

# Potential probiotic *Bifidobacterium animalis* ssp. *lactis* 420 prevents weight gain and glucose intolerance in diet-induced obese mice

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Received: 3 February 2014 / Accepted: 19 June 2014

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## RESEARCH ARTICLE

### Abstract

Alterations of the gut microbiota and mucosal barrier are linked with metabolic diseases. Our aim was to investigate the potential benefit of the potential probiotic *Bifidobacterium animalis* ssp. *lactis* 420 in reducing high-fat diet-induced body weight gain and diabetes in mice. In the obesity model, C57Bl/6J mice were fed a high-fat diet (60 energy %) for 12 weeks, and gavaged daily with *B. lactis* 420 ( $10^9$  cfu) or vehicle. In the diabetes model, mice were fed a high-fat, ketogenic diet (72 energy % fat) for 4 weeks, with a 6-week subsequent treatment with *B. lactis* 420 ( $10^8$ - $10^{10}$  cfu/day) or vehicle, after which they were analysed for body composition. We also analysed glucose tolerance, plasma lipopolysaccharide and target tissue inflammation using only one of the *B. lactis* 420 groups ( $10^9$  cfu/day). Intestinal bacterial translocation and adhesion were analysed in a separate experiment using an *Escherichia coli* gavage. Body fat mass was increased in both obese ( $10.7 \pm 0.8$  g (mean  $\pm$  standard error of mean) vs.  $1.86 \pm 0.21$  g,  $P < 0.001$ ) and diabetic mice ( $3.01 \pm 0.4$  g vs.  $1.14 \pm 0.15$  g,  $P < 0.001$ ) compared to healthy controls. Treatment with *B. lactis* 420 significantly decreased fat mass in obese ( $7.83 \pm 0.67$  g,  $P = 0.007$  compared to obese with vehicle) and diabetic mice ( $1.89 \pm 0.16$  g,  $P = 0.02$  for highest dose). This was reflected as reduced weight gain and improved glucose tolerance. Furthermore, *B. lactis* 420 decreased plasma lipopolysaccharide levels ( $P < 0.001$ ), liver inflammation ( $P = 0.04$ ), and *E. coli* adhesion in the distal gut ( $P < 0.05$ ). In conclusion, *B. lactis* 420 reduces fat mass and glucose intolerance in both obese and diabetic mice. Reduced intestinal mucosal adherence and plasma lipopolysaccharide suggest a mechanism related to reduced translocation of gut microbes.

**Keywords:** diabetes, obesity, intestinal permeability, mice, probiotics

### 1. Introduction

A dramatic rise in the incidence of obesity over the last decades has given rise to an array of obesity-associated metabolic disturbances. Many of these are suspected to stem from chronic low-grade inflammation, which has become a well-recognised risk factor for metabolic disease (Hotamisligil, 2006). The causes of low-grade inflammation have long been unknown, but recent advances have revealed a mechanism related to the leakage of inflammatory molecules from bacterial origin, such as lipopolysaccharides (LPS) (Cani *et al.*, 2007a) or peptidoglycan (Amar *et al.*, 2011; Schertzer *et al.*, 2011) from the gut. The rise in the basal concentration of blood LPS is known as metabolic

endotoxaemia. However, a constant mildly elevated plasma concentration of LPS is proposed to cause low-grade inflammation and predispose to metabolic disease as causally demonstrated in the triggering of metabolic inflammation in mice (Cani *et al.*, 2007a) and associated to the disease in human (Pussinen *et al.*, 2011).

Metabolic endotoxaemia can be induced in mice using a high-fat diet (HFD) (Cani *et al.*, 2007b, 2008; Carvalho *et al.*, 2012; De la Serre *et al.*, 2010; Everard *et al.*, 2012; Kim *et al.*, 2012; Serino *et al.*, 2012), but the mechanisms for increased LPS absorption and translocation towards tissues associated with HFD are unknown. Proposed hypotheses include chylomicron-facilitated LPS-transport

(Ghoshal *et al.*, 2009), decreased mucosal clearance of LPS by alkaline phosphatase (De la Serre *et al.*, 2010), and gut barrier disruption by various mechanisms related to the gut microbiota, such as an overly active gut endocannabinoid system (Muccioli *et al.*, 2010), or altered luminal bile acid profile (Stenman *et al.*, 2012, 2013). Recent findings from our laboratory demonstrated the key role of metabolic endotoxaemia in increasing preadipocyte and macrophage proliferation in adipose tissue (Luche *et al.*, 2013).

