Title: Can chronic probiotic intake modulate psychological profile, gut microbiota and body composition of women affected by normal weight obese syndrome and obesity? A double blind randomized clinical trial.

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Abstract

Objective: Evidence of probiotics effects on gut function, brain activity and emotional behavior were provided. Probiotics can have dramatic effects on behavior through the microbiome-gut-brain axis, through vagus nerve. We investigated whether chronic probiotic intake could modulate psychological state, eating behavior, and body composition of normal weight obese (NWO) and preobese-obese (PreOB/OB) compared to normal weight lean women (NWL).

Methods: 60 women were enrolled. We categorized the subjects according to body mass index (BMI), and % of total body fat (TBFat) in: NWL; NWO; PreOB/OB. At baseline and after three-week of a probiotic oral suspension (POS) intake all subjects underwent to evaluation of body composition and gut microbiota and were also assessed by means of self-report questionnaries (i.e. EDI-2, SCL90R, and BUT).

Results: Of the 60 women initially recruited, 48 participated in the study. We found a 24% of NWO, 26% of NWL, and 50% of PreOB/OB women. Significant differences ($p<0.05$) were highlighted between: NWL and NWO (TBFat, total body Lean, TBLean); NWO and PreOB/OB (Weight, BMI, TBFat, TBLean); NWL and PreOB/OB (Weight, BMI, TBFat, TBLean). After POS treatment, reduction ($p<0.05$) of TBFat and syndrome of bacterial overgrowth, as well as lower psychopathological scores (i.e. depression, anxiety, body dissatisfaction, bulimia) ($p<0.05$) were observed in NWO and PreOB/OB.

Conclusion: Three-week intake of selected probiotic modulate body composition, bacterial contamination, psychopathological scores of NWO and PreOB/OB. Further research is needed on a larger population and for longer period of treatment before definitive conclusions can be made.

Trial Registraton: ClinicalTrials.gov Id: NCT01890070

Keywords

NWO Syndrome, Probiotic, Gut Microbiota, Psychological Profile, Body Composition

Abbreviation list

GI = gastrointestinal; LPL = lipoprotein lipase; LPS = lipopolysaccharide; BMI = body mass index; NWO = normal weight obese; TBFat = total body fat; TBLean = total body lean; LBT = lactulose breath test; NWL = normal weight lean; DXA = dual X-ray absorptiometry; IG = intervention group; CG = control group; SCL90R = Symptom checklist 90; BUT = body uneasiness test; IC = body image; POS = probiotic oral suspension; TBW = total body water; ECW = extracellular water; ICW = intracellular water; PA = phase

angle; BCM = body cell mass; BCMI = body cell mass index; PBF = body fat percentage; TBBone = total body bone; DT = drive for thinness; BD = body dissatisfaction; B = bulimia; I = Ineffectiveness; IA = Interoceptive awareness; MF = Maturity Fears; A = Asceticism; IR = Impulse Regulation; SI = Social Insecurity; P = Perfectionism; ID = Interpersonal Distrust; GSI = global severity index; PST = positive symptom total; PSDI = positive symptom distress index; WP = Weight Phobia; BIC = Body Image Concerns; A = Avoidance; CSM = Compulsive Self-Monitoring; D = Depersonalization; Som = somatization; Obs = obsessive/compulsive; Interp Sens = interpersonal sensitivity; Dep = Depression; Anx = anxious; Anger Host = anger/hostility; Phob = phobia; Psych = psychoticism; Paran = paranoia; NS = not significant

Introduction

The human gut hosts a dynamic and complex microbial ecosystem, and probiotics can have dramatic effects on behavior, through vagus nerve acting on the microbiome-gut-brain axis, which constitutes a bidirectional communication network. Probiotics produce a variety of neurochemicals, analogs of mammalian hormones involved in mood and behavior. Therefore, the visceral messages from the gut can affect brain function, and, *vice versa*, signals from the brain may affect the sensory system and the gut secretion mode.

Human gut is represented by approximately 1 kg of bacteria in the average adult, about the weight of the human brain. There are microorganisms belonging to 14 families, 45 genera and 400-500 different species, variously distributed along the entire intestinal tract. In particular the density increases exponentially from the upper portion to the lower one of the intestine [1].

The microflora in the colon performs a number of functions including those metabolic, trophic and defense. It has been estimated that there are about 500 species, belonging to the genera *Firmicutes* and *Bacteroidete* [2]. In the last few years, the importance of gut microbiota impairment in the etiopathogenesis of pathology such as autism, dementia and mood disorder, has been raised. The evidence of the inflammatory state alteration, highlighted in disorders such as schizophrenia, major depressive disorder and bipolar disorder, strongly recalls the microbiota alteration, highly suggesting an important role of the alteration of the gastrointestinal (GI) system also in neuropsychiatric disorders [3].

Different probable signalling mechanisms by which the intestinal flora and probiotics may influence brain activity, including changes in signal molecules produced by the microbiota (as metabolites, amino acids, short-chain fatty acids, and neuroactive substances), the mechanisms of the mucosal immune system, and the enterochromaffin cell-mediated vagal activation have been identified [4].

Moreover, a bidirectional association between obesity and self-reported or clinical measures of depression were observed, and conscientiousness, body dissatisfaction was robustly associated with risk factor for obesity and eating disorders [5]. *Lactobacillus* appear to reduce body fat mass, anxiety and dysphoria, and improve insulin sensitivity and glucose tolerance.

A growing body of scientific evidence supports the notion that the crosstalk between the gut microbiota, diet and immune system activates mediators and signalling pathways, which influence whole body metabolism and disease.

The metabolic activities of the intestinal flora play a decisive role in obesity, because it facilitates the extraction of calories from foods, easing the accumulation of substances, such as fatty acids, in adipose tissue, and at the same time providing energy and nutrients to the same microbial growth [6,7].

