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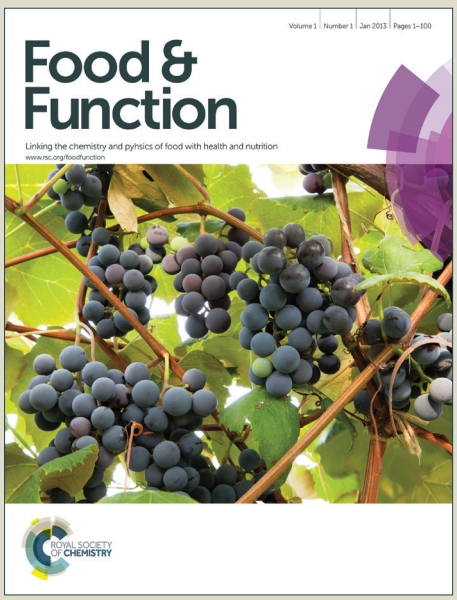

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Lactobacillus plantarum NCU116 improves liver function, oxidative stress and lipid metabolism in high fat diet induced non-alcoholic fatty liver disease rats

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Abstract

The effect of *Lactobacillus plantarum* NCU116 on liver function, oxidative stress and lipid metabolism in rats with high fat diet induced non-alcoholic fatty liver disease (NAFLD) were studied. The rats were divided into four groups: normal diet (ND) group; high fat diet (HFD) group, HFD plus *L. plantarum* NCU116 two doses (NCU116-L, 10^8CFU/mL; NCU116-H, 10^9 CFU/mL) groups. Treatment of *L. plantarum* NCU116 for 5 weeks was found to restore liver function and oxidative stress in rats with NAFLD, and decrease the levels of fat accumulation in liver. In addition, the bacterium significantly reduced endotoxin and proinflammatory cytokines, and regulated bacterial flora in the colon and the expression of lipid metabolism in the liver. These results suggest that possible underlying mechanisms for beneficial effect of *L. plantarum* NCU116 on NAFLD may include two pathways of downregulating lipogenesis and upregulating lipolysis and fatty acid oxidation related genes expression.

Keywords: *Lactobacillus plantarum* NCU116; Non-alcoholic fatty liver disease; High fat diet; liver function; oxidative stress; lipid metabolism.

1 Introduction

Non-alcoholic fatty liver disease (NAFLD), the major reason for abnormal liver function worldwide, is considered to be an integral part of the metabolic syndrome that is associated with obesity, hyperlipidemia and diabetes.[1, 2] NAFLD includes a spectrum of pathologies from simple steatosis (fatty liver) to variable fibrosis and meets criteria for non-alcoholic steatohepatitis.[3, 4] NAFLD is defined by accumulation of liver fat > 5% of liver weight with <10 g of daily alcohol consumption.[5] A number of animal studies have investigated the influence of high fat diets on the composition of intestinal microbiota and the effects on inflammation, and development of obesity-related metabolic complications, such as NAFLD.[6]

In recent years, studies have suggested that intestinal flora could inhibit the development of obesity-associated fatty liver.[2] Alterations of microbiota in intestine seem to play a significant role in liver damage. In addition, application of probiotics has been proposed as a potential prevention strategy for different types of chronic liver damage, for their ability to improve intestinal barrier function.[7] Probiotics are live microorganisms which when administered in adequate amounts confer a promoting property to the host health and disease modulating intestinal microbiota composition and function, improving epithelial barrier function, and reducing inflammation.[8, 9] Several strains of *Lactobacillus* have been reported to exhibit protective effects on NAFLD in rodent models, but the mechanisms of lipid metabolism in liver has not been fully understood yet.[10]

L. plantarum NCU116, a newly identified probiotic, was isolated from pickled

vegetables in our laboratory.[11] Previous studies have shown that the probiotic is characterized with good performance *in vitro* and the cholesterol lowering effect *in vivo*.[12, 13] To our knowledge, these properties may associate with the improvement of NAFLD. Therefore, the aim of this study was to investigate the effects of *L. plantarum* NCU116 in high fat diet induced liver steatosis and oxidative stress in an animal model.

2 Materials and methods

2.1 Experimental animals

Forty male Sprague-Dawley rats (120 to 150 g) were obtained from Vital River Lab Animal Technology Co., Ltd (Certificate number: SCXK (Jing) 2012-0001, Beijing, China). Before starting the experiments, all animals were housed at an ambient temperature of 23 ± 1 ℃, 12/12 h of light–dark cycle with *ad libitum* food and water to acclimatize the laboratory conditions for one week.

All animals used in this study were cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication 85-23, 1996), and all experimental procedures were approved by the Nanchang University Medical College Animal Care Review Committee.

2.2 Experimental design

After acclimation, 10 rats were fed a normal diet as the ND group, the others were fed with high fat diet. Rats fed on high fat diet were randomly divided into three

groups: high fat diet (HFD) group; rats on HFD plus oral administration 10^8 CFU/mL *L. plantarum* NCU116 (NCU116-L, 10 mL per kilogram body weight) group and HFD plus oral administration 10^9 CFU/mL *L. plantarum* NCU116 (NCU116-H, 10 mL per kilogram body weight) group. Rats in ND and HFD groups received the same volume of vehicle per day during the same period. *L. plantarum* NCU116 were suspended in sterile saline solution and diluted to the designated doses. The dietary treatments continued for remaining days of the study. The high fat diet consists of normal diet (66.5%, w/w), lard (10.0%), sucrose (20.0%), cholesterol (2.5%) and sodium cholate (1.0%). Both of the normal and high fat diets were provide by Medical College of Nanchang University.

