

DESCRIPTION of the invention having title:**"*Lactiplantibacillus plantarum* IMC 510 for use in a method for the prevention and treatment of obesity"****Summary of the Invention**

The present invention has as its object the use of the bacterial strain *Lactiplantibacillus plantarum* IMC 510 for use in a method for the prevention and treatment of overweight and obesity conditions, particularly for use in a method for body weight reduction, in a method for body mass index (BMI) reduction, in a method for waist circumference reduction, in a method for the reduction of the waist circumference to height ratio, in a method for fat mass reduction, or in a method for blood glucose reduction.

Technical Background

Obesity is a multifactorial disease caused by socioeconomic, hormonal and neuronal mechanisms, unhealthy lifestyles, genetic and epigenetic factors. In addition, overweight and obesity are closely related with other chronic diseases such as, for example, type 2 diabetes, cardiovascular disease, some types of cancer, musculoskeletal disorders, and infertility. The World Health Organization (WHO) defines obesity as an abnormal or excessive accumulation of fat that can compromise health, and is the result of an imbalance between caloric intake and energy expenditure; it is estimated that 39% of the world's adult population will become obese by 2035. The criterion used by WHO to diagnose obesity is the body mass index (BMI), which considers an individual with a $BMI \geq 25$ to be overweight, and obese when the BMI is ≥ 30 . This index is a useful indicator of a subject's overall adiposity, but it should be kept in mind that different adipose compartments are associated with different possible metabolic risks. For this reason, the assessment of abdominal circumference represents, in some cases, a more accurate classification of obesity: there is subcutaneous obesity in case the abdominal circumference is greater than 88 cm for women and greater than 102 cm for men.

As mentioned, overweight and obesity are the result of a positive energy balance, namely increased energy intake and decreased energy expenditure. However, many recent studies have highlighted how the human intestinal microbiota (referred to as the *Gut Microbiota* or GM) may also play a role in increased obesity, particularly in aspects of energy storage and nutrient absorption. Furthermore, it has been shown how the gut microbiota is different in obese individuals, compared with people of lean/normal weight constitution. The GM of adult subjects, in fact, is mainly characterized by taxa belonging to two phyla, *Firmicutes* and *Bacteroidetes*: in normal-weight subjects, there is a greater presence of *Bacteroidetes*, while the alteration of this ratio has been correlated with the state of obesity. Indeed, it appears that significant variations within the *Firmicutes* population, where the specific alteration in the composition of the microbiota is characterized by an increase in *Firmicutes* and a corresponding decrease in *Bacteroidetes*, may contribute to a more efficient extraction of energy from food sources and the consequent increase in its accumulation in host adipose tissue.

Other clinical studies have revealed that individuals with lower bacterial diversity have higher levels of leptin and protein C, are prone to forms of insulin resistance, dyslipidemia, adiposity, and exhibit an inflammatory phenotype; all of which result in a tendency to gain more weight than individuals with higher bacterial diversity.

Among their functions, intestinal bacteria ferment indigestible carbohydrate residues, synthesize short-chain fatty acids (SCFAs) and amino acids; these operations can help increase the amount of energy supplied to the host. On the other hand, however, keep in mind that the byproducts of bacterial fermentation can also reduce appetite and increase satiety, as well as lead to a decrease in body weight and BMI.

For all these reasons, the gut microbiota is considered to be a factor, potentially modifiable, that can be associated with the prevention and treatment of excessive body weight but whose full mechanisms of action and consequent implications for human health have not yet been conclusively established.

In fact, based on the actions exerted by the intestinal microbiota in humans, three main mechanisms of action in the treatment of obesity by probiotics can be schematically recognized: antagonistic effects on the growth of pathogenic microorganisms and competitive adherence to the intestinal mucosa and epithelium (antimicrobial activity), increased production of the intestinal mucus layer and reduced intestinal permeability (barrier function), and modulation of the gastrointestinal immune system (immunomodulation).

Experimental evidence has shown that the gut microbiota plays a role in the protection and health of the gastrointestinal tract, regulates the degree of mucosal permeability, aids in the metabolism of dietary polysaccharides by promoting fermentation and absorption, and is responsible for increased catabolism and increased SCFAs production, consequently acting on the management of fat accumulation and the resulting possible condition of obesity.

Specifically, the gut microbiota can modulate body weight and energy metabolism by acting on SCFAs production, control of bile acid metabolism, and induction and/or protection from metabolic endotoxemia.

With these assumptions in mind, therefore, in addition to the evaluation of nutrients and dietary patterns to be followed, the influence of probiotic intake has also been studied in relation to its possible ability to positively modulate the composition of the microbiota, stimulate the immune system, and maintain the integrity of the intestinal barrier through competitive effects against certain pathogenic strains. Studies have shown that probiotics possess positive effects toward some obesity-related side aspects, such as, for example, reduction of inflammation within adipose tissue, endotoxemia, adiposity, overall body mass, leptin levels as well as energy absorption.

Based on initial studies conducted in experimental animals, most of the to date available preparations later used in clinical trials contain bacterial strains of the genera *Lactobacillus* and *Bifidobacterium*. Said clinical studies aimed to evaluate the impact of these specific probiotics on the state of obesity, and in particular on weight changes, while considering the use of other strains as disadvantageous.

Therefore, there is still a need to have available a particular bacterial strain that can act on the specific factors in the gut microbiota that influence the propensity or development of overweight or obesity conditions.

It is still necessary to date to have available alternative and specific probiotic therapies that can act at the level of the gut microbiota in obese or overweight subjects, and that are effective in inducing a reduction in body weight and its maintenance at correct levels. Said therapies must possess high acceptability by the subjects involved, be safe to use, and also be able, when necessary, to act positively at the level of biomarkers of obesity-associated diseases such as diabetes, hypertension, and vascular disease.

Aims of the Invention

It is an aim of the present invention to make available a new microorganism of human origin isolated and characterized for probiotic properties.

It is an aim of the present invention to make available a strain of probiotic bacteria for use in a method for the prevention and treatment of overweight and/or obesity.

A further aim of the present invention is to make available a probiotic to rebalance the gut microbiota, preferably in a subpopulation of obese or overweight subjects.

These and other aims are achieved by the object of the present invention, which provides a bacterial strain as an effective probiotic for use in a method for the prevention and treatment of overweight and obesity conditions, particularly for use in a method for body weight reduction, in a method for body mass index (BMI) reduction, in a method for waist circumference reduction, in a method for the reduction of the waist circumference to height ratio, in a method for fat mass reduction, or in a method for blood glucose reduction.

Description of the Invention

It is an object of present invention a new isolated, non-genetically modified, human-derived microorganism named *Lactobacillus plantarum* IMC 510, deposited by Synbiotec S.r.l. at the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) under the deposit number DSM 32548.

