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ORIGINAL CONTRIBUTION

Impact of a short-term synbiotic supplementation on metabolic syndrome and systemic inflammation in elderly patients: a randomized placebo-controlled clinical trial

Arrigo F. G. Cicero[1] · Federica Fogacci[1] · Marilisa Bove[1] · Marina Giovannini[1] · Claudio Borghi[1]

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Abstract

Purpose The connection between gut microbiota imbalance, inflammation and its role in the pathogenesis of metabolic syndrome (MetS) clustering factors has been increasingly recognized. However, data on the efficacy of probiotics supplementation on MetS components are few and almost lacking in the elderly. To address this issue, we conducted a randomized, double-blind, placebo-controlled, parallel-group, clinical study on a large sample of MetS elderly patients.

Methods After 14 days of diet and physical activity standardization, 60 elderly patients were randomized to treatment with a synbiotic formula of *Lactobacillus plantarum* PBS067, *Lactobacillus acidophilus* PBS066 and *Lactobacillus reuteri* PBS072 with active prebiotics or placebo. Patients were evaluated anamnestically and by the execution of a physical examination and laboratory and haemodynamic analyses at the baseline and after 60 days of treatment. At enrollment and at the end of the trial, all enrolled patients complete the EuroQol-5 Dimension (EQ-5D) questionnaire.

Results Through the 2-month period of treatment, patients who received active treatment experienced a statistically significant improvement in waist circumference and in fasting plasma insulin, total cholesterol, high-density lipoprotein cholesterol, non-HDL-C, triglycerides (TG), low-density lipoprotein cholesterol, high-sensitivity C-reactive protein and tumor necrosis factor alpha serum levels, compared both to the baseline and the control group. Visceral adiposity index improvement in the synbiotic treatment group was significantly greater than in placebo group. Compared to baseline, treatment with synbiotics also significantly reduced mean arterial pressure and fasting plasma glucose.

All treatment groups demonstrated a significant decrease in TG. TG reduction in the synbiotic group was significantly greater than in the control group.

The EQ-5D VAS questionnaire significantly improved only in probiotics-treated subjects.

Conclusion Treatment with a synbiotic formula of *L. plantarum* PBS067, *L. acidophilus* PBS066 and *L. reuteri* PBS072 with active prebiotics decreased MetS syndrome prevalence, several cardiovascular risk factors and markers of insulin resistance in elderly patients.

Keywords Elderly · Probiotics · Metabolic syndrome · Inflammation

Background

An increasing body of evidence indicates that dysbiosis (defined as microbial imbalance) of the gut is implicated not only in the pathogenesis of intestinal disorders, but also in a number of extra-intestinal diseases including non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome (MetS) [1–3], which worsen patients' quality of life and have a significant economic impact on public health expenditure [4].

In the general population, components of MetS—including obesity, dyslipidemia, glucose intolerance and hypertension—are associated with a twofold increase in the risk of coronary heart disease (CHD) and cerebrovascular disease [5]. Moreover, in the elderly, MetS leads to a greater risk of cardiovascular (CV) and all-cause mortality (by 24 and 23%, respectively), accordingly with the findings of a meta-analysis including 57,202 subjects [6].

✉ Arrigo F. G. Cicero
arrigo.cicero@unibo.it

[1] Department of Medical and Surgical Sciences, Alma Mater Studiorum, Università di Bologna, Bologna, Italy

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The growing prevalence of MetS in the last decades has encouraged the search for strategies for its prevention and treatment [7]. As a matter of fact, the average prevalence of MetS is currently approximately 31% in the general population [5]. In particular, its frequency is likely to be high among elderly individuals (reaching up to 55% in some study populations), being considered a major public health challenge [8, 9].

The connection between gut microbiota imbalance, inflammation and its role in the pathogenesis of MetS components has recently been the focus of attention [10]. As a matter of fact, it is well known as the gut microbiota is the product of a complex interaction between host's genetics and environment [11].

Intestinal microorganisms are involved in the bioconversion of food components and lead to the production of several bioactive molecules (e.g., short-chain fatty acids, vitamins and metabolites) with multiple activities, finally playing a crucial role in different aspects of our physiology [12]. Considering the current evidence, gut bacterial profiles may represent new disease predictors and manipulation of the gut microbiota could be a promising approach for the prevention and management of metabolic diseases, especially in elderly where changes in the gut microbiota are frequently associated with a concomitant decline in the cognitive and immune functions [13–15]. For this reason, nutritional strategies directed at restoring the microbiota in the elderly have to be addressed from a global perspective, considering not only the microbiota but also other extra-intestinal targets of action [15].