At the end of the 5 weeks feeding experiment, the rats were humanly anesthetized with chloral hydrate via peritoneal injection. Blood samples were obtained by cardiac puncture and centrifuged at 1000 × g for 10 min and the serum was removed for further analyses. Samples of liver, adipose tissue, spleen and feces in colon were quickly removed, and stored at -80 ºC until used. Liver and adipose tissue indices were calculated by the following formula: An organ index =Weight of an organ (g)/Weight of a body (g) × 100.

2.3 Analyses of liver function and oxidative stress

Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), and levels of total bilirubin (TBil), malondialdehyde (MDA) and total anti-oxidant capacity (T-AOC) were determined using commercial kits (Jiancheng Bioengineering,

Nanjing, China). The absorbance values were measured by a Varioskan Flash (Thermo Scientific, Waltham, MA, USA).

2.4 Analyses of lipopolysaccharide and cytokines

Contents of serum lipopolysaccharide (LPS), interleukin (IL)-6, IL-10 and tumor necrosis factor (TNF)-α were determined by ELISA kits (Westang Bio-Tech, Shanghai, China) according to manufacturer's instructions.

2.5 Measurement of fatty acids, cholesterol and triacylglycerols in liver

Liver samples were smashed into fine powder in liquid nitrogen. 1 g of every sample was extracted by using chloroform/methanol (1:1) and the total lipids were methylated using sodium methoxide.[14] 6890N gas chromatograph (GC) system equipped with a flame ionization detector (FID), a GC column (CP-Sil 88, 100 m x 0.25 mm I.D. coated with 0.20 μm film thickness, Agilent Technologies Inc., USA) were used to analyze the fatty acid methyl esters (FAME). The initial temperature of the program was 60 °C (held for 5 min), and then increased at a rate of 11.5 °C/min to 170 °C (held for 25 min), further increased to 200 °C at 5 °C/min (held for 5 min), and finally rose at a rate of 2 °C/min to 215 °C and held for 20 min. The temperatures of the FID and injection port were 250 °C. The flow rates of hydrogen and air were 26 and 300 mL/min, respectively. The injected sample volume for GC analysis was 1 μL. The analysis method of fatty acids was used as described previously.[15, 16] Levels of liver lipids including total cholesterol (TC) and triacylglycerols (TG) were determined using an assay kit (Beihua-Kangtai, Beijing, China) according to manufacturer's instructions.

125 **2.6 RT-qPCR analyses**

126 The expression levels of lipid metabolism (lipolysis, fatty acid oxidation and
127 lipogenesis) in the liver were analysed by RT-qPCR. The colon feces were removed
128 for *Lactobacillus*, *Bifidobacterium*, *Enterobacteriaceae* and *Bacteroides* groups
129 expression. The liver and colon feces samples were transferred to TRIzol reagent
130 (Life Technologies, Carlsbad, CA, USA) for total RNA extraction. 2 μg of total RNA
131 were used to synthesize first strand cDNA by reverse transcription using the
132 RevertAid™ First Strand cDNA Synthesis Kit (Thermo Scientific Fermentas, Vilnius,
133 Lithuania) according to the manufacturer's instructions. PCR reactions were
134 conducted by 7900HT real-time PCR System (Applied Biosystems, Foster City, CA,
135 USA) using SYBR® Premix Ex Taq™ (Takara, Kusatsu, Japan). Data analysis was
136 carried out using the $2^{-\Delta\Delta CT}$ method. The sequences of the primers used are listed in
137 Supplementary Table 1 (Invitrogen China Limited, Beijing, China).

138 **2.7 Bacterial translocation**

139 Bacterial translocation to liver and spleen samples were determined as a previous
140 study.[17] Briefly, the samples were aseptically removed, weighed and homogenized in
141 sterile 0.1% (w/v) peptone solution. Serial dilutions of the homogenate were plated in
142 triplicate to detect a wide range of microorganisms in the following media: MRS, BHI
143 and, MacConkey (Land Bridge Technology, Beijing, China) a wide range of
144 microorganisms. Microbial growth was evaluated after incubation at 37 °C for 48-72
145 h.

146 **2.8 Statistical analysis**

Results were expressed as mean ± standard error of mean, and the data were analyzed by SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to compare the differences among various groups. Difference with P value < 0.05 was considered statistical significant.

3. Results and discussion

3.1 Effect of *L. plantarum* NCU116 treatment on liver function

In this study, we have demonstrated that *L. plantarum* NCU116 effectively ameliorated the steatosis and attenuated damage in liver in a HFD induced NAFLD rat model. When individuals experience the disease, the levels of hepatic indicators, such as AST, ALT and TBil, are significantly increased.[18]

Activities of ALT, AST and level of TBil of HFD group were markedly higher than those of the ND group (P < 0.05, Figure 1), which were decreased by supplementation of *L. plantarum* NCU116. These results suggested that *L. plantarum* NCU116 improved liver function in the rats with HFD-induced NAFLD.

Lactic acid bacteria have been shown to improve these parameters of liver function in some animal models.[19, 20] It has been reported that lower ALT and AST levels meant better liver function.[21] It might be because that *L. plantarum* NCU116 improved the intestinal barrier effect and decreased the aggravating injury.