It is noted that a new reclassification of the genus *Lactobacillus* occurred, published by Zheng et al. in the scientific journal *Int. J. Syst. Evol. Microbiol.*, 70(4):2782-2858, 2020. Therefore, the *Lactobacillus plantarum* bacteria strain IMC 510 can be identified as *Lactiplantibacillus plantarum* IMC 510. The above two designations are interchangeable with each other because they always refer to the same deposited strain of bacteria.

It is a microaerophilic, Gram-positive, immobile, elongated bacterium. IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is facultatively heterofermentative and produces L-lactic acid from glucose fermentation.

The colony (culture medium HHD, anaerobic conditions, 37°C, 2 days) is circular, medium to large, white, shiny, smooth and raised, with a gray, transparent border; under the microscope (MRS broth, 37°C, 12 hours) the microorganisms appear as short to medium-length rods with rounded ends; they are single, in pairs or in short chains, with palisade arrangement.

The optimal growth temperature of *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is 37°C, it has good tolerance to pancreatic acids and juices (85 to 95% survival) as well as excellent tolerance to bile and oxidative stress (greater than 95% survival).

Methods to test contact survival to acids, pancreatic juice and bile were conducted on cells derived from 12-hour cultures in MRS medium at 37°C, washed and resuspended in sterile saline, about at 10⁹ CFU/ml. In the case of acids, survival at pH 3 (citrate buffer) for 5 hours at 37°C is considered, while for pancreatic juices (pH 8) survival for 3 hours at 37°C is considered. In the case of bile, the method includes culture in MRS agar with or without bile salts (at concentration 0.1; 0.3; 0.5%) and in MRS agar with or without No. 3 bile salts (at concentration 0.05; 0.1; 0.2%). The survival percentage is calculated as log CFU/ml in the culture exposed to the adverse conditions compared with a culture grown under the same conditions but without the stressor.

Instead, oxidative stress is assessed from cells cultured in MRS broth at 37°C to a turbidity grade of McFarland 4, using a paper disk wetted with a 3% hydrogen peroxide solution resting on the colony in MRS soft agar and calculating the resulting free zone (MRS soft agar, anaerobic conditions, 37°C).

EFSA (*European Food Safety Authority*) has assigned QPS (qualified presumption of safety) status to this microbial strain; the microorganisms have no identified antibiotic resistance-related genes or plasmids. Antibiotic sensitivity is also within the parameters recommended by EFSA.

According to another of its aspects, it is an object of the invention a strain of probiotic bacteria based on *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) for use in a method for the prevention and treatment of overweight and obesity.

It is a further object of the present invention the bacterial strain *Lactiplantibacillus plantarum* IMC 510 for use in the treatment of dysbiosis of the gut microbiota of overweight or obese individuals for the purpose of reducing body mass and also intervening, when desired or necessary, on biomarkers of obesity-associated diseases such as diabetes, hypertension, and vascular disease.

In the present description, dysbiosis of the gut microbiota is understood to identify a condition of alteration in the amount and type of microorganisms present in the gastro-intestinal tract compared to normal physiological conditions.

As previously indicated, a nonlimiting example of dysbiosis present in obese and overweight individuals involves a high F/B (*Firmicutes/Bacteroidetes*) ratio that appears to be related to more efficient extraction of energy from food sources and the consequent increase in its accumulation in host adipose tissue.

Overweight and obese subjects are defined as those who meet the parameters of BMI and abdominal circumference as previously stated.

Preferably, the bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for body weight reduction; preferably in overweight and/or obese subjects; more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota; even more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota wherein said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.

Preferably, the bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for body mass index (BMI) reduction; preferably in overweight and/or obese

subjects; more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota; even more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota wherein said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.

Preferably, the bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for waist circumference reduction; preferably in overweight and/or obese subjects; more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota; even more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota wherein said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.

Preferably, the bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for reducing the waist circumference to height ratio; more preferably in overweight and/or obese subjects; more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota; even more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota wherein said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.

Preferably, the bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for fat mass reduction.

Preferably, the bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for blood glucose reduction; more preferably in overweight and/or obese subjects; more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota; even more preferably in overweight and/or obese subjects who have dysbiosis of the gut microbiota wherein said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.

It is an object of the present invention a composition or formulation comprising a mixture that comprises or, alternatively, consists of a bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) and, optionally, a pharmaceutical or food grade additive and/or carrier and/or excipient, for use in a reduction treatment method:

- (i) of body mass or body weight,
- (ii) of waist circumference and BMI,
- (iii) of waist circumference to height ratio,
- (iv) of fat mass,
- (v) of blood glucose,

preferably, reductions (i) to (v) are observed in a representative subpopulation of overweight or obese subjects.

The probiotic for use according to the invention is preferably administered orally in the form of capsules, tablets or sachets, preferably in capsule form, containing not less than 15 billion of *Lactiplantibacillus plantarum* IMC 510 live cells (CFU), preferably 15 billion, e.g., from not less than 15 billion to 100 billion of *Lactiplantibacillus plantarum* IMC 510 live cells (CFU).

According to one aspect of the present invention said administration occurs preferably once a day. According to a further aspect of the invention said administration takes place in the morning indifferent to meals. The contents of the capsule can be dispersed in a liquid at the temperature of intake (e.g., milk or fruit juices) or in semi-solid food (e.g., yogurt).

The probiotic for use according to the invention is gluten-free and lactose-free, contains no soy, GMOs, aspartame, acesulfame and added flavorings, and is compatible with a vegetarian and vegan diet.

As will be amply demonstrated in the experimental part below, the probiotic bacterial strain *Lactiplantibacillus plantarum* IMC 510 for use according to the invention is capable of:

- (i) reducing body mass or body weight,
- (ii) reducing waist circumference and BMI,
- (iii) reducing the waist circumference to height ratio,
- (iv) reducing fat mass,
- (v) reducing blood glucose,

preferably, reductions (i) to (v) are observed in a representative subpopulation of overweight or obese subjects.

The bacterial strain *Lactiplantibacillus plantarum* IMC 510 for use according to the invention has been shown to be effective in influencing the gut microbiota of subjects to whom it was orally administered, especially with reference to the populations of *Lactobacillus* spp. and *Bifidobacterium* spp. and in reducing the F/B ratio.

In addition, the bacterial strain *Lactiplantibacillus plantarum* IMC 510 for use according to the invention has been shown to be effective in influencing the intestinal microbiota of subjects to whom it has been orally administered, especially with reference to the populations of bacteria belonging to the genus *Hafnia-Obesumbacterium* and *Rambutsia* a significant reduction is observed, while with reference to the population of bacteria belonging to the genus *Succiniclasticum* spp. an increase is observed.

According to another of its aspects, it is an object of the invention a method for decreasing body weight in overweight or obese subjects, which comprises administering to said subjects in need, one or more oral formulations, preferably capsules, containing *Lactiplantibacillus plantarum* IMC 510 for use according to the invention. Subject is herein understood to mean, preferably, a human being.

According to another of its aspects, it is an object of the invention a method for body mass index (BMI) reduction in overweight or obese subjects, which comprises administering one or more oral formulations, preferably capsules, containing *Lactiplantibacillus plantarum* IMC 510 to said subjects in need, for use according to the invention. Subject is herein understood to mean, preferably, a human being.