Circulating LPS has been causally linked to the development of mouse obesity (Cani *et al.*, 2007a). As germ-free mice are resistant to obesity caused by a HFD (Backhed *et al.*, 2007; Rabot *et al.*, 2010), the gut microbiota and gut-derived endotoxins have been proposed to participate in the development of diet-induced obesity in mice. The mouse knock-out for CD14, which is required for the recognition of LPS by toll-like receptor 4, is resistant to obesity caused by subcutaneously administered LPS (Cani *et al.*, 2007a). The LPS-TLR4 pathway may thus play a role in the development of obesity in HFD-fed mice. Hence, we recently proposed that the translocation of bacteria from the intestinal mucosa towards tissues such as the adipose depot was responsible for the triggering of metabolic inflammation (Amar *et al.*, 2011). The translocated bacteria and bacterial fragments stimulated the proliferation of adipose tissue precursors and favoured body weight gain through a process associated with LPS-induced inflammation of adipose tissue macrophages (Luche *et al.*, 2013). Hence, the former and latter arguments strongly suggest that treating mucosal microbial ecology would ameliorate energy metabolism and body weight management.

It is an on-going struggle to find effective treatment strategies for obesity and metabolic syndrome. Among newly emerging approaches, probiotics have shown a potential efficacy in improving glucose tolerance (Andreasen *et al.*, 2010; Asemi *et al.*, 2013) and weight gain (Kadooka *et al.*, 2010, 2013) in humans. As we have shown in a mouse model of HFD-induced diabetes, *Bifidobacterium lactis* 420 (B420) may improve glucose tolerance and reduce tissue inflammatory status (Amar *et al.*, 2011), suggesting a benefit in metabolic disease. Our aim was to study whether B420 may treat or prevent adiposity by reducing metabolic endotoxaemia and gut bacterial translocation in mice. These were investigated in diet-induced obese and diabetic animal models.

## 2. Materials and methods

### Animals

Male C57Bl/6J mice were purchased from Charles River (Sulzfeld, Germany), and housed in a standard animal facility with food and water *ad libitum*. At eight weeks of age, mice were allocated to one of the two experimental designs. Obesity and weight-related outcomes were studied in both

models, while mechanisms were explored in the diabetic model only. The experimental procedures were approved by the local ethics committee of the Rangueil Hospital.

### Obesity model

Mice were fed a HFD containing 60 energy % fat or a low-fat diet with 12 energy % fat for 12 weeks (Research Diets, New Brunswick, NJ, USA). For the entire duration of the experiment, mice were gavaged daily with the probiotic B420 (ATCC: SD6685) ( $10^9$  cfu/day) or water (controls).

### Diabetes model

Diabetes was induced with a ketogenic diet (KD) containing 72 energy % fat (maize oil and lard), 28 energy % protein and <1 energy % carbohydrate (Safe, Augy, France), which was given for four weeks. This diet has been previously shown to cause fasting hyperglycaemia, glucose intolerance and insulin resistance after one month of feeding (Burcelin *et al.*, 2002). This diet impairs glucose induced insulin secretion. The mice are considered hypoinsulinemic, which strongly reduces HFD-induced obesity. The animals of this model can be considered to have 'lean diabetes'. They can thus be compared with the obesity model to study the effects of probiotic treatment on glucose metabolism without the impact of body weight gain. Control mice were fed standard chow. After the four-week consumption of the experimental diets, mice were gavaged with either B420 ( $10^8$ ,  $10^9$  or  $10^{10}$  cfu/day) or water (control), for six more weeks. Only body composition analyses were made for all doses. Only the dose  $10^9$  cfu/day was used for further experimentation.

### Body composition measurements by EchoMRI

The body composition of the mice, including the fat and lean masses, was analysed by NMR using EchoMRI-100TM equipment (Echo Medical Systems, Houston, TX, USA) after six weeks (Obesity model) or four weeks (Diabetes model) of treatment with or without probiotics.

### Intraperitoneal glucose tolerance tests

An intraperitoneal glucose-tolerance test was performed after six (Obesity model) or four (Diabetes model) weeks of treatment with B420 to obtain an index of glucose management in mice. Briefly, 6-h fasted mice were injected with glucose (1 g/kg) into the peritoneal cavity. The glycaemia was followed 30 min before the glucose challenge and then every 30 min using a glucose meter (Roche Diagnostics, Basel, Switzerland). An index for glucose-induced glycaemia was calculated as  $\mu\text{M}/\text{min}$  by dividing the mean blood glucose at 30-90 by 60 min.

### Analysis of metabolic endotoxaemia

Plasma LPS levels were measured with a kit based on a Limulus ameocyte extract (LAL kit; Cambrex BioScience, Walkersville, MD, USA); samples were diluted 1:50 and heated for 10 min at 70 °C.

