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## **Pro-and prebiotic effects on oxidative stress and inflammatory markers in non-alcoholic fatty liver disease**

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**Running title:** Probiotic, prebiotic and NAFLD

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## ABSTRACT

**Background and Objectives:** Non-alcoholic fatty liver disease (NAFLD) is related to inflammation and oxidative stress. Probiotics and prebiotics are considered anti-inflammatory and antioxidative factors. In this study, we evaluated the effects of probiotic and/or prebiotic on oxidative stress and inflammatory markers in patients with NAFLD. **Methods and Study Design:** Seventy-five NAFLD subjects were divided into four groups. The first group received a pro-biotic capsule of *Bifidobacterium longum* (B.L) and *Lactobacillus acidophilus* (L.A) ( $2 \times 10^7$  CFU/day), the second group received prebiotic (10g/day inulin), the third group received pro-biotic and prebiotic, and the fourth group received placebo, for three months. Anthropometric, inflammatory and oxidative/anti-oxidative indices were measured in all patients before and after the intervention. **Results:** We showed that consumption of pro- and/or prebiotic compared to placebo is able to significantly decrease body weight, body mass index, waist and hip circumferences, tumour necrosis factor- $\alpha$  and increase serum levels of total antioxidant capacity in patients with NAFLD ( $p < 0.01$ ). There were not any significant differences between probiotic, prebiotic and co-administration of them on the mentioned parameters. Co-administration of pro- and prebiotic caused significant decrease of high-sensitive C-reactive protein (hs-CRP) compared to the placebo and other groups ( $p < 0.01$ ). Interleukin-6 and malondialdehyde were not significantly different among groups at the end of study. **Conclusions:** Probiotic or/and prebiotic supplementation can be effective for improvement of some anthropometric, inflammatory and oxidative indices in patients with NAFLD. Co-administration of pro- and prebiotic is more effective than probiotic and prebiotic alone in modifying hs-CRP in patients with NAFLD.

**Key Words:** prebiotic, probiotic, oxidative stress, inflammatory markers, liver diseases

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic hepatic disorder worldwide. NAFLD can progress from simple steatosis to non-alcoholic steatohepatitis (NASH), and can lead to cirrhosis and hepatocellular carcinoma.<sup>1</sup> Recent studies show that global prevalence of NAFLD is 25.24% with highest prevalence in the Middle East and South America and lowest in Africa.<sup>2</sup> Increased levels of endogenous and exogenous toxins, increased oxidative stress and subsequent lipid peroxidation, insulin resistance and elevated levels of pro-inflammatory cytokines including interleukin-6 (IL-6), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and adipokines, facilitate the progression of steatosis to non-alcoholic steatohepati-

tis.<sup>3</sup> TNF- $\alpha$  plays a key role in the pathogenesis of NAFLD/NASH by inducing insulin resistance, increasing the formation of reactive oxygen species, and promoting hepatocyte apoptosis.<sup>4</sup> Oxidative stress occurs in patients with NAFLD due to an imbalance in the body's pro-oxidant/anti-oxidant status, which is normally regulated by the liver. Activated oxygen species stimulate membrane lipid peroxidation and production of malondialdehyde (MDA).<sup>3,4</sup>

There is no proven treatment for the disease.<sup>5</sup> Previous studies have showed that the gut microbiota is associated with host metabolism, and may play a major role in the pathogenesis, initiation and progression of NAFLD.<sup>6-8</sup> The microflora can cause displacement of endo-toxin from the intestines to the mesenteric bloodstream, and thus induce the production of IL-6 and TNF- $\alpha$  through activation of Kupffer cells.<sup>1,6</sup> The composition of the intestinal flora also affects energy extraction from foods, mucosal immunity, intestinal permeability; and systemic inflammation.<sup>7-9</sup> Probiotics are dietary supplements containing live bacteria that may have beneficial effects on human health.<sup>9</sup> Beneficial effects on health have been reported specially for *Bifidobacterium* and *Lactobacillus* species.<sup>10</sup> Many studies have showed that *Bifidobacterium longum* (*B.L*) and *Lactobacillus acidophilus* (*L.A*) are effective in the reduction of inflammatory cytokines.<sup>11,12</sup> Prebiotics are fermentable but non-digestible food supplements that provide benefit to the host by stimulating the growth and activity of probiotic bacteria.<sup>13,14</sup>

Inulin is a soluble fibre which has prebiotic effects. In many studies the potency of inulin to stimulate the growth and activity of probiotic bacteria as well as promoting the liver and gut function has been showed.<sup>13-15</sup> As probiotics and prebiotics have positive effects on obesity, systemic inflammation, NAFLD- and the regulation of lipid metabolism, they can be considered an ideal strategy for treating and preventing of obesity and NAFLD.<sup>16,17</sup>

Clinical trials on the effects of probiotics and prebiotics in NAFLD are limited, so more complete studies have been recommended.<sup>16-18</sup> Therefore, the aim of this study was to evaluate the effects of probiotic (*B.L* and *L.A*) and prebiotic (inulin HP: High performance; Frutafit Tex, with an average chain length of  $\geq 22$  fructose monomers), alone and in combination, on anthropometric, oxidative and inflammatory indices in patients with NAFLD.

