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Article *in* Journal of the American College of Nutrition · October 2015

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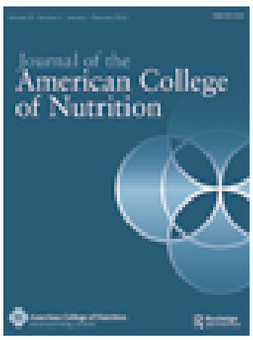

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Journal of the American College of Nutrition

ISSN: 0731-5724 (Print) 1541-1087 (Online) Journal homepage: http://www.tandfonline.com/loi/uacn20

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To cite this article: Abbaszadeh Sepideh PhD, Parastouei Karim PhD, Afshari Hossein MS, Rostami Leila MS, Mahmoudi Hamdollah MS, Ghamarchehreh Mohammad E PhD, Sepandi Mojtaba PhD, Samadi Mohammad PhD, Ghanizadeh Ghader PhD & Alavian Seyed Moayed PhD (2015): Effects of Multistrain Probiotic Supplementation on Glycemic and Inflammatory Indices in Patients with Nonalcoholic Fatty Liver Disease: A Double-Blind Randomized Clinical Trial, Journal of the American College of Nutrition, DOI: 10.1080/07315724.2015.1031355

To link to this article: http://dx.doi.org/10.1080/07315724.2015.1031355

Published online: 02 Oct 2015.

Original Research

Effects of Multistrain Probiotic Supplementation on Glycemic and Inflammatory Indices in Patients with Nonalcoholic Fatty Liver Disease: A Double-Blind Randomized Clinical Trial

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Key words: probiotic, fatty liver, blood glucose, cytokines

Objective: Nonalcoholic fatty liver disease (NAFLD) is a condition defined by exceeding triglycerides accumulation in the liver. The condition can develop into fibrosis, cirrhosis, and hepatocellular carcinoma. Considering the ever-increasing prevalence of NAFLD, the aim of the present study was to investigate the effects of probiotic supplementation on glycemic and inflammatory indices in patients with NAFLD.

Methods: This randomized clinical trial was conducted on 42 patients with NAFLD who had been referred to a gastroenterology clinic. Subjects in the intervention and control groups consumed 2 capsules/day probiotic or placebo, respectively, for 8 weeks. Fasting blood sugar (FBS), insulin, insulin resistance, tumor necrosis factor alpha (TNF-α), and interleukin 6 (IL-6) were measured at baseline and at the end of the study.

Results: Means of FBS, insulin, insulin resistance, and IL-6 were significantly different between groups after intervention ($p < 0.05$), whereas TNF-α was not significantly modified ($p > 0.05$). In the probiotic group, insulin, insulin resistance, TNF-α, and IL-6 decreased significantly at the end of the study compared to the beginning of study.

Conclusion: Considering the effects of probiotic supplementation on the reduction of glycemic and inflammatory indices in patients with NAFLD, consumption of probiotics is recommended as a complementary therapy in these patients.

INTRODUCTION

Fatty liver disease, the most common liver disease worldwide, is a condition defined by exceeding triglycerides accumulation in the liver [1]. Nonalcoholic fatty liver disease (NAFLD) ranges from benign steatosis to nonalcoholic steatohepatitis, which can develop into fibrosis, cirrhosis, and hepatocellular carcinoma [2,3]. The prevalence of NAFLD is rapidly increasing worldwide in parallel with the increase in obesity and type 2 diabetes [3]. The global prevalence of NAFLD in the general population is estimated to be 10%–24% [1]. The prevalence of liver diseases in Asian countries is increasing [4]. The overall prevalence of NAFLD in the general population of Asian countries is estimated to be 9%–40%, mostly linked with obesity [5]. In 2013, the prevalence of NAFLD in Iranian adult general population was reported to be 21.5% [6]. NAFLD is strongly associated with obesity, dyslipidemia, and type 2 diabetes [7,8]. No licensed treatments are currently available for NAFLD, but lifestyle modification aimed at weight loss and increased physical activity is important in managing these patients [9]. Small intestine bacterial imbalance, having extensive effects on the liver health, occurs

