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Efficient Weight Loss and Type II Diabetes Control in Overweight and Obese Patients Consuming the Probiotic *Bacillus Subtilis* DG101: A Randomized Double-Blind Placebo-Controlled Study

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Abstract

Objective: Obesity is a worldwide endemic disease with limited options for treatment. Studies have indicated that the probiotic *Bacillus subtilis* can reduce weight gain and serum lipid levels in animal models. However, there are no publications showing positive results on the anti-obesity effect in humans. Here, we investigated the anti-obesity effect of the probiotic *B. subtilis* DG101 (i.e., reductions in weight, corporal fat content, and BMI) on overweight/obese subjects and the management of their HbA1c and insulin values.

Design: A randomized, double-blinded, placebo-controlled study.

Methods: A total of 294 subjects were divided into two groups according to sex. The subjects were randomly assigned to receive a daily dose of the probiotic *B. subtilis* DG101 or pure water (placebo) in all cases combined with a low-caloric/low-fat diet for 12 weeks. Weight, fat percentage, and BMI were measured, as well as HbA1c and insulin levels before and after the intervention.

Results: The probiotic *B. subtilis* DG101 significantly reduced weight, fat percentage, and BMI after 12 weeks of the intervention compared to the placebo ($p<0.05$). There was a significant reduction of HbA1c and insulin levels between the groups ($p<0.05$).

Conclusions: Ingestion of the probiotic *B. subtilis* DG101 as an adjuvant in the anti-obesity therapy compared with placebo for 12 weeks significantly reduce obesity related parameters. Our finding of lower HbA1c and insulin levels in pre-diabetic and diabetic patients during ingestion of the probiotic *B. subtilis* DG101 validated one of our previous reports.

Keywords

Obesity, Weigh Lose, Body Fat, Body Mass Index, Type II Diabetes, Insulin Resistance, Probiotics, *Bacillus Subtilis*

Introduction

Obesity, traditionally defined as an excess of body fat causing prejudice to health, is usually assessed in clinical practice by the body mass index (BMI), which is expressed as the ratio of body weight in kilograms divided by height in square meter (kg/m^2). Since its introduction, many large population studies have reported a J-shaped relationship between the BMI and mortality risk, i.e., a BMI above 30 kg/m^2 is clearly associated with an increased morbidity/mortality risk [1]. Despite considerable efforts devoted to understanding the biology of obesity and energy balance, it has become obvious that available knowledge has to date been of little help to curb the obesity epidemic and that no region of the world has been spared from this phenomenon. It has been estimated by WHO that 39% of individuals older than 18 years are overweight, and nearly 2.8 million deaths per year are a consequence of overweight and obesity-associated conditions (e.g., blood hypertension, dyslipidemia, coronary heart disease, ischemic stroke, osteoarthritis, and type 2 diabetes mellitus) [1,2]. Based on such widespread growth, it is obvious that our evolving scientific knowledge about the management of obesity at the individual level has not been translated into successful, large-scale clinical programs. The prescription of moving more and eating less has now been proven as a crude oversimplification of a complex interaction between physiology and the presence of obesity-promoting environments. Thus, beyond a better understanding of the biology of energy balance and obesity, the major social, environmental, and economic drivers of this epidemic must be understood, and alternative solutions targeting them should be evaluated. Worsening the situation, there are few available strategies to fight obesity rather than the adoption of low-calorie diets, daily exercise routines, pharmacotherapy, and anti-obesity dietary supplements supplied by medical prescription or a dietician, respectively, and in extreme situations bariatric surgery [1,2]. Therefore, overweightness and obesity represent one of the most serious economic and health problems of our era. During the last years, the incorporation of probiotic bacteria into the human diet has been proposed as a new element to fight overweight and obesity [3,4]. Probiotics are defined as "live microorganisms that, when administrated in adequate amounts, confer a health benefit on the host" [3,4]. The spore-forming *Bacillus subtilis*, *Bacillus coagulans* and *Bacillus clausii* are probiotic bacteria with multiple beneficial effects over the host once established in the intestine [5-8]. Probiotic bacilli are preferred over lactic bacteria (i.e., *Lactobacillus* and *Bifidobacterium*) because of their higher resistance and stability (prolonged shelf life) [6]. The probiotic *B. subtilis* is able to reduce weight gain and the level of serum lipids in an animal models [9], but it is unknown if the *B. subtilis* anti-obesity effects observed in animal models could be translated to overweight / obese persons [4]. In this work, we report the results of a randomized, double-blind trial consisting of the co-administration of the probiotic *Bacillus subtilis* DG101 (natto strain) [5,10] to the diet of overweight / obese people carrying obesity-related comorbidities (i.e., pre-diabetes and type II diabetes mellitus).

