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Oral Probiotic Bifidobacterium Longum Supplementation Improves Metabolic Parameters and Alters the Expression of the Renin-Angiotensin System in Obese Mice Liver

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Abstract

Background: Obesity and non-alcoholic fatty liver disease (NAFLD) have been increasing at an alarming rate worldwide. *Bifidobacterium longum* (BL), a common member of the human gut microbiota, has important health benefits through several mechanisms. **Objectives:** We evaluated the BL supplementation effects on body metabolism and renin-angiotensin components hepatic expression in mice fed a high-fat diet. **Methods:** Thirty-two male mice were divided into four groups: standard diet + placebo (ST), standard diet + *Bifidobacterium longum* (ST + BL), high-fat diet + placebo (HFD) and high-fat diet + *Bifidobacterium longum* (HFD + BL). Following the obesity induction period, the ST + BL and HFD + BL groups were supplemented with *Bifidobacterium longum* for 4 weeks. Then, body, biochemical, histological and molecular parameters were evaluated. **Results:** HFD + BL mice had a significant decrease in adipose tissue mass and blood glucose levels, as well as a significant reduction in blood glucose during an intraperitoneal glucose tolerance test. The treatment also resulted in reduced levels of total cholesterol and hepatic fat accumulation. Moreover, we observed an increase in angiotensin converting enzyme 2 (ACE2) and Mas receptor (MASR) expression levels in BL-treated obese mice. **Conclusions:** These data demonstrate that BL may have the potential to prevent obesity and NAFLD by modulating the mRNA expression of renin-angiotensin system components.

Keywords

renin-angiotensin system, obesity, non-alcoholic fatty liver disease, gut microbiota

Obesity represents one of the greatest public health problems worldwide, afflicting more than 600 million people (Collaboration, 2016). The obesity etiopathogenesis is multifactorial, involving genetic and environmental factors (for example, hypercaloric diets and sedentarism), and is characterized by excessive body fat accumulation, which commonly results from an imbalance between energy intake and expenditure (Bluher, 2019). The white adipose tissue accumulation predisposes one to a low-grade systemic chronic inflammatory condition that contributes to the development of several diseases, such as diabetes mellitus, atherosclerosis, stroke, non-alcoholic fatty liver disease (NAFLD), and some types of cancer (Choquet & Meyre, 2011).

Non-alcoholic fatty liver disease is a pathological clinical entity characterized by the accumulation of triglycerides in the hepatocytes, with or without inflammation or fibrosis. One of the main risk factors for NAFLD obesity (Brunt et al., 2015; Cobbina & Akhlaghi, 2017) and 30%–40% of the individuals with obesity also have NAFLD. NAFLD is a public health problem with growing prevalence and increased potential to progress to more severe hepatic diseases (Polyzos et al., 2019). Furthermore, NAFLD is associated with several cardiometabolic

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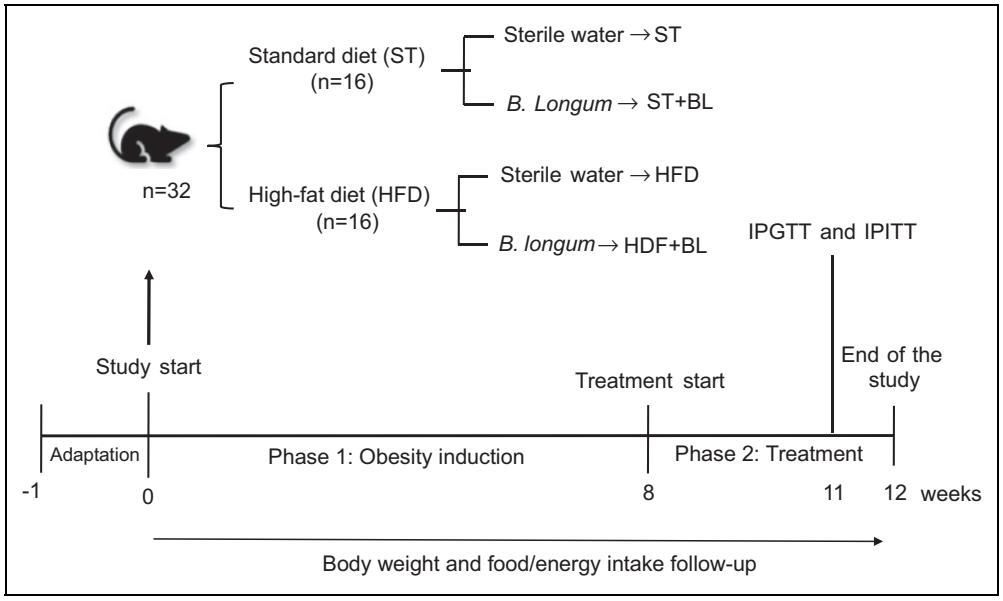

Figure 1. Experimental timeline. The experiment was performed on 32 male mice divided in four groups (n = 8 animals/group; 4 animal/cage): standard diet + placebo (ST), standard diet+ Bifidobacterium longum (ST + BL), high-fat diet + placebo (HFD) and high-fat diet + Bifidobacterium longum (HFD + BL). At week -1 they were kept in adaptation. Following, the animals were submitted to phase 1: obesity induction period (8 weeks), and then, phase 2: treatment period (4 weeks). At week 11, IPGTT and IPITT tests were performed and at week 12 the animals were killed for further analysis.

abnormalities, including type 2 diabetes mellitus (T2DM), metabolic syndrome, and coronary heart disease (Brunt et al., 2015).

The link between obesity and NAFLD comprises several factors. Recently, the literature has drawn attention to the roles of the renin-angiotensin system (SRA) and gut microbiota in relation to the molecular aspects associated with obesity and NAFLD (Aron-Wisnewsky et al., 2020; Borem et al., 2018; Goh et al., 2015; Safari & Gerard, 2019). The SRA is a complex network, formed by several components that together exert important functions in body homeostasis, including hydroelectrolytic balance, arterial pressure control and body metabolism, as well as in the function of several organs, such as the liver, adipose tissue, and intestine (Miller & Arnold, 2019; Patel et al., 2017; Rein & Bader, 2017).