The mechanisms through which the gut microbiota affect the extraction of calories from foods are different. The bacterial flora is able to produce many of glycosidic hydrolases that are involved in digestion of complex polysaccharides derived from plants, in this way complex carbohydrates are metabolized into monosaccharaides and short-chain fatty acids, an important source of energy for the organism [8]. The intestinal flora can suppress the expression of a circulating Lipoprotein lipase (LPL) inhibitor, the Fasting-induced adipose factor. The increased levels of LPL leads to an increased cellular uptake of fatty acids from triacylglycerols associated to lipoproteins.

Obesity and insulin resistance are associated with a systemic chronic inflammatory condition. Lipopolysaccharide (LPS) of Gram-negative bacteria represent a factor triggering inflammation and obesity induction. The LPS is continually being produced, at the intestinal level, through the lysis of the bacteria and subsequently absorbed and transported from the intestine to the tissue, by a lipoprotein dependent mechanism. The LPS induces the secretion of pro inflammatory cytokines (IL-1, IL-6, TNFα) when it binds to the complex CD14/TLR4 on the surface of cells of the immune system [9].

Therefore, obesity is characterized by a different microbiota than normal, and the microbiota itself together with the host genotype and its style life could contribute to the development of this metabolic dysfunction.

Different obese phenotype have been described, based on body fat composition and distribution, rather than the simply increase of body weight, and the Body Mass Index (BMI), and genetic [10]: (1) normal weight obese (NWO); (2) metabolically obese normal weight [11]; (3) metabolically healthy obese [12]; and (4) metabolically unhealthy obese or "at risk" obese [13].

A significant body of research has documented the existence of subjects suffering of NWO syndrome [14], a status of normal weight and total body fat (TBFat) accumulation, characterized by higher oxidative stress level, early inflammatory status and few metabolic abnormalities.

NWO women, despite having body weight and BMI (<25 kg/m2) values within the normal range, were characterized by high TBFat percentage (≥30%) accompanied by total body lean (TBLean) mass deficiency, based on a genetic predisposition [15-17].

In previous study it was highlighted that in the NWO syndrome not only conveys an increased risk of cardiovascular and metabolic disease, but they are worn down over time by the will to control their own body weight, and to reveal the suppressed vocation for obesity, scoring in the intermediate range between normal weight lean women and pre-obese or obese women on the Eating Disorder Inventory–2 (EDI-2), particularly in terms of body dissatisfaction and drive for thinness [18].

Given the link between gut microbiota, body composition, and the risk of psychiatric illness, in the current study, we hypothesized that a change in the gut microbiota induced by chronic probiotic intake could modulate psychological state, eating behavior, and body composition of NWO and preobese-obese (PreOB/OB) compared to normal weight lean women (NWL).

We comprehensively analyzed body composition, by anthropometric and dual X-ray absorptiometry (DXA) evaluation, gut microbiota evaluation by LBT. Moreover, all patients were also assessed by means of self-report questionnaires.

Patients and Methods

Clinical Study Design

The clinical study used a randomized, controlled, cross-over design, conducted through the CONSORT flowchart (Figure 1), between October 2015 and February 2016. Subjects were consecutively recruited within a program of routine medical check-up at the Section of Clinical Nutrition and Nutrigenomic, at the University of Rome "Tor Vergata", at "Nuova Annunziatella" Clinic, and General Hospital Foundation, Catholic University of Sacred Heart, Rome, Italy. Informed consent was obtained from all subjects, in accordance with principles of the Declaration of Helsinki.

Exclusion criteria included pregnancy, breast-feeding, type 1 diabetes, presence of intestinal bacterial overgrowth, acute diseases, endocrine disorders, liver, heart or kidney dysfunctions, history of chronic degenerative or infectious diseases, medication, smoke, drug or alcohol abuse, participation in another diet trial. Subjects could not have taken antibiotics or probiotics in the month before the study and were willing to avoid use of probiotics for the duration of the study.

Sixty women were enrolled. Subjects were screened for eligibility at visit 1, and underwent body composition analysis. We categorized the subjects according to BMI, and % of TBFat into: NWL women,

with a BMI <25kg/m^2 and TBFat(%)<30) NWO,with a BMI <25 kg/m^2, and TBFat(%) ≥30) PreOB/OB women, with a BMI ≥25 kg/m^2 and TBFat(%) ≥30 [17].

Subjects, who were eligible for the study, were randomly divided into two groups. One intervention group (IG) and one control group (CG) were utilized. The randomization was determined by an external contract research organization and coordinated with the Section of Clinical Nutrition and Nutrigenomic, at the University of Rome "Tor Vergata", independently of the investigators.

After three weeks of washout period, to avoid additive effects on treatments to follow, the IG and CG were reversed. The IG and CG arms were double-blinded.

The subjects had a repeat visit 3 weeks after intervention initiation of each arms (± 3 days).

To all patients were administered an anonymous questionnaire, self-compiled, for the collection of socio-demographic data, the Symptom Checklist 90 (SCL90R) [19], for the evaluation of general psychopathology and the Body Uneasiness Test (BUT) the evaluation of the perception of body image (IC) [20], and the EDI-2, for Eating behavior [21].

IG and CG were subjected to a LBT to determine orocaecal transit time and consequently bacterial overgrowth. They all also completed a questionnaire evaluating gastrointestinal symptoms (meteorism, abdominal pain, number of defecations/week).

No patients with known alterations to intestinal transit following organic pathologies (abdominal surgery, diabetes mellitus, scleroderma, hypothyroidism, etc.) were included in the study. At the end of the therapy, the patients were re-assessed by repeating the lactulose breath test and once again completing the questionnaire on gastrointestinal symptoms.

