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An evaluation of the effects of *Lactobacillus ingluviei* on body weight, the intestinal microbiome and metabolism in mice

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ABSTRACT

Background: Food can modify the intestinal flora, and *Lactobacillus ingluviei* has been shown to cause weight gain in chicks and ducks but not in mammals.
Methodology: Female BALB/c mice were divided into a control and two experimental groups and were inoculated either once or twice with *L. ingluviei* or with PBS. Faecal samples were collected and tested using qPCR in order to detect and quantify Lactobacillus spp., Bacteroidetes spp. and Firmicutes spp. Gene expression was examined in liver and adipose tissue by microarray and qPCR. Metabolic indicators in the plasma were also measured.
Results: Mice that were inoculated with 4×10^{10} *L. ingluviei* presented a significant increase in weight gain and liver weight and significant increases in *Lactobacillus* spp. and *Firmicutes* DNA copy numbers in their faeces. The mRNA levels of fatty acyl synthase (*Fas*), sterol regulatory element binding factor 1 (*Srebp1c*), tumour necrosis factor alpha (*Tnf*), cytochrome P450 2E1 (*Cyp2e1*), 3-phosphoinositide-dependent protein kinase-1 (*Pdpk1*), acyl-Coenzyme A dehydrogenase 11 (*Acad11*), ATP-binding cassette sub family member G (*ABCG2*) and DEAD box polypeptide 25 (*Ddx25*) were significantly elevated in the liver tissues of animals in the experimental group. In gonadal adipose tissue, the expression levels of leptin, peroxisome proliferator-activated receptor γ (*Pparg*) and *Srebp1c* were significantly higher in animals from the experimental group, whereas the expression of adiponectin was significantly lower in these animals.
Conclusions: The inoculation of *L. ingluviei* in mice resulted in alterations in the intestinal flora, increased weight gain and liver enlargement, accelerated metabolism and increased inflammation.

1. Introduction

Food is a major source of bacteria and viruses and modifies the microbial balance in the intestine [1]. Probiotics are defined as live microbial food supplements that have a beneficial effect on human health via delivery through the gastrointestinal tract. According to the Food and Drug Administration (FDA), probiotics are classified as "live biotherapeutics" and fall under the category of "Generally Regarded as Safe" (GRAS) substances (April 17 1997, Substances Generally Recognised as Safe; Proposed Rule). Recently was found that functional food and yoghurts contained very large numbers of living bacteria varying from 10^6 to 10^9 CFU/g [2]. Probiotics do not always result in health benefits, and it was recently discovered that probiotic-based prophylaxis is associated with an increased risk of mortality in patients with acute pancreatitis [3]. Moreover, it has been proposed that the ingestion of large concentrations of bacteria can modify the intestinal microflora [4,5], indicating the necessity for further investigation into the effects of routinely adding large amounts of bacteria to food [6].

Most probiotic products that are used in agriculture contain lactic acid bacteria, including *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pediococcus* and *Streptococcus*, as well as yeast strains, including *Saccharomyces cerevisiae* and *Kluyveromyces* spp [7]. Probiotics have been used to promote growth in farm animals for at least 30 years and are regulated by the FDA in the USA [8] and by the European Commission in Europe [7]. Probiotics were initially used to prevent episodic diarrhea in poultry, as they reduce intestinal colonisation by *Salmonella* [9] and *Clostridium perfringens* [10]; however, it was observed that *Lactobacillus* spp. probiotics promote weight gain even in the absence of diarrheal outbreaks, and the presence of

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these species in the intestinal microflora has been shown to dramatically increase food conversion [11]. *L. acidophilus*, *Lactobacillus casei*, *Lactobacillus farciminis*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* are the most common *Lactobacillus* species that are used in agriculture to stimulate fattening, reproduction and milk production in livestock [7]. Recently, we demonstrated that differences in the intestinal microbiota following inoculation with *Lactobacillus ingluviei* may lead to weight increases in new-born ducks and chickens [12]. To further examine the growth-promoting effects of this probiotic strain, our objective in this study was to determine whether inoculating mice with *L. ingluviei* also promotes growth. We also sought to determine whether changes in the intestinal microflora are associated with changes in known metabolic markers.

2. Materials and methods

2.1. Animals

After approval from the institutional ethics committee, individually weighed 3-week-old female BALB/c mice (10–15 animals per group) were given *L. ingluviei* that had been suspended in 1 mL of phosphate-buffered saline (PBS; pH 7.0) by gastric lavage as previously described [11]. Control animals were inoculated with PBS only. The *L. ingluviei* (CIP 102980) was originally isolated from an ostrich [11,12].