Our understanding of gut microbiota modulation as a possibility for the prevention/treatment of metabolic disturbances associated with obesity is growing (Karlsson et al., 2013; Lynch & Pedersen, 2016). Gut microbiota, or probiotics provide many crucial functions for the host during health and disease and are now recognized as playing key roles in obesity by regulating energy balance and inflammation (Lynch & Pedersen, 2016; Tilg et al., 2020). Probiotics are reported to improve metabolic diseases such as obesity and diabetes through modulating gut microorganisms (Bleau et al., 2015; Brusaferro et al., 2018). *Bifidobacterium longum* (*B. longum*) is a gram-positive anaerobic bacteria belonging to *Bifidobacterium spp*. Recent studies have shown several positive effects of *Bifidobacterium longum* (*B. longum;* An et al., 2011; In Kim et al., 2019; Krumbeck et al., 2018).

Therefore, we hypothesized that supplementation of probiotic *B. longum* for obese animals would modulate the renin-angiotensin system in the liver and improve systemic metabolic parameters. Thus, the objective of this study was to evaluate the effect of *B. longum* supplementation on body metabolism and the renin-angiotensin system's expression in the livers of high-fat fed mice.

Methods

Study Design

All experimental procedures were approved by the Ethics Committee of the State University of Montes Claros and were conducted by following the regulations described in the Committee Guiding Principles Manual (Protocol number 103/2016). The experiment was conducted with 4-week-old male Swiss mice divided into four groups (n = 8 animals/group): standard diet + placebo (ST), standard diet + *Bifidobacterium longum* (ST + BL), high-fat diet + placebo (HFD) and high-fat diet + *Bifidobacterium longum* (HFD + BL). The animals were kept in propylen cages (41 × 34 × 18 cm) with four animals each, with 12 h light/dark cycle, temperature 22 °C ± 2 °C, and free access to food and water during treatment. The study was divided into two phases: phase 1) obesity induction: the animals were fed for 8 weeks with the respective diets (ST or HFD); phase 2) treatment: the groups ST and HFD maintained their diets and received placebo for 4 weeks, while the ST + BL and HFL + BL groups received *B. longum* probiotic for the same period (Figure 1).

Diets and Drugs

The standard diet is composed of approximately 50% carbohydrates, 32% proteins, and 18% lipids, presenting 2.2 Kcal/g (Ração Labina Presence®, Brazil), and the high-fat diet is composed by 24.5% carbohydrate, 14.5% protein and 61% fat,

presenting a total of 5.28 Kcal/g diet (Haslam & James, 2005; Rocha & Libby, 2009). The *B. longum* probiotic was administered by gavage at a dose of 50 billion bacteria/kg body weight/daily (Rashid et al., 2014). ST and HFD groups received vehicle daily—sterile water as placebo.

Measurements of Body Weight and Food/Energy Intake

The body weight (in grams), food intake (diet in grams/animal/day), and energy intake (Kcal/animal/day) were measured three times a week at fixed times and days during all experimental procedures. Mice were fasted overnight (10 hours) and killed by decapitation. Samples of blood, adipose tissues (epididymal, mesenteric, and retroperitoneal), and liver were collected, weighed, immediately frozen in liquid nitrogen and stored at −80 °C for subsequent analyses. The tissue weights were corrected by the animal's body weight (tissue weight (g) ÷ animal body weight (g). Visceral adiposity (g) was calculated by the sum of all adipose tissues ($\sum$ epididymal, mesenteric and retroperitoneal adipose tissues weight (g) ÷ animal body weight (g). For the food intake analysis, the mean food intake of each animal (g and Kcal) was also adjusted by the body weight.

Histology Staining

Liver samples were fixed in 10% neutral-buffered formalin at 4 °C overnight, dehydrated by escalating grades of alcohol, xylene, and paraffin, and then embedded in paraffin. Sections of 5 micrometers were prepared for Hematoxylin & Eosin staining. The slides were analyzed in an FSX100 Inverted Microscope (Olympus®, Japan).

Glucose Tolerance and Insulin Sensitivity Tests

For the glucose tolerance test (IPGTT), a 50% weight/volume hypertonic glucose solution (2 g/kg animal) was intraperitoneally administered after a 10-hour overnight fast. Blood glucose levels were measured in blood samples taken from the caudal vein, in the times 0 (baseline), 15, 30, 60, and 120 minutes after injection. The insulin sensitivity test (IPITT) was performed with the animals in the fed state, being administered an injection of insulin (0.75 U/Kg body weight); tail blood samples were taken at the time points 0 (baseline), 15, 30, and 60 minutes after injection for the measurement of blood glucose levels. The glucometer Accu-Chek Active (Roche®, Suíça) was used for both tests.

Determination of Blood Measurements

Serum was obtained by centrifugation (3,200 rpm for 10 minutes at 4 °C) of the blood samples obtained when the animals were killed. Fasting glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides levels were assessed using enzymatic kits (Wiener®, Argentina). Measurements were performed on a Wiener BT-3000 plus ChemistryAnalyzer (Wiener®, Argentina).

Table 1. Specific mouse primers sequences.

Primer target		Primer sequences (5'-3')
ACE	F	CTC CTG GGA CTT CTA CAAC
	R	CTC CAT GTT CAC AGA GGT ACA CT
ACE2	F	GGA TAC CTA CCC TTC CTA CAT CAG C
	R	CAT CCC CAC ATA TCA CCA AGCA
MASR	F	ACT GCC GGG CGG TCA TCA TC
	R	GGT GGA GAA AAG CAA GGA GA

Note. ACE = angiotensin-converting enzyme; ACE2 = angiotensin-converting enzyme 2; MASR = Mas receptor; F = forward; R = reverse.

Reverse Transcription and qRT-PCR

Total RNA extracted from the liver was prepared using TRIzol reagent (Invitrogen Corp.®, USA), treated with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.®, USA). The endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH), angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE2), and Mas receptor (MASR) were assessed using specific primers and SYBR green reagent (AppliedBiosystems®, USA) in a Plus-One Platform (Applied Biosystems®). Relative comparative CT method was applied to compare gene expression levels between groups, using the $2^{-\Delta\Delta CT}$ equation (Livak & Schmittgen, 2001). Sequences of primers are shown in Table 1.