Recently, a meta-analysis of 18 randomized clinical trials with 1544 included patients has concluded that probiotic foods and supplements with *Lactobacillus* and *Bifidobacterium* could be considered as interventions to improve anthropometric and biochemical outcomes in MetS [16]. However, to date the number of human intervention studies considering the effect of probiotics and synbiotics on every component of MetS is very limited and sometimes contradictory [10], especially in the elderly, though treating MetS would be particularly useful to prevent disability and promote a normal aging [17].

To address this issue, the present study aimed to explore the potential effects of a synbiotic supplementation on an adequate sample of MetS elderly subjects, in the context of a randomized, double-blind, placebo-controlled, parallel-group, clinical trial.

Methods

Study design and participants

This was a double-blind, randomized, placebo-controlled, parallel-group clinical trial carried out in a group of elderly patients (aged 65–80 years) with a diagnosis of MetS based on the IDF criteria [18] and consecutively enrolled in the ambulatory service of cardiovascular disease prevention of the Medical and Surgical Sciences Department of the University of Bologna. Enrolled volunteers did not have any serious or disabling diseases (e.g., severe organ failure—hepatic, renal, cardiac, respiratory—previous major cardiovascular event, active viral hepatitis, inflammatory bowel disease, malignancy and dementia). Furthermore, they did not experience optimizable chronic therapies.

Before enrollment, all patients underwent the mini nutritional assessment (MNA) examination, which provides a single and rapid assessment of nutritional status in frail elderly patients, evaluating the risk of malnutrition. Patients with protein-calories undernutrition (MNA < 17) or at risk for malnutrition (MNA ≥ 17 or MNA ≤ 23.5) were excluded from the trial [19, 20].

The entire study included a 14-day run-in period of diet standardization and a 60-day treatment period.

At enrollment, patients had an interview with a specialist physician, who developed flexible diet plans in line with the general indications of a Mediterranean diet. Patients were suggested to provide around 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/day and 35 g/day of fiber. They were suggested to maintain an overall balanced diet, avoiding an excessive intake of dairy and red meat derived products and reducing the dietary excesses. Furthermore, they were asked to maintain a constant intake of fruits, vegetables, olive oil and wine throughout the study, to reduce the variability in the dietary content of fibers and polyphenols. Finally, individuals were encouraged to increase their physical activity by walking briskly or cycling from three to five times for week, at least 20 min every time.

Nutrients' intake was estimated from the 4 days records before the randomization and at the end of the intervention period. The nutritional evaluation was performed by an expert nutritionist biologist with Software MètaDieta® (Me.Te.Das.r.l., San Benedetto del Tronto, Italy) using Italian Food Composition databases.

Adherence to exercise prescription was evaluated according to a metabolic equivalent task (MET) assessment using a short version of the global physical activity questionnaire (GPAQ).

Patients were evaluated anamnestically and by the execution of a physical examination and laboratory and hemodynamic analyses at the baseline and after 60 days of treatment. At enrollment and at the end of the trial, all enrolled patients complete the EuroQol-5 Dimension (EQ-5D) questionnaire.

All the measurements were carried out following standardized protocols by specially trained staff.

The study fully complied with the ethical guidelines of the Declaration of Helsinki and with The International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Harmonized Tripartite Guideline for Good Clinical Practice (GCP). The study protocol was approved by the Ethical Committee of the University of Bologna. All volunteers signed a written informed consent to participate and were free to withdraw from the study at any time.

Treatment

After 14 days of diet and physical activity standardization, each patient was randomly allocated to either placebo or active treatment. Intervention group was asked to take one liquid vial every day for 60 days. Each biphasic vial contained a symbiotic formula and was identical to placebo in terms of size, shape, color, weight, odor and taste. *Lactobacillus plantarum* PBS067—DSM 24,937 (colony forming units (CFU) per dose = 2×10^9), *Lactobacillus acidophilus* PBS066—DSM 24,936 (CFU per dose = 2×10^9) and *Lactobacillus reuteri* PBS072—DSM 25,175 (CFU per dose = 2×10^9) (total CFU/dose = 6×10^9 CFU) were enclosed in the dosing cap, while prebiotic fibers, inulin and fructooligosaccharides (FOS) were dissolved in the liquid phase (Table 1).