3.2 Effect of *L. plantarum* NCU116 treatment on oxidative stress

Previous studies have shown that fat rich diets increase free radicals and cause

oxidative stress, which is a key role in the progression of NAFLD.[22] The increased production of reactive oxygen species generates lipid peroxides, leading to subsequent damage to hepatic membranes, proteins, and DNA.[23] In the present study, the rats in HFD group showed lower activities of SOD, GSH-Px and CAT, a lower level of T-AOC, and higher MDA content compared with ND group ($P < 0.05$; Table 1). The treatment of *L. plantarum* NCU116 improved those parameters to varying degrees. Especially, the NCU116-H group showed higher activities of GSH-Px (4031.01 U/mL) and CAT (14.51 U/mL), and a higher level of T-AOC (5.24 U/mL), and lower MDA content (5.61 nmol/mL) than HFD group ($P < 0.05$).

Oxidative stress is considered to be a significant factor affecting the process of aging and species longevity, although aging is a multicausal complex process.[24] Each type of organisms has its own antioxidant defense system, such as SOD, GSH-Px, CAT and T-AOC.[25, 26] MDA is toxic to DNA and protein, and often used as a marker of lipid peroxidation.[27] In the present study, the administration of *L. plantarum* NCU116 caused as a significant increase in activities of SOD, GSH-Px and CAT and level of T-AOC as well as reduction in MDA content (Table 1). Thus, *L. plantarum* NCU116 might act as a potential anti-oxidant reagent and reduce oxidative stress.

3.3 *L. plantarum* NCU116 improves the fat accumulation in liver

HFD diet induced metabolic syndrome is characterized by greater fat mass, especially visceral adipose tissue mass.[28] As shown in Figure 2 A and B，HFD diet was found to obviously raise liver and adipose tissue indices than the normal diet, suggesting that HFD induced NAFLD in the rats. Interestingly, the treatment of *L.*

191 *plantarum* NCU116 led to a significant reduction of liver and adipose tissue indices in
192 the rats with HFD induced NAFLD. Reports demonstrated that an excess nutrient
193 supply caused adipocyte hypertrophy and adipocyte insulin resistance. A previous
194 study showed that *L. plantarum* NCU116 effectively improved insulin sensitivity and
195 restored liver and adipose tissues damage in HFD diet fed rats.[13] Although the
196 mechanism involved could be not clarified, we speculated that *L. plantarum* NCU116
197 exerted anti-NAFLD effects by preventing metabolic disturbances in liver and adipose
198 tissues.[29]

199 In addition, the levels of most fatty acids in liver of animals fed the HFD diet
200 were significantly higher than those in rats fed ND diet (Table 2). ∑SFA (18.539
201 mg/g), ∑MUFA (28.156 mg/g), ∑PUFA (24.405 mg/g), ∑trans (0.097 mg/g) and ∑FA
202 (71.197 mg/g) in HFD group were 1.91, 13.60, 2.07, 5.71 and 3.02 fold higher than
203 that of ND group. In addition, *trans* fatty acids of ctt/cct/ctc/tcc 18:3 in ND group
204 were not detected. The concentrations of fatty acids in NCU116-L and NCU116-H
205 groups reduced differently. Similarly, liver TC and TG levels differed significantly
206 among the four groups (Figure 2 C and D). The liver TC and TG levels of rats fed a
207 HFD diet had greatly increased compared with ND group. *L. plantarum* NCU116
208 could significantly reduce the liver TC and TG levels comparing with HFD group;
209 and the dose of 10^9 CFU/mL was more effective than 10^8 CFU/mL.

210 This study showed that administration of high fat dietary substrate changed the
211 fat accumulation of liver in rats. Interestingly, dietary supplementation with *L.*
212 *plantarum* NCU116 decreased the concentrations of total fatty acids in liver. This

study showed that fatty acid composition, TC and TG in liver was influenced by oral administration of a metabolically active commensal acting on a dietary substrate. Excess fat accumulation in hepatocytes may lead to hepatocellular injury mediated by oxidative stress and lipid peroxidation.[30] From the results ALT, AST, organs indices, TC, TG and fatty acids composition in liver, it was concluded that *L. plantarum* NCU116 was effective in the protection against hepatocellular injury.

3.4 Modulation of LPS and cytokines by *L. plantarum* NCU116

Previous studies demonstrated a causal relation between HFD diets increased serum LPS concentrations.[6] LPS of Gram-negative bacteriais known to stimulate proinflammatory cytokines production. Proinflammatory cytokines, including TNF-α, and IL-6, are produced by the host in response to bacterial colonisation or invasion and hence are central to the host defense mechanism against pathogens.[31] IL-10 is generally considered as anti-inflammatory cytokines, which are a series of immunoregulatory molecules that control the proinflammatory cytokine response.[32] Several probiotic effects are mediated through immune regulation, particularly through improving a balance between pro-and anti-inflammatory cytokines in the immune dysfunction.[31]

In this study, as a consequence of HFD diet feeding in this study, the levels of LPS, IL-6 and TNF-α of HFD group were strongly increased compared with ND group. In addition, the levels of IL-10 was significantly decreased than that of ND group (Figure 3). Interestingly, the treatment of *L. plantarum* NCU116 could ameliorate these parameters close to the normal levels, and the NCU116-H group had

the statistical significant compared to the HFD group of all the immune indices ($P < 0.05$).