According to another of its aspects, it is an object of the invention a method for waist circumference reduction in overweight or obese subjects, which comprises administering one or more oral formulations, preferably capsules, containing *Lactiplantibacillus plantarum* IMC 510 to said subjects in need, for use according to the invention. Subject is herein understood to mean, preferably, a human being.

According to another of its aspects, it is an object of the invention a method for the waist circumference to height ratio reduction in overweight or obese subjects, which comprises administering to said subjects in need, one or more oral formulations, preferably capsules, containing *Lactiplantibacillus plantarum* IMC 510 for use according to the invention. Subject is herein understood to mean, preferably, a human being.

According to another of its aspects, it is an object of the invention a method for fat mass reduction in overweight or obese subjects, which comprises administering to said subjects in need, one or more oral formulations, preferably capsules, containing *Lactiplantibacillus plantarum* IMC 510 for use according to the invention. Subject is herein understood to mean, preferably, a human being.

According to another of its aspects, it is an object of the invention a method for blood glucose reduction in overweight or obese subjects, which comprises administering to said subjects in need, one or more oral formulations, preferably capsules, containing *Lactiplantibacillus plantarum* IMC 510 for use according to the invention. Subject is herein understood to mean, preferably, a human being.

The probiotic for use according to the invention can be used alone or, if desired or necessary, in combination with other substances, provided those substances do not counteract the effects of the probiotic.

The following experimental section will present the results of the clinical study regarding the probiotic for use according to the invention, in which said use is compared with the results obtained from the administration of a placebo.

Preferred embodiments FRn of the present invention are shown below.

FR1. A bacterial strain named *Lactobacillus plantarum* IMC 510 deposited by Synbiotec S.r.l. at the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) under the deposit number DSM 32548 for use in a method for the prevention and treatment of overweight and/or obesity.

FR2. *Lactobacillus plantarum* IMC 510 bacterial strain for use according to FR1, characterized by the fact that said subjects have dysbiosis of the gut microbiota.

FR3. *Lactobacillus plantarum* IMC 510 bacterial strain for use according to FR2, characterized by the fact that said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.

FR4. The bacterial strain *Lactobacillus plantarum* IMC 510 for use according to the previous FRs, characterized by the fact that it is administered orally.

FR5. The bacterial strain *Lactobacillus plantarum* IMC 510 for use according to FR4, characterized by the fact that it is administered, preferably, in capsules containing an amount from 1 billion CFU to 100 billion CFU, preferably from 5 billion CFU to 50 billion CFU, even more preferably from 10 billion CFU to 25 billion CFU.

FR6. The bacterial strain *Lactobacillus plantarum* IMC 510 for use according to FR5, characterized by the fact that said administration occurs once or more times a day, preferably at breakfast.

FR7. A bacterial strain *Lactobacillus plantarum* IMC 510 (also named *Lactiplantibacillus plantarum* IMC 510), deposited at the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) under the deposit number DSM 32548.

Brief description of the drawings

Figure 1 Changes, expressed as %, in body weight (A), waist circumference (B), and BMI (C) at the predetermined measurement times (T0 empty bar, T90 days grid-filled bar, T30 follow-up days dotted-filled bar) relative to the two groups of volunteers (Probiotic and Placebo). Asterisk (*) indicates data in which there is a statistically significant difference ($P < 0.05$) from T0 (t of student's test).

Figure 2 Change in body weight (in kg), waist circumference (in cm) and BMI (in $\text{kg(m}^2)^{-1}$), at the predetermined measurement times (T0, T90 days and T30 follow-up days). For each time, the two bars represent the data for the two groups of volunteers: Probiotic (grid-filled bar) and Placebo (dotted-filled bar).

Figure 3 Bacterial concentration in fecal samples ($\log \text{CFU/g} \pm \text{SD}$) of bacterial groups *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides-Prevotella-Porphyromonas* spp., *Staphylococcus* spp, *Clostridium coccoides-Eubacterium rectale* group, and *Enterobacteriaceae* at the predetermined measurement times (T0 empty bar, T90 days grid-filled bar, T30 follow-up days dot-filled bar) relative to the two groups of volunteers (Probiotic and Placebo). The asterisk (*) indicates data in which there is a statistically significant difference ($P < 0.05$) from T0; the hash mark (#) indicates data in which there is a statistically significant difference ($P < 0.05$) from the Placebo group (Tukey's test according to one-way ANOVA).

Figure 4 Recovery, expressed as %, of *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548), compared with total *Lactobacillus* spp., in fecal samples from subjects belonging to the Probiotic group. The asterisk (*) indicates a statistically significant difference ($P < 0.05$) from T0 (t of student's test)

Figure 5 ORWELL 97 score expressed as mean $\pm$ SD at the predetermined measurement times (T0, T90 days and T30 follow-up days) relative to the two groups of volunteers (Probiotic and Placebo). For each time, the three bars represent, in order, the total ORWELL score (gray), the ORWELL relevance score (dark gray), and the ORWELL occurrence score (light gray).

Experimental section I

Lactiplantibacillus plantarum IMC 510 for use according to the invention: double-blind randomized clinical trial versus placebo

Subjects

The study involved 19 overweight/obese volunteers, 10 males and 9 females, aged 26 to 73 years with a BMI of 25 or greater. Participants were asked to maintain their usual diet and their usual level of physical activity. All volunteer subjects gave informed consent to participate; the procedures followed during the study were in accordance with the ethical standards of the responsible committee on human experimentation and the 1975 Declaration of Helsinki, as revised in 1983.

In addition to being between the ages of 26 and 73 and having BMI values greater than 25, study participants had to be willing to cooperate by providing informed consent and having the ability to follow the guidelines: demonstrating, in particular, willingness to complete questionnaires, records, and diaries associated with the

study. Participants were also asked to discontinue consumption of functional foods, dietary supplements containing probiotics, laxatives and/or body weight control substances throughout the study. In the four weeks prior to treatment with the probiotic according to the present invention or placebo, the use of other treatments (drugs or nutritional programs) that could affect body weight, food intake and/or energy expenditure was also prohibited. Postmenopausal, pregnant or breastfeeding women were excluded from selection for the present study, as were those who had enrolled in another obesity treatment program.

In addition, exclusion criteria during the study include: non-adherence to the probiotic/placebo intake protocol, the onset of a serious illness, and the need for continuous use of antibiotics and/or laxatives or other probiotics.

Study design

The randomized, placebo-controlled study was conducted with an initial phase (run-in) of 4 weeks, followed by a 12-week intervention phase (T90 days) and a final phase of another 4 weeks (T30 follow-up days). Subjects did not undergo antibiotic treatment during the month prior to the start of the study.

Already during the first phase, subjects were asked to stop any consumption of laxatives, dietary fiber and/or probiotics. At the end of the first 4 weeks (T0), volunteers were divided into an intervention group (treated with the probiotic for use according to the invention) and a control group (placebo).