Randomization sequence were performed centrally, by computer-generated codes (using R package), and block were stratified by sex and age. All participants, study staffs and data analysis were blinded to the group assignment. Codes were kept in a sealed envelope, which was not opened until the end of the trial.

All patients were recommended to take the treatment regularly, every day early in the morning. Patients were asked to record every day the time of administration of the supplement, and document any missing dose in a daily diary. At the end of the study, all unused vials were retrieved for inventory. Participants' compliance was counter tested by counting the number of vials returned at the last visit of the trial.

Assessments

Clinical data and anthropometric measurements

Patients' personal history was evaluated taking particular attention to CVD and other diseases, dietary and smoking habits assessment (both evaluated with validated semiquantitative questionnaires) [21], physical activity and pharmacological treatment.

Waist circumference (WC) was measured at the end of a normal expiration, in a horizontal plane at the midpoint between the inferior margin of the last rib and the superior iliac crest. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, with subjects standing erect with eyes directed straight wearing light clothes, and with bare feet. Body mass index (BMI) was calculated as body weight in kilograms, divided by height squared in meters (kg/m^2). The index of central obesity (ICO) resulted from WC to height ratio [22].

EuroQol-5 Dimension questionnaire

The EQ-5D is an instrument that evaluate the quality of life. The EQ-5D descriptive system is a preference-based HRQL measure with one question for each of the five dimensions that include mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The answers given to EQ-5D permit to find 243 unique health states or can be converted into EQ-5D index, a utility scores anchored at zero for death and one for perfect health. The questionnaire includes a Visual Analog Scale (VAS), by which respondents can report their perceived health status with a grade ranging from 0 (the worst possible health status) to 100 (the best possible health status) [23].

Each patient was asked to answer the questionnaire before and at the end of the treatment period, to assess their self-perception of the current physical state and quality of life in response to the treatment received.

Table 1 Percentage composition of active and placebo treatment

	Active treatment	Placebo treatment
Lactobacillus plantarum LP PBS067 (DSM 24,937)	12.18	–
Lactobacillus acidophilus LA PBS066 (DSM 24,936)	30.44	–
Lactobacillus reuteri LR PBS072 (DSM 25,175)	30.44	–
Inulin	12.97	–
Fructooligosaccharides	12.97	–
Vegetable magnesium stearate	1	1
Maltose	–	99

Laboratory analyses

All measurements were centrally performed in the laboratory of our department. The biochemical analyses were carried out on venous blood and all subjects were fasted for at least 12 h at the time of sampling. Serum used was obtained by addition of Na_2EDTA (1 mg/mL) and centrifuged at 3000 RPM for 15 min at 25 °C. Immediately after centrifugation, trained personnel performed laboratory analysis according to standardized methods [24]. The following parameters were obtained or calculated through the appropriate formula: total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), non-HDL cholesterol, low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG), fasting plasma insulin (FPI), homeostatic model assessment of insulin resistance (HOMA-IR), serum uric acid (SUA), creatinine, estimated glomerular filtration rate (eGFR), high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor alpha (TNF-alpha), adiponectin, leptin, gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), fatty liver index (FLI), lipid accumulation product (LAP), hepatic steatosis index (HSI) and visceral adiposity index (VAI). TNF-alpha was measured by enzyme-linked immunosorbent assay (ELISA).

LDL-C was obtained by the Friedewald formula. Non-HDL-C resulted from the difference between TC and HDL-C. HOMA-IR was calculated as the product of FPG and FPI (respectively, expressed in mmol/L and U/mL) divided by 22.5 [25]. GFR was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CK-epi) equation [26]. FLI was obtained by dividing $(e^{0.953} \times \log_e(TG) + 0.139 \times BMI + 0.718 \times \log_e(GGT) + 0.053 \times WC—15.745)$ by $(1 + e^{0.953} \times \log_e(TG) + 0.139 \times BMI + 0.718 \times \log_e(GGT) + 0.053 \times WC—15.745)$ and then by multiplying by 100. LAP was calculated as follows: (WC—65) × TG (expressed in mmol/L) for men, and (WC—58) × TG (expressed in mmol/L) for women. HSI resulted from 8 × ALT/AST ratio + BMI (+ 2 for women; + 2 if type two diabetes) [27]. Finally, VAI was the resulting product from {WC/[39.68 + (1.88 × BMI)]} × [TG (expressed in mmol/L)/1.03] × [1.31/HDL-C (expressed in mmol/L)] for men, and from {WC/[36.58 + (1.89 × BMI)]} × [TG (expressed in mmol/L)/0.81] × [1.52/HDL-C (expressed in mmol/L)] for women [28].