The raise of proinflammatory cytokines is one of the early events in NAFLD. Particularly, TNF-α and IL-6 are two prototypic inflammatory cytokines involved in metabolic impairment.[8] The *L. plantarum* NCU116 was found to markedly reduce the TNF-α and IL-6 levels and oxidative damage (Table 1), interfering with the key pathogenetic mechanisms responsible of the onset of liver damage.[33]

3.5 mRNA expression of colonic bacterial flora

Intestinal microbiota composition is related to weight gain, host energy and lipid metabolism. Reports suggested that HFD diet feeding leads changes of intestinal microbiota which was associated with an increased intestinal permeability and consequently triggered inflammation and metabolic disorders.[34] In this study, colonic bacterial flora of *Lactobacillus* spp., *Bifidobacterium* spp., *Enterobacteriaceae* spp. and *Bacteroides* spp. mRNA expression resulted in different levels (Figure 4). The inclusion of HFD resulted in a significant downregulation of *Lactobacillus* spp. and *Bifidobacterium* spp. and upregulation of *Enterobacteriaceae* spp. compared with ND group ($P < 0.05$). Whereas oral supplementation of *L. plantarum* NCU116 upregulated the *Lactobacillus* spp. and *Bifidobacterium* spp. and downregulated *Bacteroides* spp. mRNA expressions compared with the HFD group ($P < 0.05$). The high fat diet reduced gene copy counts of gram-positive bacteria, including *Lactobacillus* spp. and *Bifidobacterium* spp., as well as increased the gene copy counts of *Enterobacteriaceae* spp. and *Bacteroidetes* spp., in accordance with a

previous study.[35]

Intestinal bacterial flora is increasingly recognized to play an essential role in the development of NAFLD, and it is involved in several biological functions, such as inhibiting pathogens, maintaining mucosal immune system and intestinal barrier integrity.[2] Several studies reported the beneficial effects of probiotics on lipid metabolism. The possible mechanism involves both assimilation of cholesterol and deconjugation of bile salts.[36] The total bile acids and cholesterol in fecal was dramatically increased in rats treated with *L. plantarum* NCU116.[13] The probiotic might assimilate lipids by incorporating it into the cellular membranes and then via fecal excretion, suggesting that intestinal flora contributes to energy harvesting. [36]

3.6 mRNA expression of lipid metabolism

Lipid metabolism in liver is mainly regulated by lipid regulatory proteins, such as β-oxidation-related and lipogenic proteins. β-oxidation is the key pathway of fatty acid metabolism, which is the indicator of liver lipid accumulation.[37, 38] To explore the possible mechanisms whereby *L. plantarum* NCU116 decreases liver lipid accumulation, expression levels of the genes involved in lipolysis and fatty acid oxidation (PPARs, PGC1α and CPT1α) and lipogenesis (FAS, ACC and SCD1) were investigated.

The expression of these genes levels in the liver were changed obviously in HFD group compared with ND group ($P < 0.05$, Figure 5). With the oral administration of *L. plantarum* NCU116 for 5 weeks, an altered lipometabolism including increased expression of PPARα, PPARγ, PPARδ, PGC1α and CPT1α mRNA levels of in HFD

279 rats were observed ($P < 0.05$). Meanwhile, the mRNA levels of FAS, ACC and SCD1
280 were notably decreased in NCU116 groups, leading to lower hepatic steatosis (Table
281 2 and Figure 2) compared with the HFD group. In *L. plantarum* NCU116 groups, this
282 phenomenon was linked to the reduction of inflammation related to indicators, such as
283 visceral fat mass and serum LPS, were accompanied by the downregulation of TNF-α
284 (Figure 3). The results indicated that *L. plantarum* NCU116 is likely to inhibit
285 inflammation and hepatic oxidative stress (Table 1) induced by HFD diet.[23]

286 In addition, liver mRNA levels involved in fatty acid oxidation (PPARs, PGC1α
287 and CPT1α) were significantly increased in *L. plantarum* NCU116 treated rats.
288 Conversely, the probiotic decreased the expression of genes involved in lipogenesis
289 (FAS, ACC and SCD1). These results suggest that *L. plantarum* NCU116 reduces
290 liver lipid accumulation via the two pathways of downregulating lipogenesis and
291 upregulating lipolysis and fatty acid oxidation related genes expression.

292 **3.7 Safety evaluation of *L. plantarum* NCU116**

293 Bacterial translocation was not observed in all rats (data not shown). These
294 results indicate that the *L. plantarum* NCU116 do not cause alterations in the
295 intestinal mucosa, and may be considered as an indicator of the biological safety of
296 the product and the probiotic used in its preparation.[17]

297 In summary, *L. plantarum* NCU116 was found to restore liver function,
298 oxidative stress, colonic bacterial flora in rats with HFD-induced NAFLD, regulate
299 fatty acids composition of liver and decreased LPS and proinflammatory cytokines,
300 and regulated the expression levels of lipid metabolism. In addition, *L. plantarum*

NCU116 was considered a safe probiotic and was not found bacterial translocation in other organs. Further, our data suggest that possible underlying mechanism for beneficial effects of *L. plantarum* NCU116 on NAFLD may include two pathways of downregulating lipogenesis and upregulating lipolysis and fatty acid oxidation related genes expression.

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Conflict of Interest

The author declares that there are no conflicts of interest.