### Quantification of tissue inflammation markers

RNA was extracted from subcutaneous adipose tissue, liver and skeletal muscle (*vastus lateralis*), reverse transcribed, and analysed with qPCR targeting tumour necrosis factor alpha (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , IL-6 and plasminogen activator inhibitor-1 (PAI-1), with ribosomal protein PL19 (RPL19) as endogenous control for relative quantification. The primers used were: TNF- $\alpha$  forward CATCTTCTCAAAATTCGAGTGACAA, reverse TGGGAGTAGACAAGGTACAACCC; IL-1 $\beta$  forward TCGCTCAGGGTCACAAGAAA, reverse CATCAGAGGCAAGGAGGAAAAC; PAI-1 forward ACAGCCTTTGTCATCTCAGCC, reverse CCGAACCACAAAGAGAAAGGA; IL-6 forward TAGTCCTTCCTACCCCAATTTCC, reverse TTGGTCCTTAGCCACTCCTTC; RPL19 forward GAAGGTCAAAGGGAATGTGTTCA, reverse CCTTGCTGCCTTCAGCTTGT. An index was calculated as the mean expression of all inflammation markers for each tissue separately.

### Quantification of the translocation and mucosal adherence of *Escherichia coli*

A separate experiment with four mice per group was performed for translocation and adherence experiments. Mice were fed with KD and gavaged with B420 ( $10^9$  cfu/day) or vehicle. After five weeks of treatment, mice were gavaged with  $10^9$  cfu *Escherichia coli* (isolated from mouse colon), and sacrificed 2 h later. Liver, spleen, subcutaneous and mesenteric adipose tissue, and the corresponding ganglions were harvested, and luminal and mucosal contents of each intestinal segment were separated. Tissues were homogenised in Luria Broth (Gibco; Life Technologies, Darmstadt, Germany), plated onto ampicillin-supplemented (100  $\mu$ g/ml) Luria Broth agar, and yellow colonies were enumerated after overnight incubation at 37 °C.

### Statistical analysis

Data were analysed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) and SPSS Statistics 21 (IBM, Armonk, NY, USA). All data were analysed with ANOVA. If the global *P* was significant, Bonferroni's multiple comparisons test was used to assess differences between groups. For adherence and translocation, zero values were replaced with half of the smallest possible value, and a logarithm transformation was run to obtain

Normal distribution before statistical testing. All data are expressed as mean  $\pm$  standard error of mean, and significances are two-sided. Differences were considered statistically significant when *P* < 0.05.

## 3. Results

### Influence of *Bifidobacterium lactis* 420 on weight and body fat mass

The effect of B420 on adiposity was studied in two mouse models. In the obese prevention model, treatment with the potential probiotic is initiated together with the HFD (60 energy % fat). In this model, B420 significantly reduced weight gain compared to the high-fat control (Figure 1A). An analysis of body composition by EchoMRI revealed that the increase in body fat mass in diet-induced obese mice was markedly prevented by B420 during six weeks of feeding (*P* = 0.007), whereas there was no effect on lean mass (*P* = 1.00) (Figure 1B). At baseline, there had been no differences in body fat mass (Control  $1.11 \pm 0.05$  g, HFD  $1.23 \pm 0.14$  g, HFD+B420  $1.29 \pm 0.19$  g) or lean mass (Control  $19.09 \pm 0.34$  g, HFD  $20.44 \pm 0.24$  g, HFD+B420  $20.21 \pm 0.28$  g). In the diabetic treatment-model, mice become diabetic following a four-week induction phase eating a KD (72 energy % fat). In this model, the KD group had significantly more fat mass (*P* < 0.001) (Supplemental Figure S1B), despite the lack of body weight change (Supplemental Figure S1A). Fat mass expansion was ameliorated by treatment with B420 at  $10^{10}$  cfu/day (*P* = 0.020), and there was a marked trend of fat mass reduction by  $10^9$  cfu/day (*P* = 0.066). Lean mass was significantly reduced in all mice on a KD (*P* < 0.01 for all vs. control), and was not affected by B420.

### Glucose tolerance in *Bifidobacterium lactis* 420-treated mice

We performed intraperitoneal glucose tolerance tests to HFD mice after six weeks of fatty diet with or without B420, and to KD mice after four weeks of treatment. In diet-induced obese mice, the B420-treated group demonstrated a significantly lower glycaemia compared to fat-fed mice (Figure 2A). Respectively, glucose-stimulated glycaemia was reduced by B420 treatment (Figure 2B). Interestingly, also the fasting blood glucose in B420-treated mice ( $6.9 \pm 0.4$  mM) was significantly lower compared to HFD mice ( $8.2 \pm 0.4$  mM, *P* < 0.05), and comparable to that of control mice ( $6.7 \pm 0.3$  mM). In the diabetic model with KD, the glucose-stimulated glycaemia in B420-treated mice was similar to that of control mice (*P* = 1.00), although it did not significantly differ from that of KD mice without B420 (*P* = 0.16) (Figure 2C-D).