Blood pressure measurements

Systolic (SBP) and diastolic blood pressure (DBP) measurements were detected in each subject supine and at rest, by the use of a standard mercury sphygmomanometer (Erkameter 3000, ERKA, Bad Tolz, Germany; Korotkoff I and V), with a cuff of the appropriate size applied to the right upper arm. To implement detection's accuracy, three BP readings were sequentially obtained at 1-min interval. Then, the first one was discarded, and the average between the other ones was considered as study variable [29].

Mean pulse pressure (PP) was calculated as the difference between SBP and DBP (PP = SBP−DBP). Mean arterial pressure (MAP) was obtained by adding one-third of PP–DBP (MAP = 1/3 PP + DBP) [30].

Endothelial reactivity

Endothelial function was evaluated though Endocheck® (BC Biomedical Laboratories Ltd, Vancouver, BC, Canada), a method embedded within the Vicorder® device, which is supposed to record brachial pulse volume (PV) waveforms, at baseline and during reactive hyperemia. Reactive hyperemia usually is provoked through PV displacement, obtained by inflating a cuff positioned distally around the forearm. After a 10-min rest, brachial blood pressure is evaluated and PV waveforms are recorded at the baseline for 10 s. Then, the cuff is inflated to 200 mmHg for 5 min and PV waveforms are recorded for 3 min after cuff released. PV displacement is calculated as a percent change in the PV waveform area, comparing waveforms before and during hyperemia through the equation PV2/PV1, where PV1 represents PV at the baseline and PV2 represents PV during hyperemia [31].

The Vicorder apparatus is quite simple to use and guarantees a very good intra- and inter-operator reliability [32].

Assessment of safety

During the designated study period, volunteers were carefully monitored by investigators for any clinical or laboratory adverse event. According to GCP requirements, investigators registered tolerability and safety issues eventually occurred during the trial—regardless whether or not they were considered to be related to the study intervention—and their severity or outcome.

Statistical analysis

Data were analyzed by the Statistical Package for Social Science (SPSS) 21.0 (IBM Corporation, Armonk, NY, USA), version for Windows. A sample size of 28 subjects per group was needed to detect a mean treatment difference in MetS prevalence of 5%, with a power of 0.90 and an alpha error of 0.05. This estimation was also valid to detect significant changes in FPI and laboratory markers of inflammation. As per protocol, we decided a priori to check the efficacy of treatments in subjects assuming at least the 90% of the tested products doses foreseen by the trial design. The normality distribution of the tested parameters was evaluated by the Kolmogorov–Smirnov

test. Baseline between group characteristics were compared using the independent *t* test for normally distributed variables and the Kruskal–Wallis *H* test for non-parametric variables. Gender distribution was compared by χ^2 test followed by Fisher's exact test. Between group differences were assessed by the ANOVA followed by the Tukey's post hoc test. All data were expressed as means and related standard deviations. All tests were two sided. A p level of < 0.05 was considered significant for all tests.

Results

Sixty patients were consecutively enrolled and randomized to receive synbiotic ($n = 30$; M 14, F 16) or placebo treatment ($n = 30$; M 13, F 17) for 60 days. Subjects' characteristics at the screening visit are summarized in Table 2. The final intergroup distribution between men and women did not show any significant difference ($p > 0.05$). Baseline clinical features and laboratory analyses were also similar between the treatment groups (Table 2).

All participants completed the trial according to the study design (dropout rate = 0%) and no patient experienced any subjective or laboratory adverse event (Fig. 1). The compliance to the treatment was similar in the groups (99.7% overall; $p > 0.05$ between groups).

From the randomization visit until the end of the study, enrolled subjects maintained similar dietary habits, without significant changes in total energy, salt intake and coffee and alcohol consumption (Table 3), and they regularly performed moderate intensity physical activity.

Through the 2-month period of treatment, patients who received active treatment experienced a statistically significant improvement in WC and in FPI, TC, HDL-C, non-HDL-C, TG, LDL-C, hsCRP and TNF-alpha serum levels, compared to the baseline and to the control group (Table 2). VAI improvement in the synbiotic treatment group was significantly greater than in placebo group ($p < 0.05$). Compared to the baseline, treatment with synbiotics also significantly reduced MAP and FPG (Table 2).