Statistical Analysis

Data were expressed as means ± standard error of mean (SEM) and analyzed using GraphPad Prism version 5.0 (GraphPad Software, United States). All data were analyzed for normality of distribution using the Kolmogorov-Smirnov test and were found to be normal. Multiple comparisons were performed using one-way ANOVA with Tukey post-hoc analysis. IPGTT and IPITT were analyzed by two-way ANOVA test. Statistical significance was set at $p < 0.05$.

Results

Food/energy Intake and Adiposity

As expected, after 8 weeks (phase 1: obesity induction period) the mice fed on high fat-diet gained more weight than did the ST fed mice (gain weight: ST, 79%; ST + BL, 89%; HFD, 109%; and HFD + ST, 106%; Figure 2A). During the second phase (treatment period), no significant differences were observed in mice body weight; mice treated with *B. longum* displayed a slightly higher weight loss than that of respective control mice (ST, 0.53%; ST + BL, −1.3%; HFD, 0.18; HFD + BL, −1.7%; Figure 2B). Furthermore, *B. longum* had no significant effect on food intake (Figure 2C).

The results evidenced an important reduction in visceral adiposity between HFD and HFD + BL mice (HFD, 0.047 ± 0.005; HFD + BL, 0.028 ± 0.004; reduction in approximately 42.5% in HFD + BL; Figure 3D). Similar results were observed to epididymal adipose tissue (HFD, 0.029 ± 0.007; HFD + BL,

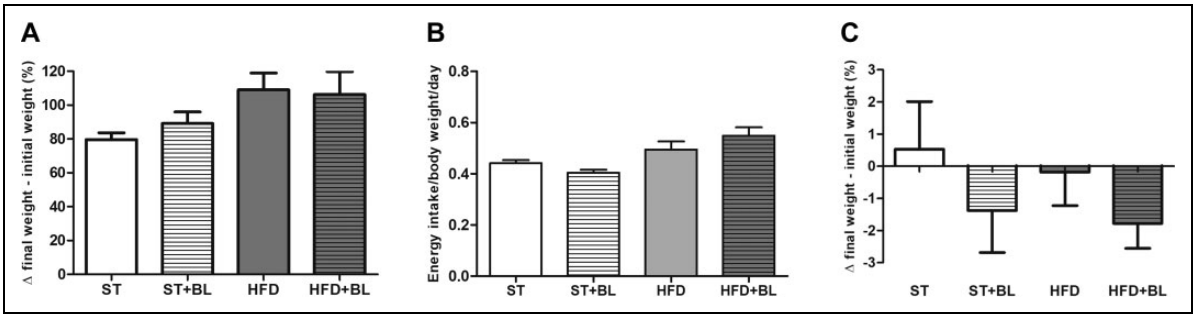

Figure 2. Effect of *B. longum* on food intake and body weight of mice fed a high fat-diet. Body weight difference (day 56—day 1 → 8 weeks) in percentage (A), Body weight difference (day 84 – day 56 → treatment period—4 weeks) in percentage (B), energy intake (C). Data are presented as means ± SEM (n = 8, each group). Statistically significant differences between groups are indicated as *p < 0.05, **p < 0.01, ***p < 0.001 as compared to the HFD. Data were analyzed by One-way ANOVA test.

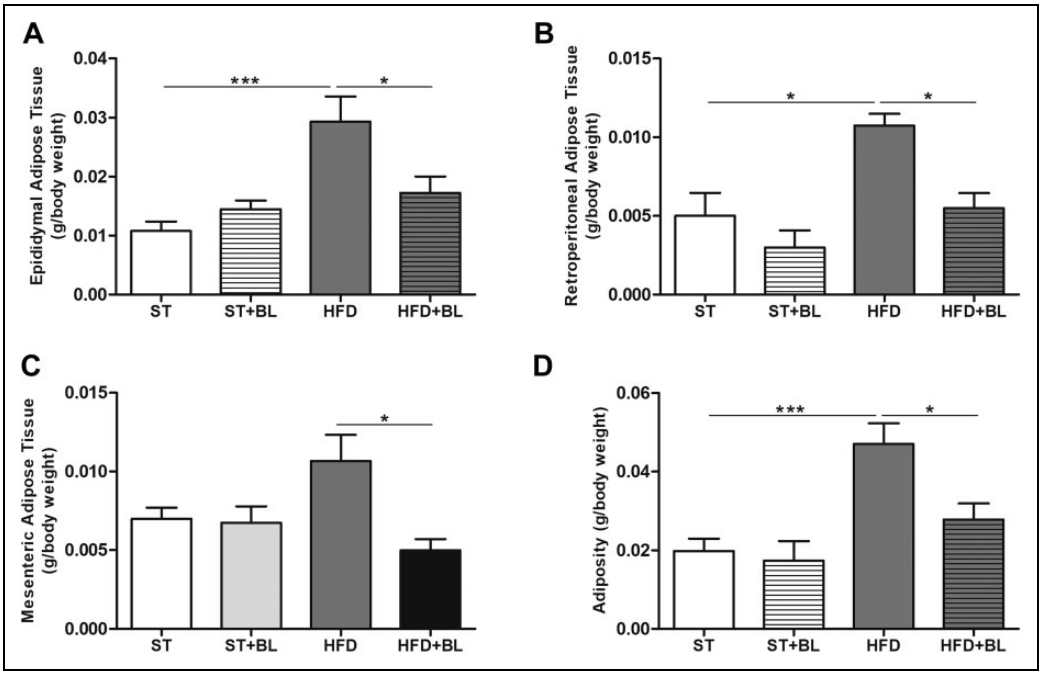

Figure 3. Effect of *B. longum* on adiposity of mice fed a high fat-diet. Visceral adipose tissue weight in different anatomical sites. These values were normalized to body weight. Epididymal adipose tissue (A), retroperitoneal adipose tissue (B), mesenteric adipose tissue (C), total visceral adiposity (D). Data are presented as means ± SEM (n = 8, each group). Statistically significant differences between the groups are indicated as *p < 0.05, **p < 0.01, ***p < 0.001 as compared to the HFD group. Data were analyzed by One-way ANOVA test.