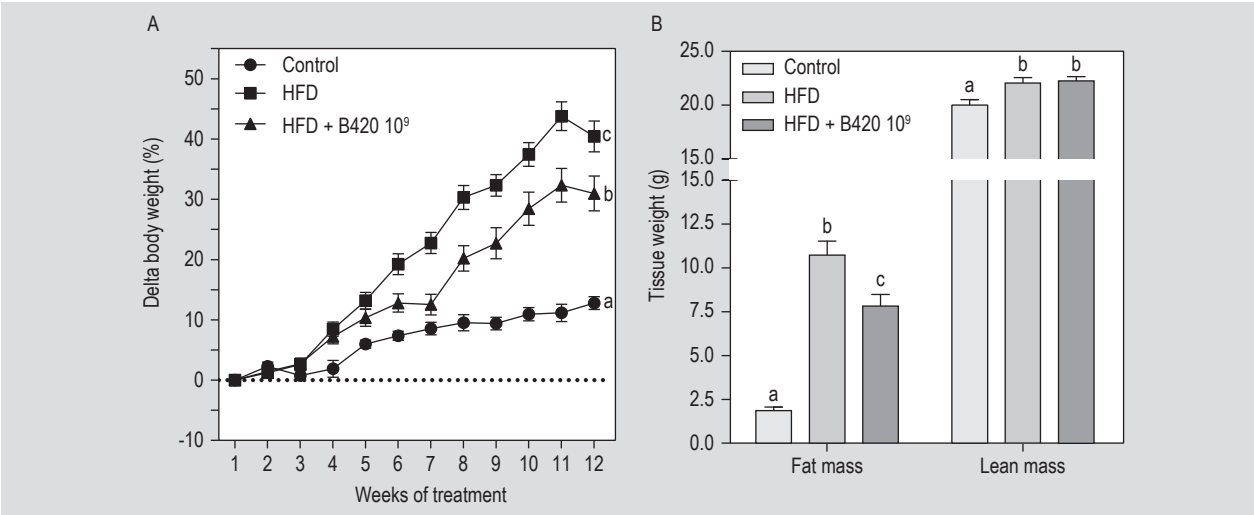


Figure 1. (A) Body weight change and (B) body composition during *Bifidobacterium animalis* ssp. *lactis* 420 (B420) treatment in diet-induced obese mice. Obese mice were fed a high-fat diet (HFD) with 60 energy % fat. Body composition was measured with EchoMRI. Values are mean  $\pm$  standard error of the mean from 10 mice per group. Groups without a common letter differ significantly from each other (ANOVA and Bonferroni's multiple comparisons).