All treatment groups demonstrated a significant decrease in TG. TG reduction in the synbiotic group was significantly greater than in the control group ($p < 0.05$) (Table 2).

At the end of the study, 23% of subjects assigned to the active treatment group did not comply anymore with the diagnosis of MetS versus 10% of subjects randomized to placebo ($p < 0.01$).

The EQ-5D VAS significantly improved only in synbiotic-treated subjects ($p < 0.05$ vs baseline and placebo) (Table 2).

No significant change was observed in the other considered parameters (Table 2).

Discussion

In this 2-months clinical trial, treatment with a synbiotic formula of *L. plantarum* PBS067—DSM 24,937, *L. acidophilus* PBS066—DSM 24,936 and *L. reuteri* PBS072—DSM 25,175 and prebiotic fibers inulin and FOS decreased several important CV risk factors and markers of insulin resistance related to MetS. Consequently, at the end of the study, the prevalence of patients reaching the MetS diagnostic criteria was significantly lower in the synbiotic-treated group rather than placebo. The tested formulation also exerted an anti-inflammatory effect by reducing hsCRP and TNF-alpha serum levels. For this reason, this intervention may broaden the area of non-medication strategies to be employed to ameliorate the components of MetS and insulin resistance, which currently include healthy nutrition (with large quantities of foods high in beneficial antioxidants and polyunsaturated fatty acids) and regular physical activity [10, 33].

The probiotic species used in the current study have just shown to exert multiple beneficial effect on host health [34]. However, to the best of our knowledge, this is the first clinical trial evaluating the metabolic and anti-inflammatory effect of the specific probiotic strains *L. plantarum* PBS067—DSM 24,937, *L. acidophilus* PBS066—DSM 24,936 and *L. reuteri* PBS072—DSM 25,175, along with prebiotic fibers inulin and FOS.

According to the lately in vitro data published, it is likely that the simultaneous supplementation of lactose-based probiotics and prebiotics (as carbon sources for the primary and secondary metabolism of the probiotic strains) offers some further advantages compared to each component individually, due to their synbiotic activity [35, 36]. As a matter of fact, the co-administration of lactose-based probiotics along with FOS and inulin is widely recognized to help improving the survival of lactobacilli under stress conditions [37, 38]. Furthermore, this approach is well tolerated and safe, since FOS has not been associated with an increased risk of intestinal discomfort in elderly [39].

Certainly, the main limitation of the current study is related to the relatively short period of observation, which was, however, sufficient to allow the occurrence of a number of metabolic changes. Furthermore, during the trial, no instrumental measurement of patients' fat mass (i.e., impedentiometry) was performed and a limited number of inflammatory biomarkers and adipokines were evaluated. Then, our study is preliminary and further researches are needed to more deeply investigate the long-term effect of the tested synbiotic formula on a broader number of parameters. However, our results support the clinical evidence of a beneficial effect of the administration of

Table 2 Baseline and post-treatment parameters in placebo- and synbiotic-treated subjects