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in a large percentage of patients with NAFLD. This disturbance results in an increase in intestinal permeability. Bacterial and endotoxin translocation trigger the production of pro-inflammatory molecules and cytokines [10,11]. According to the definition by the World Health Organization, probiotics are "live commensal microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [12]. In fact, probiotics help to maintain the balance of microbiota in the gut. Lipopolysaccharides from gram-negative bacteria interact with toll-like receptors and induce nuclear factor kappa–light chain enhancer of activated B cells (NF-κB) activation on the hepatic cells and up-regulate inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), which lead to hepatic damages [13].

Considering the high prevalence of NAFLD and the lack of a comprehensive human study on multistrain probiotic effects on patients with NAFLD in the Islamic Republic of Iran, the present study was performed to investigate the effects of probiotic supplementation on glycemic and inflammatory indices in patients with NAFLD.

METHODS

Study Design

This was a randomized, double-blind, placebo-controlled trial in patients with ultrasonographically proven NAFLD. Patients who had been referred to gastroenterology and liver clinics at Baqiyatallah hospital between June 2013 and December 2013 were included. The study was approved by the Baqiyatallah University Ethics Committee and was registered at Iranian Registry of Clinical Trials (IRCT: 2012122911920N1).

Participants were randomly assigned to the intervention or control group (1:1 ratio). Randomization and allocation were concealed from the researchers and patients until the statistical analysis was completed. Participants received either placebo or probiotic for 2 months, 1 g daily.

All patients gave written informed consent to participate in the study. Patients were called every 14 days to remind them about the supplement consumption and ask whether there were any side effects.

Subjects

Patients between the ages of 18 and 65 years were screened initially by medical history and ultrasonographic results. Fifty met inclusion criteria and were randomly assigned to receive probiotic or placebo, and 42 completed the study (Fig. 1). The diagnosis of NAFLD was based on ultrasound examination, including the presence of a bright hepatic echo-texture (compared to the renal cortex), deep attenuation, and vascular blurring either singly or in combination, to diagnose hepatic steatosis. Exclusion criteria included the presence of liver disease due to any of the following: alcohol consumption, hypothyroidism, Wilson's disease, viral hepatitis (HBV, HCV), acute systemic disease, cystic fibrosis, coeliac disease, metabolic inherited diseases, autoimmune hepatitis and drug toxicity. In addition, those who suffered from cardiovascular, immunodeficiency, hemochromatosis, and kidney diseases or were pregnant or lactating were excluded. Finally, the use of nonsteroidal anti-inflammatory drugs, medicinal plants, antibiotics, and probiotics within 2 months preceding enrollment was also considered as exclusion criteria.

Study Medication

Lactocare (Zist-takhmir Co., Tehran, Iran) contains 7 strains of naturally occurring beneficial bacteria: Lactobacillus casei 3×10^9 CFU/g, Lactobacillus acidophilus 3×10^{10} CFU/g, Lactobacillus rhamnosus 7×10^9 CFU/g, Lactobacillus bulgaricus 5×10^8 CFU/g, Bifidobacterium breve 2×10^{10} CFU/g, Bifidobacterium longum 1×10^9 CFU/g, and Streptococcus thermophilus 3×10^8 CFU/g. The placebo (Zist-takhmir Co.) was a capsule that is similar in color, shape, size, taste, and packaging, containing maltodextrin, lactose, and magnesium stearate. The placebo and probiotic were packaged in identical sealed boxes, identified by a code number only. Patients were instructed to keep the study medications refrigerated (between 2 and 7°C) throughout the study. We called patients once every 14 days to remind them about the supplements and ask whether there were any side effects.