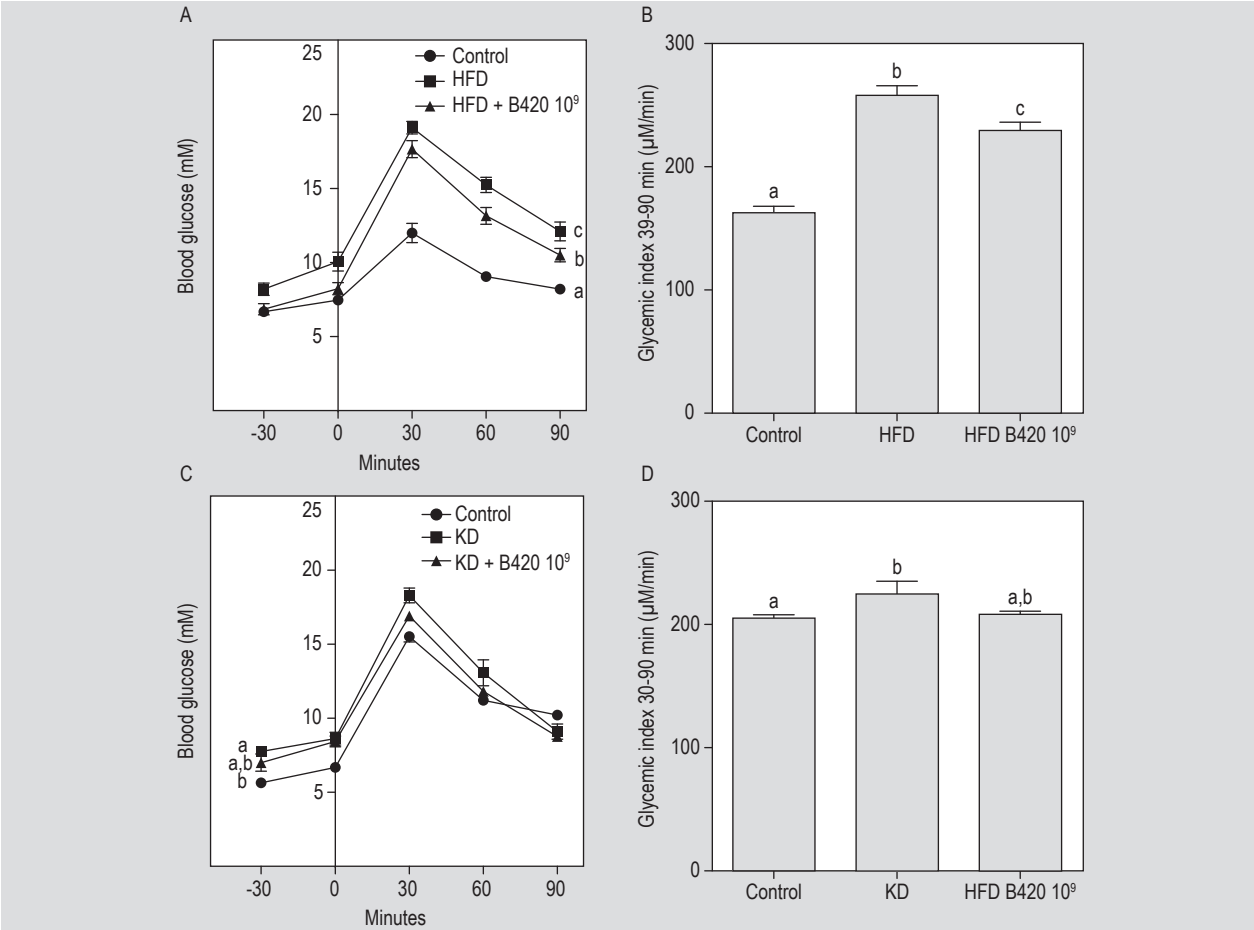


Figure 2. Glucose tolerance of diet-induced obese mice after six weeks of treatment with *Bifidobacterium animalis* ssp. *lactis* 420 (B420) (A-B) and of diet-induced diabetic mice after four weeks of treatment (C-D) in an intraperitoneal glucose tolerance test. Obese mice were fed a high-fat 60 energy % fat diet (HFD), whereas diabetic mice were fed a ketogenic 72 energy % fat diet (KD). Diabetes was induced with KD for four weeks before probiotic treatment. An index of glucose-induced glycaemia was calculated as mean blood glucose per minutes during 30-90 min after glucose injection. Values are mean  $\pm$  standard error of the mean from 10 mice per group (A-B) or 5-10 mice per group (C-D). Groups without a common letter differ significantly from each other (ANOVA and Bonferroni's multiple comparisons).

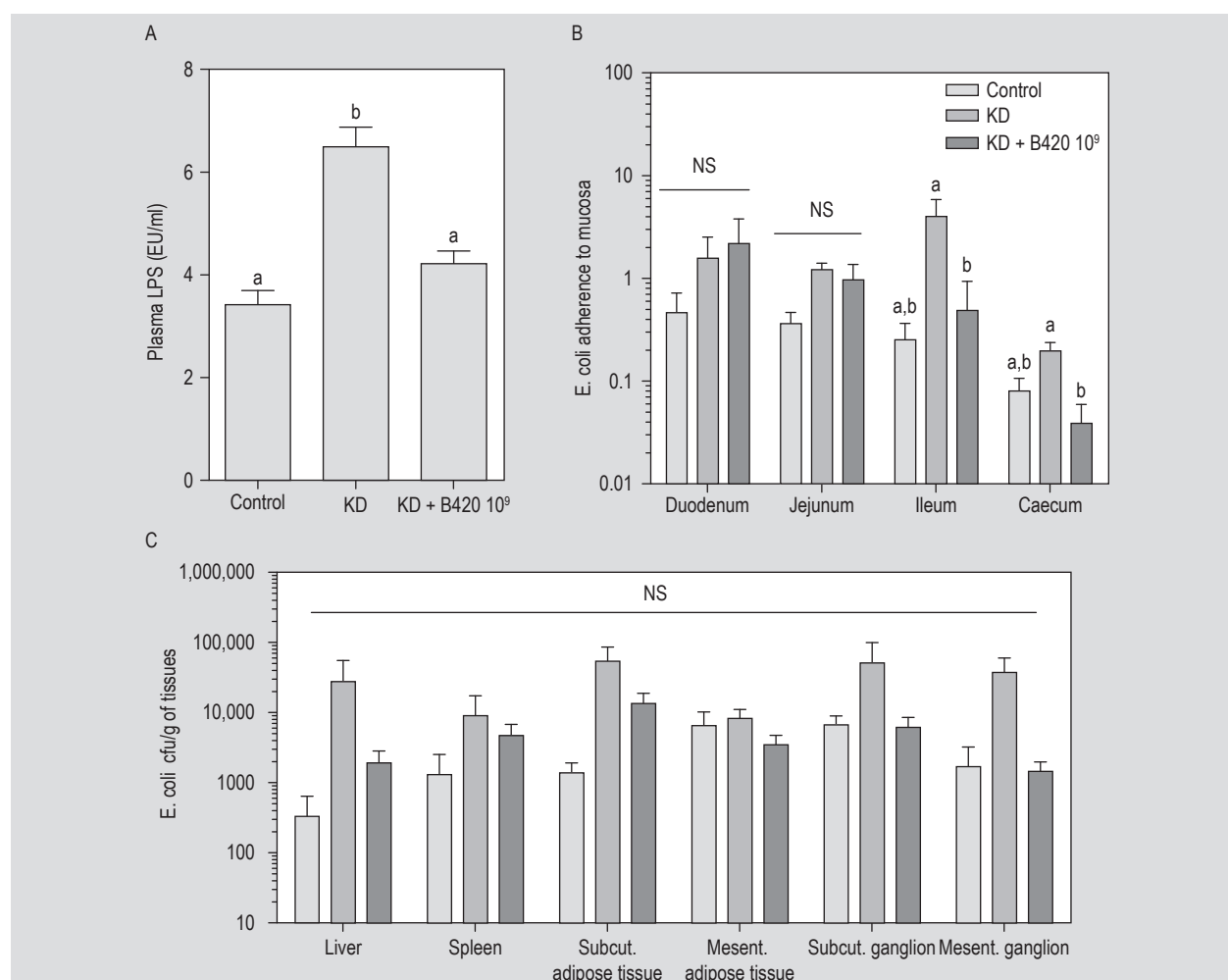
# Mechanism of action: intestinal bacterial adherence and translocation