In the first set of experiments, mice were inoculated with one of three different doses of *L. ingluviei* (3×10^7, 1×10^8 or 5×10^9 bacteria suspended in 1 ml of PBS per animal) or with PBS alone. These animals were euthanised 28 days after inoculation. Three other groups of mice were inoculated once (LB1 group) with 4×10^{10} bacteria/animal or PBS (control group) and euthanised 20, 40 or 90 days after inoculation. For a second experimental group (LB2), a second inoculation with the same dose of bacteria was repeated on day 7, and the mice were euthanised either 40 or 90 days after the first inoculation. For all of the mice that were inoculated with 4×10^{10} bacteria per animal and euthanised after 40 or 90 days, faecal samples were collected before inoculation, 24 h after inoculation and 2, 4, 8, 16, 23 and 30 days after inoculation. The animals' diet was a standard rat and mouse maintenance mix [UAR-SAFE RM, 2760 kcal/kg (68.1% carbohydrates, 9.1% lipids, 22.8% proteins)], and the mice had free access to food and water at all times. Food intake and body weight were measured weekly. The light schedule consisted of 10 h of darkness and 14 h of light, and the room temperature and humidity were maintained at 22 ± 2 °C and 55 ± 5%. All animal procedures were conducted according to local regulations governing animal welfare.

All of the mice that were inoculated with 4×10^{10} *L. ingluviei* per animal were euthanised under anaesthesia by isoflurane inhalation following 6 h of fasting. Cardiac blood was collected into tubes with 0.01 M trisodium citrate, and plasma was isolated after of centrifugation at $1200 \times g$ for 15 min. Inguinal and gonadal adipose tissue and liver were collected, weighed and immediately frozen in liquid nitrogen.

2.2. Plasma

Plasma glucose levels were measured using a glucometer (Accu-Check Performa, Roche, Mannheim, Germany), and insulin levels were measured using radioimmunoassays (Linco Research, St. Louis, MO). The leptin and adiponectin levels were assayed using an ELISA (Linco Research, St. Louis, MO and R&D Systems, Abingdon, UK, respectively).

2.3. Liver

The protein levels of the tumour necrosis factor alpha (*Tnf-α*) levels were assayed using an ELISA (R&D Systems, Abingdon, UK) in the liver of mice euthanised 20 days after inoculation. Liver lipids were extracted according to the method of Folch et al. [13], and triglyceride levels were measured using routine chemical methods.

2.4. Molecular techniques

DNA was extracted from the faeces using a NucleoSpin Tissue Mini kit (Macherey Nagel, Hoerdt, France) according to the manufacturer's protocol. DNA was eluted in 100 μL of elution buffer and stored at −20 °C until use. A negative control using 250 μL of sterile water was included in each series of DNA extractions. The DNA was tested using quantitative real-time PCR (qPCR), which was performed in an MX3000 system (Stratagene Europe, Amsterdam). The detection and quantification of *Lactobacillus* spp. were performed as reported by Menard et al. [14]. *Bacteroidetes* and *Firmicutes* were quantified using a quantification plasmid that was constructed as previously described by Carcopino et al. [15].

The total RNA from 20 to 40 mg of liver or 150–200 mg of adipose tissue was extracted using TRIzol (Invitrogen, Cergy-Pontoise, France) as per the manufacturer's instructions. The RNA concentration and purity were spectrophotometrically determined (NanoDrop 1000, NanoDrop Technologies, Wilmington, DE). Following RNA isolation, cDNA was synthesised from 1 μg of total RNA from either liver or adipose tissue using MMLV transcriptase (Invitrogen, Cergy-Pontoise, France). PCR was performed using an ABIPrism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). A control without cDNA was included in each experiment. The relative amount of each assayed mRNA was normalised to the mRNA level of *36B4*, which encodes a housekeeping ribosomal protein and is expressed relative to the mean value from the control group (User Bulletin # 2, Applied Biosystems, Foster City, CA) [16].

2.5. Microarray experiments

In order to identify differences in gene expression, microarray analysis was performed on the livers of two LB1 and two control mice that had been euthanised 20 days after inoculation. We used a whole mouse genome oligomicroarray 4×44K kit (44,000 60-mer oligonucleotides) and performed a one-color microarray-based gene expression analysis as previously described [17]. Labelled RNAs (Low RNA Input Fluorescent Amplification kit, Perkin Elmer) were deposited on slides and hybridised using an *in situ* Hybridisation Plus kit (Agilent Technologies) for 17 h. The arrays were scanned using a DNA Microarray Scanner G2505B (Agilent Technologies), and the image analysis and correction of intra-array signals were performed using Feature Extraction Software A.9.1.3 (Agilent Technologies). Microarray data analysis was performed using GeneSpring 10.01 with the default settings for inter-array normalisation and inter-replicate corrections. To identify genes that were differentially expressed, we used Student's *t*-test with $p < 0.05$ and considered an absolute fold change (FC) of greater than 2.0 to be significant. An analysis of Gene Ontology (GO) terms was performed in order to identify any altered biological processes. Additional data analysis was performed using R software, v2.8.1 (R: A Language and Environment for Statistical Computing, *R Development Core Team*, Vienna, Austria: R Foundation for Statistical Computing 2009).

Based on the microarray analysis results, we tested the expression of specific genes. RNA extraction and cDNA synthesis were performed as described above. Primers and probes that

targeted the mouse genes and glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*, used as a housekeeping gene) were purchased from Applied Biosystems. qPCR reactions were performed as described above. The relative amounts of each examined mRNA were normalised to the *Gapdh* mRNA expression level and expressed relative to the mean amount of the same mRNA in the control group.