Abbreviations

ACC, Acetyl-coenzyme A carboxylase; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CAT, Catalase; CPT1α, Carnitine palmitoyltransferase-1α; ∑FA: Total fatty acids; FAS, Fatty acid synthetase; GSH-Px, Glutathione peroxidase; IL-6, interleukin-6; LPS, Lipopolysaccharide; MDA: Malondialdehyde; ∑MUFA, Total monounsaturated fatty acids; NAFLD, Non-alcoholic fatty liver disease; PGC1α, PPARγ coactivator-1α; PPAR, Peroxisome

proliferator-activated receptor; ∑PUFA, Total polyunsaturated fatty acids; SCD1, Coenzyme A desaturase 1; ∑SFA, Total saturated fatty acids; SOD: Superoxide dismutase; T-AOC: Total anti-oxidant capacity; TBil: Total bilirubin; TC, Total cholesterol; TG, Triacylglycerols; TNF-α, Tumor necrosis factor-α; ∑trans, Total trans fatty acids.

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Table 1 Effect of *L. plantarum* NCU116 treatment on oxidative stress in rats

Oxidative stress	ND	HFD	NCU116-L	NCU116-H
SOD(U/mL)	191.61 ± 6.83^{c}	133.70 ± 8.71^{a}	168.66 ± 4.33^{b}	163.82 ± 3.72^{b}
GSH-Px(U/mL)	4177.58 ± 101.36^{b}	3428.65 ± 115.64^{a}	4002.70 ± 23.61^{b}	4031.01 ± 52.28^{b}
MDA(nmol/mL)	4.78 ± 0.41^{a}	7.35 ± 0.46^{c}	6.63 ± 0.49^{bc}	5.61 ± 0.45^{ab}
CAT(U/mL)	17.51 ± 1.60^{c}	9.14 ± 0.72^{a}	12.93 ± 1.63^{ab}	14.51 ± 1.46^{bc}
T-AOC(U/mL)	7.10 ± 0.76^{c}	3.08 ± 0.56^{a}	5.45 ± 0.46^{b}	5.24 ± 0.31^{b}

ND: rats on the normal diet; HFD: rats on the high fat diet; NCU116-L: rats on the high fat diet $+10^{8}$ CFU/mL *L. plantarum* NCU116; NCU116-H; rats on the high fat diet $+10^{9}$ CFU/mL *L. plantarum* NCU116. Results are expressed as the means ± SEM (n = 10).Values within a row with different superscripts are significantly different ($P < 0.05$).

Table 2 Fatty acids composition in liver (mg/g)

Fatty acids	ND	HFD	NCU116-L	NCU116-H
C14:0	0.045 ± 0.005^{a}	0.250 ± 0.062^{c}	0.165 ± 0.029^{b}	0.171 ± 0.079^{b}
9cC14:1	0.006 ± 0.002^{a}	0.015 ± 0.003^{b}	0.013 ± 0.001^{b}	0.017 ± 0.009^{b}
C15:0	0.029 ± 0.003^{a}	0.090 ± 0.018^{c}	0.062 ± 0.014^{b}	0.059 ± 0.017^{b}
C16:0	4.585 ± 0.303^{a}	12.354 ± 2.039^{c}	9.783 ± 1.302^{b}	8.972 ± 1.879^{b}
9cC16:1	0.206 ± 0.045^{a}	1.287 ± 0.301^{c}	1.123 ± 0.292^{bc}	0.942 ± 0.328^{b}
C17:0	0.093 ± 0.011^{a}	0.136 ± 0.013^{b}	0.129 ± 0.013^{b}	0.107 ± 0.012^{a}
9cC17:1	0.085 ± 0.014^{a}	0.167 ± 0.051^{b}	0.133 ± 0.046^{ab}	0.131 ± 0.070^{ab}
C18:0	4.806 ± 0.282	5.333 ± 0.650	5.556 ± 0.946	5.038 ± 0.359
9t/11tC18:1	0.010 ± 0.004^{a}	0.061 ± 0.011^{b}	0.068 ± 0.008^{b}	0.056 ± 0.012^{b}
9cC18:1	1.135 ± 0.264^{a}	24.130 ± 3.945^{c}	21.793 ± 3.099^{bc}	18.387 ± 3.254^{b}
11cC18:1	0.606 ± 0.090^{a}	1.997 ± 0.316^{c}	1.589 ± 0.251^{b}	1.496 ± 0.367^{b}
9c12t/9t12cC18:2	0.007 ± 0.002^{a}	0.021 ± 0.004^{b}	0.014 ± 0.008^{ab}	0.021 ± 0.009 b
9c12cC18:2n-6	3.809 ± 0.404^{a}	14.785 ± 2.615^{c}	12.109 ± 1.771^{b}	11.329 ± 1.807^{b}
6c9c12cC18:3n-6	0.024 ± 0.005^{a}	0.127 ± 0.029^{c}	0.098 ± 0.007^{b}	0.091 ± 0.027^{b}
ctt/cctC18:3	nd	0.007 ± 0.001	0.008 ± 0.001	0.007 ± 0.002
C20:0	0.015 ± 0.005^{a}	0.033 ± 0.008^{b}	0.032 ± 0.003^{b}	0.029 ± 0.005^{b}
ctc/tccC18:3	nd	0.009 ± 0.004^{b}	0.006 ± 0.002^{a}	0.006 ± 0.001^{a}
9c12c15cC18:3n-3	0.036 ± 0.012^{a}	0.373 ± 0.057^{c}	0.302 ± 0.046^{b}	0.275 ± 0.067^{b}
11cC20:1	0.024 ± 0.003^{a}	0.499 ± 0.122^{c}	0.364 ± 0.095^{b}	0.308 ± 0.066^{b}
C20:2n-6	0.062 ± 0.010^{a}	0.425 ± 0.111^{c}	0.299 ± 0.067^{b}	0.285 ± 0.071^{b}
C20:3n-6	0.187 ± 0.040^{a}	0.896 ± 0.173^{c}	0.701 ± 0.124^{b}	0.656 ± 0.150^{b}
C22:0	0.035 ± 0.002^{a}	0.045 ± 0.006^{b}	0.041 ± 0.009^{ab}	0.042 ± 0.009^{ab}
C20:4n-6	5.651 ± 0.131^{c}	4.755 ± 0.516^{b}	4.579 ± 0.599^{ab}	4.186 ± 0.257^{a}
C20:5n-3	0.084 ± 0.020^{a}	0.192 ± 0.042^{c}	0.157 ± 0.024^{bc}	0.154 ± 0.030^{b}
C24:0	0.093 ± 0.016^{a}	0.298 ± 0.085^{c}	0.220 ± 0.067^{b}	0.198 ± 0.042^{b}
C22:5n-3	0.221 ± 0.044^{a}	0.457 ± 0.113^{b}	0.315 ± 0.075^{a}	0.298 ± 0.075^{a}
C22:6n-3	1.723 ± 0.131^{a}	2.358 ± 0.439^{b}	1.932 ± 0.385^{a}	1.759 ± 0.182^{a}
∑SFA	9.701 ± 0.531^{a}	18.539 ± 2.313^{c}	15.988 ± 1.803^{b}	14.617 ± 2.037^{b}
∑MUFA	2.071 ± 0.333^{a}	28.156 ± 4.500^{c}	25.083 ± 3.500^{bc}	21.337 ± 4.016^{b}
∑PUFA	11.804 ± 0.597^{a}	24.405 ± 3.663^{c}	20.520 ± 2.780^{b}	19.066 ± 2.340^{b}
∑trans	0.017 ± 0.006^{a}	0.097 ± 0.008^{b}	0.095 ± 0.016^{b}	0.089 ± 0.020^{b}
∑FA	23.593 ± 1.390^{a}	71.197 ± 10.161^{c}	61.686 ± 7.456^{b}	55.108 ± 8.078^{b}