In other words, the study involves 3 phases: *Phase 1* evaluation period (1 month/4 weeks) in which recruited subjects must meet the previously described inclusion criteria in order to enter the next phase; *Phase 2* intervention period (3 months/12 weeks) in which subjects were given one capsule per day, containing *Lactiplantibacillus plantarum* IMC 510 or placebo; *Phase 3* follow-up period (1 month/4 weeks).

At the end of each phase, at times designated T0 (end of *Phase 1*), T90 days (end of *Phase 2*) and T30 follow-up days (end of *Phase 3*), a medical and nutritional examination was performed as well as blood and fecal samples were collected. In addition, all subjects underwent questionnaires on gut well-being, psychological well-being, and overall quality of life assessment at each phase of the study, with specific attention directed toward obesity-related aspects.

Primary objectives

The safety evaluation of the selected probiotic (*Lactiplantibacillus plantarum* IMC 510), as well as its effect on gut microflora and body weight reduction while maintaining the same dietary habits, was carried out at the time of first intake (T0), after 3 months of treatment (T90 days) and after the next month of follow-up (T30 follow-up days).

Secondary objectives

Assessments of changes in BMI, waist circumference, blood parameters such as blood glucose, lipid levels, liver function (glutamic oxaloacetic transaminase - GOT and glutamic pyruvic transaminase - GPT in U/L), gut well-being, quality of life, general well-being, and level of discomfort generated by overweight and obesity status were performed.

Administration

Lactiplantibacillus plantarum IMC 510 was administered orally inside capsules, each containing no less than 1.5×10^{10} bacterial cells of the strain under consideration, previously isolated from healthy human subjects and deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) by Synbiotec S.r.l. (Camerino, Italy).

Compared with the capsules containing the probiotic, the placebo capsules carried only the inert diluent and were the same in size, shape, and taste as those containing the probiotic.

Physiological measurements

The volunteers' body weight was measured using a calibrated scale after shoes and jackets were removed. Waist circumference was determined at the height of two fingers below the navel; height was measured after shoes were removed. All measurements were taken at the same time of day, at each control time point: T0 (end of Phase 1), T90 days (end of Phase 2) and T30 follow-up days (end of Phase 3).

Health status assessment using questionnaires

Gut wellness

Gut well-being and stool consistency, a key parameter in describing normal or impaired bowel habit, were self-assessed by individual subjects at the end of Phase 2, through questions regarding "change in the number of daily defecations" and "change in the number of eggs (large stool portions) corresponding to equal volume by visual estimation". Specifically, then, subjects were asked to record whether, during the three months of probiotic (or placebo) administration and the subsequent month of follow-up, their gut well-being (in terms of bowel regularity and stool volume) remained the same, improved, or worsened from the previous period. The degree of change was recorded on a combined scale leading to a 10-point Likert scale (-5, 0, +5).

Using the same Likert scale of values, secondary parameters such as ease of defecation, bloating, constipation, abdominal pain, intestinal cramping, feeling of incomplete defecation, incontinence and halitosis were also investigated. Stool consistency was defined by the Bristol Stool Form Scale.

Health status-related quality of life

Subjects' health-related quality of life (HRQoL) was assessed by self-administration of the *Psychological General Well-Being Index* (PGWBI), a questionnaire that measures, in general, psychological well-being and degree of discomfort, and consists of 22 items covering six dimensions (anxiety, depression, self-control, positive attitude, general health and vitality). The questions allow multiple-choice answers with scores ranging from 0 to 5 (with 5 being the best score). The overall PGWBI score is then made up of the sum of all items in the various dimensions; the higher the score (100 being the maximum) the higher the psychological well-being.

ORWELL Questionnaire

The Obesity Related Wellbeing (ORWELL) questionnaire is a psychometric test to assess obesity-specific quality of life. The areas investigated include both physical well-being and emotional state and relational life. For each item considered, participants were asked to rate on a 4-point Likert scale (0, not at all; 1, a little; 2, not so much; 3, a lot) the symptom's occurrence/gravity (*occurrence*) and the subjective relevance of the symptom-related

impairment in their lives (*relevance*). The score for each item is then calculated as the product of occurrence and relevance. The total score (ORWELL 97) is obtained as the sum of the scores for the individual items; the sums of the scores for the occurrence (ORWELL 97-O) and relevance (ORWELL 97-R) of the symptoms in the individual items were also calculated separately. Higher ORWELL scores are related to a lower perception of quality of life.

Characterization of the gut microbiota

Fecal samples

Fecal samples were collected from each subject, both from the placebo group and the probiotic-treated group, at time 0 (T0), corresponding to the day of initiation of probiotic/placebo supplementation (end of *Phase 1* - beginning of *Phase 2*), after 90 days of probiotic/placebo supplementation (T90 days, end of *Phase 2*) and after the 30 days of follow-up period (T30 follow-up days, end of *Phase 3*). The feces were frozen at -80°C and so stored until the microbiota analysis was performed (enumeration of selected bacterial groups by quantitative real-time PCR and colonization of the probiotic bacterial strain).

DNA extraction

DNA extraction from all fecal samples was performed using a DNA isolation kit from feces (NorgenBiotek Corp., Thorold, Canada) with a modified protocol following the manufacturer's instructions specific for fecal samples. The amount and purity of extracted DNA were checked with the NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and then stored at -20°C until use for molecular analysis.

Real-Time PCR

A quantitative Real-Time PCR (qPCR) procedure was used to quantify selected bacterial groups within the feces of all subjects. Specifically, the bacterial groups of interest are: *Lactobacillus* spp., *Bifidobacterium* spp., *Bacteroides-Prevotella-Porphyromonas* spp., *Staphylococcus* spp., *Clostridium coccoides-Eubacterium rectale* group, and *Enterobacteriaceae*. Specific primers and SYBR Green quantitative real-time PCR amplification were used, using an iCycleriQ real-time detection system (Stratagene) associated with MXP software employing the conditions and standard curves for each bacterial group.

Lactiplantibacillus plantarum IMC 510 in feces

In order to confirm the presence of the probiotic strain for use according to the invention in the gut of the treated subjects, fecal samples were analyzed by counting lactobacilla on MRS agar (de Man, Rogosa and Sharpe agar, Oxoid, Basingstoke, UK) by a base-10 serial dilution method. After aerobic incubation at 37°C for 48-72 hours, ten to twenty percent of the total colonies per sample, randomly selected from counted agar plates, were isolated and tested for purity. DNA extracted from the selected colonies using a modified benzyl chloride method was analyzed by RAPD (Random Amplification of Polymorphic DNA) technique.

Blood samples

Blood samples were collected, from each subject, at the same times as the fecal samples: at time 0 (T0), corresponding to the day of initiation of probiotic/placebo supplementation (end of *Phase 1* - beginning of *Phase 2*), after 90 days of probiotic/placebo supplementation (T90 days, end of *Phase 2*) and after the 30 days of follow-up period (T30 follow-up day, end of *Phase 3*).