To investigate the possible role of metabolic endotoxaemia in the reduction of adiposity by B420, we measured plasma LPS levels, as well as mucosal adherence and translocation of gavaged *E. coli* in the diabetic mouse model with KD. Plasma LPS levels were doubled in the KD group ( $P<0.001$ ) compared to control, but restored to normal levels by B420 ( $P<0.001$  compared to KD) (Figure 3A). Adherence was calculated as the ratio of mucosal to luminal *E. coli* after gavage. B420 substantially decreased mucosal adherence of gavaged bacteria in ileum and caecum ( $P=0.034$  and  $P=0.021$ , respectively), but not in duodenum and jejunum (Figure 3B). There were no significant differences in translocation of gavaged *E. coli*, although all tissues

from B420-treated mice tended to give lower cfu counts compared to vehicle-treated mice (Figure 3C).

# Influence of *Bifidobacterium lactis* 420 on hepatic inflammation

The KD increased the expression of inflammatory markers in liver (index +68% KD vs. Control,  $P=0.001$ ) and skeletal muscle (+64%,  $P=0.036$ ), with a tendency seen also in subcutaneous adipose tissue (+95%, global  $P=0.099$ ) (Table 1). In B420-treated mice, all mean inflammatory indices were lower than in vehicle-treated KD mice (subcutaneous fat -41%, liver -27%, skeletal muscle -8.3%), but the difference was significant only in liver ( $P=0.041$ ).



**Figure 3.** Gut barrier function in diabetic mice fed with a ketogenic diet (KD) and treated with the potential probiotic *Bifidobacterium animalis* ssp. *lactis* 420 (B420). (A) Plasma lipopolysaccharide (LPS) levels were determined at week 6 of B420 treatment (n=9-10 per group). For (B) adhesion and (C) bacterial translocation, a set of four mice per group were fed a KD with concomitant gavage of probiotic for five weeks, after which they were gavaged with *Escherichia coli*. Adherence was calculated as mucosal/luminal counts of *E. coli*. Translocation was determined as cfu of *E. coli* in target tissues. Groups without a common letter differ significantly from each other (ANOVA and Bonferroni's multiple comparisons).



Table 1. Tissue inflammatory markers in the diet-induced diabetes mouse model.<sup>1</sup>