2.6. Meta-analysis

Meta-analysis was conducted following PRISMA 2009 guidelines [18] using Comprehensive Meta-Analysis Software, v2.2 [Biostat, Englewood NJ (2005)]. Control mice and mice that had been inoculated with *L. ingluviei* were compared in the analysis.

2.7. Statistical analysis

The data are expressed as the mean ± standard deviation (SD). The statistical significance of the differences between independent variables was tested using the Mann–Whitney and Kruskal–Wallis tests in GraphPad Prism v5.00 software (GraphPad Software). A p value of less than 0.05 was considered to be significant.

3. Results

3.1. Weight gain

There were no significant differences between the weights of the mice in the experimental and control groups at the beginning of the experiment. No significant changes were observed in mice that had been inoculated with 3×10^7, 1×10^8 or 5×10^9 *L. ingluviei* per mouse (data not shown); however, inoculation with 4×10^{10} *L. ingluviei* per mouse resulted in an overall weight gain and increase in liver weight (Fig. 1), although food intake remained the same (data not shown). On day 20, the control animals weighed an average of 15.7 ± 1.0 g, whereas the average weight of the LB1 mice was 17.1 ± 0.6 g ($p < 0.0001$). On day 20, the average liver weight for the control group was 0.76 ± 0.09 g, whereas the average liver weight of the LB1 mice was 0.85 ± 0.11 g ($p = 0.06$). By day 40, the control animals weighed 20.2 ± 1.2 g, whereas the LB1 and LB2 mice weighed 22.3 ± 1.6 g ($p = 0.014$) and 21.2 ± 0.6 g ($p = 0.0064$), respectively. On day 40, the average liver weight of the control mice was 0.88 ± 0.15 g, whereas the average liver weights of the LB1 and LB2 mice were 1.12 ± 0.12 g ($p = 0.004$) and 1.00 ± 0.10 g ($p = 0.011$), respectively. Finally, the control animals that had been euthanised on day 90 weighed 22.5 ± 1.9 g, whereas the LB1 mice weighed 23.1 ± 0.7 g ($p = 0.05$) and the LB2 mice weighed 23.3 ± 0.8 g ($p = 0.03$). On day 90, the control livers weighed 1.12 ± 0.12 g, whereas the livers from the LB1 and LB2 mice weighed 1.33 ± 0.01 g ($p = 0.01$) and 1.35 ± 0.15 g ($p = 0.009$), respectively. No significant differences were observed in either the inguinal or gonadal adipose tissue weights at any of the investigated doses (Supplementary Data Table 1).

3.2. Faecal samples

Prior to inoculation, there was no difference in the *Lactobacillus*, *Firmicutes* or *Bacteroidetes* DNA copy numbers between the experimental and control groups, and the copy numbers remained constant in the control group throughout the 30 days of the experiment (Fig. 2); however, on day 2, a significant increase in the *Lactobacillus* spp. DNA copy number was observed in the two experimental groups LB1 and LB2 ($p = 0.011$ and $p = 0.026$). The *Firmicutes* DNA copy number was also significantly higher in the two experimental groups relative to the control group ($p = 0.009$ and $p = 0.01$). On day 8 after the second inoculation, the LB2 mice had a significantly greater *Lactobacillus* spp. DNA copy number than either the control group ($p = 0.007$) or the LB1 group ($p = 0.01$). LB2 animals on day 8 also had a significantly greater *Firmicutes* DNA copy number than either the control group ($p = 0.01$) or the LB1 group ($p = 0.03$); however, after day 8, no differences were observed in the *Lactobacillus* or *Firmicutes* DNA copy numbers between the control group and the LB1 group ($p = 0.19$ and $p = 0.21$). After day 16, no changes were observed in the *Lactobacillus* or *Firmicutes* DNA copy numbers between the control group and the LB2 group ($p = 0.08$ and $p = 0.1$). For the *Bacteroidetes* DNA copy numbers, we found a tendency to decrease after each *L. ingluviei* inoculation; however this difference was not significant between the control group and the LB1 or LB2 groups during the 30-day experiment.

The ratio of *Firmicutes* to *Bacteroidetes* DNA copy numbers in the control group remained constant over the course of the 30-day experiment, whereas, in the experimental groups, inoculation with *L. ingluviei* increased this ratio. This difference was associated with the significant increase of *Firmicutes* and the non significant decreased of *Bacteroidetes*. In LB1 mice, the largest difference between the *Firmicutes* and *Bacteroidetes* copy numbers was observed on day 2, wherein the *Firmicutes*:*Bacteroidetes* ratio was 1.77-fold greater than that observed in the control group. After day 8, the *Firmicutes*:*Bacteroidetes* ratio was similar between the LB1 group and the control animals. We observed the largest difference in the *Firmicutes*:*Bacteroidetes* ratio in LB2 animals on day 8, wherein the *Firmicutes*:*Bacteroidetes* ratio was 1.9-fold greater than that of the control group. After day 23, no difference was observed in the *Firmicutes*:*Bacteroidetes* ratio between the LB2 group and the control animals.