"nd" means not detected. ∑SFA, total saturated fatty acids; ∑MUFA, total monounsaturated fatty acids; ∑PUFA, total polyunsaturated fatty acids; ∑trans, total trans fatty acids; ∑FA: total fatty acids. Results are expressed as the means ± SEM (n

= 10).Values within a row with different superscripts are significantly different ($P <$ 0.05).

FIGURE CAPTIONS

Figure 1 Effect of *L. plantarum* NCU116 treatment on liver function in rats

Results are expressed as the means ± SEM (n = 10). Values with different superscripts are significantly different ($P < 0.05$).

Figure 2 Liver (A) and adipose tissue (B) indices, TC and TG in Liver

Results are expressed as the means ± SEM (n = 10). Values with different superscripts are significantly different ($P < 0.05$).

Figure 3 LPS and cytokines in serum

Results are expressed as the means ± SEM (n = 10). Values with different superscripts are significantly different ($P < 0.05$).

Figure 4 mRNA expression of colonic bacterial flora

Results are expressed as the means ± SEM (n = 10). Values with different superscripts are significantly different ($P < 0.05$).

Figure 5. mRNA levels of lipolysis, lipogenesis and fatty acid oxidation genes in liver

ACC, Acetyl-coenzyme A carboxylase; CPT1α, Carnitine palmitoyltransferase-1α; FAS, Fatty acid synthetase; PGC1α, PPARγ coactivator-1α; PPAR, Peroxisome proliferator-activated receptor; SCD1, Coenzyme A desaturase 1.

Results are expressed as the means ± SEM (n = 10). Values with different superscripts are significantly different ($P < 0.05$).