## **MATERIALS AND METHODS**

### ***Trial design***

The study was a double-blind randomised, parallel, placebo-controlled clinical trial. The study was conducted according to the guidelines of the Declaration of Helsinki and all procedures involving human patients were approved by the Ethics Committee of the Tabriz University of

Medical Sciences (university ethical code: 5/4/7041, 1392/9/2). The study was registered as a clinical trial with the Iranian Registry of Clinical Trials (registration number: IRCT201301223140N6, <http://www.irct.ir>).

### ***Subjects***

Participants received a description of the study, and informed consent was obtained from all patients. The inclusion criteria were as follow: patients with NAFLD willing to participate, men and women aged 20–60 years and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels higher than the normal range (reference range for ALT and AST: 0–37 units/L, and 0–40 units/L, respectively). NAFLD was diagnosed via ultrasound (Medison Sonoace X6) of the liver and bile ducts. In the study, an experienced radiologist at the Ultrasonic center of Tabriz University Medical Science performed the liver ultrasound. The liver was evaluated for size, echogenicity, structure and penetration of the ultrasound beam. A normal liver and absent of steatosis was defined as having a normal liver echo texture. Based on echogenicity, beam penetration and portal vessel wall distinction, nonalcoholic fatty livers were defined.<sup>19</sup>

The exclusion criteria were as follow: pregnant and lactating women, individuals with cardiovascular, thyroid, kidney, inflammatory or autoimmune disease, individuals with diabetes, hepatitis A, B or C, individuals with hemochromatosis, Wilson's disease or inflammation; use of vitamin supplements, including vitamins A, E and C, use of prebiotic/probiotic supplements and alcohol consumption. Patients who were developed diabetes or hepatitis during the study were excluded too.

### ***Sample size***

Sampling for this study was performed by the convenience method. The participants were divided between study groups (probiotic, prebiotic, pro- and prebiotic and the placebo group) by random allocation. The required sample size was at least 19 patients per each group calculated based on the mean change in TNF- $\alpha$ , according to Malaguarnera et al study.<sup>20</sup> To allow for a dropout rate of 10%, the sample size was increased to 21 in each group.

### ***Interventions***

Participants were matched for age and sex and randomly divided into four groups, including three intervention and one control group, using a computer-generated randomisation scheme with block sizes of four and eight and an allocation ratio of 1:1:1:1. The first group (n=21)

received probiotic capsule (*B.L* and *L.A*:  $2 \times 10^7$  CFU/day; the probiotics were prepared and assessed for their probiotic properties and viability in Pharmaceutical Nanotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran) and prebiotic placebo. Prebiotic placebo, as sachet, was filled with maltodextrin powder (Huirong Trade Company Limited). The second group (n=21) received a prebiotic as inulin HP (Sensus, Borchwerf, 34704 RG Roosendaal, The Netherlands) and probiotic placebo, as capsule, was filled with fat and lactose-free milk (Nestle S.A; Vevey, Switzerland). The third group (n=21) received probiotic and prebiotic (*B.L* and *L.A*:  $2 \times 10^7$  CFU/day, plus 10 g/day inulin HP). The fourth group (n=21) received prebiotic and probiotic placebo. The probiotic and its placebo were administered as 250 mg capsules, and the prebiotic and its' placebo were administered as 5 g packaged sachets, to be taken twice a day in the morning and evening. All treatments were administered for three months. The distribution of the supplements and placebo was in the beginning and middle of the study. We followed up supplements and placebo consumption every two weeks by phone call. The dose of the pro- and prebiotic was according to WHO guidelines and studies on the pro- and prebiotic studies.<sup>15,18</sup>

### **Measurements**

All of the measurements including anthropometric assessments, dietary intake records and blood tests, were done before and after the intervention. The primary outcome of the study was difference of TNF- $\alpha$  serum levels. The remaining variables (i.e. IL-6, high-sensitive C-reactive protein (hs-CRP), MDA, total antioxidant capacity (TAC), body mass index (BMI), weight, waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR), fat mass (FM), fat-free mass (FFM) and total body water (TBW)) were considered as secondary outcomes.