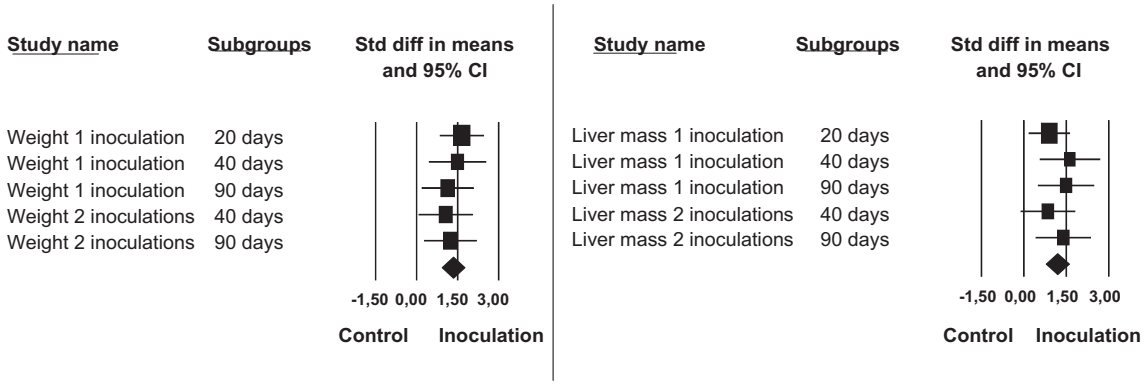

Fig. 1. Meta-analysis results for body and liver weight in control group mice and mice inoculated with *L. ingluviei*.

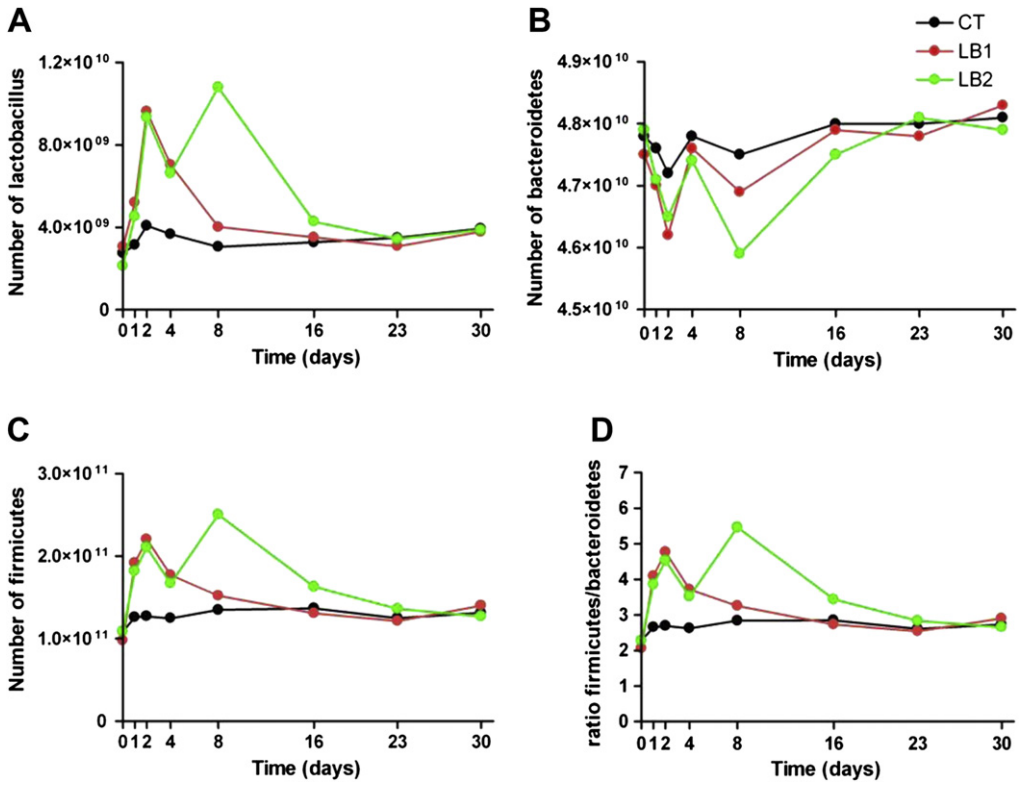

Fig. 2. Changes in the proportion of *Lactobacillus* spp., *Firmicutes* and *Bacteroidetes* between the control and experimental groups. The results are based on the mean copy number of a quantification plasmid [15]. CT, control group; LB1, mice inoculated once with *L. ingluviei*; LB2, mice inoculated twice with *L. ingluviei*.

3.3. Liver transcriptomics

Based on the transcriptomic results, we tested 16 genes by qPCR. We confirmed that inoculated mice demonstrated significantly greater mRNA expression of cytochrome P450 2E1 (*Cyp2e1*), 3-phosphoinositide-dependent protein kinase-1 (*Pdpk1*), acyl-Coenzyme A dehydrogenase 11 (*Acad11*), ATP-binding cassette sub family member G (*ABCG2*) and DEAD box polypeptide 25 (*Ddx25*) in comparison to the control group [$p = 0.001$, $p = 0.009$, $p = 0.02$, $p = 0.03$ and $p = 0.008$, respectively; (Fig. 3)]. No significant differences were identified for the other genes examined (Supplementary Table 1).