Measurements

Height, weight, and waist and hip circumference of each patient were measured at baseline and at the end of study. Height was measured without shoes using a stadiometer with a precision of 0.1 cm. Weight was measured by a weight measuring device in light clothing with a precision of 0.1 kg. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in square meters). Waist-to-hip ratio was calculated as waist circumference divided by hip circumference using a flexible tape. Subjects were advised to maintain their usual diet and activity levels. To assess dietary intake, a 3-day (2 weekdays and a weekend day) daily food record was used before and after the intervention. The average daily nutrient intake was calculated by modified Nutritionist IV software (First Databank Inc., Hearst Corp., San Bruno, CA). Moreover, physical activity of each participant was assessed using a physical activity questionnaire monitor at baseline and at the end of study. Biochemistry parameters were obtained from a centralized laboratory after a 12-hour overnight fast. We determined blood glucose and glycated hemoglobin (HbA1c) levels with colorimetric and ion-exchange high-performance liquid chromatography methods, respectively. Serum insulin was measured by

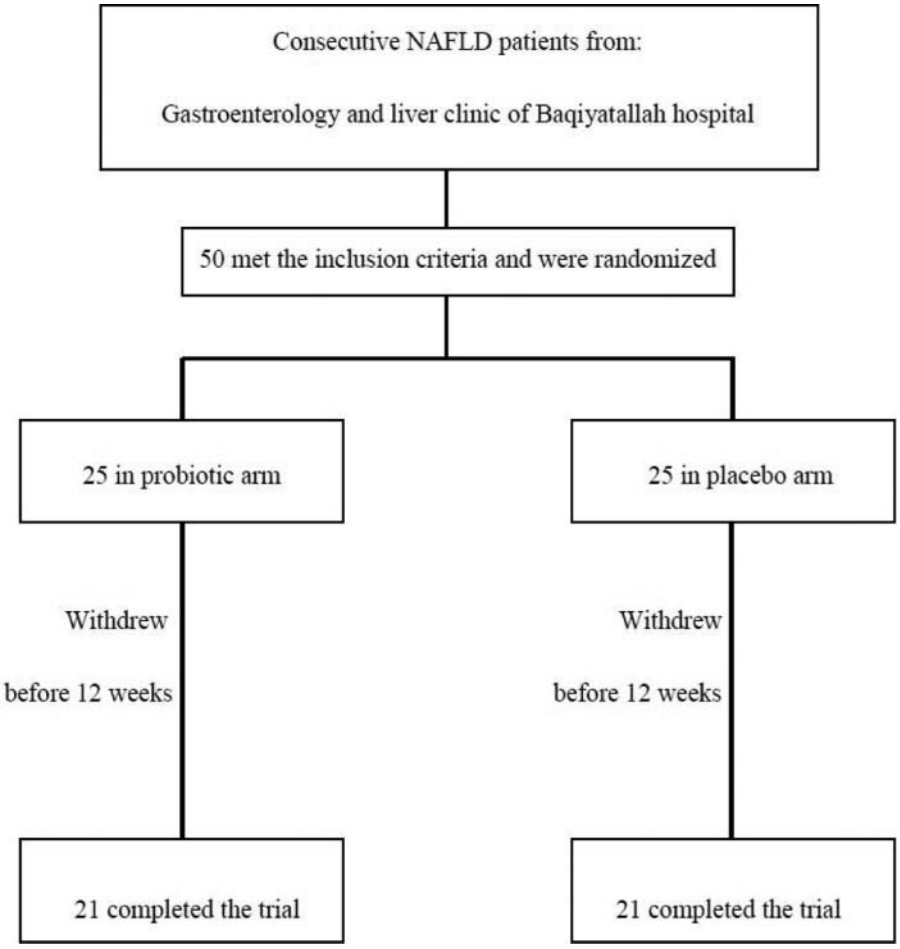

Fig. 1. Flowchart of trial participants.

a chemiluminescent immunoassay. Homeostatic model assessment–insulin resistance (HOMA-IR), a marker of insulin resistance, was calculated according to the following formula [14]: HOMA-IR = FBS (mg/dl) × Fasting insulin (μU/ml)/405. In addition, serum TNF-α and IL-6 levels were assessed with the ELISA-sandwich method using commercially available immunoassay kits (e-Bioscience, BMS223/4CE/4TENCE, US; e-Bioscience, BMS213/2CE/2TENCE, US, respectively) according to the manufacturer's guidelines. All clinical and laboratory data were collected in a double-blind manner.