Statistical analysis

Results are expressed as mean $\pm$ standard deviation (SD) or standard error (SE). Statistical significance of differences between the probiotic group and the placebo group was analyzed using Student's t test. Significant differences between mean values were determined by Tukey's test after one-way analysis of variance ANOVA (1-way ANOVA) using the GraphPad PRISM program® 5.1. A P value of less than 0.05 was considered statistically significant. For bowel habits, where $P < 0.05$ reflects a significant difference, a test of four multiple comparisons was used.

Results

Nineteen volunteers were recruited for the clinical study. There were no dropouts, exclusions, or adverse events in either group (Probiotic and Placebo), and compliance with supplementation was 100%. Baseline demographics of participants who completed the study are shown in Table 1.

Table 1: Demographic and baseline characteristics of the study population. Values are expressed as Mean $\pm$ DS

	Probiotic (n° = 12)	Placebo (n° = 7)	P*
Age (years)	44.2 $\pm$ 11.8	59.3 $\pm$ 11.6	<0.05
Gender			
Males (n°. (%))	5 (41.7%)	5 (71.4%)	
Females (n°. (%))	7 (58.3%)	2 (28.6%)	
Body weight (kg)	84.7 $\pm$ 20.3	98.2 $\pm$ 16.4	>0.05
Height (m)	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1	>0.05
Waist circumference (cm)	101.8 $\pm$ 20.0	115.1 $\pm$ 15.5	>0.05

* significance level of $P < 0.05$ by Student's t test

As evident from the data in the table, the subjects had balanced characteristics between the two groups, Probiotic and Placebo, with respect to baseline characteristics such as gender, height, waist circumference and weight. Only the age parameter showed a significant difference between the two groups ($P < 0.05$, student's t-test).

Changes in body weight, BMI and waist circumference (mean values) are summarized in Figure 1. The Probiotic group revealed statistically significant ($P < 0.05$) reductions from T0 for all parameters. In contrast, the Placebo group never showed a statistically significant decrease.

As shown in Figure 2, at the end of 90 days of treatment, subjects in the Probiotic group experienced a significant average reduction in weight (-3.39 kg), waist circumference (-4.29 cm) and BMI (-1.15 kg/m²). The trend of maintaining the weight loss and waist circumference reduction was maintained even after the next 30 days of follow-up.

A quantitative Real-Time PCR (qPCR) procedure was used for quantification of selected bacterial groups from volunteers' feces. Specifically, the bacterial groups of interest were *Bacteroides-Prevotella-Porphyromonas* spp., *Staphylococcus* spp., *Clostridium coccoides-Eubacterium rectale* group, *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterobacteriaceae*. Figure 3 shows the changes in microbiota composition at the end of the treatment period (T90 days) and after the next 30 days of follow-up (T30 follow-up days), in the Probiotic and Placebo groups.

In Figure 3, it can be seen that supplementation of the probiotic for use according to the present invention significantly increased the concentration of *Lactobacillus* spp. ($P < 0.05$) at T90 days, compared with T0. In addition, it can be shown that for *Bifidobacterium* spp. the level was also higher after 90 days of treatment; although not statistically significant compared to T0, but statistically significant compared to the values recorded for the Placebo group. In contrast, no significant change was found in the case of the other bacterial species between the two groups during the treatment period. However, the proportion of subjects with increased or decreased *Bifidobacterium* spp. concentration after the treatment period tended to be different between the two groups (Chi square, $P = 0.01$). Specifically, the level of *Bifidobacterium* was found increased in 10 out of 12 subjects (83.3%) in the Probiotic group while an opposite trend, with decreasing values, was observed in 5 out of 7 subjects (71.4%) in the placebo group.

As previously reported, *Firmicutes* and *Bacteroidetes*, two key bacterial phyla of the human gut microbiota, have been shown to interact with homeostasis in obesity; in particular, the F/B ratio has been found to be higher in obese people. The present study reveals an increase in the relative abundance of *Firmicutes* (which include *Staphylococcus* spp., *Clostridium coccoides-Eubacterium rectale* group, and *Lactobacillus* spp.) and a consequent higher F/B ratio in overweight and obese subjects in the Placebo group, while in the Probiotic group the administration of the probiotic for use according to the invention seems to be able to keep the F/B ratio lower (also due to an increase in *Bacteroides-Prevotella-Porphyromonas* spp.).

To confirm the survival of the administered probiotic, during intestinal transit in the treated subjects, recovery of *Lactiplantibacillus plantarum* IMC 510 was performed from all fecal samples (collected at T0, T90 days and T30 follow-up days). As can be seen from the histogram in Figure 4, the strain was found to be present in the feces of all participants in the Probiotic group, not only at the end of the 90-days treatment period (T90 days) but also at the end of the follow-up period (T30 follow-up days). The specific treatment strain was not present in any of the fecal samples collected at T0, before the start of administration.

In more detail: the mean value of the recovery percentages of the probiotic *Lactiplantibacillus plantarum* IMC 510 reached 31.12% of the total *Lactobacillus* spp. at the end of the administration period (T90 days) and remained at

a high level, 18.50%, even after the 30-day follow-up phase (T30 follow-up days), compared with the total *Lactobacillus* spp.

Significant changes in bowel habits, considered as primary outcomes by the gut wellness questionnaire, are listed in Table 2.

Table 2: Mean absolute changes in bowel habit frequency score, stool consistency, and overall Psychological General Well-Being Index (PGWBI) questionnaire score after the 3-month probiotic/placebo supplementation period (T90 days) and after the next month of follow-up (T30 follow-up days)

Bowel habits*	Probiotic (mean±confidence limits)		Placebo (mean±confidence limits)	
	T90 days	T30 follow-up days	T90 days	T30 follow-up days
Bowel regularity	1.8±0.3 ^{#†}	1.1±0.3 [#]	-0.1±0.3	0.0±0.4
Stool volume	1.8±0.4 ^{#†}	1.3±0.3 [#]	-0.1±0.4	0.1±0.5
Stool consistency	2.2±0.3 ^{#†}	1.8±0.3 ^{#†}	0.0±0.1	0.0±0.1
Ease of defecation	1.8±0.3 ^{#†}	1.3±0.3 [#]	0.0±0.5	-0.1±0.5
Gastric noises	1.8±0.3 [#]	1.4±0.3	-0.3±0.4	-0.1±0.5
Bloating	1.6±0.5 [#]	1.3±0.3 [#]	-0.3±0.1	-0.3±0.1
Flatulence	1.2±0.3 [#]	1.3±0.4	0.0±0.1	0.0±0.1
Constipation	0.9±0.4	0.0±0.2	0.0±0.5	0.0±0.5
Diarrhea	0.2±0.1 [†]	0.0±0.1 [†]	-0.1±0.5	0.0±0.6
Dermatitis	0.7±0.2	0.4±0.1	-0.1±0.4	0.0±0.5
Food intolerance	0.8±0.1	0.6±0.1	0.0±0.5	0.0±0.5
Stool consistency^a				
Type	3±0.2 [#]	3±0.2 [#]	3±0.5	4±0.5
PGWBI^b				
Overall score	76.2±3.2 [#]	76.3±3.8 [#]	73.7±3.3	72.0±2.8

* changes in bowel habits were assessed with a ten-point Likert scale (-5, 0, 5);

^a evaluation was obtained by following the Bristol Stool Chart;

^b overall score scale from 0 to 100 (100 the best);

[#] statistically different from T0; [†] statistically different from Placebo at T90 days; [‡] statistically different from Placebo at T30 follow-up days (Student's t test, P<0.05).