### **Anthropometric assessments**

Each patient's height, weight, WC and HC were measured using standard anthropometric techniques.<sup>21</sup> The BMI and WHR were calculated. FM, FFM, and TBW were measured simultaneously using a body composition analyser (BC-418 MA).

### **Dietary intakes**

The participants' dietary intake was assessed by use of a three-day food record (two weekdays and one weekend day). The dietary data were analysed using the Nutritionist IV software

program (First Databank, Inc., Hearst Corporation) using the database from tables of content and nutritional value of Iranian food products.

### **Blood tests**

At the beginning and at the end of study, 10 mL of venous blood samples were obtained after an overnight fast (12 h). Sera were separated at 4°C and stored at -70°C for later analysis. More details of blood sampling and sera preparation procedures are given in Javadi et al study.<sup>22</sup> Hs-CRP levels were determined using a BIOSYS kit (Biosystems S.A. Costa Brava, 30.08030 Barcelona, Spain) and an Alcyon 300 auto analyser. TNF- $\alpha$  and IL-6 levels were determined using a Diasource kit (Immuno Assays S.A.–Rue du Bosquet, 2-b-1384 Louvain-la-Neuve-Belgium) via ELISA (ELISA plate reader, Statfax-2100 and ELISA plate washer, Statfax-2600 model). MDA levels, as a marker of lipid peroxidation, were measured using 2-Thiobarbituric Acid Reactive Substances (TBARS) and a spectrophotometer (UV-Vis Spectrophotometer, Ultra-Spect 2000, Pharmacia, Pfizer, USA). TAC levels were measured as an indicator for different antioxidants in the body using an LDN Labor Diagnostika Nord GmbH & Co. KG kit via ELISA (Elisa plate reader, Model Statfax-2100, Awareness Technology, USA).

### **Statistical analysis**

The data were analysed using SPSS 21.0 (SPSS, Japan, Inc.). The normal distribution of all variables was confirmed by residual plot. Analysis of variance for basal comparisons and analysis of covariance (ANCOVA) for final comparisons were used. We considered in the ANCOVA model basal figures, and age, sex, BMI, energy intake as confounding factors. A paired t-test was used for intra-group changes. The least significant difference test was used for inter-group comparisons. Statistical significance was set at a  $p$ -value of <0.05.

## **RESULTS**

### **Subject characteristics**

The participants were recruited from May 2013 to March 2014 and were followed up with until April 2015. The study diagram is shown in Figure 1. All included subjects for statistical analysis consumed supplements. No side effects were reported by the subjects. The mean (standard deviation, range) of age and BMI in the studied subjects were: 42.0 (8.95, 20–60 year) and 30.84 (4.1, 25.46–35.51 kg/m<sup>2</sup>), respectively (Table 1).

### ***Anthropometric indices***

Probiotic, prebiotic and co-administration of pro- and prebiotic caused significant decrease of BMI, weight, HC and WC at the end of the study (Table 2). In the probiotic, prebiotic, probiotic plus prebiotic and placebo groups, BMI changes were as: -0.65, 95% CI: -0.99–0.32 kg/m<sup>2</sup>; -0.58, 95% CI: -0.96–0.19 kg/m<sup>2</sup>; -0.83, 95% CI: -1.24–0.42 kg/m<sup>2</sup> and 0.18, 95% CI: -0.03–0.39 kg/m<sup>2</sup>, respectively. The effects of probiotic, prebiotic and probiotic plus prebiotic on the assessed anthropometric indices were not significantly different. WHR, FM, FFM and TBW were not significantly different among the four studied groups at the end of study (Table 2). In our study, ALT and AST levels decreased in intervention groups compared to the placebo group. Grade of fatty liver in probiotic and prebiotic plus probiotic group decreased compared to the placebo group (data not shown). Related results are given in detail in another article.<sup>22</sup>

### ***Dietary intakes***

Dietary intakes, including: energy, carbohydrate, protein and fat intakes were not significantly different at the basal and at the end of study among groups. Zinc intake was different, while vitamin E, vitamin C and selenium were not different at the end of study among the four groups (Table 3).

### ***Inflammatory and oxidative/antioxidative status parameters***

The inflammatory and oxidative/anti-oxidative indices in the four groups are shown in Table 4. We found significant differences in mean of hs-CRP, TNF- $\alpha$  and TAC among the four studied groups at the end of study. Co-administration of pro- and prebiotic caused significant decreases in hs-CRP (-0.93, 95% CI: -1.32–0.53 mg/L) compared to the placebo, probiotic and prebiotic groups. Mean of TNF- $\alpha$  in probiotic, prebiotic and probiotic plus prebiotic groups were significantly lower than placebo at the end of the study (Table 4).