Statistical Methods

Normal distribution of data was assessed by Kolmogorov-Smirnov test. Data are reported as mean ± standard error. Comparisons between groups were made using independent t tests in numerical variables. Changes within each group over the study period were evaluated using the paired t test. Chi-square test was used to assess qualitative variables such as sex. Analysis was conducted using SPSS version 16 statistical software (SPSS Inc., Chicago, IL). Statistical significance was set to 0.05.

RESULTS

Study Population

Fifty participants were allocated to the trial groups; 8 subjects withdrew from the 8-week study and 21 in each group completed the follow-up period. Mean age was 44.71 ± 1.64 for subjects with NAFLD. Women represented 38.1% of the probiotic group and 28.6% in the placebo group.

Anthropometric and Nutritional Parameters

Anthropometric and nutritional baseline measurements of all subjects, in both study groups, are described in Tables 1 and 2, respectively. There were no significant differences at baseline between the groups in weight, BMI, waist circumference, waist-to-hip ratio, physical activity, and macro- and micronutrient intake ($p > 0.05$). In addition, anthropometric indices, energy and nutrient intake, and physical activity levels were not significantly modified during the study or between the groups postintervention (data not shown).

Table 1. Demographic Characteristics of Participants at Baseline

Variable	All	Probiotic	Placebo	*p* Value
N	42	21	21	
Age (year)	44.71 ± 1.64	42.10 ± 1.99	47.33 ± 2.53	0.11
Gender (male, %)	28 (66.66%)	13 (61.9%)	15 (71.4%)	0.51
Weight (kg)	85.66 ± 2.03	85.09 ± 2.90	86.33 ± 2.96	0.78
BMI (kg/m^2)	29.92 ± 0.72	30.34 ± 1.17	29.50 ± 0.84	0.57
Waist circumference (cm)	98.88 ± 1.65	98.14 ± 2.37	99.62 ± 2.36	0.66
Waist-to-hip ratio (W/H)	0.91 ± 0.01	0.90 ± 0.01	0.92 ± 0.01	0.49

BMI = body mass index.

Biochemical Parameters

In Table 3, the baseline and 2-month values of the main outcomes of the study are shown. Among biochemical parameters, there were no significant differences between the groups at baseline in blood glucose, serum insulin, HbA1C, HOMA-IR, TNF-α, and IL-6 levels. FBS, HOMA-IR, IL-6, and serum insulin levels were significantly lower in the probiotic group compared to the placebo group at the end of the 2-month intervention ($p < 0.05$). Furthermore, within-group changes showed that supplementation with probiotics significantly decreased serum insulin, HOMA-IR, TNF-α, and IL-6 levels during the 2-month intervention ($p < 0.05$). However, after finishing the study, there were no differences in TNF-α levels between the study groups ($p = 0.10$). Additionally, although HbA1C levels decreased during the intervention period in the probiotic group compared to the placebo group, no significant difference was observed in within- and between-group comparisons ($p > 0.05$).