0.017 ± 0.003), retroperitoneal adipose tissue (HFD, 0.01075 ± 0.001; HFD + BL, 0.005 ± 0.001), mesenteric adipose tissue (HFD, 0.007 ± 0.002; HFD + BL, 0.005 ± 0.001) weights (Figure 3A–C).

IPGTT, IPITT and Plasmatic Analyses

Upon glucose challenge, blood glucose levels in HFD + BL mice were significantly lower than those of the HFD group in the IPGTT test (AUC: HFD, 40.543 ± 5.215 vs. HFD + BL, 26.178 ± 2.882; Figure 4A–B), similarly to the tendency observed in fasting glucose levels (HFD, 143.8 mg/dL ± 12.24 vs. HFD + BL, 77.00 mg/dL ± 13.8; Figure 4E). No differences were found between groups in the IPITT test (Figure 4C-D).

When serum lipid parameters were analyzed, a decrease in total cholesterol was observed HFD + BL versus HFD mice (HFD, 156.7 mg/dL ± 23.73 vs. HFD + BL, 105.7 mg/dL ± 5.78; Figure 4F). However, the levels of HLD-c, LDL-c and triglycerides did not differ between the experimental groups and their respective controls (Figure 4G–I).

Histological and qRT-PCR liver analysis

No statistically significant differences were found regarding the liver weight of the mice (Figure 5A). Hence, we performed a histological analysis to examine the effect of BL on the

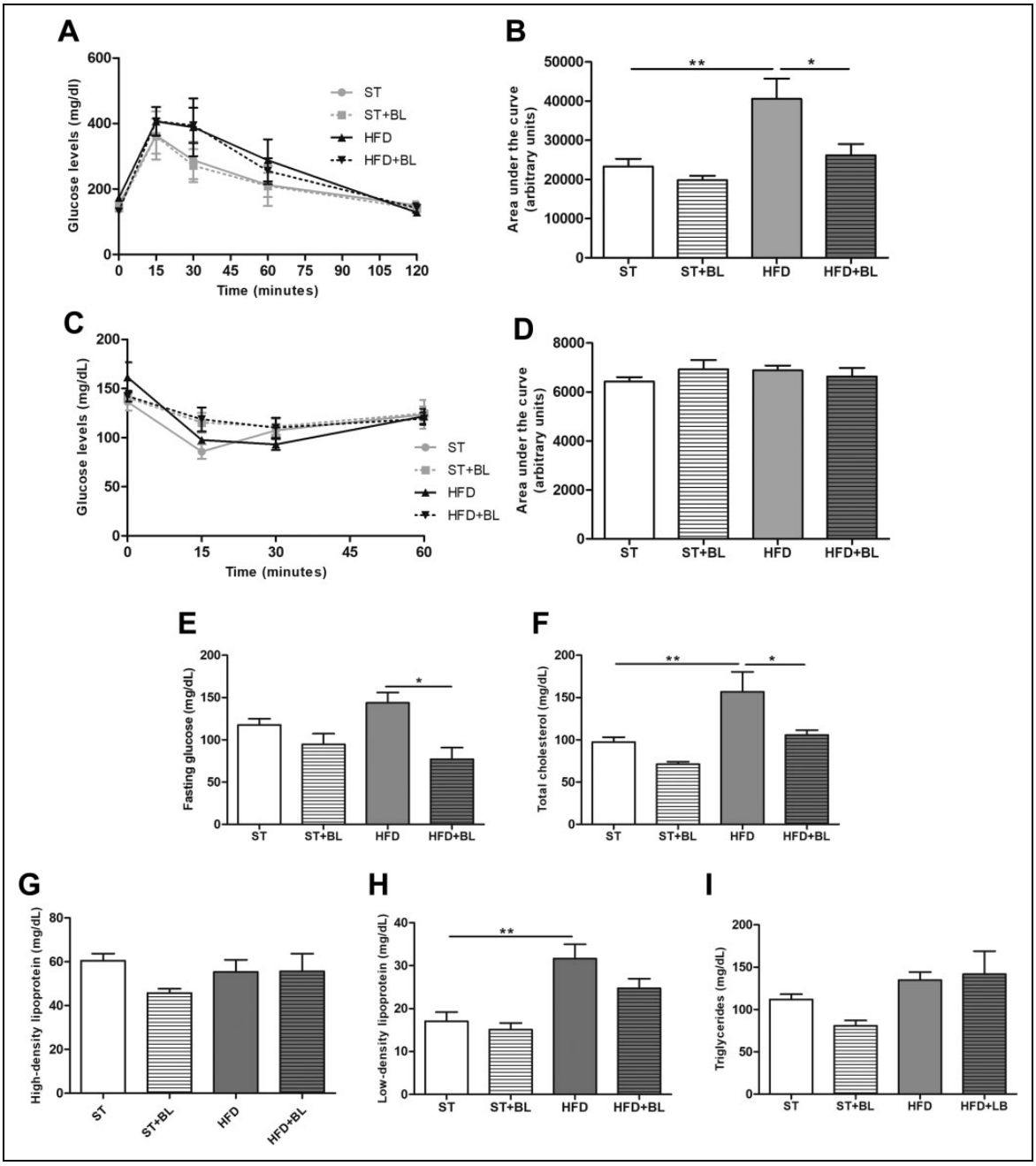

Figure 4. Effect of *B. longum* in glucose tolerance, insulin sensibility and plasmatic metabolic profile of mice fed a high fat-diet. Serum glucose levels following intraperitoneal glucose tolerance test (IPGTT) challenge (A), area under the curve (AUC) for IPGTT (B), serum glucose levels following intraperitoneal insulin tolerance test (IPITT) challenge (C), area under the curve (AUC) for IPITT (D), fasting glucose levels (E), total cholesterol (F), high-density lipoprotein cholesterol (HDL-c) (G), low-density lipoprotein cholesterol (LDL-c) (H) and triglycerides (I). Data are presented as means $\pm$ SEM ($n = 8$, each group). Statistically significant differences between the groups are indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to the HFD group. Data were analyzed by One-way ANOVA test (b, d, and e-i) and Two-way ANOVA test a, and c).

development of intrahepatic fat deposition. Large hepatic lipid droplets were diffusely present in the liver of the HFD group mice as compared with the other groups (Figure 5B).

qRT-PCR analysis showed increased mRNA expression of ACE2 (HFD, 3.143 ± 1.244 vs. HFD + BL, 7.158 ± 1.315) and MASR (HFD, $1.597 \pm 0{,}584$ vs. HFD + BL, $3.137 \pm$ BL, 0.186) in HFD + BL versus HFD mice (Figure 5D–E). No significant differences in the ACE mRNA expression were observed between groups and their respective controls (Figure 5C).