Variables	Placebo ($n=30$; M = 13, F = 17)			Synbiotic treatment ($n=30$; M = 14, F = 16)		
	Pre-diet standardization	Baseline	Post-treatment	Pre-diet standardization	Baseline	Post-treatment
Age (years)	71 ± 3	71 ± 3	71 ± 3	72 ± 3	72 ± 3	72 ± 3
Body mass index (kg/m^2)	27.3 ± 2.5	27.3 ± 2.5	27.3 ± 2.4	27.4 ± 2.8	27.4 ± 2.6	27.3 ± 2.2
Waist circumference (cm)	95 ± 7	95 ± 5	96 ± 4	95 ± 5	94 ± 6	92 ± 3*°
ICO	0.56 ± 0.07	0.56 ± 0.08	0.57 ± 0.09	0.57 ± 0.09	0.57 ± 0.09	0.55 ± 0.08
Systolic blood pressure (mmHg)	138.7 ± 9.2	138.6 ± 9.3	138.1 ± 9.1	137.8 ± 7.5	137.9 ± 8.8	136.1 ± 8.7
Diastolic blood pressure (mmHg)	89.5 ± 2.7	89.4 ± 3.8	89 ± 3.7	88.7 ± 2.9	88.5 ± 3.5	88.2 ± 3.2
Pulse pressure (mmHg)	49.2 ± 1.7	49.3 ± 1.9	50.4 ± 1.8	49.1 ± 3.7	49.8 ± 1.8	48.3 ± 1.6
Mean arterial pressure (mmHg)	105.9 ± 5.2	105.8 ± 5.4	104.9 ± 5.6	105.2 ± 6.7	106.7 ± 5.1	104.1 ± 4.3*
Heart rate (bpm)	72.4 ± 4.2	72.3 ± 4.3	72.0 ± 4.1	71.5 ± 4.2	71.9 ± 4.8	70.8 ± 4.6
Pulse volume change (%)	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.3	1.9 ± 0.4	1.9 ± 0.3	2.1 ± 0.2
Fasting plasma glucose (mg/dL)	102.4 ± 3.9	101.4 ± 4.8	100.8 ± 4.9	103.9 ± 4.1	103.1 ± 3.7	99.5 ± 3.6*
Fasting plasma insulin (μIU/ml)	19.1 ± 2.7	18.9 ± 2.9	18.1 ± 3.1	18.4 ± 2.9	17.6 ± 3.1	16.3 ± 2.5*°
HOMA-IR	4.7 ± 1.1	4.6 ± 1.1	4.4 ± 1.2	4.4 ± 1.7	4.3 ± 1.2	4.6 ± 1.3
Total cholesterol (mg/dL)	212 ± 16.5	208.3 ± 17.3	205.9 ± 17.7	223.2 ± 17.1	211.8 ± 16.8	199.6 ± 15.8*°
LDL-cholesterol (mg/dL)	122.4 ± 18.2	121.6 ± 12.8	121.1 ± 12.1	132.9 ± 12.9	123.8 ± 13.1	114.7 ± 10.9*°
HDL-cholesterol (mg/dL)	43.6 ± 2.9	43.5 ± 3.8	43.3 ± 3.3	43.1 ± 2.8	42.9 ± 3.9	46.1 ± 3.1*°
Non HDL-cholesterol (mg/dL)	168.4 ± 13.9	165.8 ± 14.7	163.2 ± 14.2	180.1 ± 19.4	168.4 ± 14.1	154.2 ± 13.1*°
Triglycerides (mg/dL)	230 ± 15.6	221.3 ± 24.8	212.8 ± 21.1*	235.8 ± 14.3	229.1 ± 26.2	201.9 ± 19.6*°
Aspartate transaminase (U/L)	24.1 ± 4.2	23.9 ± 4.3	24.2 ± 3.9	24.3 ± 2.8	24.1 ± 4.2	22.9 ± 4.5
Alanine transaminase (U/L)	24.6 ± 3.5	24.1 ± 3.9	25.3 ± 3.3	24.7 ± 2.9	24.1 ± 3.7	23.5 ± 3.6
Gamma-glutamyl transferase (U/L)	29.8 ± 8.5	29.4 ± 8.4	27.6 ± 8.8	28.9 ± 7.8	28.2 ± 9.2	26.9 ± 8.1
VAI	1.7 ± 1.2	1.7 ± 1.1	1.8 ± 1.2	1.6 ± 1.3	1.6 ± 1.2	1.5 ± 0.9°
LAP	44.9 ± 17.3	44.8 ± 17.5	45.1 ± 16.9	46.3 ± 15.9	46.2 ± 16.2	44.8 ± 17.0
HSI	37.7 ± 5.6	37.8 ± 5.8	36.9 ± 6.1	37.1 ± 5.7	36.9 ± 5.9	37.2 ± 5.4
FLI	29.1 ± 2.4	29.3 ± 2.9	29.2 ± 2.8	28.4 ± 4.5	28.9 ± 3.1	27.3 ± 3.3
Serum uric acid (mg/dL)	5.9 ± 1.2	5.9 ± 1.1	5.8 ± 0.9	6.1 ± 0.8	6.0 ± 1.0	5.8 ± 0.8
Creatinine (mg/dL)	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.2	1.0 ± 0.3	1.0 ± 0.4	1.0 ± 0.5
eGFR (ml/min)	78.3 ± 4.1	78.3 ± 4.1	78.4 ± 4.0	78.2 ± 5.1	77.9 ± 4.3	77.8 ± 3.9
hsCRP (mg/L)	2.99 ± 0.27	2.98 ± 0.25	2.86 ± 0.31	3.11 ± 0.43	2.90 ± 0.25	2.71 ± 0.23*°
TNF-alpha (pg/mL)	6.8 ± 2.5	6.6 ± 3.4	6.5 ± 3.1	6.7 ± 1.8	6.4 ± 2.9	5.9 ± 2.7*°
Adiponectin (pg/mL)	8.4 ± 1.5	8.3 ± 1.7	8.1 ± 1.8	8.1 ± 1.9	8.0 ± 2.1	8.1 ± 1.7
Leptin (pg/mL)	1.1 ± 0.7	1 ± 0.9	1.2 ± 0.8	1.2 ± 0.7	1.1 ± 0.9	1.1 ± 0.7
Leptin/adiponectin ratio	0.13 ± 0.04	0.12 ± 0.04	0.15 ± 0.06	0.12 ± 0.09	0.13 ± 0.05	0.13 ± 0.05
EQ-5D VAS[a]		80 (75–90)	78 (75–86)		82 (75–88)	88 (76–94)*°