Probiotic, prebiotic and co-administration of pro- and prebiotic caused significant increase of TAC compared to the placebo group (0.44, 95% CI: 0.25–0.63 mmol/L; 0.18, 95% CI: 0.03–0.38 mmol/L; 0.40, 95% CI: 0.19–0.6 mmol/L, and -0.1, 95% CI: -0.23–0.03 mmol/L, respectively). Probiotic was more effective than prebiotic, but similar to probiotic plus prebiotic in TAC increase (Table 4). The mean of IL-6 and MDA were not significantly different among four groups at the end of study ( $p > 0.05$ , Table 4).

## **DISCUSSION**

The aim of the study was to evaluate the singular and combined effects of probiotic (*B.L* and *L.A*) and prebiotic (inulin HP) on oxidative stress and inflammatory markers in patients with NAFLD. Body weight and BMI are important factors in the pathogenesis of NAFLD. It is shown that 7%-10% reduction in body weight is considered a way to manage NAFLD.<sup>5</sup> Our study showed that probiotic, prebiotic and co-administration of them are effective in BMI, weight, HC and WC reduction. Probiotic, prebiotic and co-administration of pro- and prebiotic had no effect on energy, carbohydrate, fat and protein intakes. There were no significant differences in FM, FFM and TBW between the groups at the end of study. Decreases in BMI were reported by Alisi et al<sup>23</sup> in the children with NAFLD treated with VSL#3. VSL#3 was a mixture of eight probiotic strains including *B.L* and *L.A* Yadav et al<sup>24</sup> in a study on mouse model showed that probiotic VSL#3 is able to prevent and treat obesity. Ferolla et al<sup>25</sup> in nonalcoholic steatohepatitis (NASH) patients, showed that symbiotic supplementation (*Lactobacillus reuteri* with guar gum and inulin) for three months caused a significant reduction in body weight, BMI and WC. In contrast to our results, some other studies have failed to show any significant effect of probiotics or prebiotics on anthropometric parameters, including BMI in NAFLD subjects.<sup>26,27</sup>

The exact mechanism(s) of probiotic and prebiotic in weight and BMI reduction are not completely clarified. Some studies showed that composition of the intestinal flora affects energy extraction from foods.<sup>7,9</sup> Suppression of ghrelin and enhancement of peptide YY and glucagon-like peptide1, appetite suppression, altered lipid metabolism, altered choline and bile acid metabolism and an increase in energy expenditure are the other probable mechanisms by which probiotics and prebiotics exert their anti-obesity effects.<sup>7,23,24,28-30</sup> Parnell and Reimer<sup>30</sup> clarified that suppression of ghrelin and enhancement of peptide YY may contribute to the reduction of energy intake in overweight adults who were supplemented with 21 g oligofruco-tose for 12 weeks. In contrast to this, in the Eslamparast et al<sup>27</sup> study, symbiotic supplementa-tion had no effect on energy intake of the NAFLD subjects.

Dysbiosis of the gut microbiota can result in altered immune system responses. These altered immune responses can affect many pathways which are related to the NAFLD.<sup>1,8</sup> Increased levels of endogenous and exogenous toxins, elevated levels of pro-inflammatory and oxidative stress parameters are common in NAFLD.<sup>3,4</sup> We showed that co-administration of pro- and prebiotic caused significant decreases in hs-CRP. Probiotic, prebiotic and probiotic plus prebiotic had significant effects on TNF-a and TAC compared to the placebo group. We did not detect any significant differences on the levels of IL-6 and MDA at the end of study among the four groups.

The results of the present study for hs-CRP are similar to the many studies on the subject.<sup>20,27</sup> Malaguarnera et al<sup>20</sup> and Eslamparast et al<sup>27</sup> in patients with NAFLD, showed that supplementation with symbiotic (*B.L* with fructooligosaccharides and seven strains of probiotic bacteria with fructooligosaccharide, respectively) caused significant decreases in CRP and hs-CRP. The effects of symbiotic supplementation on the hs-CRP reduction also have been reported in patients with type 2 diabetes mellitus.<sup>31,32</sup> Unlike these studies, Asgharian et al<sup>33</sup> failed to show any significant effect of symbiosis (contained of seven strains of probiotic bacteria, including *L.A* and *B.L* together with fructooligosaccharide) in NAFLD patients.