Discussion

The present findings indicate the potential beneficial effects of *B. longum* in mice fed a high-fat diet: reduction of the body

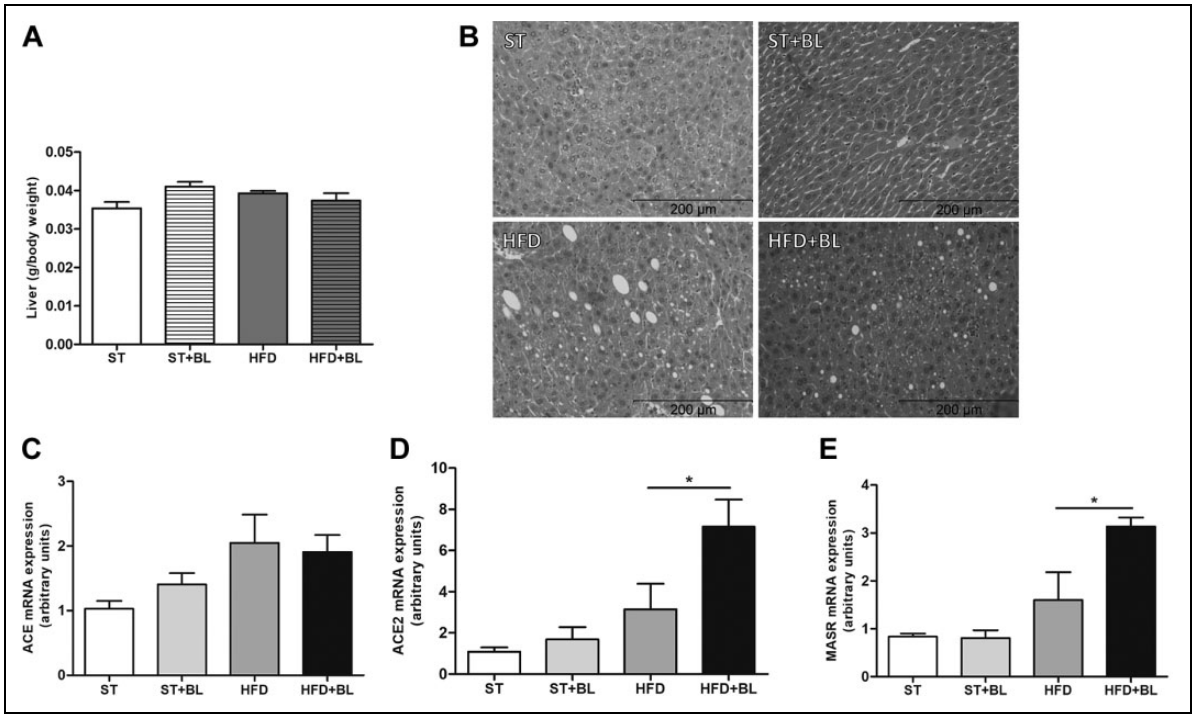

Figure 5. Effect *B. longum* in intracellular lipid accumulation and expression hepatic of the renin-angiotensin system by qRT-PCR in mice fed a high-fat diet. Liver weight (A), Hematoxylin & Eosin (HE)-staining liver sections—scale bar, 200 µm. (B), angiotensin-converting enzyme (ACE) mRNA levels (C), angiotensin-converting enzyme 2 (ACE2) mRNA levels (D), and Mas receptor (MASR) mRNA levels (E). Data are presented as means ± SEM (n = 8, each group). Statistically significant differences between groups are indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to the HFD group. Data were analyzed by one-way ANOVA test.

adiposity, improvement in the glycemic/lipid profiles, and the hepatic histological pattern of fat deposition. Furthermore, increased mRNA expression of ACE2 and MASR, were observed. Therefore, even in a preliminary way, *B. longum* may modulate the renin-angiotensin system components in the liver and by consequence lead to improvements in the metabolic profile, thus supporting our hypothesis.

Experimental and clinical studies have shown that supplementation with probiotics has different metabolic effects and that these effects are strain-dependent (Dong et al., 2012; Million et al., 2012). There are several mechanisms by which bacterial strains perform their functions (Firouzi et al., 2013; Parvaneh et al., 2015). In particular, the genus *Bifidobacterium spp.* consists of anti-obesity microorganisms and comprises a group of gram-positive bacteria, representing the most common genus in the human gastrointestinal tract, exerting a key role in the promotion of beneficial health effects (An et al., 2011; In Kim et al., 2019; Le et al., 2014). *B. longum*, one species found in this group, deserves special attention. These therapeutic options are especially important considering the common gastrointestinal and metabolic abnormalities founding in daily nursing practice.

Our study demonstrated an important reduction in the body adiposity of mice fed a high-fat diet supplemented with *B. longum*, despite the absence of differences in energy intake. The *B. longum* capacity to decrease body adiposity without affecting food intake might be linked to the modulation of genes involved in the processes of adipogenesis, lipolysis, and lipogenesis. Karimi et al. found that *B. longum* bacteria supplementation displayed better results than other bacterial species in obese mice in terms of modulating fat mass and adipocyte size (Karimi et al., 2017). An et al. (2011) corroborated these same findings, providing evidence for the *B. longum's* ability to decrease body adiposity. Furthermore, in the elderly, *B. longum* was capable of reducing body mass index (Inoue et al., 2018). Accordingly, strong evidence exists for the renin-angiotensin system's role in white adipose tissue modulation. Studies suggest that the activation of the Ang-(1-7)/ACE2/MASR axis may lead to decreased body adiposity (de Macedo et al., 2015; Oliveira Andrade et al., 2014). Supporting these findings, Santos et al. (2008) observed increased adipocytes size in MASR knockout mice.