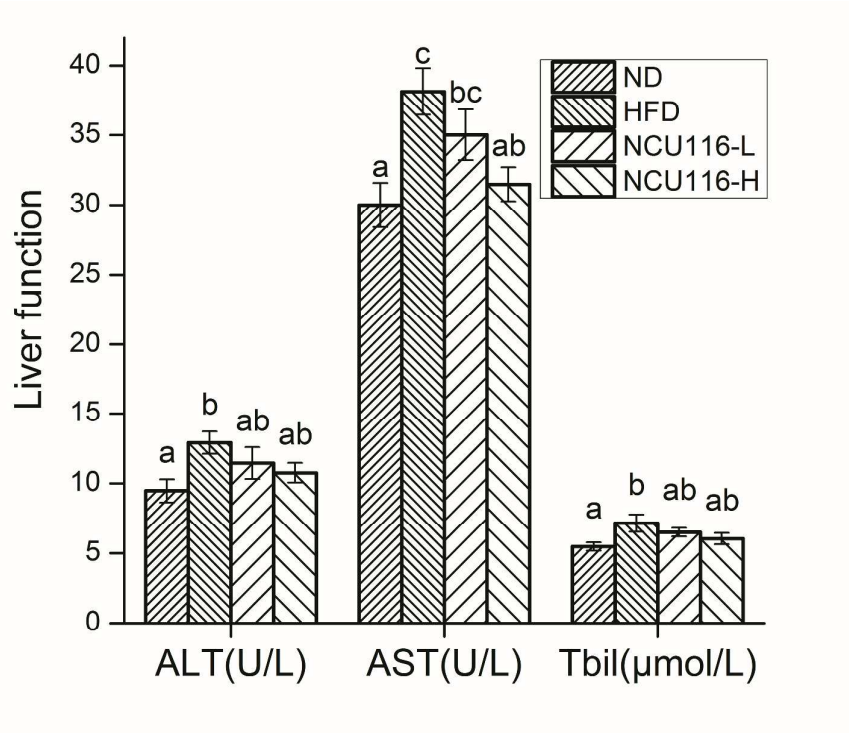


Figure 1

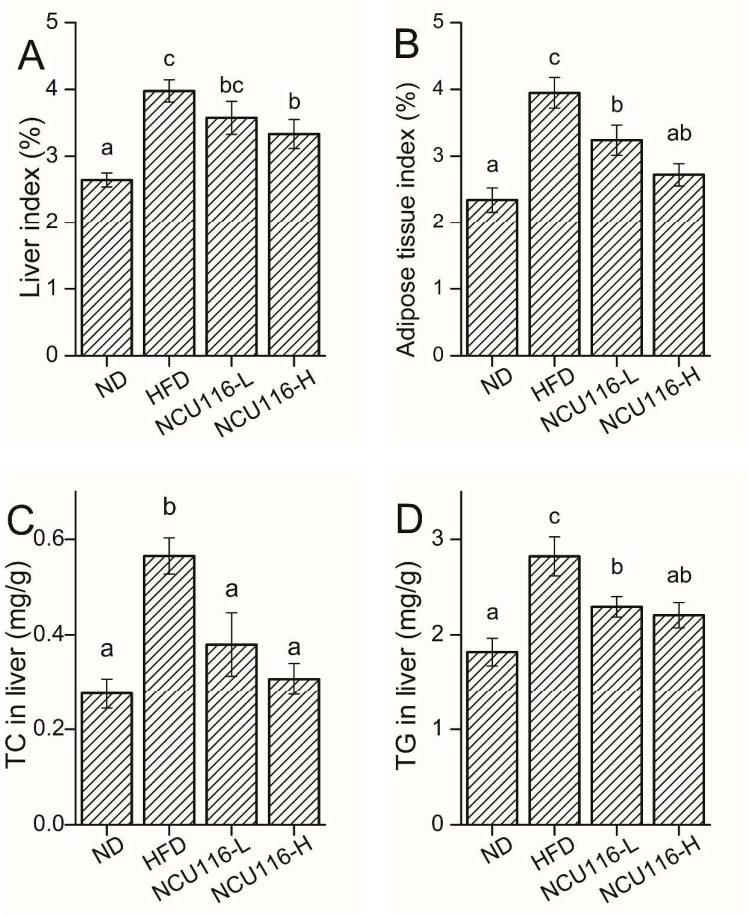
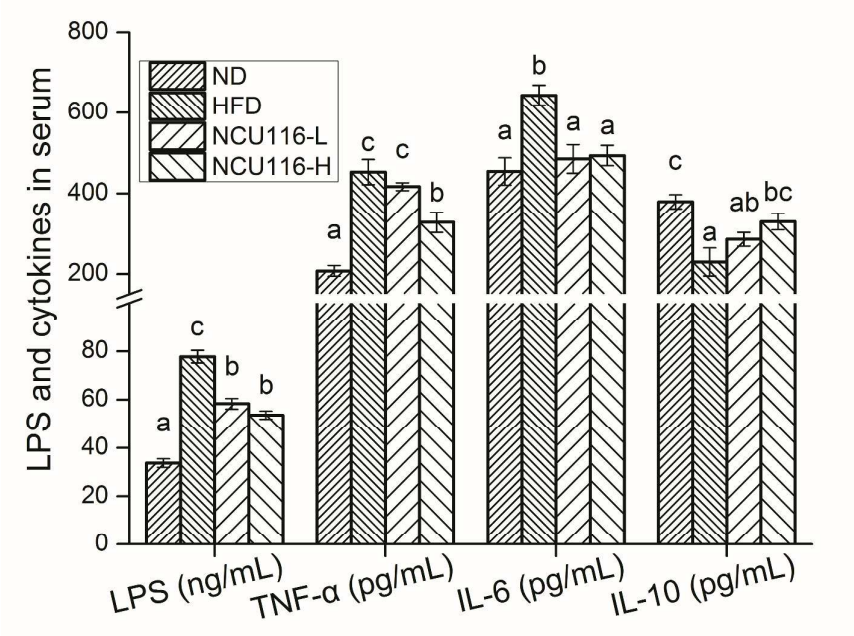