The IG received daily a probiotic oral suspension (POS), contained: *Streptococcus thermophiles* (1.5×10^{10} colony-forming unit CFU), *Bifidobacterium animalis subsp Lactis* (1.5×10^{10} colony-forming unit CFU), *Streptococcus thermophiles* (1.5×10^{10} colony-forming unit CFU), *Lactobacillus bulgaricus* (1.5×10^{10} colony-forming unit CFU), *Lactococcus lactis subsp Lactis* (1.5×10^{10} colony-forming unit CFU), *Lactobacillus acidophilus* (1.5×10^{10} colony-forming unit CFU), *Lactobacillus Plantarum* (1.5×10^{10} colony-forming unit CFU), *Lactobacillus Reuteri* (1.5×10^{10} colony-forming unit CFU) (Italfarmacia, Rome, Italy).

The CG received the placebo represented by inert material (flour type 00).

It was asked to the subjects not to change their lifestyle habits. Any adverse effect has been properly signed. At the end of each arms, a clinician assessed any adverse effects from the interventions by going through a checklist of symptoms, including bloating, fullness, or indigestion, altered bowel habit, dizziness, and other symptoms that were possibly associated with the interventions. All patients completed the study.

Study design was clearly written in lay person language and provided to each study subject. The participants received no financial compensation or gifts.

Trial registration: ClinicalTrials.gov NCT01890070.

Anthropometric measurements

At T1, after a 12-hour overnight fast, all subjects underwent anthropometric evaluation (body weight, height, waist and hip circumferences), according to standard method [22]. All the individuals were instructed to take off their clothes and shoes before undergoing the measurements.

BMI was calculated using the formula: BMI= body weight (Kg)/height $(m)^2$.

Bioelectrical Impedance Analysis (BIA)

Resistance, reactance, impedance and phase angle at 50 kHz frequency were measured using a BIA phase sensitive system (BIA 101S, Akern/RJL Systems-Florence, Italy).

Measurements were taken according to Di Renzo et al. [23].

Dual X-ray Absorptiometry (DXA)

To assess body composition analysis, that give the possibility to measure TBFat and TBLean, DXA (i-DXA, GE Medical Systems, Milwaukee, WI, USA) evaluation was performed at baseline, according to De Lorenzo et al. [17].

Breath testing

The Lactulose breath test was performed by administering 20g lactulose dissolved in 100 cc water to the subjects. Breath samples were obtained by asking the patients to blow into suitable containers at time 0 (before ingesting the lactulose) and then every 15 minutes thereafter for the 4 hours following lactulose administration. Gas chromatography was used to assess the presence and quantity of hydrogen in the breath (Quintron Milwaukee, Wisconsin USA). Orocaecal transit time was calculated for each patient by

constructing the curves of hydrogen in the breath over time. This therefore showed the time necessary for the bolus to reach the caecum [24].

Psychodiagnostic Instruments

Eating behavior was assessed using the Italian version of the EDI-2, standardized in an Italian population [25-26]. The subscales of the EDI-2 include drive for thinness (DT), Bulimia (B), Body Dissatisfaction (BD), Ineffectiveness (I), Interceptive awareness (IA), Maturity Fears (MF), Asceticism (A), Impulse Regulation (IR), Social Insecurity (SI), Perfectionism (P) and Interpersonal Distrust (ID).

Body Uneasiness Test (BUT). It's a self-assesment scale, used for body image studies and related pathologies. Beyond the total score, BUT allows to calculate the Global Severity Index (GSI) or total average score, which is obtained from the sum of clinical scores (BUT a), divided by their number (34). Items number with score ≥1 correspond to Positive Symptom Total (PST). The sum of items scores ≥1 divided by PST, produces the Positive Symptom Distress Index (PSDI) [20].
Five factors were defined: WP-Weight Phobia, BIC- Body Image Concerns, A- Avoidance, CSM-Compulsive Self-Monitoring, D-Depersonalization. In our study, we considered as positive for altered perception of body image a GSI score ≥1.2.
Symptom Check List - Revised (SCL90R). It's a general evaluation scale of the psychopathology, based on patients self-evaluation. This scale is composed by 90 items, which investigate the presence of symptoms in the week before the test check. These 90 items, which have 5 levels Likert answers, have 10 reference factor: 1) somatization (Som); 2) obsessive/compulsive (Obs); 3) interpersonal sensitivity (Interp Sens); 4) Depression (Dep); 5) anxious (Anx); 6) anger/hostility (Anger Host); 7) phobia (Phob); 8) psychoticism (Psych); 9) paranoia (Paran); 10) sleep disorders. The score goes from 0 to 4, and a score above 1 is a index of pathology [19].

Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp). Data are expressed as mean±standard deviation (SD), and minimum and maximum. A paired t test or a non-parametric Wilcoxon test were performed to evaluate differences between baseline and after POS. A one-way ANOVA was carried out to compare the average of the responses obtained in all three groups. Where applicable, the Chi square or Student's t-test were used to assess symptoms.

A difference of $p<0.05$ was considered significant.

Results

Of the 60 women initially recruited, 8 did not meet inclusion criteria, 4 dropped out of the study voluntarily, leaving a total of 48 subjects for final analysis.

The characteristics of the study population in terms of age, weight, height, BMI, TBFat (%), psychological profile (EDI-2, BUT, and SCL90R) are shown in Supplementary Table 1.

In particular, between the NWL and the NWO groups, significant differences ($p<0.001$), in terms of Weight (Δ% 14.31), BMI (Δ% 16.95), hip circumpherence (Δ% 9.4), TBW (%) (Δ% -10.05), and TBFat (%) (Δ% 32.77), TBFat (g) (Δ% 43.43), were observed.

Between the NWL and the PreOB/OB there were significant differences ($p<0.001$) in terms of Weight (Δ% 25.06), waist (Δ% 18.93) and hip circumpherence (Δ% 14.50), BMI (Δ% 35.22), PA (°), TBW (%), (Δ% -17.49), ECW and ICW (L, %), BCMI, and TBFat (%) (Δ% 64.97), TBFat (g) (Δ%125.08).