Improvement in glucose tolerance and reduction in serum glucose levels of obese animals that received *B. longum* correspond to the results of other studies (Backhed et al., 2007; Cani et al., 2007; Chen et al., 2011; Ley et al., 2005) and may be directly associated with particularities of the interaction of this microorganism with the intestinal microbiota of the host (Mazloom et al., 2019). The increase in the *Bifidobacterium* population is directly related to the lean phenotype and metabolic improvement (Backhed et al., 2005; Ley et al., 2006). Microorganisms colonizing the intestinal microbiota have the ability to degrade polysaccharides and dietary fiber, producing short-chain fatty acids (SCFAs). In the intestine, SCFAs bind to

the short-chain fatty acid receptor (GPR43) leading to secretion of anorexigenic peptides, including glucagon-like peptide-1 (GLP-1) and YY peptide (PYY), resulting in improved glucose tolerance and increased energy use, which may explain the decrease in body adiposity (Mazloom et al., 2019).

Recent studies have found that bacteria producing lactic acid, including *Bifidobacterium longum* have hypocholesterolemic effects in rats and humans (Xiao et al., 2003). Possible mechanisms have been suggested. For example, fermentation products of lactic acid bacteria might inhibit enzymes involved in the synthesis of cholesterol, thus reducing their production. Alternatively, lactic acid bacteria could facilitate the elimination of cholesterol in feces, or bacteria may inhibit the absorption of cholesterol back into the body by binding to cholesterol. In addition, bacteria might interfere with the process of recycling bile salt (a metabolic product of cholesterol) and facilitate its elimination, which increases the demand for bile salt produced from cholesterol, resulting in the consumption of body cholesterol (Beena & Prasad, 1997; Fukushima & Nakano, 1995; Kim et al., 2017).

Obese animals that received *B. longum* had a lower deposition of liver fat. Recently, Xu et al. demonstrated that *B. longum* supplementation attenuated the accumulation of hepatic fat in a model of non-alcoholic fatty liver disease (Xu et al., 2012). Furthermore, supplementation with VSL#3, a preparation composed of a mixture of four strains of *Lactobacillus* and *Bifidobacterium,* including *B. longum* improved the fat-induced dietary hepatic steatosis and insulin resistance in mice by modulating hepatic natural killer T cells (NKT cells), suppressing the TNF-alpha/IKK signaling pathway, and reducing the inflammatory signaling (Ma et al., 2008). Some studies have also suggested that *B. longum* can mitigate hepatic injuries caused by endotoxin-induced activation of macrophages (Fuller, 1991; Liu et al., 2004).

Increased hepatic ACE2 and MASR expression observed in obese animals treated with *B. longum* describes, for the first time, a modulation of the ACE2/Ang-(1-7)/MASR axis by a probiotic strain. In addition, the metabolic improvement observed in treated animals is associated with RAS since the increased expression of ACE2 and MASR is directly related to the increase in Ang-(1-7) (Santos & Simoes e Silva, 2014). The first study showing the metabolic potential of Ang-(1-7)/MASR, demonstrated a worsening of metabolic efficiency, as well as dyslipidemia, insulin resistance, and decreased expression of adiponectin and GLUT-4 in transgenic mice with MASR suppression (Santos et al., 2008).

Regarding hepatic homeostasis, an oral administration of Ang-(1-7) may decrease gluconeogenesis in the liver, improving glucose and lipid metabolism (Bilman et al., 2012). In addition, Ang-(1-7) decreased inflammatory marker levels by downregulation of the resistin/TLR4/NF-KB pathway in obese rats (Santos et al., 2013). Another remarkable finding was that obese mice treated with an oral formulation of Ang-(1-7) had a significant reduction in levels of TNF-alpha and IL-6, as well as decreased markers related to adipogenesis, such as ACC, PPAR-Y, and SREBP-1c (Feltenberger et al., 2013).

In summary, we conclude that oral supplementation with *B. longum* may improve important metabolic parameters, especially in the liver, of mice fed a high-fat diet. Furthermore, the beneficial effects observed by the use of *B. longum* may be attributed, even partially, to the modulation of the renin-angiotensin system ACE2/Ang-(1-7)/MASR axis. Although further studies are necessary for firmer conclusions, the present study provides important perspectives in the comprehension of the *B. longum* anti-obesity effects.

Abbreviations

ACE	angiotensin-converting enzyme
ACE2	angiotensin-converting enzyme 2
B. longum	*Bifidobacterium longum*
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GLP-1	glucagon-like peptide-1
GPR43	short chain fatty acid receptor
GLUT-4	glucose transporter type 4
HDL	high-density lipoprotein
HFD	high-fat diet
IKK-β	IκB kinase beta
LPS	lipopolysaccharides
Mas	Mas Receptor
NAFLD	non-alcoholic fatty liver disease
NF-KB	factor nuclear kappa B
NKT	natural killer cells
qRT-PCR	quantitative real time-PCR
PYY	peptide YY
SCFAs	short-chain fatty acids
SCFAs	short chain fatty acid
SEM	standard error
ST	standard diet
TNF-α	tumor necrosis factor alpha
TRL4	toll-like receptor 4.

Declaration of Conflicting Interests

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References

An, H. M., Park, S. Y., Lee, D. K., Kim, J. R., Cha, M. K., Lee, S. W., Lim, H. T., & Kim, K. J. (2011). Antiobesity and lipid-lowering effects of Bifidobacterium spp. in high fat diet-induced obese rats. *Lipids in Health and Disease*, *10*, 116.

Aron-Wisnewsky, J., Vigliotti, C., Witjes, J., Le, P., Holleboom, A. G., Verheij, J., Nieuwdorp, M., & Clément, K. (2020). Gut microbiota and human NAFLD: Disentangling microbial signatures from metabolic disorders. *Nature Reviews Gastroenterology & Hepatology*, *17*(5), 279–297.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*, *307*(5717), 1915–1920.

Backhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(3), 979–984.