In general, subjects administered with the probiotic for use according to the present invention presented significantly higher values (P<0.05) in scores related to the scale related to bowel habits. Health status-related quality of life was estimated as the overall PGWBI score; the Probiotic group, after 90 days of treatment (T90

days) and after the subsequent 30 days of follow-up (T30 follow-up days), showed statistically higher ($P < 0.05$) values than the PGWBI index values recorded at the beginning of treatment (T0).

To assess obesity-related quality of life, patients were asked to perform a self-assessment using the ORWELL 97 questionnaire to assess the subjective relevance and intensity of physical and psychological discomfort. The questionnaire was then evaluated for all 19 overweight/obese volunteers by determining the change over time (T0, T90 days T30 follow-up days) of symptom onset and/or severity (occurrence) and subjective relevance of symptom-related impairment in one's life (relevance). The data obtained are shown in Figure 5.

The total ORWELL 97 score is obtained as the sum of the scores of individual items, also, to understand the results shown in Figure 5, it is important to keep in mind that higher ORWELL 97 scores are correlated with lower quality of life, analyzed using Student's t-test with significance level $P < 0.05$.

The value of the total score, in the case of subjects in the Probiotic group, has a decreasing trend over time, although not significant ($P > 0.05$), resulting in a slight improvement in quality of life. This trend is not noticeable in the Placebo group.

In conclusion, the experimental data presented indicate that the administration of the probiotic *Lactiplantibacillus plantarum* IMC 510 for use according to the present invention resulted in statistically significant reductions in body weight, BMI, and waist circumference in subjects in the Probiotic group, contrary to what was recorded in the case of the Placebo group. In particular, reduction in waist circumference is an important factor because it is involved in a direct measurement of fat distribution and is closely correlated with atherogenic lipid profiles.

Confirming the safety of the treatment, the present study recorded no significant alterations or physiological abnormalities in volunteers, in lipid metabolism-related parameters such as triglycerides, total cholesterol, low-density lipoprotein, or high-density lipoprotein.

The clinical study also showed that the ratio of *Firmicutes* to *Bacteroidetes* was lower in the Probiotic group at the end of the administration period; while the Placebo group had higher values of the F/B ratio, indicative of the tendency to overweight/obesity. This fact represents further confirmation of the potential of the probiotic strain *Lactiplantibacillus plantarum* IMC 510 for use according to the present invention to prevent/treat overweight and obesity, and in particular by treating dysbiosis of the gut microbiota of overweight or obese subjects by acting on the F/B ratio.

Finally, it is well known how obesity and overweight conditions affect the quality of life of subjects with these conditions by implying a decreased ability to perform activities, early fatigue, as well as increased anxiety, depression, and general low self-esteem. The self-assessment questionnaires used in the conduct of this study made it possible to demonstrate how there were also significant improvements in these aspects related to the psychological and relational spheres of the subjects; in fact, the scores calculated for the Probiotic group recorded a positive evolution in aspects related to overall well-being, health/energy/mood status, and general quality of life.

Experimental Section II

The Applicant performed a second study in which the aim was to evaluate the effect of supplementation with *Lactiplantibacillus plantarum* IMC 510 on anthropometric and biochemical parameters, gut microbiota (GM) composition and function, gastrointestinal and general symptoms of overweight/obese subjects.

A group of 40 overweight/obese subjects were randomly assigned to consume the probiotic *Lactiplantibacillus plantarum* IMC 510 or placebo daily for 3 months. Before and after the administration period, anthropometric and biochemical parameters, self-administered questionnaires, plasma and fecal samples were obtained from each participant. GM characterization was performed by 16S rRNA sequencing, while short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs) in feces were analyzed by a gas chromatography-mass spectrometry protocol.

Compared with placebo, supplementation with the probiotic resulted in a significant reduction in body weight, body mass index (BMI), waist circumference, waist circumference-to-height ratio, and blood glucose. In addition, probiotic administration resulted in a significant decrease in the genera *Hafnia-Obesumbacterium* and *Rombutsia* and an increase in *Succiniclasticum* spp.; in contrast, placebo administration resulted in a decrease in *Actinomycetaceae* and an increase in *Alloprevotella* spp. and proinflammatory hexanoic and heptanoic acid levels.

Due to its effect of increasing certain beneficial gut bacteria and its effects of reducing waist circumference, fasting glucose levels, and gastrointestinal symptoms in obese subjects, *Lactiplantibacillus plantarum* IMC 510 supplementation could be an encouraging future strategy for the prevention or treatment of obesity.

The present clinical study aimed to evaluate the effect of a probiotic formulation based on *Lactiplantibacillus plantarum* IMC 510 on anthropometric and biochemical parameters, GM composition and function, and gastrointestinal and systemic clinical manifestations of overweight/obese subjects.

MATERIALS AND METHODS

Study population

Forty subjects (20 males and 20 females) with a mean age of 51.6 ± 13.2 years were enrolled in the study. Participants were considered eligible for inclusion if they were between 18 and 65 years of age and had a BMI ≥ 25 kg/m². Exclusion criteria were as follows: continued use of probiotics in the two months prior to enrollment; use of other treatments (drugs or nutritional programs) that affect body weight, food intake and/or energy expenditure; pregnancy or lactation; enrollment in another obesity treatment program.

Study design

The present study is a parallel, randomized, double-blind controlled trial. Before randomization, there was a 2-week run-in period to assess participants' commitment, motivation, and willingness. After the run-in period, eligible participants were randomly divided into two groups, each assigned to consume the probiotic *Lactiplantibacillus plantarum* IMC 510 or a placebo for 3 months. During the intervention period, all participants were encouraged not to change their lifestyle, exercise or eating habits. The study procedures were approved by

the Ethics Committee of the Tuscany Region, Azienda Ospedaliera Universitaria Careggi (14592_spe), they were registered on clinicaltrials.gov (identifier: NCT05358301), and adhered to the principles of the Declaration of Helsinki and the Data Protection Act.

Body weight and body composition were measured at each clinical evaluation. Weight and height were measured with a stadiometer. BMI was calculated as weight (kg)/height (m²), and WHtR (waist circumference/height ratio) was calculated as waist circumference (cm)/height (cm). Participants were classified as overweight if their BMI was ≥ 25 kg/m² and < 30 kg/m², and obese if their BMI was ≥ 30 kg/m². Body composition was determined using a bioelectrical impedance analysis device (AKERN, Model 101 Sport Edition).