Significant differences ($p<0.001$) between the NWO and the PreOB/OB groups in terms of Weight (Δ% 9.40), BMI (Δ% 15.62), Reactance (Ohm), (Δ% 19.73), PA (°), (Δ% 20.72), TBW (%),(Δ% -8.27), ECW (Δ % -11.70), and ICW (L), (Δ% 9.50), ICW (%), (Δ% 9.78), BCMI, and TBFat (%), (Δ% 24.26), TBFat (g), (Δ% 56.93) were observed (Supplementary Table 2).

After POS treatment, a significant reduction of Weight, BMI, hip and waist circumpherence, ICW (L), TBW (%), and TBFat (%) ($p<0.001$) was observed in the total population as described in Table 1.

After POS treatment, significant differences ($p<0.001$) were observed among the NWL, NWO and PreOB/OB groups in body composition parameters as showed in Table 2.

At baseline, the total sample tested was negative to SCL90R_GSI scale, and the 33.30% of population was positive at BUT_ GSI scale (GSI≥1.2; mean 0.96±standard deviation 0.66).

After POS treatment all population remained negative to SCL90R_GSI scale, and the positive to BUT scale was significantly reduced ($p<0.01$) at 8.33% (GSI≥1.2; mean 0.59±standard deviation 0.52).

At baseline, among the 33.3% of the positive to BUT_GSI and BUT_CSM scale the 12.5% were NWL, the 50% were NWO, and 37.55% were PreOB/OB.

After POS treatment, among the 8.33% of the positive, were identified only NWO (100%).

The average scores of the various dimensions and the total score of SCL90R scale, BUT_GSI and EDI-2 of are represented in Table 3.

After POS treatment, in the general population significant differences ($p<0.001$) in terms of the responses to the subscales of the EDI-2 were observed: -37.98 Δ% of B (T0, 1.74±3.01; T1, 1.08±2.06), the -15.95 Δ% of DT (T0, 1.74±3.01; T1, 1.08±2.06), the -40.15 Δ% of I (T0, 1.74±3.01; T1, 1.08±2.06).

After POS treatment, in the NWO group significant differences ($p<0.001$) in terms of the responses to the subscales of the EDI-2 were observed: -41.94 Δ% of B (T0, 0.91±1.64; T1, 0.53±1.16), the -19.30 Δ% of DT (T0, 9.29±7.81; T1, 7.50±7.18), the -50.45 Δ% of I (T0, 3.26±4.29; T1, 1.62±2.85).

After POS treatment, in the PreOB/OB group significant differences ($p<0.001$) in terms of the responses to the subscales of the EDI-2 were observed: -31.25 Δ% of B (T0, 3.64±4.12; T1, 2.50±2.89), the -15.48 Δ% of DT (T0, 14.09±6.35; T1, 11.91±5.76), the -36.72 Δ% of I (T0, 5.82±4.92; T1, 3.68±4.51) (Table 4).

Table 5 reported the orocaecal transit time in all patients and 3 healthy the subjects at baseline and after therapy: significant improvement were observed ($p<0.001$) in the patients respect to control.

Symptoms before and after POS therapy were reported in Table 5. Significant differences were observed for meteorism ($p<0.001$) and number of defecation ($p<0.001$).

Discussion

The link between the "somatic" and "mental" is undeniably a subject that has fascinated during the course of history and until the present day many artists, philosophers, but also researchers, scientists, who daily make their contribution to reveal how this chimera, really, could probably show an albeit complex, fascinating harmonic unit.

The axis brain gut microbiota includes the central nervous system, neuroendocrine and neuro-immune system, the sympathetic and parasympathetic arms of the autonomic nervous system, the enteric nervous system and most importantly the intestinal microbiota [26].

Due to these new evidences about the fundamental role of gut microbiota in the alteration of immune, neural, and endocrine pathways, the so-called "gutbrain axis" is acquiring new significance, even if the communication routes are not still defined [27].

From clinical experience and from the literature is clear the importance of the "personalization" of treatment, respect to the individual we have in care, taking into account the body composition, the intestinal flora, feeding behavior, attitude toward the food, and the presence of emotional states that may influence them. It is interesting to note that eating disorders, in a substantial proportion of cases, they are associated with other psychiatric disorders such as mood disorders (depression in particular), anxiety disorders and personality disorders. The rate of co-morbidity is very high, varying from 42 to about 75%.

Consistent with these considerations, it has been prepared a new formula of POS, containing different strain of bacteria, such as *Streptococcus thermophiles*, *Bifidobacterium animalis subsp Lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, *Lactococcus lactis subsp Lactis*, *Lactobacillus acidophilus*, *Lactobacillus Plantarum*, *Lactobacillus Reuteri*, for a total of 120 $\times 10^{10}$ colony-forming unit CFU.

The paper has two main purposes: 1) to investigate the correlation between body composition and the presence of psychological disorders and psychopathological symptoms, in people affected by NWO syndrome and obesity, respect to normal lean individual; 2) to check whether a hiring of POS could change all the examined parameters, in order to make an early diagnosis and to block any nascent development of a psychopathological disorder, taking into account all its consequences and impacts future.

Recent data show the strong correlation between dysbiosis and conditions such as obesity, allergies, autoimmune disorders, Irritable Bowel Syndrome, Inflammatory Bowel Disease, and psychiatric disorders [28].

Bifidobacterium and *Atopobium* were significantly less abundant in obese animals compared to the non-obese rats, in conjunction with significantly higher levels of the *Clostridium cluster* XIVa and *Lactobacillus* group [29]. In the mean time, Cani et al. reported a reduction in the *Clostridium cluster* XIVa (*Clostridium coccoides*) group, along with lower *Bifidobacterium* and *Bacteroides* levels in mice fed high-fat diet. An increase of *Firmicutes* levels was observed high-fat fed mice, while Bacteroides phylum decreased overtime in obese [30].