DISCUSSION

The role of the probiotics in NAFLD has been intensely evaluated during the last decade, and several studies in animal models emphasis on the important role of intestinal microbiota composition in NAFLD development and progression [15,16]. Although it is now well known that in humans, changes in microbiota composition are strongly associated with aminotransferases changes in NAFLD [17,18], limited clinical trials were found regarding the effects of probiotics on glycemic and inflammatory indices in patients with NAFLD. The gut gram-negative bacteria are associated with increased exposure to lipopolysaccharides (LPSs). LPSs were found to enhance the release of pro-inflammatory molecules. LPSs are also implicated in the etiology of insulin resistance and NAFLD [19,20]. The aim of this single-center, randomized, double-blind, placebo-controlled trial was to determine whether supplementation with probiotics would be effective on glycemic and pro-inflammatory indices in patients with NAFLD over a 2-month treatment duration. At baseline, all anthropometric indices, physical activity, and nutritional intake were similar in both groups, demonstrating similar baseline conditions. There were no serious adverse events, suggesting that the probiotic has a good safety profile and is well tolerated in patients with NAFLD. Our study showed significant improvement in FBS, insulin, HOMA-IR, and IL-6 in the probiotic group compared to placebo after the intervention period. In addition, probiotic consumption significantly decreased serum insulin, HOMA-IR, TNF-α, and IL-6 during 2 months. Bhathena et al. [16] and Ritze et al. [21] have recently found that probiotic may reduce insulin, insulin resistance, TNF-α, and IL-1β levels in rats with NAFLD. The present results, in agreement with other investigations in patients with diabetes or in animal models [14,22,23], suggested that probiotic supplementation may decrease insulin resistance and inflammatory parameters. It was previously shown that dietary counseling with probiotic supplementation decreases FBS, insulin, and HOMA-IR in normo-glycemic pregnant women and therefore may provide potential novel means for the prophylactic and therapeutic management of glucose disorders [22]. Malaguarnera et al. recently suggested that supplementation with symbiotics results in a significant

Table 2. Macronutrient Intake and Physical Activity of Participants at Baseline

Variable	All ($n = 42$)	Probiotic ($n = 21$)	Placebo ($n = 21$)	*p* Value
Energy (kcal/d)	1985.50 ± 97.28	1960.50 ± 162.01	2010.60 ± 111.75	0.88
Carbohydrate (g/d)	284.96 ± 13.50	283.40 ± 24.16	286.51 ± 12.80	0.69
Protein (g/d)	69.47 ± 3.05	65.60 ± 3.71	73.34 ± 4.77	0.96
Fat (g/d)	69.47 ± 6.01	70.22 ± 9.47	68.71 ± 7.64	0.69
Physical activity (MET h/day)	35.41 ± 0.72	35.07 ± 0.90	35.75 ± 1.14	0.64

MET = metabolic equivalent.

Table 3. Changes in Glycemic and Inflammatory Indices during the Study[a]

Variables		Probiotic ($n = 21$)	Placebo ($n = 21$)	p Value*
FBS (mg/dl)	Baseline	97.95 ± 3.71	97.81 ± 3.93	0.98
	2 Months	93.42 ± 2.21	100.43 ± 2.49	0.04
	p Value**	0.15	0.32	
Insulin (μU/ml)	Baseline	11.20 ± 1.63	12.51 ± 1.16	0.52
	2 Months	8.95 ± 1.32	14.33 ± 1.80	0.03
	p Value	0.002	0.34	
HOMA-IR	Baseline	2.69 ± 0.37	3.02 ± 0.29	0.48
	2 Months	2.18 ± 0.35	3.49 ± 0.41	0.02
	p Value	0.004	0.31	
TNF-α (pg/ml)	Baseline	36.31 ± 0.41	35.30 ± 0.66	0.20
	2 Months	32.80 ± 0.74	34.43 ± 0.64	0.10
	p Value	0.01	0.08	
IL-6 (pg/ml)	Baseline	29.32 ± 0.84	29.92 ± 0.61	0.55
	2 Months	26.39 ± 1.06	28.98 ± 0.67	0.04
	p Value	0.01	0.21	
HbA1C (%)	Baseline	5.89 ± 0.14	5.77 ± 0.11	0.50
	2 Months	5.78 ± 0.12	5.90 ± 0.10	0.42
	p Value	0.51	0.29	

FBS = fasting blood sugar, HOMA-IR = homeostatic model assessment–insulin resistance, TFN-α = tumor necrosis factor-alpha, IL-6 = interleukin-6, HbA1C = glycated hemoglobin.