3.4. The effect of L. ingluviei on metabolism

At 20 days post-inoculation, no differences in the metabolic parameters were observed between the two groups except for plasma adiponectin levels, which were significantly lower in the LB1 group as compared to the control group (5550 ± 1358 vs. 6696 ± 602 ng/mL, $p < 0.01$). The results that were obtained for the metabolic parameters for mice that were sacrificed at 40 and 90 days are shown in Supplementary Table 2. *L. ingluviei* inoculation had no effect on glycaemia in mice that were sacrificed at 40 and 90 days ($p > 0.05$). At 90 days post-inoculation, the fasting insulinemia levels tended to be higher in the LB1 mice ($p = 0.09$) and were significantly higher in the LB2 mice ($p = 0.02$) relative to the control mice. As a result, the quantitative insulin check index of insulin sensitivity [QUICKI; 1/[log(insulin) + log(glucose)] was lower in LB1 ($p = 0.06$) and LB2 ($p = 0.01$) mice, indicating a higher level of insulin resistance in mice that had been inoculated with *L. ingluviei*. Plasma leptin levels tended to be higher in LB1 and LB2 mice relative to the control group at 90 days post-inoculation, but this difference did not reach statistical significance ($p = 0.21$ and $p = 0.24$).

We studied the mRNA expression of lipogenic markers in the livers of mice that had been euthanised 90 days after inoculation and found that the mRNA expression level of fatty acyl synthase (*Fas*) was significantly higher in the LB1 ($p = 0.05$) and LB2 mice ($p < 0.02$) than in the control mice (Fig. 4A). Moreover, in the livers of LB1 and LB2 mice, the mRNA expression level of the lipogenic marker *Srebp1c* (sterol regulatory element binding factor 1) was approximately 2–2.5 times greater than that in the control group ($p = 0.0002$; Fig. 4B). This was accompanied by a low-grade inflammatory state in the liver, which was indicated by a significantly increased mRNA expression of liver *Tnf-α* in LB2 mice in comparison to the control group ($p = 0.033$; Fig. 4C). Moreover mice inoculated with *L. ingluviei* tended to express higher levels of *Tnf-α* proteins than control group mice (1.19 ± 0.13 versus 0.89 ± 0.05 respectively) ($p = 0.06$). We did not observe any significant differences in the hepatic triacylglycerol content between the experimental and control groups ($p = 0.509$ by the Kruskal–Wallis test; Fig. 4D).

In the gonadal adipose tissues of LB1 and LB2 mice that had been euthanised 40 days after inoculation, we observed an increased mRNA expression level of leptin, which is a lipogenic marker, in comparison to the control animals ($p = 0.23$ and $p = 0.01$, respectively; Fig. 5A). This observation agrees with the observed mRNA expression levels of two key lipogenic factors, *Pparg* (peroxisome proliferator-activated receptor γ; Fig. 5C) and *Srebp1c* (Fig. 5D), which were significantly increased ($p = 0.02$ and $p = 0.04$, respectively) in LB2 mice in comparison to the control group. Furthermore, we observed lower levels of adiponectin in the gonadal adipose tissues of LB1 and LB2 mice relative to the control group ($p = 0.02$ and $p = 0.06$, respectively; Fig. 5B). There were no significant differences in the expression levels of the above genes in the inguinal adipose tissues from the control and experimental groups.