Figure 2



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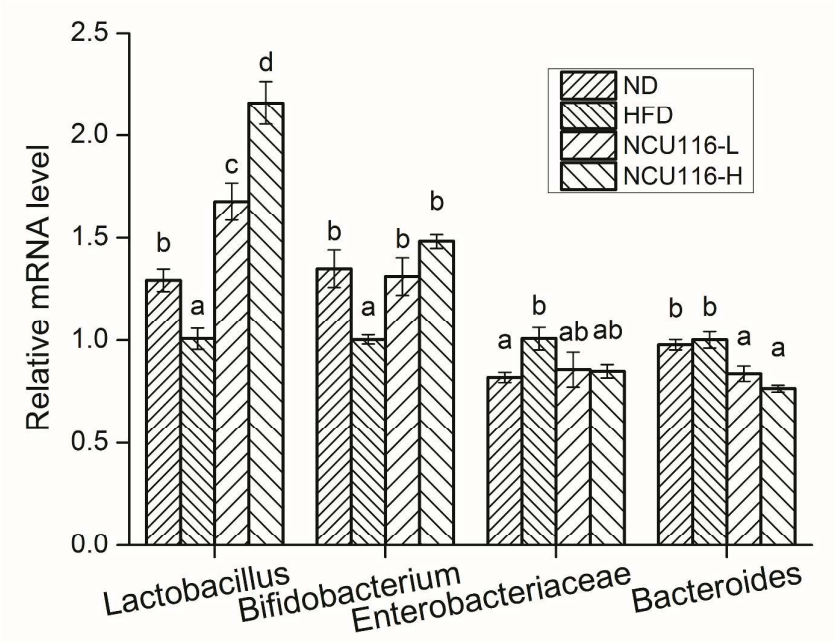

Figure 4

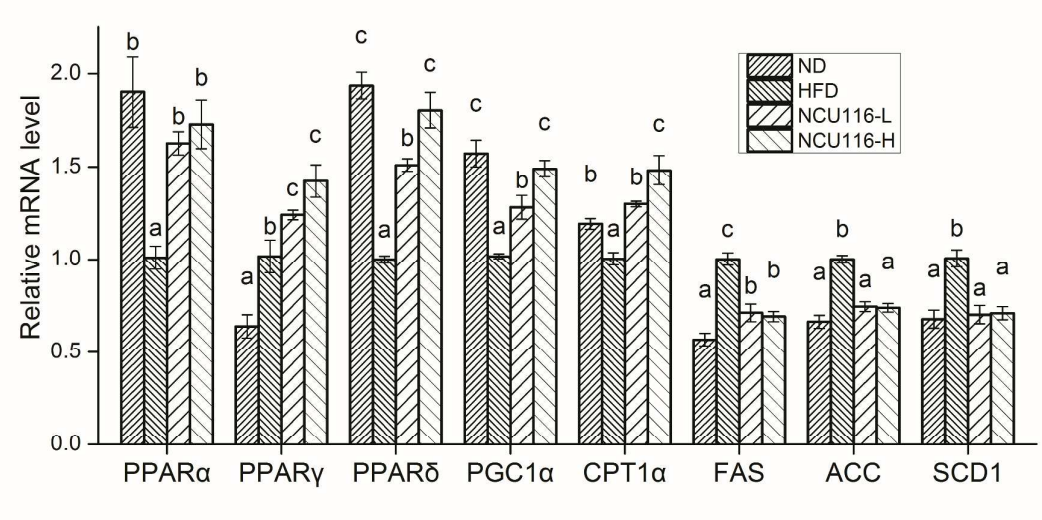

Figure 5

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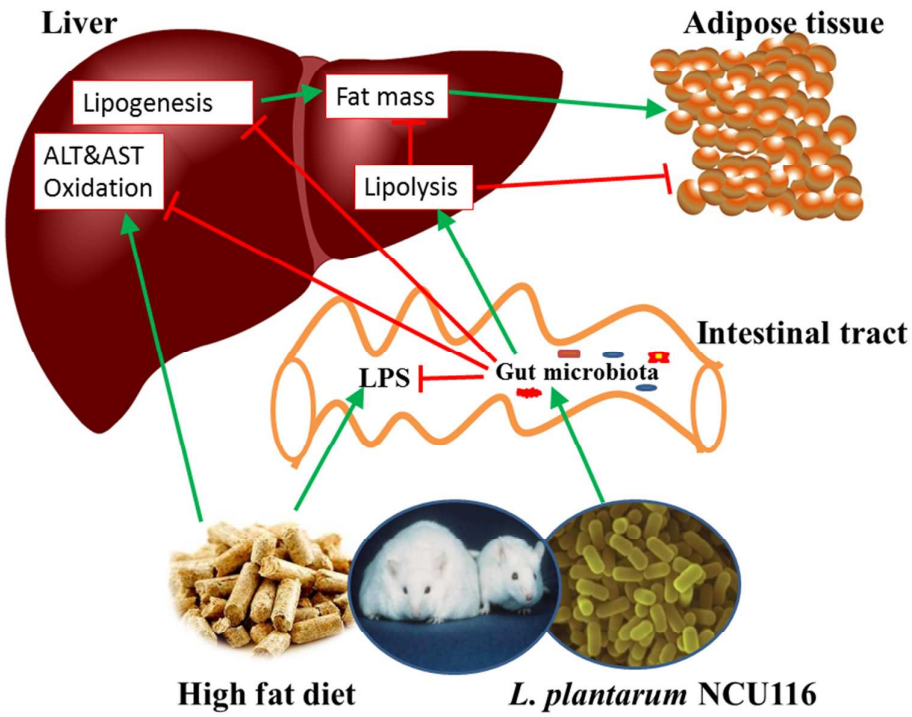

Possible mechanism involved in *Lactobacillus plantarum* NCU116 improves lipid metabolism in high fat diet induced NAFLD rats.