The results of the present study with pro- and prebiotics on TNF- $\alpha$  are like many other studies.<sup>20,27</sup> Gao et al<sup>34</sup> in a meta-analysis of randomised controlled trials for assessing of efficacy of probiotic, showed improvement of TNF-a in NAFLD patients. Like our study Vajro et al<sup>35</sup> on paediatric obesity related liver disease and Loguercio et al<sup>36</sup> on chronic liver disease patients, showed no effect of probiotic on IL-6; IL-6 is specific to and confirming of NASH.<sup>37</sup> In our study, insignificant decreases of IL-6 may be due to the relatively short treatment period and low-grade inflammatory status of the subjects.

The possible probiotic and symbiotic effects on the improvement of some inflammatory parameters may be attributed to the probiotic capacities for preventing of endotoxin displacement from the intestines to the blood.<sup>1,6</sup> Increased intestinal permeability and tight junction alterations in NAFLD patients have been reported by Miele et al<sup>38</sup> and Rodes et al<sup>11</sup> in an in vitro study showing that B.L can decrease colonic lipopolysaccharide concentration and reduce pro-inflammatory parameters. Briskey et al<sup>39</sup> in a mouse model of NAFLD, showed that probiotics modify tight junction proteins like as follows: ZO-1 and ZO-2.

The result of the present study for TAC improvement by administration of pro- and/or prebiotic supplementation is similar to Ipar et al<sup>40</sup> who showed that symbiotic supplementation caused improvement in total oxidative stress in obese children. Borges Haubert et al<sup>41</sup> in animal model study of NAFLD, showed that fructooligosaccharide had no effect on liver and heart thiobarbituric acid reactive substances (TBARS). TBARS are formed as a byproduct of lipid peroxidation. Unlike our study, Loguercio et al<sup>36</sup> showed that plasma levels of MDA decreased in NAFLD patients who received probiotic VSL#3. Pourghassem et al<sup>42</sup> showed HP inulin caused significant increases in TAC and superoxide dismutase activity in women with type 2 diabetes. The exact mechanism(s) of pro- and/or prebiotics on oxidative stress is not clearly discussed. There are several possible mechanisms, such as direct neutralisation of oxidants in the intestinal tract by the expression of antioxidant enzymes, reduction of inflammation and prevention from cytokine-induced oxidative stress, inhibition of intestinal patho-

gens, which reduces inflammation and associated oxidative damage, modification of lipid metabolism, enhancement of absorption of micro- and macronutrients and increasing antioxidant enzymes activity in the host.<sup>1,9,43</sup> The observed controversies in the studies are most likely due to the differences in pathological state of the subjects, as well as basal status of inflammatory/anti-inflammatory and oxidative/anti-oxidative of participants, study duration, dosage, type and time of supplementation, disease severity and genotype of the used bacteria.

The most important strength of the current study is that it is among few studies which assessed probiotic and prebiotic effects - alone and in combination to each other - in NAFLD subjects. In addition, some aspects of our study e.g. probiotic, prebiotic and their co-administration effects on oxidative/anti-oxidative status in NAFLD patients, to the best of our knowledge, are novel.

Our study had some limitations. The first was that we did not use liver biopsy to assess disease severity; instead, we used a noninvasive method, ultrasound imaging of the liver and bile ducts, for disease detection. This may have effects on the measurements of the interventions. The second was that we have not assessed intestinal bacteria and SCFA. Short follow up time may be considered another limitation. With considering these limitations, further clinical investigations with large sample sizes and long-term follow up are needed to better clarify the effects of probiotic, prebiotic and co-administration of pro- and prebiotics on the NAFLD patients.

### **Conclusion**

In conclusion, probiotic and/or prebiotic supplementation such as  $2 \times 10^7$  CFU/day *B.L* and *L.A* and 10 g inulin HP/day, can be effective for the reduction of some anthropometric and inflammatory markers and can increase of the total antioxidant capacity in patients with NAFLD. Co-administration of pro- and prebiotic is more effective than probiotic and prebiotic alone in modifying hs-CRP in patients with NAFLD. Probiotic and/or prebiotic can be considered as an adjuvant therapy for NAFLD patients.