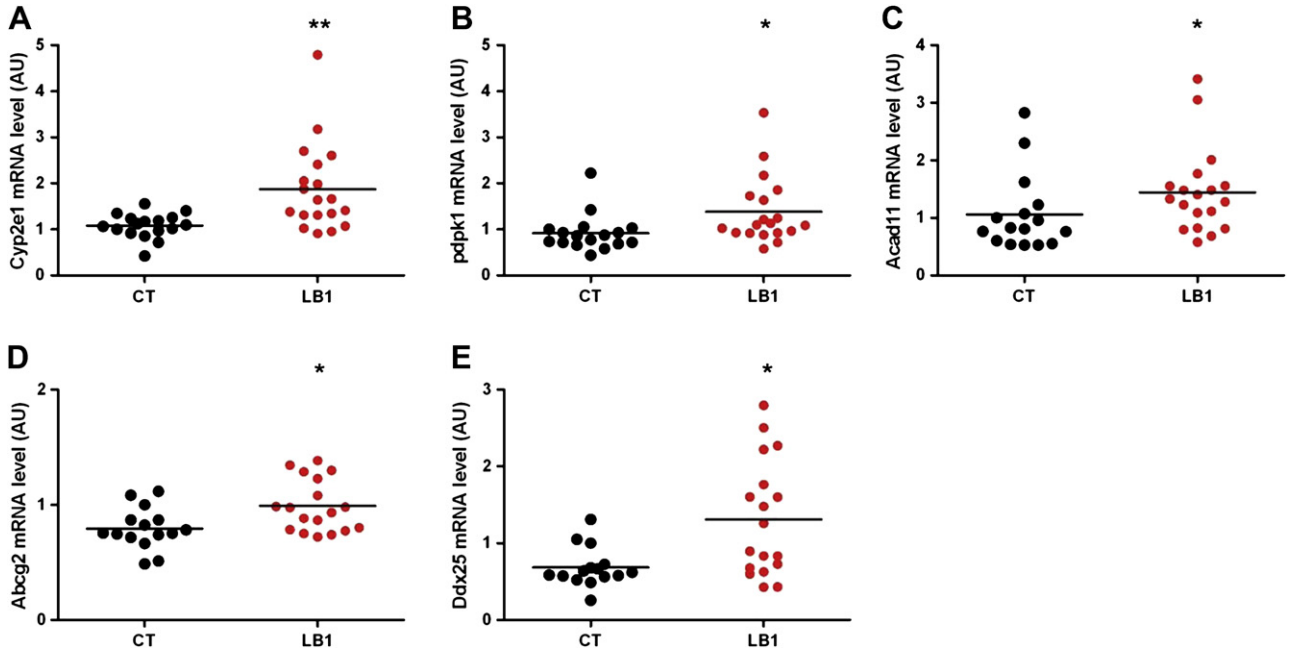

Fig. 3. mRNA expression levels for A) Cytochrome P450 family 2 polypeptide 1 (*Cyp2e1*), B) 3-phosphoinositide-dependent protein kinase-1 (*Pdpk1*), C) acyl-Coenzyme A dehydrogenase 11 (*Acad11*), D) ATP-binding cassette sub family G (*ABCG2*) and E) DEAD box polypeptide 25 (*Ddx25*) in control mice (CT) and mice inoculated with 4×10^{10} *L. ingluviei* (LB1) euthanized 20 days post-inoculation. The mRNA levels were normalized to *Gapdh* expression. *: $p < 0.05$, **: $p \leq 0.001$ by Mann–Whitney.

4. Discussion

In this experimental mouse model, we found that the delivery of one dose of 4×10^{10} *L. ingluviei* was associated with an increase in the intestinal population of *Lactobacillus* spp. and *Firmicutes* spp., as well as an increase in the *Firmicutes*:*Bacteroidetes* ratio. These results confirm our previous studies with *L. ingluviei* inoculation in ducks and chicks [12]. Moreover, we found that the increase in the *Lactobacillus* spp. population in the intestinal microflora accelerated the metabolisms of the plasma, liver and gonadal adipose

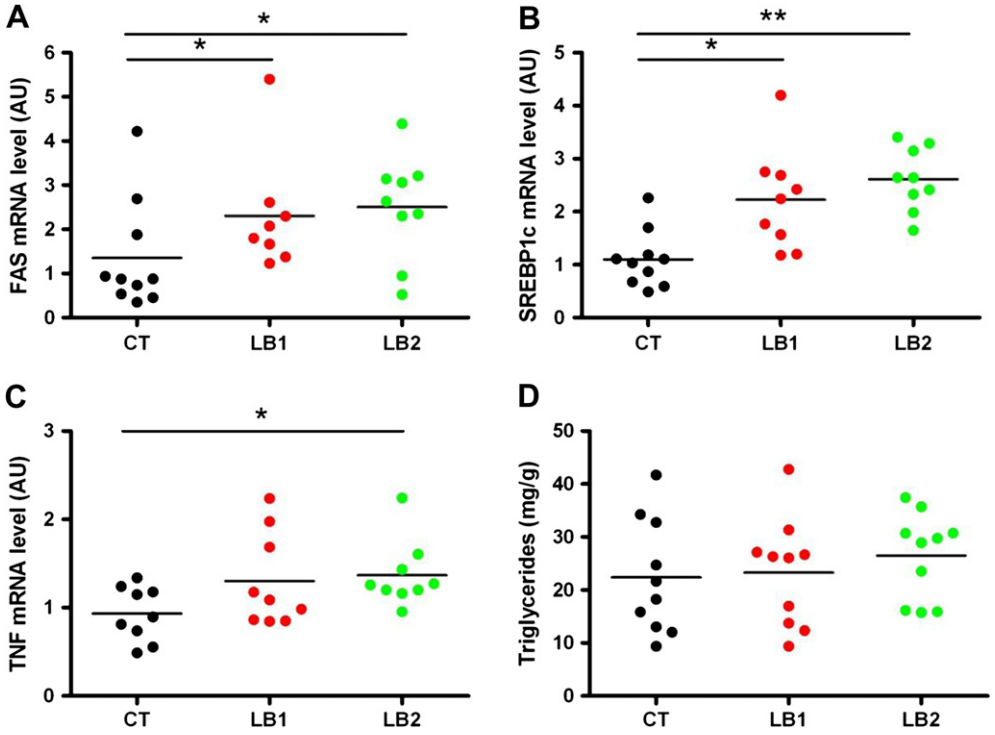

Fig. 4. The effect of *L. ingluviei* inoculation on liver tissue from mice euthanized 90 days post-inoculation. A) The mRNA levels of fatty acid synthase (*Fas*), B) sterol regulatory element binding factor 1 (*Srebp1c*) and C) tumor necrosis factor alpha (*Tnf*) and D) the hepatic triglyceride content. The mRNA levels were normalized to the expression of *36B4*. CT, control group; LB1, mice inoculated once with *L. ingluviei*; LB2, mice inoculated twice with *L. ingluviei* *: $p < 0.05$, **: $p < 0.01$ by Mann–Whitney.