	Control	KD	KD + B420 10 <sup>9</sup>	ANOVA
Subcutaneous fat				
TNF-α	1.57±0.21 <sup>2</sup>	2.03±0.23	1.98±0.79	0.79
IL-1β	1.37±0.20	2.04±0.47	1.00±0.12	0.74
PAI-1	0.43±0.06	2.11±1.19	0.68±0.17	0.25
IL-6	0.75±0.12	1.85±0.45	1.07±0.35	0.11
Index <sup>3</sup>	1.03±0.08	2.01±0.46	1.18±0.26	0.099
Liver				
TNF-α	1.32±0.17 <sup>a</sup>	2.28±0.26 <sup>b</sup>	1.60±0.18 <sup>a,b</sup>	0.012
IL-1β	1.15±0.15 <sup>a</sup>	1.88±0.11 <sup>b</sup>	1.44±0.16 <sup>a,b</sup>	0.008
PAI-1	0.98±0.12	1.77±0.29	1.35±0.37	0.17
IL-6	1.38±0.16	2.18±0.25	1.81±0.38	0.19
Index	1.21±0.09 <sup>a</sup>	2.03±0.13 <sup>b</sup>	1.48±0.18 <sup>a</sup>	0.002
Skeletal muscle				
TNF-α	0.94±0.09 <sup>a</sup>	1.82±0.26 <sup>b</sup>	1.85±0.32 <sup>b</sup>	0.015
IL-1β	1.00±0.11 <sup>a</sup>	1.63±0.16 <sup>b</sup>	1.21±0.21 <sup>a,b</sup>	0.036
PAI-1	0.82±0.10	1.46±0.46	1.25±0.23	0.27
IL-6	1.06±0.17	1.33±0.15	1.70±0.46	0.30
Index	0.95±0.05 <sup>a</sup>	1.56±0.17 <sup>b</sup>	1.43±0.23 <sup>a,b</sup>	0.021

<sup>1</sup> n=6-10 per group. KD = ketogenic diet; B420 = *Bifidobacterium animalis* ssp. *lactis* 420.

<sup>2</sup> Mean ± standard error of the mean; groups without a common letter differ significantly from each other (Bonferroni's multiple comparisons).

<sup>3</sup> Index was calculated as a mean of the relative expression of the four individual cytokines.

#### 4. Discussion

We have shown for the first time that a chronic treatment with B420 reduces body weight gain and fat mass accumulation, and improves glucose tolerance in HFD-fed mice. Furthermore, B420 reduced bacterial adherence to the intestinal mucosa, plasma LPS levels and hepatic inflammation, which together point to a mechanism related to decreased bacterial translocation by the treatment with B420. To identify the metabolic effect of a B420 treatment on metabolic diseases we compared two different animal models. In the first model, diet-induced obesity was induced by a high-fat (60 energy %) diet, and B420 was gavaged daily during the entire experiment to prevent obesity and diabetes. In the second model, the impact of B420 was tested in an animal model of diabetes which does not feature major body weight gain: the mice were fed a high-fat, KD for four weeks to induce diabetes, as previously described (Burcelin *et al.*, 2002).

The data of the present study demonstrate that B420 both prevented and treated fat mass accumulation in two different models. Our previous work has demonstrated B420 to also improve glucose tolerance in the diabetic model (Amar *et al.*, 2011), although we could not confirm it here, which could be due to a difference in the impact of the diet on this group of animals. Nevertheless, the present work shows effectiveness in a mouse model of

obesity. Hence, the metabolic effects of B420 were not linked to a specific pathogenesis. Assuming a similar effect in a clinical setting, such a feature would be beneficial in treating metabolic disease, since it enables effective use of the treatment in a broader population with a range of different pathogeneses.

The present study reinforces the concept that certain probiotics may be used in the prevention and/or treatment of obesity and adiposity. While some previous reports have shown various lactobacilli (Kim *et al.*, 2013; Park *et al.*, 2013; Sato *et al.*, 2008), bifidobacteria (Cano *et al.*, 2013; Chen *et al.*, 2011; Kondo *et al.*, 2010) and their combination (Yadav *et al.*, 2013) to reduce body weight or fat mass in rodents and humans, the present study is the first to show an effect for a *B. lactis* strain and to present a new potential mechanism for fat mass reduction. Weight reduction is a state of negative energy balance, which can be conferred by three mechanisms: (1) reducing energy intake; (2) stimulating energy expenditure; or (3) inhibiting energy harvesting or absorption by modulating gut microbiota or other luminal components. We previously demonstrated that the B420 strain reduced the impact of a fat-enriched diet on insulin resistance (Amar *et al.*, 2011). No changes in food intake were observed during the treatment (data not shown), however, the impact on glycaemia suggested that glucose was used as an energy source, which could, in case of oxidation, be dissipated and

no longer stored. This hypothesis could contribute to the prevention of body weight gain. In other instances, a recent report demonstrated that a probiotic product, VSL#3 – a combination of four different lactobacilli; three non-*Lactis* strains of bifidobacteria and one strain of *Streptococcus thermophilus* – reduced body weight gain in mice by decreasing feed intake (Yadav *et al.*, 2013). However, in this study energy expenditure was not assessed. We do believe that a combination of several mechanisms such as reduced food intake, increased energy expenditure and reduction of chronic inflammation could be responsible for the potential beneficial effect of probiotics on body weight gain. In the present study, we show new evidence suggesting that the effect of B420 on adiposity is related to an improvement of intestinal barrier function. According to a previous study, circulating endotoxins may induce weight-gain (Cani *et al.*, 2007a). Further, we have recently shown that bacterial fragments, such as LPS, can directly trigger adipose tissue precursor proliferation to increase the number of preadipocytes (Luche *et al.*, 2013). The latter will then differentiate into adipocytes in the presence of a large amount of energy available (Luche *et al.*, 2013).