Angelakis et al. [31], highlighted an high level of of *Bacteroidetes phylum*, a higher abundance of the Firmicutes phylum and higher concentrations of lactobacilli in the gut microbiota of obese and overweight adults compared to lean individuals.

Recently, it has been shown that specific bacterial gut microbiota profile with increased extraction of calories

has been associated with obesity [32], leading to a microbiota signature, characterized by a decrease of Bacteroidetes, and an increase of Lactobacillus, E. coli, Faecalibacterium.

In the present study, the cut-off points of total body fat was 30% [33]; the analysis of anthropometric and body composition values showed 24% of NWO, 26% of NWL, and 50% of PreOB/OB women.

In particular, between the NWL *versus* the NWO groups, and NWO *versus* the PreOB/OB groups significant differences ($p<0.001$), in terms of anthropometric parameters, body water content (%), and TBFat (%) were highlighted. Moreover, significant differences ($p<0.001$) between the NWO and the PreOB/OB groups in terms of Weight, BMI, Reactance (Ohm), Phase Angle (°), TBW (%), ECW and ICW (L,%), BCMI, and TBFat (%, g), were observed.

As a challenge is the development of effective strategies to prevent the increased prevalence in obesity, data reported in this study highlighting the effectiveness of the POS treatment on body composition, given the considerable improvement of the weight, BMI, waist circumpherence, hydration status and TBW (%), and TBFat (%) ($p<0.001$), suggest a safe and effective interventions for general population, with substantial benefits to public health.

Hence, the lactulose passes the stomach and small intestine intact and reaches the caecum, where the bacteria (normally present in the colon flora) break it down, leading to the production of hydrogen. Part of the hydrogen that forms is absorbed by the intestinal mucous and therefore enters the bloodstream before being released at the pulmonary alveoli and expired. By evaluating the time at which hydrogen appears in the breath, we are therefore able indirectly to determine orocaecal transit time [24].

After POS treatment, a reduction of syndrome of bacterial overgrowth ($p<0.05$), were observed in NWO and PreOB/OB as compared with controls (Table 5). All patients and 3 healthy subjects showed an alteration to orocaecal transit time. After therapy a statistically significant improvement was seen in the intestinal transit time in all patients. A slight reduction in transit time was also seen in control subjects and particularly in the 3 patients who had initially presented a reduced transit time. An increase in the number of weekly defecations was recorded in patients and a reduction of meteorism in patients affected by constipation (Table 5).

Many neurotransmitters and neuromodulators can be secreted by the bacteria, able to modulate the state of the host mood: gamma-aminobutyric acid is produced by certain *Lactobacillus* and *Bifidobacterium* species;

norepinephrine is released *Escherichia, Bacillus* and *Saccharomyces spp.*; 5Hydroxy Triptamine by Candida, Streptococcus, Escherichia and Enterococcus spp.; dopamine is produced by Bacillus produces, and acetylcholine by Lactobacillus [34]. Dinan et al. have reported that probiotic *Bifidobacerium infantis* 35624 has shown to have antidepressant action in preclinical models of depression acting as a psychobiotic with a mental health benefit [35].

Gut composition is affected also by the resilience to environmental stress, impairing the cortisol awaking response and emotional reaction in healthy subjects [36]. On the other hand, it has been shown that psychological stress itself leads to dysbiosis [37], turning in a vicious-circle.

It has been demonstrated that the oral administration of the probiotic *Bifidobacterium longum* NCC3001 (Morinaga, Japan) is able to prevent the anxiety-like behavior associated with gut inflammation in animals with an intact vagus nerve [38], as *Lactobacillus rhamnosus* and *Lactobacillus hilgardii* reduced anxiety [39].

Lactobacillus reuteri prevented the physiological signs of visceral pain, with a reduction in cardio-autonomic response [40], and *Bifidobacterium lactis* decreased visceral hypersensitivity when colorectal distention occurred in the context of psychological stress.

Our results seem to confirm the high prevalence of body image disorders in NWO and PreoOB/OB patients.

In our study, we provide evidence that POS therapy improves the psychological state, reducing the positivity to BUT of 24.90%, and the alteration of body image, as demonstrated also by the significant reduction, in terms of the responses to the subscales of the EDI-2 (-41.94 Δ% of B, the -19.30 Δ% of DT, the -50.45 Δ% of I in NWO;-31.25 Δ% of B, the -15.48 Δ% of DT in PreOB/OB).

Three-week intake of selected probiotic, by modulating body composition, bacterial contamination, psychopathological scores and eating behavior of women affected by NWO syndrome and obesity, offers a tractable approach to problems related to obesity, psychological state and unhealthy eating.

These results thus highlight the need for a more detailed psychiatric evaluation of subjects with an alteration of the body image, even when this alteration does not fit into a previous pattern refers to eating disorders.

Further research is needed on a larger population and for longer period of treatment before definitive conclusions can be made.

Despite the limitations of our study, related to the low sample size, our results seems to confirm the importance of a psychological evaluation in NWO and PreOB/OB patients, in order to make an early diagnosis, select a adequate population candidabile to therapy with POS, improve the prognosis and the outcome of the treatment itself and, also, to avoid a worsening of the psychiatric symptomatology, the establishment of a global functional impairment of the subject and improve the quality of life of patients.

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Authors Contributions

The authors' responsibilities were as follows:

ADL had primary responsibility for the final content; SG, GM, SB, MM: performed the experiments, and collected the data; PG, analyzed the data; LDR drafted the manuscript, and designed the study. All authors contributed to the interpretation of the data and revision of the manuscript, read and approved the final manuscript. The authors declare no conflict of interest.