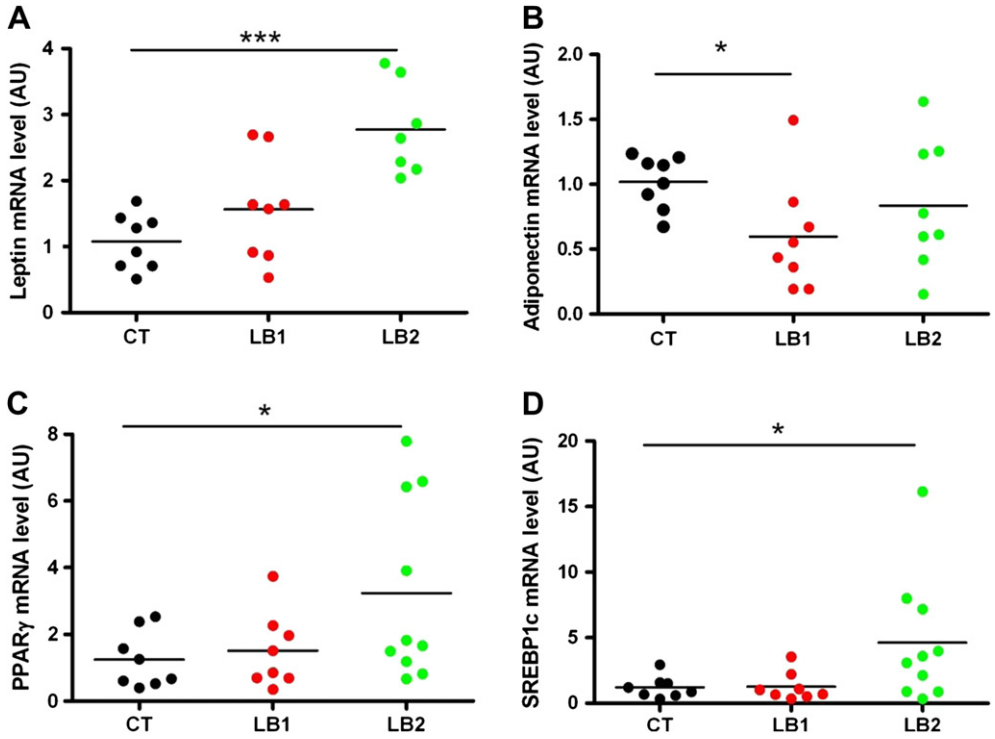

Fig. 5. The effect of *L. ingluviei* inoculation on gonadal adipose tissue from mice euthanized 40 days post-inoculation. A) The mRNA levels of leptin, B) adiponectin, C) peroxisome proliferator-activated receptor γ (*Pparg*) and D) sterol regulatory element binding factor 1 (*Srebp1c*). The mRNA levels were normalized to the expression of *36B4*. CT, control group; LB1, mice inoculated once with *L. ingluviei*; LB2, mice inoculated twice with *L. ingluviei* *: $p < 0.05$, ***: $p < 0.001$ by Mann–Whitney.

tissue and increased inflammation. As a result, mice that were inoculated with *L. ingluviei* demonstrated significant increases in body and liver weights. The differences in body weight, liver weight and metabolism were further increased following the administration of a second dose of this bacterial species. We confirmed that these growth-promoting effects were dose-dependent because when we inoculated the mice with lower doses of *L. ingluviei*, we did not observe differences in weight gain or liver weight.

Inoculating mice with *L. ingluviei* resulted in deleterious effects on metabolism in both the liver and gonadal adipose tissue. The liver weight was significantly increased in mice that had been inoculated with *L. ingluviei*, which was accompanied by an increased expression of key lipogenic markers, such as FAS and SREBP1c, which have been implicated in the development of steatosis [16], and TNF, which is a proinflammatory cytokine that is known to initiate the early stages of fatty liver [19,20]. These data indicate that the administration of *L. ingluviei* induces the expression of genes that are involved in hepatic steatosis, although we were unable to detect changes in the hepatic triacylglycerol content. Inoculation with *L. ingluviei* also had deleterious effects on gonadal adipose tissue metabolism, as evidenced by the increased mRNA levels of *Srebp1c* and *Pparg*, which are indicators of increased lipogenesis [21]. We also observed a higher level of leptin, which is a hormone that is synthesised by differentiated adipocytes, in the gonadal adipose tissues of inoculated mice. Surprisingly, the above changes in gene expression were not followed by an increase in adipose tissue weight, although this could be related to our use of young and lean mice that were not fed a high-fat diet or to the short duration of our study.