Beena, A., & Prasad, V. (1997). Effect of yogurt and bifidus yogurt fortified with skim milk powder, condensed whey and lactose-hydrolysed condensed whey on serum cholesterol and triacylglycerol levels in rats. *Journal of Dairy Research*, *64*(3), 453–457.

Bilman, V., Mares-Guia, L., Nadu, A. P., Bader, M., Campagnole-Santos, M. J., Santos, R. A., & Santos, S. H. (2012). Decreased hepatic gluconeogenesis in transgenic rats with increased circulating angiotensin-(1-7). *Peptides*, *37*(2), 247–251.

Bleau, C., Karelis, A. D., St-Pierre, D. H., & Lamontagne, L. (2015). 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/Metabolism Research and Reviews*, *31*(6), 545–561.

Bluher, M. (2019). Obesity: Global epidemiology and pathogenesis. *Nature Reviews Endocrinology*, *15*(5), 288–298.

Borem, L. M. A., Neto, J. F. R., Brandi, I. V., Lelis, D. F., & Santos, S. H. S. (2018). The role of the angiotensin II type I receptor blocker telmisartan in the treatment of non-alcoholic fatty liver disease: A brief review. *Hypertension Research*, *41*(6), 394–405.

Brunt, E. M., Wong, V. W., Nobili, V., Day, C. P., Sookoian, S., Maher, J. J., Bugianesi, E., Sirlin, C. B., & &Neuschwander-Tetri, B. A. (2015). Nonalcoholic fatty liver disease. *Nature Reviews Disease Primers*, *1*, 15080.

Brusaferro, A., Cozzali, R., Orabona, C., Biscarini, A., Farinelli, E., Cavalli, E., Grohmann, U., Principi, N., & Esposito, S. (2018). Is it time to use probiotics to prevent or treat obesity? *Nutrients*, *10*(11), 1613.

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A. M., Francesca Fava, F., Tuohy, K. M., Chabo, C., Waget, A., Delmée, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrières, J., Tanti, J.-F., Gibson, G. R., Casteilla, L., & … Burcelin, R. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, *56*(7), 1761–1772.

Chen, J. J., Wang, R., Li, X. F., & Wang, R. L. (2011). Bifidobacterium longum supplementation improved high-fat-fed-induced metabolic syndrome and promoted intestinal Reg I gene expression. *Experimental Biology and Medicine (Maywood)*, *236*(7), 823–831.

Choquet, H., & Meyre, D. (2011). Molecular basis of obesity: Current status and future prospects. *Current Genomics*, *12*(3), 154–168.

Cobbina, E., & Akhlaghi, F. (2017). Non-alcoholic fatty liver disease (NAFLD)—Pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. *Drug Metabolism Reviews*, *49*(2), 197–211.

Collaboration, N. C. D. R. F. (2016). Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet*, *387*(10026), 1377–1396.

de Macedo, S. M., Guimarares, T. A., Andrade, J. M., Guimaraes, A. L., Batista de Paula, A. M., Ferreira, A. J., & Sousa Santos, S. H. (2015). Angiotensin converting enzyme 2 activator (DIZE) modulates metabolic profiles in mice, decreasing lipogenesis. *Protein and Peptide Letters*, *22*(4), 332–340.

Dong, H., Rowland, I., & Yaqoob, P. (2012). Comparative effects of six probiotic strains on immune function in vitro. *British Journal of Nutrition*, *108*(3), 459–470.

Feltenberger, J. D., Andrade, J. M., Paraiso, A., Barros, L. O., Filho, A. B., Sinisterra, R. D., Sousa, F. B., Guimarães, A. L., de Paula, A. M., Campagnole-Santos, M. J., Qureshi, M., dos Santos, R. A., & Santos, S. H. (2013). Oral formulation of angiotensin-(1-7) improves lipid metabolism and prevents high-fat diet-induced hepatic steatosis and inflammation in mice. *Hypertension*, *62*(2), 324–330.

Firouzi, S., Barakatun-Nisak, M. Y., Ismail, A., Majid, H. A., & Nor Azmi, K. (2013). Role of probiotics in modulating glucose homeostasis: Evidence from animal and human studies. *International Journal of Food Sciences and Nutrition*, *64*(6), 780–786.

Fukushima, M., & Nakano, M. (1995). The effect of a probiotic on faecal and liver lipid classes in rats. *British Journal of Nutrition*, *73*(5), 701–710.

Fuller, R. (1991). Probiotics in human medicine. *Gut*, *32*(4), 439–442.

Goh, G. B., Pagadala, M. R., Dasarathy, J., Unalp-Arida, A., Sargent, R., Hawkins, C., Sourianarayanane, A., Khiyami, A., Yerian, L., Pai, R., McCullough, A. J., & Dasarathy, S. (2015). Renin-angiotensin system and fibrosis in non-alcoholic fatty liver disease. *Liver International*, *35*(3), 979–985.

Haslam, D. W., & James, W. P. (2005). Obesity. *Lancet*, *366*(9492), 1197–1209.

In Kim, H., Kim, J. K., Kim, J. Y., Jang, S. E., Han, M. J., & Kim, D. H. (2019). Lactobacillus plantarum LC27 and Bifidobacterium longum LC67 simultaneously alleviate high-fat diet-induced colitis, endotoxemia, liver steatosis, and obesity in mice. *Nutrition Research*, *67*, 78–89.

Inoue, T., Kobayashi, Y., Mori, N., Sakagawa, M., Xiao, J. Z., Moritani, T., Sakane, N., & Nagai, N. (2018). Effect of combined bifidobacteria supplementation and resistance training on cognitive function, body composition and bowel habits of healthy elderly subjects. *Beneficial Microbes*, *9*(6), 843–853.

Karimi, G., Jamaluddin, R., Mohtarrudin, N., Ahmad, Z., Khazaai, H., & Parvaneh, M. (2017). Single-species versus dual-species probiotic supplementation as an emerging therapeutic strategy for obesity. *Nutrition, Metabolism & Cardiovascular Diseases*, *27*(10), 910–918.

Karimi, G., Jamaluddin, R., & Parvaneh, K. (2013). the effects of probiotics on body weight and biomarkers of animal. *Pakistan Journal of Nutrition*, *12*, 793–799.