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Table 1. Comparison of body composition between T0 and T21

	T0 Mean±SD (Min-Max)	T21 Mean±SD (Min-Max)	p
Weight (Kg)	64.58±7.69 (50.50 - 78.00)	60.94±5.10 (49.20 - 66.60)	<0.001
BMI (Kg/m²)	24.98±3.35 (19.48 - 30.47)	23.61±2.31 (18.98 - 26.23)	<0.001
Waist (cm)	75.09±8.45 (64.50 - 91.00)	72.75±4.90 (63.80 - 81.00)	<0.001
Hip (cm)	102.71±6.89 (87.00 - 108.50)	100.97±6.25 (87.50 - 107.50)	0.01
Resistance (Ohm)	574.57±54.09 (501.00-735.00)	559.55±57.78 (497.00 - 690.00)	0.06
Reactance (Ohm)	68.61±10.88 (53.00-89.00)	64.94±8.86 (51.00 - 76.00)	0.94
PA (°)	6.83±0.88 (5.30-8.30)	6.65±0.95 (5.10 - 7.90)	0.39
TBW (l)	32.75±1.85 (29.10-36.60)	32.91±2.35 (29.70 - 36.90)	0.15
Total Body Water (%)	51.04±5.39 (42.00-61.30)	54.15±3.21 (50.60 - 60.40)	<0.001
ECW (l)	13.91±1.52 (12.10-17.10)	14.22±1.80 (12.40 - 17.40)	0.96
ECW (%)	42.51±3.55 (37.10-49.30)	43.02±4.08 (38.20 - 50.10)	0.23
ICW (l)	18.82±1.46 (16.10-20.60)	18.68±1.71 (16.10 - 20.80)	0.01
ICW (%)	57.51±3.54 (50.70-62.90)	56.89±4.08 (49.85 - 61.92)	0.27
TBFat (%)	36.17±6.71 (24.10 - 43.80)	35.70±5.47 (23.90 – 42.45)	0.02

Results are expressed in mean value±standard deviation, and minimum and maximum for each parameter.

For abbreviation, see Abbreviation List. Values of $p<0.05$ are considered significant.

Table 2. Comparison of normal weight lean, normal weight obese and pre-obese/obese groups of women at T21.

	NWL	NWO	PreOB/OB	
	Mean±SD	Mean±SD	Mean±SD	
	(Min - Max)	(Min - Max)	(Min - Max)	p
Weight (Kg)	54.84±5.63 [a,b]	63.10±3.27	63.35±0.16	
	(49.20 - 63.00)	(59.00 - 66.60)	(63.20 - 63.50)	<0.001
BMI (Kg/m²)	20.49±1.40 [a,b]	23.80±0.85 [c]	25.77±0.48	
	(18.98 - 22.59)	(22.92 - 24.88)	(25.32 - 26.23)	<0.001
Waist (cm)	67.98±4.60 [a,b]	75.33±4.70	73.75±2.35	
	(63.80 - 75.00)	(70.00 - 81.00)	(71.50 - 76.00)	<0.001
Hip (cm)	93.23±4.35 [a,b]	101.00±3.72 [c]	106.75±0.78	
	(87.50 - 97.00)	(98.00 - 106.00)	(106.00 - 107.50)	<0.001
Resistance (Ohm)	598.33±69.50 [b]	567.00±53.60	523.00±24.02	
	(497.00 - 690.00)	(513.00 - 636.00)	(500.00 - 546.00)	0.01
Reactance (Ohm)	65.89±8.46	59.67±8.75 [c]	69.50±6.79	
	(56.00 - 73.00)	(51.00 - 71.00)	(63.00 - 76.00)	0.02
PA (°)	6.29±0.46 [b]	6.03±0.97 [c]	7.55±0.37	
	(5.70 - 6.80)	(5.10 - 7.30)	(7.20 - 7.90)	<0.001
TBW (L)	31.66±3.00	33.40±2.56	33.35±1.10	
	(29.70 - 36.90)	(31.10 - 36.80)	(32.30 - 34.40)	0.18
Total Body Water (%)	57.82±3.19	52.90±2.01	52.65±1.83	
	(52.40 - 60.40)	(50.60 - 55.30)	(50.90 - 54.40)	<0.001
ECW (L)	14.07±1.39	15.37±2.18 [c]	13.20±0.84	
	(12.60 - 16.10)	(12.50 - 17.40)	(12.40 - 14.00)	0.01
ECW (%)	43.93±2.64 [b]	45.87±4.29 [c]	39.50±1.36	
	(40.80 - 47.20)	(40.30 - 50.10)	(38.20 - 40.80)	<0.001
ICW (L)	17.59±1.86 [b]	18.03±1.47 [c]	20.15±0.26	
	(16.20 - 20.80)	(16.10 - 19.40)	(19.90 - 20.40)	<0.001
ICW (%)	55.62±1.91 [b]	54.12±4.37 [c]	60.61±1.37	
	(52.92 - 57.58)	(49.85 - 59.81)	(59.30 - 61.92)	<0.001

Results are expressed in mean value±standard deviation, and minimum and maximum for each parameter.

For abbreviation, see Abbreviation List. Values of $p<0.05$ are considered significant.

[a] $p<0.05$ NWL vs NWO;

[b] $p<0.05$ NWL vs PreOB/OB;

* $p<0.05$ NWO vs PreOB/OB.

Table 3. Comparison of self-report questionnaries SCL90R and BUT results between T0 and T21.