Liver tissue from mice that were inoculated with *L. ingluviei* and euthanised at day 20 also demonstrated transcriptional profile differences in comparison to the control group. Specifically, we found that the inoculated mice expressed an increased level of *Pdpk1*, which is involved in insulin signalling *in vitro* [22]. It has been observed that mice with a liver-specific *Pdpk1* deficiency were markedly more glucose intolerant and were unable to normalise their blood glucose level during an insulin tolerance test [23]. *Cyp2e1*, which is involved in metabolising and deactivating many toxicologically important substrates, including ethanol, was also over-expressed in mice that had been inoculated with *L. ingluviei*. *Cyp2e1* is induced in the liver under a variety of conditions, including fasting, diabetes, obesity, a high-fat diet and some models of non-alcoholic steatohepatitis [24,25]. *ABCG2* and *Ddx25* are implicated in a number of cellular processes that involve the alteration of RNA secondary structure, such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly, whereas *Acad11* provides instructions for making an enzyme called isobutyryl-CoA dehydrogenase (IBD), which is involved in the breaking down of proteins from food.

The results from previous experimental models that have investigated probiotics use in mice vary. It was observed that the administration of *L. rhamnosus* or *Lactobacillus acidophilus* in the drinking water of weaned Swiss mice did not result in any significant differences in weight gain between the control group and groups receiving 10^6, 10^4 or 10^2 CFU/ml. In contrast, treatment with 10^8 CFU/ml resulted in increased weight gains of up to 28.9% and 31.7% for *L. rhamnosus* and *L. acidophilus*, respectively [26]. Zhou et al. found no significant difference in the growth rate of BALB/c mice that were treated with *L. rhamnosus*, *L. acidophilus*, *Bifidobacterium lactis* or a commercial strain of *L. acidophilus* over 4 weeks [27]. In contrast, when 6-week-old mice were inoculated with 10^7 and 10^9 CFU of *L. plantarum* PL62 for 8 weeks, an overall reduction in body weight was observed [28,29]. The same study also found that, although energy intake was the same, the weight gain was significantly greater for control mice than for those that were treated with *L. rhamnosus* [28,29]. As a consequence, treatment with high inocula of *L. rhamnosus*, *L. acidophilus* and

L. ingluviei probiotics is thought to be associated with weight gain. In contrast, others have shown that *L. plantarum* treatment results in anti-obesity effects.

We have demonstrated that a single *L. ingluviei* inoculation early in the life of a mouse can modify the intestinal microflora, resulting in increased body weight and metabolic changes. Our results support previous studies with mice that have demonstrated that an increase of Gram-positive *Firmicutes* bacteria in the microbiota play a significant role in weight gain. For example, genetically obese (leptin-deficient ob/ob) mice had 50% fewer *Bacteroidetes* spp. and correspondingly more *Firmicutes* spp. than their lean siblings [30]. In another study, lean, germ-free mice that received transplanted intestinal microflora from the caecum of obese donors exhibited a significantly larger increase in body fat over 2 weeks than mice that were colonised with the intestinal microflora of lean donors [31]. In addition, switching axenic mice from a low-fat diet to a high-fat diet altered their microbiota within 1 day and changed the gene expression and metabolic pathways in their microbiome [32]. Finally, it has been shown that mice that are fed a Western diet have increased adiposity and that this trait is transmissible via transplantation of the microbiota [32].

In humans, obesity has been associated with a reduction in Gram-negative bacteria, specifically *Bacteroidetes* spp., and an increase in Gram-positive *Firmicutes* bacteria [4], whereas differences in the intestinal microbiota may lead to the development of an overweight phenotype [33]. Recently, it was proposed that families of proteins that are represented in the microbiome, such as carbohydrate-active enzymes (CAZymes), carbohydrate-binding modules (CBMs) and other sugar-interacting proteins, may be involved in numerous biologically important chemical transformations, such as the degradation of complex dietary polysaccharides, and may play a role in weight gain [34]. Although few trials have been conducted, several factors suggest a role for *Lactobacillus* spp. in weight changes. Conversely, the quantification of the *Lactobacillus* spp. in lean, anorexic and obese subjects have revealed significantly higher *Lactobacillus* concentrations in almost half of the obese patients studied [35]. Type-2 diabetic patients also had a significantly lower proportion of *Firmicutes* spp. and significantly higher levels of *Lactobacillus* spp. [36]. An increased number of *Lactobacillus* spp. has also been reported in obese adolescents who followed a weight loss program [37].

5. Conclusions

In the current study using mammals, we have shown that increasing the *L. ingluviei* content in the intestinal microbiota preceded an increase in weight and resulted in metabolic changes. *L. ingluviei* is associated with significant body weight gain and liver enlargement after only a single inoculation in chickens [11], ducks [12] and mice. These growth-promoting effects were dose-dependent, and lower doses of *L. ingluviei* did not result in weight gain or an increased liver weight. This dose-dependent, growth-promoting effect of some *Lactobacillus* spp. in farm animals has also been recently proposed by others [38]; however, this link remains to be substantiated for other animal species and humans.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.micpath.2011.10.004.

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