[a]Values are mean and standard errors

*p Value for comparing the changes in variables between the groups. Two-sample t test was used.

**p Value for comparing baseline with endpoint values within each group. Paired sample t test was used.

reduction in serum glucose and insulin resistance in patients with nonalcoholic steatohepatitis [24].

In a 6-week randomized controlled trial in Iran, probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was compared with placebo in 60 patients with type 2 diabetes. Fasting blood sugar was significantly lower in the probiotic group compared to the placebo group, although no significant difference in HOMA-IR and insulin levels were observed between the 2 groups. Though probiotic yogurt decreased serum glucose levels, it had no effect on other glycemic indices, which demonstrated insufficient live probiotic cells per gram of probiotic yogurt [14]. In another previously published trial in Iran on the effect of 6 weeks of probiotic supplementation in 34 patients with type 2 diabetes, no significant effects on FBS, insulin, HOMA-IR, and IL-6 were observed [25]. It is important to note that the commercially available probiotic supplements contain different probiotic bacteria with different colony counts, which induced different effects on patients. In a trial published in 2011 on the effect of 8 weeks of Lactobacillus rhamnosus GG supplementation in 20 pediatric patients with obesity-related liver disease, there was no significant difference in serum TNF-α and ultrasonographic echogenicity of liver [23]. These controversies may be due to large variations in the study, such as the length of treatment, dosage, and type of probiotic used.

The importance of gut microbiota changes in NAFLD is now commonly accepted. Endotoxin or LPS produced by gut gram-negative microbiota can be delivered to the liver via the portal vein. Microbiota changes can induce increases in intestinal permeability that result in inflammatory signal propagation into the portal blood and the liver. High-fat diets are capable of increasing LPS concentrations by 2- to 3-fold. The intestinal microbiota of diabetic mice can be altered with prebiotic supplementation to increase *Bifidobacterium, Lactobacillus*, and Clostridium coccoides, thereby producing lower plasma LPS and cytokine levels and decreased hepatic expression of inflammatory and oxidative stress markers [26]. However, the underlying mechanisms by which intestinal microbiome patterns affect NAFLD have not been clearly defined.

CONCLUSION

Previous studies showed that the main treatment recommended for NAFLD usually consists of lifestyle changes, including diet and physical activity. Although a need for further randomized clinical trials remains, the data from this study and the available studies suggest that intestinal flora manipulation may represent an adjuvant therapeutic tool to improve glycemic and pro-inflammatory indices in patients with NAFLD.

REFERENCES

1. Labrecque D, Abbas Z, Anania F, Ferenci P, Ghafoor Khan A, Goh KL: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. World J Gastroenterol 1:1–29, 2012.