	T0	T21	
	Mean±SD	Mean±SD	
	(Min-Max)	(Min-Max)	p
SCL90R_Som	0.75±0.49	0.33±0.19	
	(0.08 - 1.67)	(0.08 - 0.67)	<0.001
SCL90R_Obs Comp	0.58±0.34	0.27±0.11	
	(0.20 - 1.30)	(0.20 - 0.60)	<0.001
SCL90R_Interp Sens	0.55±0.31	0.36±0.20	
	(0.11 - 1.33)	(0.00 - 0.56)	0.06
SCL90R_Dep	0.47±0.53	0.25±0.20	
	(0.08 - 1.62)	(0.00 - 0.69)	0.35
SCL90R_Anx	0.54±0.38	0.19±0.16	
	(0.03 - 1.19)	(0.00 - 0.50)	<0.001
SCL90R_Anger Host	0.61±0.41	0.18±0.14	
	(0.00 - 1.33)	(0.00 - 0.33)	<0.001
SCL90R_Phob	0.30±0.43	0.11±0.11	
	(0.00 - 1.29)	(0.00 - 0.29)	0.18
SCL90R_Paran	0.43±0.46	0.15±0.21	
	(0.00 - 1.67)	(0.00 - 0.67)	<0.001
SCL90R_Psych	0.30±0.31	0.10±0.11	
	(0.00 - 0.98)	(0.00 - 0.31)	<0.001
SCL90R_GSI	0.52±0.31	0.24±0.08	
	(0.14 - 1.13)	(0.13 - 0.36)	<0.001
BUT_ GSI	0.96±0.66	0.59±0.52	
	(0.09 - 2.09)	(0.12 - 1.62)	<0.001
BUT_ WP	1.72±0.93	1.14±1.02	
	(0.13 - 2.88)	(0.13 - 3.13	<0.001
BUT_ BIC	1.16±1.04	0.71±0.68	
	(0.11 - 3.00)	(0.00 - 2.00)	<0.001
BUT_ A	0.18±0.19	0.11±0.15	
	(0.00 - 0.50)	(0.00 - 0.33)	0.01
BUT_ CSM	0.94±0.67	0.60±0.62	
	(0.00 - 1.83)	(0.00 - 2.00)	<0.001
BUT_ D	0.35±0.58	0.08±0.14	
	(0.00 - 1.80)	(0.00 - 0.40)	<0.001
EDI-2_DT	5.62±6.62	5.58±6.58	
	(0.00 - 21.00)	(0.00 - 20.00)	0.62
EDI-2_B	1.74±3.01	1.08±2.06	
	(0.00 - 16.00)	(0.00 - 10.00)	<0.001
EDI-2_BD	9.74±7.65	8.19±6.95	<0.001
	(0.00 - 27.00)	(0.00 - 25.00)	
EDI-2_I	3.57±4.38	2.14±3.38	<0.001
	(0.00 - 19.00)	(0.00 - 16.00)	
	4.16±3.36	4.04±3.31	

Table 4. Comparison between T0 and T21 for each group: NWL, NWO and PreOB/OB.

	NWL		NWO		PreOB/OB	
	T0	T21	T0	T21	T0	T21
	Mean±SD (Min-Max)	Mean±SD (Min-Max)	Mean±SD (Min-Max)	Mean±SD (Min-Max)	Mean±SD (Min-Max)	Mean±SD (Min-Max)
EDI-2_DT	2.89±4.63 (0.00 - 20.00)	2.94±4.40 (0.00 - 19.00)	5.26±6.33 (0.00 - 21.00)	5.09±6.08 (0.00 - 20.00)	8.41±7.58 (0.00 - 18.00)	8.50±7.84 (0.00 - 19.00)
EDI-2_B	1.00±2.45 (0.00 - 10.00)	0.39±1.24 (0.00 - 5.00)	0.91±1.64 (0.00 - 7.00)	0.53±1.16 [a] (0.00 - 5.00)	3.64±4.12 (0.00 - 16.00)	2.50±2.89 [b] (0.00 - 10.00)
EDI-2_BD	5.28±6.09 (0.00 - 23.00)	4.94±6.01 (0.00 - 23.00)	9.29±7.81 (0.00 - 27.00)	7.50±7.18 [a] (0.00 - 25.00)	14.09±6.35 (2.00 - 24.00)	11.91±5.76 [b] (0.00 - 20.00)
EDI-2_I	1.39±2.20 (0.00 - 6.00)	1.22±1.90 (0.00 - 5.00)	3.26±4.29 (0.00 - 17.00)	1.62±2.85 [a] (0.00 - 10.00)	5.82±4.92 (1.00 - 19.00)	3.68±4.51 [b] (0.00 - 16.00)
EDI-2_P	3.83±3.54 (0.00 - 14.00)	3.61±3.16 (0.00 - 13.00)	4.85±3.00 (0.00 - 11.00)	4.74±2.99 (0.00 - 12.00)	3.36±3.67 (0.00 - 14.00)	3.32±3.81 (0.00 - 15.00)
EDI-2_ID	2.33±2.47 (0.00 - 9.00)	2.06±2.39 (0.00 - 9.00)	3.53±3.55 (0.00 - 16.00)	3.47±3.67 (0.00 - 17.00)	3.68±3.37 (0.00 - 10.00)	3.59±3.45 (0.00 - 10.00)
EDI-2_IA	3.61±3.96 (0.00 - 12.00)	3.22±3.61 (0.00 - 11.00)	4.47±5.93 (0.00 - 23.00)	4.41±6.03 (0.00 - 24.00)	5.36±5.02 (0.00 - 16.00)	5.27±4.73 (0.00 - 15.00)
EDI-2_MF	7.89±5.14 (1.00 - 21.00)	7.67±4.98 (1.00 - 20.00)	5.76±3.24 (0.00 - 14.00)	5.59±3.28 (0.00 - 15.00)	5.50±4.59 (1.00 - 20.00)	5.45±4.27 (1.00 - 19.00)
EDI-2_A	3.50±2.46 (0.00 - 8.00)	3.56±2.50 (0.00 - 9.00)	3.53±1.97 (0.00 - 7.00)	3.38±1.97 (0.00 - 8.00)	4.50±2.09 (1.00 - 8.00)	4.27±2.07 (0.00 - 7.00)
EDI-2_IR	1.61±2.48 (0.00 - 9.00)	1.67±2.28 (0.00 - 8.00)	3.50±3.86 (0.00 - 14.00)	3.68±4.09 (0.00 - 15.00)	2.32±2.92 (0.00 - 9.00)	2.27±2.81 (0.00 - 9.00)
EDI-2_SI	2.39±2.17 (0.00 - 7.00)	2.33±2.00 (0.00 - 6.00)	4.03±3.38 (0.00 - 15.00)	3.94±3.20 (0.00 - 14.00)	4.00±3.41 (0.00 - 14.00)	3.91±3.38 (0.00 - 15.00)

Results are expressed in mean value±standard deviation, and minimum and maximum for each parameter.