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## CONFLICT OF INTEREST AND FUNDING DISCLOSURE

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**Table 1.** Demographic characteristics of the studied subjects

Demographic data	Probiotic (n=20)	Prebiotic (n=19)	Probiotic + Prebiotic (n=17)	Placebo (n=19)	Total (n=75)
Age (year) <sup>†</sup>	43.9±9.02	38.68±10.0	43.24±6.95	42.21±9.11	42.0±8.95
Sex (%)					
Men	17 (85%)	16 (84.2%)	14 (82.4%)	13 (68.4%)	60 (80%)
Women	3 (15%)	3 (15.8%)	3 (17.6%)	6 (31.6%)	15 (15%)
Education (%)					
Illiterate	3 (15%)	0 (0%)	1 (5.9%)	1 (5.3%)	5 (6.7%)
Less than high school	1 (5%)	3 (15.8%)	3 (17.6%)	6 (31.6%)	13 (17.3%)
High school	11 (55%)	8 (42.1%)	7 (41.2%)	4 (21.1%)	30 (40%)
High master	5 (25%)	8 (42.1%)	6 (35.3%)	8 (42.1%)	27 (36%)

<sup>†</sup>Expressed as mean and standard deviation. No significant difference between groups was seen on the age, sex and education levels.

**Table 2.** Anthropometric characteristics of the studied subjects

Anthropometric characteristics	Probiotic (n=20)	Prebiotic (n=19)	Probiotic + Prebiotic (n=17)	Placebo (n=19)	pv <sup>‡</sup>
BMI (kg/m <sup>2</sup> )					
Before	29.91±3.88	30.96±4.39	32.30±4.78	30.38±2.88	
After	29.26±3.59 <sup>a</sup>	30.38±4.63 <sup>a</sup>	31.47±4.58 <sup>a</sup>	30.56±2.88 <sup>b</sup>	0.01
pv <sup>‡</sup>	0.01	0.01	0.01	0.09	
Weight (kg)					
Before	86.92±12.37	88.45±10.36	89.88±11.92	86.00±11.98	
After	85.08±12.25 <sup>a</sup>	86.45±10.54 <sup>a</sup>	87.91±12.08 <sup>a</sup>	86.51±12.05 <sup>b</sup>	0.01
pv <sup>‡</sup>	0.01	0.01	0.01	0.07	
WC (cm)					
Before	101.15±8.83	102.84±7.22	106.99±9.38	101.03±5.74	
After	99.85±8.41 <sup>a</sup>	101.34±7.47 <sup>a</sup>	105.85±10.05 <sup>a</sup>	101.92±5.78 <sup>b</sup>	0.01
pv <sup>‡</sup>	0.01	0.04	0.06	0.03	
HC (cm)					
Before	109.33±6.03	109.55±8.79	113.68±8.60	109.76±6.36	
After	108.22±5.98 <sup>a</sup>	109.39±8.77 <sup>a</sup>	112.24±8.19 <sup>a</sup>	111.16±5.75 <sup>b</sup>	0.01
pv <sup>‡</sup>	0.01	0.77	0.01	0.01	
WHR					
Before	0.93±0.06	0.94±0.03	0.95±0.06	0.92±0.05	
After	0.92±0.06	0.93±0.04	0.94±0.06	0.92±0.05	0.06
pv <sup>‡</sup>	0.24	0.01	0.21	0.16	
FM (kg)					
Before	22.18±6.07	24.39±7.94	27.68±8.45	25.60±6.40	
After	22.37±5.37	24.37±8.22	26.84±8.45	25.86±6.54	0.29
pv <sup>‡</sup>	0.76	0.94	0.04	0.21	
FFM (kg)					
Before	64.45±10.03	65.79±9.11	63.56±7.70	60.33±12.16	
After	64.09±9.97	64.37±6.59	62.56±8.36	61.22±12.17	0.17
pv <sup>‡</sup> <sub>2</sub>	0.35	0.17	0.09	0.15	
TBW (kg)					
Before	47.27±7.39	48.36±6.77	49.15±13.34	44.67±8.61	
After	46.27±7.11	45.77±4.48	46.52±6.85	45.06±8.55	0.89
pv <sup>‡</sup>	0.22	0.01	0.21	0.26	

BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; FM: fat mass; FFM: fat-free mass; TBW: total body water.

Data are expressed as mean± standard deviation (SD).

<sup>†</sup>pv resulted from analysis of covariance in the adjusted model for basal figures and sex, age, energy intake and body mass index.

<sup>‡</sup>pv resulted from paired sample t tests.

<sup>a, b, c</sup> Data with different superscript letters are significantly different to the ANCOVA statistical analysis.