Values are expressed as mean ± standard deviation unless otherwise specified

eGFR Estimated glomerular filtration rate, *EQ-5D* EuroQol-5 Dimension, *FLI* Fatty liver index, *GGT* Gamma glutamil transferasi, *HDL* High-density lipoprotein, *HOMA-IR* Homeostatic model assessment for insulin resistance, *hsCRP* high-sensitivity C-reactive protein, *HSI* Hepatic steatosis index, *ICO* Index of central obesity, *LAP* Lipid accumulation product, *LDL* Low-density lipoprotein, *TNF* Tumor necrosis factor, *VAI* Visceral adiposity index, *VAS* Visual analog scale

*$p < 0.05$ vs. baseline

°$p < 0.05$ vs. placebo

[a]Expressed as median (minimum–maximum)

specific strains of *L. plantarum* (PBS067—DSM 24,937), *L. acidophilus* (PBS066—DSM 24,936) and *L. reuteri* (PBS072—DSM 25,175) in elderly subjects affected by MetS.

The main strength of our study is its controlled design and the relatively large and well-characterized sample size, especially when compared with previous studies addressing the association between synbiotic supplementation

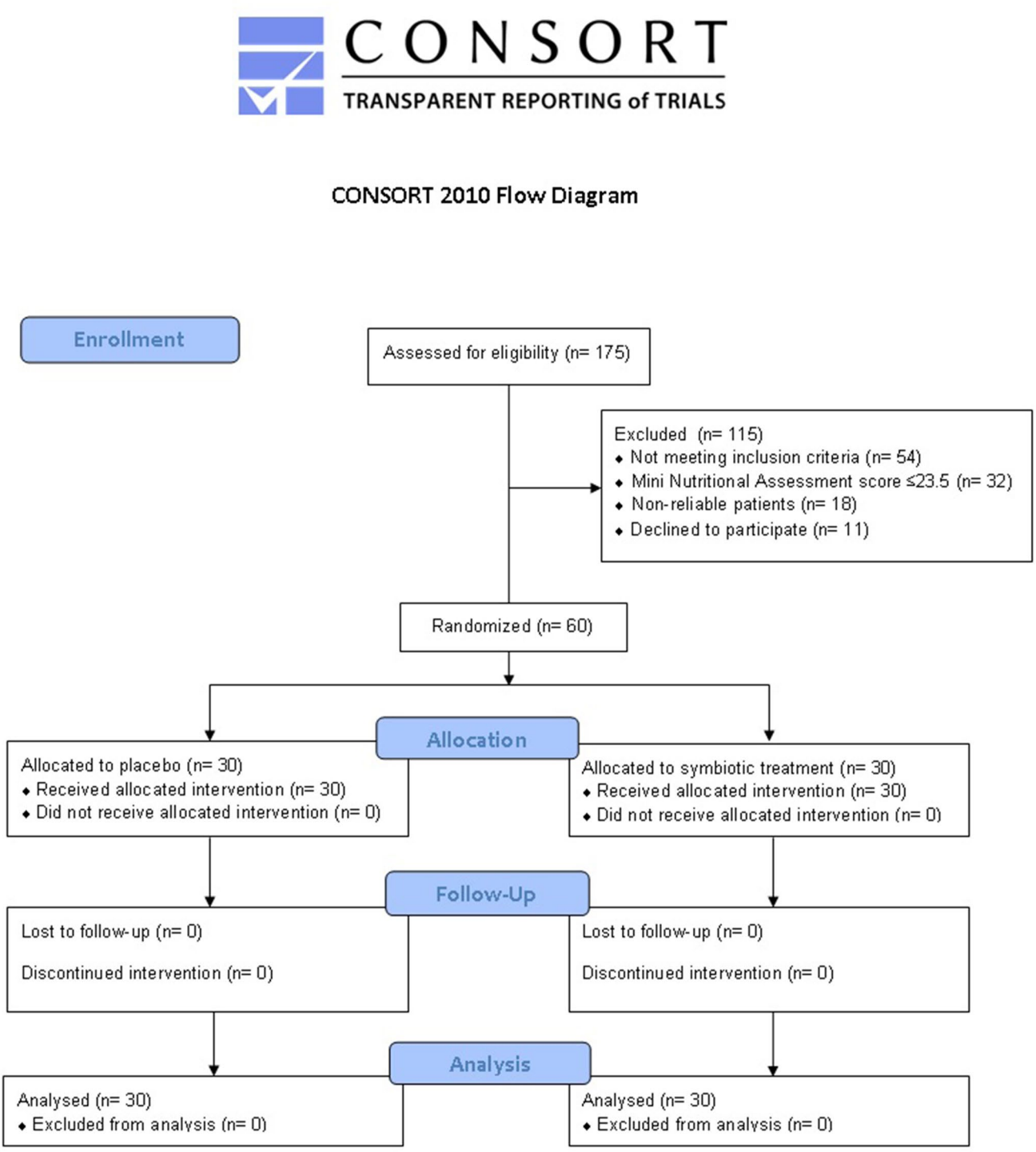

Fig. 1 CONSORT flow diagram

and changes in serum biomarkers. In particular, we were able to select a relatively homogeneous sample of healthy elderly subjects who were not taking medications potentially able to modify the gut microbiota. Finally, the tested treatment has proven to be very well tolerated, and this is evident also from the high treatment persistence over time (close to 100%).

Certainly, the characterization of baseline microbiome composition in patients' enrolled in future clinical trials may help to understand the individual responses to synbiotic