Karlsson, F., Tremaroli, V., Nielsen, J., & Backhed, F. (2013). Assessing the human gut microbiota in metabolic diseases. *Diabetes*, *62*(10), 3341–3349.

Kim, S. J., Park, S. H., Sin, H. S., Jang, S. H., Lee, S. W., Kim, S. Y., Kwon, B., Yu, K. Y., Kim, S. Y., & Yang, D. K. (2017). Hypocholesterolemic effects of probiotic mixture on diet-induced hypercholesterolemic rats. *Nutrients*, *9*(3).

Krumbeck, J. A., Rasmussen, H. E., Hutkins, R. W., Clarke, J., Shawron, K., Keshavarzian, A., & Walter, J. (2018). Probiotic Bifidobacterium strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. *Microbiome*, *6*(1), 121.

Le, T. K., Hosaka, T., Le, T. T., Nguyen, T. G., Tran, Q. B., Le, T. H., & Pham, X. D. (2014). Oral administration of Bifidobacterium spp. improves insulin resistance, induces adiponectin, and prevents inflammatory adipokine expressions. *Biomedical Research*, *35*(5), 303–310.

Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(31), 11070–11075.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: Human gut microbes associated with obesity. *Nature*, *444*(7122), 1022–1023.

Liu, Q., Duan, Z. P., Ha, D. K., Bengmark, S., Kurtovic, J., & Riordan, S. M. (2004). Synbiotic modulation of gut flora: Effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology*, *39*(5), 1441–1449.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, *25*(4), 402–408.

Lynch, S. V., & Pedersen, O. (2016). The human intestinal microbiome in health and disease. *The New England Journal of Medicine*, *375*(24), 2369–2379.

Ma, X., Hua, J., & Li, Z. (2008). Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. *Journal of Hepatology*, *49*(5), 821–830.

Mazloom, K., Siddiqi, I., & Covasa, M. (2019). Probiotics: How effective are they in the fight against obesity? *Nutrients*, *11*(2).

Miller, A. J., & Arnold, A. C. (2019). The renin-angiotensin system in cardiovascular autonomic control: recent developments and clinical implications. *Clinical Autonomic Research*, *29*(2), 231–243.

Million, M., Angelakis, E., Paul, M., Armougom, F., Leibovici, L., & Raoult, D. (2012). Comparative meta-analysis of the effect of Lactobacillus species on weight gain in humans and animals. *Microb Pathog*, *53*(2), 100–108.

Oliveira Andrade, J. M., Paraiso, A. F., Garcia, Z. M., Ferreira, A. V., Sinisterra, R. D., Sousa, F. B., Guimarães, A. L., de Paula, A. M., Campagnole-Santos, M. J., dos Santos, R. A., & Santos, S. H. (2014). Cross talk between angiotensin-(1-7)/Mas axis and sirtuins in adipose tissue and metabolism of high-fat feed mice. *Peptides*, *55*, 158–165.

Parvaneh, K., Ebrahimi, M., Sabran, M. R., Karimi, G., Hwei, A. N., Abdul-Majeed, S., Ahmad, Z., Ibrahim, Z., & Jamaluddin, R. (2015). Probiotics (Bifidobacterium longum) increase bone mass density and upregulate Sparc and Bmp-2 genes in rats with bone loss resulting from ovariectomy. *BioMed Research International*, *2015*, 897639.

Patel, S., Rauf, A., Khan, H., & Abu-Izneid, T. (2017). Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. *Biomedical Pharmacotherapy*, *94*, 317–325.

Polyzos, S. A., Kountouras, J., & Mantzoros, C. S. (2019). Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. *Metabolism*, *92*, 82–97.

Rashid, S. K., Idris-Khodja, N., Auger, C., Alhosin, M., Boehm, N., Oswald-Mammosser, M., & Schini-Kerth, V. B. (2014). Probiotics (VSL#3) prevent endothelial dysfunction in rats with portal hypertension: role of the angiotensin system. *PLoS One*, *9*(5), e97458.

Rein, J., & Bader, M. (2017). Renin-angiotensin system in diabetes. *Protein & Peptide Letters*, *24*(9), 833–840.

Rocha, V. Z., & Libby, P. (2009). Obesity, inflammation, and atherosclerosis. *Nature Reviews Cardiology*, *6*(6), 399–409.

Safari, Z., & Gerard, P. (2019). The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cellular and Molecular Life Sciences*, *76*(8), 1541–1558.

Santos, S. H., Andrade, J. M., Fernandes, L. R., Sinisterra, R. D., Sousa, F. B., Feltenberger, J. D., Alvarez-Leite, J. I., & Santos, R. A. (2013). Oral angiotensin-(1-7) prevented obesity and hepatic inflammation by inhibition of resistin/TLR4/MAPK/NF-kappaB in rats fed with high-fat diet. *Peptides*, *46*, 47–52.

Santos, S. H., Fernandes, L. R., Mario, E. G., Ferreira, A. V., Porto, L. C., Alvarez-Leite, J. I., Botion, L. M., Bader, M., Alenina, N., & Santos, R. A. (2008). Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. *Diabetes*, *57*(2), 340–347.

Santos, S. H., & Simoes e Silva, A. C. (2014). The therapeutic role of renin-angiotensin system blockers in obesity-related renal disorders. *Current Clinical Pharmacology*, *9*(1), 2–9.

Tilg, H., Zmora, N., Adolph, T. E., & Elinav, E. (2020). The intestinal microbiota fuelling metabolic inflammation. *Nature Reviews Immunology*, *20*(1), 40–54.

Xiao, J. Z., Kondo, S., Takahashi, N., Miyaji, K., Oshida, K., Hiramatsu, A., Iwatsuki, K., Kokubo, S., & Hosono, A. (2003). Effects of milk products fermented by Bifidobacterium longum on blood lipids in rats and healthy adult male volunteers. *Journal of Dairy Science*, *86*(7), 2452–2461.

Xu, R. Y., Wan, Y. P., Fang, Q. Y., Lu, W., & Cai, W. (2012). Supplementation with probiotics modifies gut flora and attenuates liver fat accumulation in rat nonalcoholic fatty liver disease model. *Journal of Clinical Biochemistry and Nutrition*, *50*(1), 72–77.