**Table 3.** Dietary intakes of the studied subjects

Dietary intakes	Probiotic (n=20)	Prebiotic (n=19)	Probiotic + Prebiotic (n=17)	Placebo (n=19)	pv <sup>†</sup>
Energy (kcal/day)					
Before	2369.70±515.66	2296.26±282.16	2153.18±459.56	2158.21±463.99	
After	2305.75±616.80	2244.74±174.53	2102.12±254.26	2080.16±408.72	0.69
pv <sup>‡</sup>	0.27	0.11	0.68	0.44	
CHO (g/day)					
Before	369.33±93.70	341.91±60.27	321.54±70.72	311.41±52.75	
After	361.97±96.84	352.12±58.10	311.29±52.64	297.89±53.96	0.11
pv <sup>‡</sup>	0.49	0.41	0.59	0.36	
Pro (g/day)					
Before	97.31±24.22	89.86±13.14	73.83±23.46	76.61±23.83	
After	101±24.85	90.00±20.89	77.74±19.87	78.28±18.60	0.26
pv <sup>‡</sup>	0.22	0.96	0.42	0.68	
Fat (g/day)					
Before	67.07±12.66	60.32±10.57	60.65±20.55	60.37±19.73	
After	68.99±19.32	52.97±4.15	62.43±17.95	64.23±24.45	0.06
pv <sup>‡</sup>	0.52	0.01	0.75	0.35	
Zinc (mg/day)					
Before	8.35±2.82	8.66±1.50	7.75±2.65	7.40±2.47	
After	8.55±2.52 <sup>a</sup>	7.81±2.10 <sup>b</sup>	6.64±1.44 <sup>a, c</sup>	6.581.32 <sup>d</sup>	0.02
pv <sup>‡</sup>	0.69	0.05	0.12	0.13	
Vit E (mg/day)					
Before	13.63±7.37	9.56±5.58	14.23±11.12	9.34±5.04	
After	11.02±5.93	10.01±5.22	9.61±5.02	9.11±4.86	0.59
pv <sup>‡</sup>	0.04	0.71	0.05	0.82	
Vit C (mg/day)					
Before	121.12±62.49	117.04±57.77	83.50±58.47	113.86±68.21	
After	89.69±63.70	93.92±51.22	98.35±51.01	86.70±49.48	0.22
pv <sup>‡</sup>	0.01	0.10	0.18	0.04	
Selenium (mg/day)					
Before	0.12±0.04	0.13±0.04	0.17±0.08	0.13±0.04	
After	0.12±0.05	0.13±0.04	0.12±0.06	0.13±0.05	0.58
pv <sup>‡</sup>	0.55	0.97	0.01	0.58	

CHO: carbohydrates; Pro: protein; Vit: vitamin.

Data are expressed as mean ± SD.

<sup>†</sup>pv resulted from analysis of covariance in the adjusted model for basal figures and sex, age, energy intake and body mass index.

<sup>‡</sup>pv resulted from paired sample t tests.

<sup>a, b, c</sup> Data with different

**Table 4.** Effects of probiotic and/or prebiotic on serum levels of inflammatory and oxidative/anti-oxidative indices

Inflammatory and oxidative/antioxidative indices	Probiotic (n=20)	Prebiotic (n=19)	Probiotic + Prebiotic (n=17)	Placebo (n=19)	pv <sup>†</sup>
hs-CRP (mg/L)					
Before	1.95±1.8	1.87±1.43	2.78±1.17	2.66±1.49	
After	1.78±1.49 <sup>a,b</sup>	1.57±1.03 <sup>a,b</sup>	1.85±0.81 <sup>b</sup>	2.36±1.05 <sup>a,c</sup>	0.01
pv <sup>‡</sup>	0.35	0.26	0.01	0.12	
TNF- $\alpha$ (pg/mL)					
Before	8.10±2.15	8.33±2.61	8.24±1.96	9.13±2.62	
After	7.59 ±2.43 <sup>a</sup>	8.12±1.55 <sup>a</sup>	8.10±1.36 <sup>a</sup>	10.24±4.24 <sup>b</sup>	0.03
pv <sup>‡</sup>	0.11	0.68	0.77	0.12	
IL-6 (pg/mL)					
Before	26.26±18.84	27.43±13.52	26.50±20.13	19.73±8.52	
After	22.43±13.82	25.48±13.32	24.31±16.67	21.18±12.71	0.51
pv <sup>‡</sup>	0.07	0.27	0.31	0.37	
MDA (nmol/L)					
Before	14.29±2.75	14.87±3.81	13.93±3.11	12.11±3.15	
After	11.41±2.57	12.76±3.82	11.65±3.21	11.67±2.14	0.42
pv <sup>‡</sup>	0.01	0.02	0.01	0.58	
TAC (mmol/L)					
Before	1.89±0.41	1.99±0.49	1.90±0.43	2.01±0.41	
After	2.33±0.29 <sup>a</sup>	2.17±0.39 <sup>b</sup>	2.30±0.28 <sup>a,b</sup>	1.91±0.39 <sup>c</sup>	0.01
pv <sup>‡</sup>	0.01	0.08	0.01	0.14	

hs-CRP: high-sensitive C reactive protein; TNF- $\alpha$ :tumor necrosis factor- $\alpha$ ; IL-6:interleukin-6; MDA: malondialdehyde; TAC: total anti-oxidant capacity.