2. Monsour HP, Frenette CT, Wyne K: Fatty liver: a link to cardiovascular disease its natural history, pathogenesis and treatment. Methodist Debakey Cardiovasc J 8:21–25, 2012.
3. Lomonaco R, Chen J, Cusi K: An endocrine perspective of nonalcoholic fatty liver disease (NAFLD). Ther Adv Endocrinol Metab 2:211–225, 2011.
4. Lee SS, Byoun YS, Jeong SH, Kim YM, Gil H, Min BY, Seong MH, Jang ES, Kim JW: Type and cause of liver disease in Korea: single-center experience, 2005–2010. Clin Mol Hepatol 18:309–315, 2012.
5. Amarapurkar D, Kamani P, Patel N, Gupte P, Kumar P, Agal S: Prevalence of non-alcoholic fatty liver disease: population based study. Hepatology 6:161–163, 2007.
6. Lankarani KB, Ghaffarpasand F, Mahmoodi M, Lotfi M, Zamiri N, Heydari ST: Non-alcoholic fatty liver disease in southern Iran: a population based study. Hepat Mon 13:1–7, 2013.
7. Chalasani N, Younossi Z, Lavine G, Diehl A, Brunt E, Cusi K: The diagnosis and management of non-alcoholic fatty liver disease: practice guideline. Am J Hepatol 55:2005–2023, 2012.
8. Leamy AK, Egnatchik RA, Young JD: Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. Prog Lipid Res 52:165–174, 2013.
9. Dyson JK, Anstee QM, McPherson S: Non-alcoholic fatty liver disease: a practical approach to treatment. Front Gastroenterol 1:1–10, 2014.
10. Abenavoli L, Scarpellini E, Rouabhia S, Balsano C, Luzza F: Probiotics in NAFLD: which and when? Ann Hepatol 12:357–363, 2013.
11. Alisi A, Ceccarelli S, Panera N, Nobili V: Causative role of gut microbiota in non-alcoholic fatty liver disease pathogenesis. Front Cell Infect Microbiol 2:1–4, 2012.
12. Food and Agriculture Organization/World Health Organization: Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. 2001. Accessed at: http://www.who.int/foodsafety/publications/fs. . ./en/probiotics.pdf
13. Chatterjee A, Bhattacharya H, Kandwal A: Probiotics in periodontal health and disease. J Indian Soc Periodontol 15:23–28, 2011.
14. Ejtahed H, Mohtadi Nia J, Homayouni Rad A, Niafar M, Asghari Jafarabadi M, Mofid V: The effects of probiotic and conventional yoghurt on diabetes markers and insulin resistance in type 2 diabetic patients: a randomized controlled clinical trial. Iran J Endocrinol Metabol 13:1–7, 2011.
15. Compare D, Coccoli P, Rocco A: Gut–liver axis: the impact of gut microbiota on non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 22:471–476, 2012.
16. Bhathena J, Martoni C, Kulamarva A, Tomaro-Duchesneau C, Malhotra M, Paul A: Oral probiotic microcapsule formulation ameliorates non-alcoholic fatty liver disease in bio f1b golden Syrian hamsters. PloS ONE 8:1–9, 2013.
17. Aller R, Deluis DA, Izaola O, Conde R, Gonzalez MS, Primo B: Effect of a probiotic on liver amino transferase in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rew Med Pharmacol Sci 15:1090–1095, 2011.
18. Shavakhi A, Minakari M, Firouzian H, Assali R, Hekmatdoost A, Ferns G: Effect of a probiotic and metformin on liver aminotransferases in non-alcoholic steatohepatitis: a double blind randomized clinical trial. Int J Prevent Med 4:531–537, 2013.
19. Manco M, Putignani L, Bottazzo GF: Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr Rev 31:817–844, 2010.
20. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D: Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56:1761–1772, 2007.
21. Ritze Y, Bardos G, Claus A, Ehrmann V, Bergheim I, Schwiertz A, Bischoff SC: Lactobacillus rhamnosus GG protects against non-alcoholic fatty liver disease in mice. PLoS ONE 9:1–8, 2014.
22. Laitinen K, Poussa T, Isolauri E: Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomized controlled trial. Br J Nutr 101:1679–1687, 2009.
23. Vajro P, Mandato C, Licenziati MR, Franzese A, Vitale DF, Lenta S: Effect of Lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. J Pediatr Gastroenterol Nutr 52: 740–743, 2011.
24. Malaguarnera M, Antic T, Giordano M, Chisari G, Acquaviva R, Mastrojeni S: *Bifidobacterium longum* with fructo-oligosaccharides in patients with non-alcoholic steatophepatitis. Dig Dis Sci 57:1884–1887, 2012.
25. Mazloom Z, Yousefinejad A, Dabbaghmanesh MH: Effect of probiotics on lipid profile, glycemic control, insulin action, oxidative stress, and inflammatory markers in patients with type 2 diabetes: a clinical trial. Iran J Med Sci 38:38–43, 2013.
26. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O: Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58:1091–1103, 2009.

Received December 14, 2014; accepted March 16, 2015.