For abbreviation, see Abbreviation List. Values of $p<0.05$ are considered significant.

[a] NWO T0 vs T21 $p<0.05$; [b] PreOB/PB T0 vs T21 $p<0.05$ c NWL T0 vs T21

Table 5. Results of Lactulose Breath Test and symptoms in patients before and after therapy.

Orocaental Transit Time	**T0** **(Mean±SD)**	**T21** **(Mean±SD)**	**p**
Patients	120.00±12.00	97.00±8.00	<0.001
Controls	85.50±14.00	81.00±8.00	NS
p	<0.001	<0.001	
Symptoms	**% (n/n)**	**% (n/n)**	
Meteorism	90.00±3.53 (43/48)	20.00±26.87(10/48)	<0.001
Abdominal Pain	40.00±20.50 (19/48)	23±17.67 (11/48)	
	Mean T0	Mean T21	Ns
No. Defecations/Week	1.7	3.3	<0.001

For abbreviation, see Abbreviation List. Values of $p<0.05$ are considered significant.

Figure 1. CONSORT Flowchart.

CONSORT
TRANSPARENT REPORTING of TRIALS

ClinicalTrials.gov Id: NCT01890070

CONSORT 2010

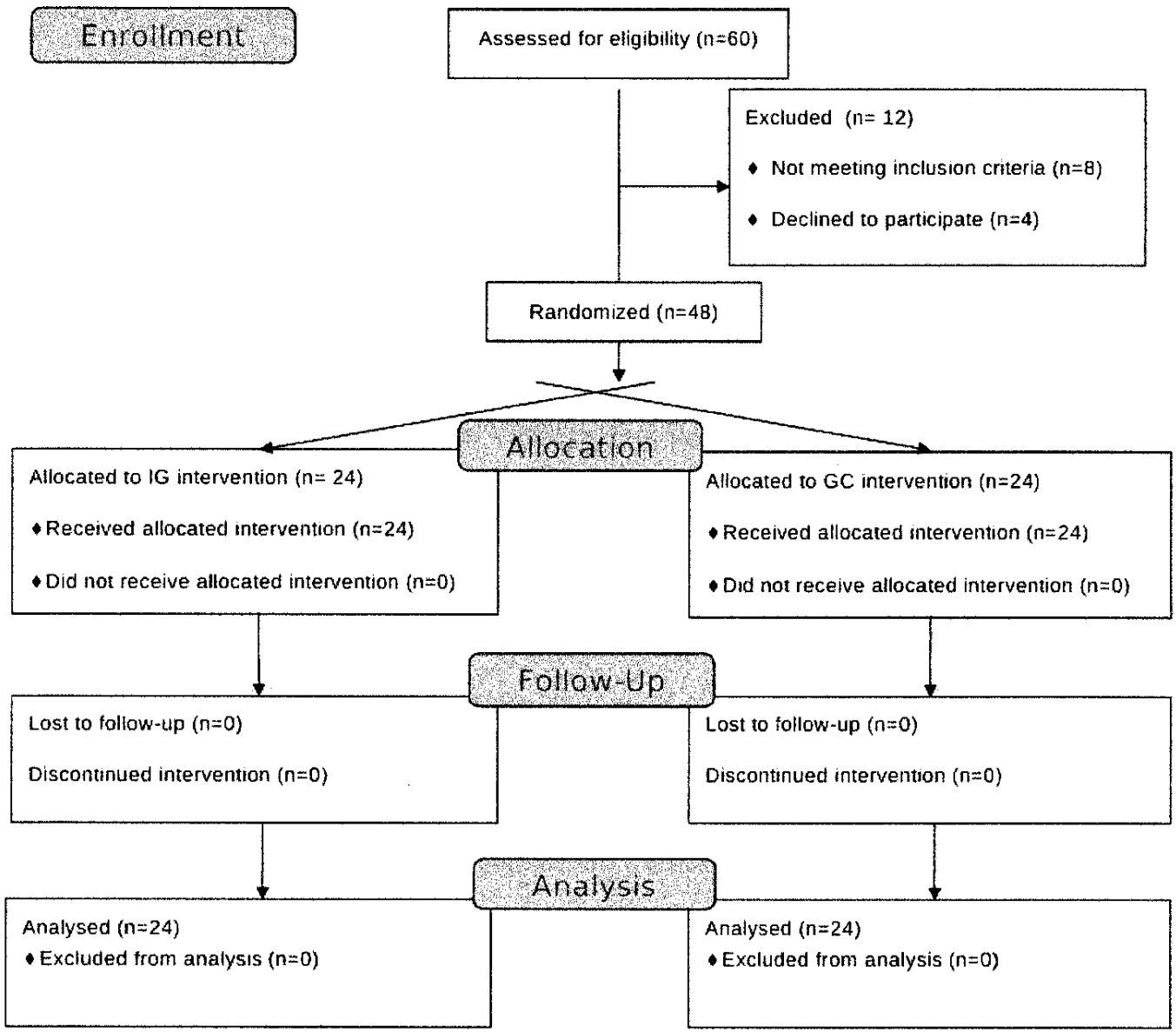

Figure 1. CONSORT Flowchart.

CONSORT
TRANSPARENT REPORTING of TRIALS

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CONSORT 2010