Here we show that B420 remarkably reduced metabolic endotoxaemia, which may have contributed to the accompanying fat mass reduction and amelioration of weight gain. In previous studies, B420 prevented mucosal pathogen adhesion and improved tight-junction integrity *in vitro* (Collado *et al.*, 2007; Putaala *et al.*, 2008). To test our hypothesis *in vivo*, we used a commensal *E. coli* isolated from mouse intestinal microbiota. Two hours after gavage, B420 had significantly reduced mucosal adherence of the *E. coli* in the distal intestine, which points to the improvement of the mucosal barrier in probiotic-treated mice.

Luminal LPS may translocate through the gut epithelium via at least two different routes: the paracellular route through the tight-junctions, or transcellularly through enterocytes and engaged with chylomicron synthesis (Ghoshal *et al.*, 2009). Generally, molecules as large as LPS are believed to translocate only transcellularly. Indeed, in healthy patients LPS translocates only transcellularly, whereas in ileal Crohn's disease patients LPS is also found in the paracellular space (Keita *et al.*, 2008). This would point towards an opening of the paracellular pathway in a pathological state. It has not been definitively shown which pathway, or both, are activated in diet-induced obesity. However, a previous report shows that reduced expression of intestinal tight-junction proteins correlates with elevated intestinal permeability when measured with a fluorescent probe in the diabetic mouse model (Cani *et al.*, 2008). We have also studied the effect of B420 on intestinal permeability in cell culture using transepithelial electrical resistance as an indicator of the intestinal barrier (Putaala *et al.*, 2008). A cell-free supernatant of B420 increased resistance by 240%, indicating that B420 produces a compound that

enhances the epithelial barrier. This compound, however, was not any short-chain fatty acid, since the short-chain fatty acid concentration of the supernatant did not differ from those in the supernatants of less effective strains. Putative mechanisms by which probiotic strains may decrease intestinal permeability include upregulation of tight-junction protein expression, antiapoptotic activity, promotion of mucous secretion, induction of defensin release, and modulation of the submucosal immune system (Bermudez-Brito *et al.*, 2012). All these effects are communicated via soluble peptides or surface ligands produced by the bacterium. Such mechanisms for B420 are still uncovered.

In addition to improving gut barrier function, it is likely that there are several complicated mechanisms behind the effect of B420 on adiposity and glucose tolerance. Improved glucose tolerance and slightly reduced weight gain was recently reported for *Lactobacillus rhamnosus* GG in mice fed with a 60 energy % HFD (Kim *et al.*, 2013). The effect was explained with increased levels of adiponectin and consequent phosphorylation of AMP-activated protein kinase (AMPK) in skeletal muscle. AMPK activation induces fatty acid oxidation, which could have led to increased energy expenditure contributing to weight loss. Interestingly, it was shown that the conventionalisation of germ free mice modulated muscle AMPK activity demonstrating a role of the microbiota on this master regulator of energy metabolism (Backhed *et al.*, 2007). Involvement of similar mechanisms in the efficacy of B420 cannot be ruled out by the present study.

## 5. Conclusions

The findings of this study give rise to the hypothesis that B420 could be used as a complementary treatment for obesity and impaired glucose tolerance. Our results imply a benefit for the potential probiotic B420 in reducing obesity as well as glucose intolerance in diet-induced obese mice. Improved gut barrier function may contribute to the mechanisms of action. Such metabolic effects and parameters will still need to be evaluated in a clinical setting.

## Supplemental material

Supplementary material can be found online at <http://dx.doi.org/10.3920/BM2014.0014>.

Figure S1. Body weight change and body composition during *Bifidobacterium animalis* ssp. *lactis* 420 treatment in diabetic mice with a ketogenic diet.

## Conflict of interest

Dr. Stenman and Dr. Lahtinen are employees of DuPont, the manufacturer of B420.

## Acknowledgements

We thank Y. Barreira and S. LeGonidec from the Animal Care Facility of Rangueil Hospital (UMS US006/Inserm) and the Phenotyping facilities of Rangueil Hospital (UMS US006/ANEXPLO). We also thank J.-J. Maoret and F. Martins from the Quantitative Transcriptomic Facility (I2MC/UMR1048 part of Toulouse Genopole). This study was sponsored by DuPont.

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