Interventions

The experimental probiotic dietary supplement used in the present clinical study is manufactured by Synbiotec S.r.l. (Camerino, Macerata, Italy). Each capsule contains the probiotic strain *Lactiplantibacillus plantarum* IMC 510 at a concentration of 15 billion live cells (CFU/capsule). Intake is N=1 capsule/day, preferably at breakfast, for 3 months. Capsules can be opened and their contents dispersed in a cold liquid or semisolid food. Capsules should be stored in the original container or in a clean, covered container, out of the reach of children. When stored in a clean, dry environment out of direct sunlight, the probiotic product has a shelf life of at least 24 months. Samples of probiotic supplements in closed and sealed boxes were provided to participants at the beginning of the study, in sufficient quantity for the entire duration of the study (90 capsules for 3 months).

The placebo dietary supplement (i.e., capsules without added probiotics) was administered as N=1 capsule/day with the same intake pattern as the experimental probiotic. The probiotic and placebo appeared identical to ensure blinding of physicians and participants.

Table 3

Variable	All (n=40)	Probiotic (n=20)	Placebo (n=20)	p° value
Age, y	51 $\pm$ 13.5	51 $\pm$ 12.7	51 $\pm$ 14.7	0.946
Female gender, n (%)	20 (50)	9 (52.9)	11 (47.1)	0.999
Weight, Kg	86.1 $\pm$ 14	90 $\pm$ 10.4	82.4 $\pm$ 16.2	0.038
BMI, Kg/m ²	29.7 $\pm$ 4.2	30.8 $\pm$ 3.8	28.7 $\pm$ 4.5	0.085
Waist circumference, cm	101.6 $\pm$ 12.9	104.7 $\pm$ 13.1	98.5 $\pm$ 13.9	0.131
WHtR	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.092
Smoking habit, n (%)	6 (17.6)	0 (0)	6 (35.3)	0.018
Physical activity, n (%)	13 (38.2)	7 (41.2)	6 (35.3)	0.999
Fat mass, Kg	29.3 $\pm$ 9.6	31.4 $\pm$ 9.5	27.0 $\pm$ 9.5	0.208
Fat mass, %	33.8 $\pm$ 8.3	34.8 $\pm$ 9.3	32.6 $\pm$ 7.3	0.525
Fat-free mass, Kg	56.9 $\pm$ 11.4	58.6 $\pm$ 10.9	55.0 $\pm$ 11.9	0.257

Total body water, L	41.7 ± 8.3	42.9 ± 8	40.3 ± 8.7	0.257
White blood cells, x10 /L ⁹	7.0 ± 2.0	6.5 ± 1.3	7.6 ± 2.5	0.099
Red blood cells, x10 /L ¹²	4.9 ± 0.4	5.0 ± 0.4	4.8 ± 0.4	0.433
Hemoglobin, g/dL	14.7 ± 1.2	14.9 ± 1.3	14.4 ± 1.1	0.231
Glucose, g/L	97.3 ± 14.4	98.7 ± 16.7	95.9 ± 12.0	0.973
Urea, g/L	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.191
Creatinine, mg/dL	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.657
Sodium, mEq/L	141.6 ± 1.7	142 ± 1.6	141.2 ± 1.7	0.182
Potassium, mEq/L	4.6 ± 0.4	4.6 ± 0.3	4.6 ± 0.4	0.838
Calcium, mg/dL	9.4 ± 0.6	9.4 ± 0.8	9.4 ± 0.4	0.290
Magnesium, mg/dL	2 ± 0.1	2 ± 0.1	2 ± 0.1	0.946
AST, U/L	22.0 ± 10.9	19.0 ± 5	24.9 ± 14.3	0.160
ALT, U/L	23.0 ± 12.6	21.5 ± 9.2	24.6 ± 15.5	0.919
Triglycerides, mg/dL	125.2 ± 105.5	106.0 ± 45.8	144.3 ± 141.7	0.786
Total cholesterol, mg/dL	194.4 ± 33.7	193.3 ± 29.4	195.6 ± 38.3	0.919
HDL-cholesterol, mg/dL	55.0 ± 13.6	56.6 ± 14.9	53.9 ± 12.5	0.786
LDL-cholesterol, mg/dL	116.1 ± 29.9	117.3 ± 24.9	114.7 ± 35.6	0.728
Uric acid, mg/dL	5.2 ± 1.2	5.2 ± 1.1	5.3 ± 1.3	0.892
eGFR	84.3 ± 8.7	83.7 ± 7.6	84.9 ± 9.9	0.309

Effects of intervention on body composition

Table 4 shows the changes in anthropometric parameters after the intervention and control periods. No significant differences were found between the two groups. The group undergoing probiotic supplementation revealed a significant ($p < 0.05$) decrease in body weight (-0.7 kg, $p = 0.026$), BMI (-0.2, $p = 0.032$), waist circumference (-0.9 cm, $p = 0.043$) and WHt ratio (-0.01, $p = 0.041$). In addition, although not statistically significant, but bordering on significance, there was also a decrease in fat mass (-0.9 kg, $p = 0.075$) in the probiotic group.

Variable	pre Probiotic	post Probiotic	p-value	pre Placebo	post Placebo	P- value	Probiotic Change	Placebo change	P-value°
Weight, Kg	90 (84.6; 95.3)	89.3 (84.2; 94.5)	0.026	82.4 (74; 90.7)	82.5 (73.6; 91.3)	0.791	-0.7 (-1.2; - 0.1)	0.1 (- 0.7; 0.9)	0.215
BMI, Kg/m ²	30.8 (28.8; 32.7)	30.5 (28.6; 32.4)	0.032	28.7 (26.4; 31.0)	28.7 (26.3; 31.1)	0.932	-0.2 (-0.4; 0)	0 (-0.3; 0.3)	0.269

				31)	31.2)				
Waist circumference, cm	104.6 (98.7; 110.5)	103.8 (97.9; 109.6)	0.043	98.5 (91.4; 105.7)	97.9 (90.4; 105.4)	0.135	-0.9 (-1.7; 0)	-0.6 (-1.4; 0.2)	0.916
WHR	0.6 (0.6; 0.6)	0.6 (0.6; 0.6)	0.041	0.6 (0.5; 0.6)	0.6 (0.5; 0.6)	0.118	0 (0; 0)	0 (0; 0)	0.876
Fat mass, Kg	31.4 (26.3; 36.4)	30.5 (25.9; 35.1)	0.075	27 (21.5; 32.5)	27.3 (21; 33.5)	0.550	-0.9 (-1.9; 0.1)	0.3 (-0.8; 1.5)	0.164
Fat mass, %	34.8 (29.9; 39.8)	34.2 (29.5; 38.9)	0.157	32.6 (28.4; 36.8)	32.8 (28.2; 37.3)	0.757	-0.7 (-1.6; 0.3)	0.1 (-0.8; 1.1)	0.177
Fat-free mass, Kg	58.6 (52.8; 64.5)	58.9 (53.2; 64.5)	0.594	55 (48.1; 61.9)	54.8 (47.9; 61.8)	0.575	0.2 (-0.6; 1)	-0.2 (-0.8; 0.5)	0.479
Total body water, L	42.9 (38.6; 47.2)	42.6 (38.5; 46.7)	0.535	40.3 (35.2; 45.3)	40.1 (35.1; 45.2)	0.634	-0.3 (-1.5; 0.8)	-0.1 (-0.6; 0.4)	0.851

Table 4

Impact of the intervention on biochemical parameters

Regarding biochemical parameters, a general linear model for repeated measures showed that the probiotic led to a significant decrease in blood glucose (-3.9 g/L; p=0.033) and a significant increase in uric acid (0.2 mg/dL; p=0.040) after 3 months of intervention. (Table 5).