Table 3 Daily energy and nutrient intakes assessed by patient food diaries, before and after the treatment period, expressed as mean ± standard deviation

Variables	Placebo ($n=30$; M = 13, F = 17)		Synbiotic treatment ($n=30$; M = 14, F = 16)	
	Baseline	Post-treatment	Baseline	Post-treatment
Energy value (Kcal/day)	1583 ± 135	1589 ± 142	1580 ± 140	1589 ± 136
Carbohydrates (% of energy)	53.5 ± 3.1	54.1 ± 3.8	53.3 ± 2.8	52.2 ± 2.7
Proteins (% of energy)	18.3 ± 1.9	18.1 ± 1.7	18.4 ± 1.8	17.2 ± 1.9
Animal proteins (% of energy)	10.6 ± 1.8	10.3 ± 1.2	10.5 ± 0.6	9.7 ± 0.8
Vegetal proteins (% of energy)	6.6 ± 0.6	6.9 ± 0.9	6.7 ± 0.7	7.3 ± 0.9
Total fat (% of energy)	28.2 ± 1.6	29.1 ± 1.8	29.2 ± 2.0	28.7 ± 2.5
Saturated fatty acids (% of energy)	9.3 ± 0.9	9.2 ± 0.6	9.1 ± 0.9	9.2 ± 1.1
MUFAs (% of energy)	12.8 ± 1.1	12.6 ± 1.3	12.0 ± 1.1	12.2 ± 1.3
PUFAs (% of energy)	6.6 ± 0.4	6.7 ± 0.6	6.7 ± 0.4	6.3 ± 0.4
Dietary Fibres (g)	18.1 ± 2.5	18.0 ± 1.9	17.9 ± 2.2	18.1 ± 2.6
Cholesterol (mg)	193.9 ± 13.9	189.9 ± 15.3	188.4 ± 12.5	188.2 ± 13.3

MUFAs monounsaturated fatty acids, *PUFAs* polyunsaturated fatty acids

supplementation, leading to more effective weight-management treatments and results interpretation [40]. For this reason, future studies will be needed to include stool analysis for changes in microbiota composition. Finally, there is a great research potential in this field, though very little has been established also regarding the dose required to achieve health benefits.

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Compliance with ethical standards

Conflict of interest No author has any conflict of interest in the publication of this study.

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