Data are expressed as mean±SD.

<sup>†</sup>pv resulted from ANCOVA in the adjusted model for basal figures and sex, age, energy intake and body mass index (in TAC adjusted models for vitamin E, C, zinc and selenium also).

<sup>‡</sup>pv resulted from paired sample t tests.

<sup>a, b, c</sup> Data with different.

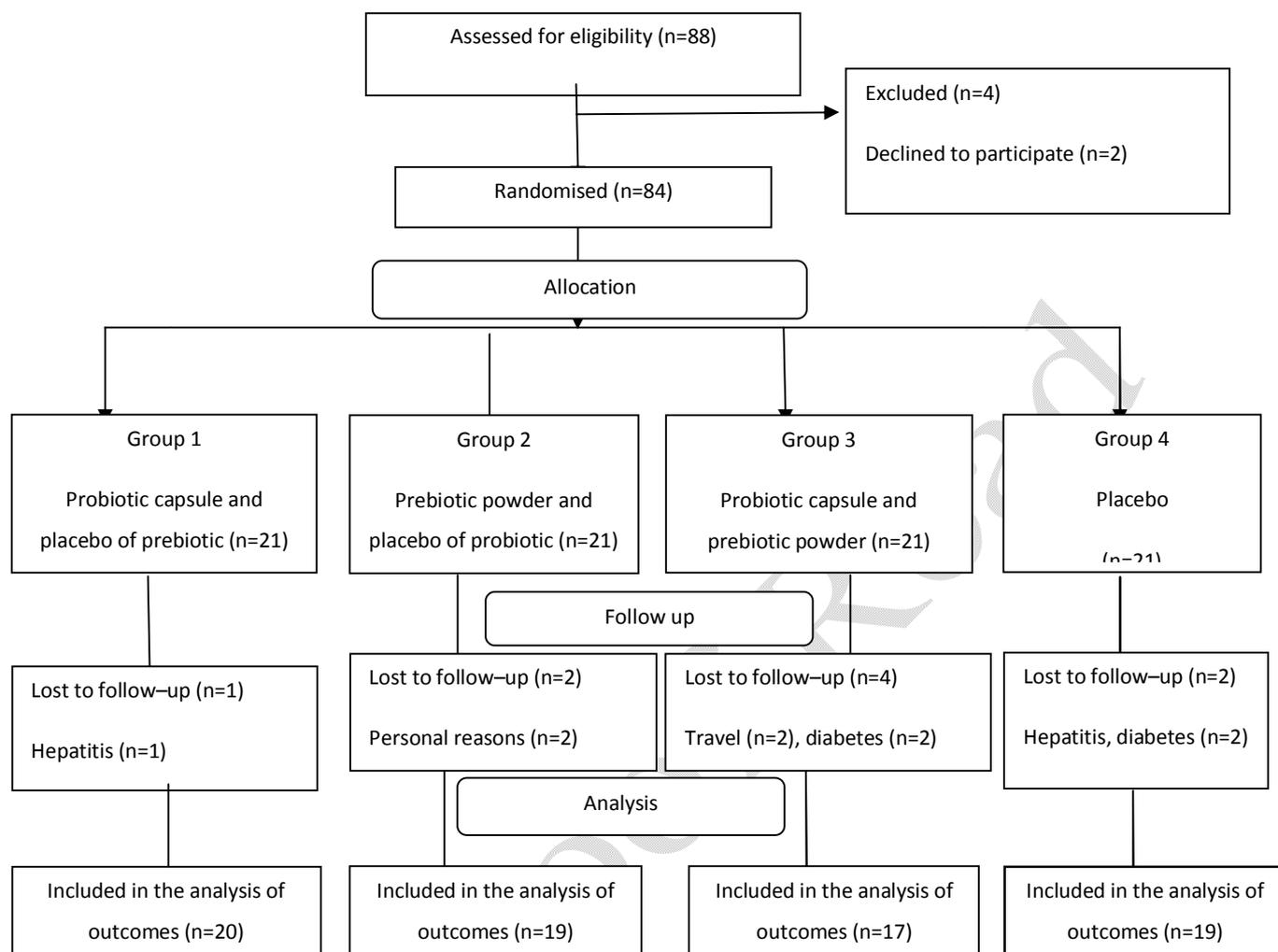


Figure 1. Flowchart of the study.