Variable	pre Probiotic	post Probiotic	p-value	pre Placebo	post Placebo	p-value	Change Probiotic	Change Placebo	p-value°
Glucose, g/L	98.6 (90.1; 107.2)	94.8 (86.4; 103.1)	0.033	95.9 (89.7; 102.1)	97.5 (89; 105.9)	0.537	-3.9 (-7.4; -0.4)	1.6 (-3.7; 6.9)	0.084
Uric acid, mg/dL	5.2 (4.6; 5.7)	5.4 (4.8; 5.9)	0.040	5.3 (4.6; 6)	5.5 (4.8; 6.1)	0.281	0.2 (0; 0.4)	0.2 (-0.2; 0.6)	0.535

Table 5

Therefore, in this study we evaluated the effects of a new probiotic formulation based on *Lactiplantibacillus plantarum* IMC 510 on anthropometric and biochemical parameters, GM composition and function, and gastrointestinal and general symptoms of overweight/obese subjects.

First, with regard to anthropometric parameters, we found that supplementation of the probiotic resulted in a slight reduction in body weight, BMI, WHt ratio, and fat mass, confirming our previous results that documented a significant net reduction in body weight gain, of BMI and white adipose tissue weight in obese rats after 84 days of supplementation with *Lactiplantibacillus plantarum* IMC 510 and in human subjects after 3 months of *Lactiplantibacillus plantarum* IMC 510 administration.

Furthermore, in agreement with Coman and colleagues, probiotic supplementation also resulted in a significant reduction in waist circumference in volunteers compared with placebo. The reduction in waist circumference provides important cardiovascular benefits to obese subjects, and the anti-obesogenic role of several *Lactobacillus* and *Bifidobacterium* strains has also been reported in obese adults. On the other hand, waist circumference and WHtR are used in clinical practice as markers of visceral adiposity, which are closely associated with obesity and related metabolic diseases. Waist circumference and WHt ratio, not BMI, are crucial measures of obesity severity and are more closely associated with obesity-related metabolic diseases. Probiotics seem to be able to alter fat distribution without significantly changing body weight in obese/overweight subjects, which is reflected in the reduction of waist circumference and WHt ratio, meaning that the use of probiotics seems to be beneficial for this type of patients.

The 3-month supplementation seems to be the starting point of the trend to observe positive effects on body weight and/or fat mass (waist circumference and WHt ratio) with the use of probiotics. It should be noted that a $\geq 1\%$ decrease in initial body weight over a 3-month period is clinically relevant and would be associated with a significant reduction in some cardiovascular risk factors, such as a decrease in blood glucose, as revealed by our data. Thus, compared with placebo, the probiotic intervention resulted in a significant reduction in blood glucose, whereas there were no significant changes for other blood parameters. Consistent with our results, a meta-analysis conducted by Kocsis and colleagues reported that supplementation with probiotics resulted in a significant reduction in fasting plasma glucose levels in patients with type 2 diabetes mellitus.

However, our data clearly report that the potential probiotic strain *Lactiplantibacillus plantarum* IMC 510 showed, in addition to the increase in some beneficial gut bacteria, effects of reduction of body weight, waist circumference, fasting glucose levels, and gastrointestinal symptoms of obese individuals.

CLAIMS

1. A bacterial strain named *Lactobacillus plantarum* IMC 510 deposited by Synbiotec S.r.l. at the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) under the deposit number DSM 32548 for use in a method for the prevention and treatment of overweight and/or obesity in subjects; wherein said subjects have dysbiosis of the gut microbiota.
2. The bacterial strain *Lactobacillus plantarum* IMC 510 according to claim 1, wherein said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.
3. The bacterial strain *Lactobacillus plantarum* IMC 510 according to claim 1 or 2, wherein said bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for body weight reduction.
4. The bacterial strain *Lactobacillus plantarum* IMC 510 according to any one of the preceding claims, wherein said bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for body mass index (BMI) reduction.
5. The bacterial strain *Lactobacillus plantarum* IMC 510 according to any one of the preceding claims, wherein said bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for waist circumference reduction.
6. The bacterial strain *Lactobacillus plantarum* IMC 510 according to any one of the preceding claims, wherein said bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for the waist circumference to height ratio reduction.
7. The bacterial strain *Lactobacillus plantarum* IMC 510 according to any one of the preceding claims, wherein said bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for fat mass reduction.
8. The bacterial strain *Lactobacillus plantarum* IMC 510 according to any one of the preceding claims, wherein said bacterial strain *Lactiplantibacillus plantarum* IMC 510 (*Lactobacillus plantarum* IMC 510 DSM 32548) is for use in a method for blood glucose reduction.

9. The bacterial strain *Lactobacillus plantarum* IMC 510 for use according to any one of the preceding claims, wherein said strain is administered orally.
10. The bacterial strain *Lactobacillus plantarum* IMC 510 for use according to any one of the preceding claims, wherein said strain is administered, preferably, in capsules containing an amount from 1 billion CFU to 100 billion CFU, preferably from 5 billion CFU to 50 billion CFU, even more preferably from 10 billion CFU to 25 billion CFU.
11. The bacterial strain *Lactobacillus plantarum* IMC 510 for use according to claim 9 or 10, wherein said administration occurs once or more times a day, preferably at breakfast.
12. The bacterial strain *Lactobacillus plantarum* IMC 510 for use according to any one of the preceding claims, wherein said administration occurs preferably in overweight and/or obese subjects; more preferably in overweight and/or obese subjects presenting a dysbiosis of the gut microbiota; even more preferably in overweight and/or obese subjects presenting a dysbiosis of the gut microbiota wherein said dysbiosis is characterized by a high F/B (*Firmicutes/Bacteroidetes*) ratio.
13. A composition comprising a mixture that comprises or, alternatively, consists of a bacterial strain *Lactobacillus plantarum* IMC 510 (also referred to as *Lactiplantibacillus plantarum* IMC 510) according to any one of the preceding claims and, optionally, a pharmaceutical or food grade additive and/or carrier and/or excipient.
14. A bacterial strain *Lactobacillus plantarum* IMC 510 (also named *Lactiplantibacillus plantarum* IMC 510), deposited at the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) under the deposit number DSM 32548.