm. Des.* 15 (13) (2009) 1546–1558.
- [34] J.A. Parnell, R.A. Reimer, Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR:LA-cp rats, *Br. J. Nutr.* 107 (4) (2012) 601–613.
- [35] A.T. Bjerg, M. Kristensen, C. Ritz, J.J. Holst, C. Rasmussen, T.D. Leser, A. Wellejus, A. Astrup, *Lactobacillus paracasei* subsp. *paracasei* L. *casei* W8 suppresses energy intake acutely, *Appetite* 82 (2014) 111–118.