Methods

Subjects and Study Design:

All patients were recruited using the electronic medical database record at the Instituto Cardinali de Nutrición. They were invited by letter to participate in the study. Inclusion criteria were age between 18 to 78 years old with at least 1-year prediabetes or diabetes condition, HbA1c levels between 8.0 and 10.5%, glycemic levels between 110 and 190 mg/dl, insulin levels between 12.0 and 14.0 μIU/ml, and overweight or obesity (fat percentage >25 and BMI >30). All the participants of this study presented a prediabetes or diabetes condition, ~ 90% and ~ 10%, respectively. The exclusion criteria for this study were individuals with malignant cancer or thyroid disorders and pregnant women. The total number of subjects recruited for this study (patient population) was three hundred and one people (n = 301) and were subsequently enrolled in the study. However, six (6) patients were excluded before randomization because of not fulfilling the inclusion criteria. Only one person declines to participate for personal reasons. The remaining two hundred and ninety-four (294) participants were divided into two groups by sex: one hundred and twelve men (n = 112) and one hundred and eighty-two women (n = 182). The subjects were randomly assigned by a computerized randomization system to incorporate the probiotic *B. subtilis* DG101 into their diets (intervention group) or the placebo (placebo group). The research staff and study participants remained blinded to the type of treatment during the entire study. The probiotic *B. subtilis* DG101 [5,10] was prepared in an aqueous solution at a concentration of 1×10^8 colony forming units (CFU) per ml and supplied to each participant of the intervention group in dropper cap bottles of 250 ml capacity. Patients of the placebo group received the same type of bottles that only contained pure water. The probiotic *B. subtilis* DG101 was consumed in a daily dose of one-hundred million colony forming units (1×10^8 CFU / day) administered in liquid form as drops (twenty drops correspond to the daily dose of the probiotic) added to a beverage. The study was carried out for 12 weeks (three months) and during that time all the participants (intervention and placebo groups) followed a low-caloric/low-fat diet based on proteins and carbohydrates of the low glycemic index.

The participants visited the clinic twice a month. At each visit, corporal weight, total body fat (measured by bioelectrical impedance), blood pressure, pulse rate, common blood and urinary tests including levels of glucose in the blood, insulin, and other biochemical or clinical parameters of relevance for each patient were documented. Glycosylated hemoglobin (HbA1c) was measured before the initiation of the trial and one month after the end of it (fourth month). For the duration of the study, the subjects were asked to maintain their habitual mode of living and otherwise comment any change in their daily routine to the doctor at the time of visiting the clinic. No adverse effects attributed to the probiotic consumption were observed and no subject dropped out during the study.

Statistical Analysis:

Linear regression analysis (ANOVA) was used to test differences in response to treatment between groups (treatment versus placebo and sex). Normally distributed variables are presented as means ±S.D. unless otherwise specified. A p-value < 0.05 was considered statistically significant. All analyses were performed using the Statistical Analysis System (SAS 9.2; SAS Institute, Cary, NC, USA).

Table-1: Population Features

Gender	Probiotic Intervention	n (individuals)	Weight (Kg)	Fat (%)	BMI (Kg/m²)
Male	No	56	107.53±5.37	33.18±1.66	35.62±1.78
	Yes	56	110.31±5.53	33.08±1.65	35.25±1.70
Female	No	90	86.71±4.34	41.34±2.07	33.71±1.69
	Yes	92	83.67±4.28	43.32±2.17	32.35±1.65

Population features. Average values of corporal weight, body fat content and BMI at the start of the study. Data are means ± S.D.; p<0.005

Results

In total, 294 overweight or obese adult persons (112 men and 182 women) participated in the study from May 3rd to September 3rd of 2021. To assess the potential benefit of *B. subtilis* DG101 consumption as an adjuvant in the anti-obesity therapy, three obesity-related parameters were measured: the average loss of corporal weight (kg/month), the percentage of total body fat reduction/month, and the percentage of BMI decrease after the 12 weeks of intervention (May 3rd to August 3rd). In addition, the level of HbA1c of each participant was measured at the beginning of the study (during the week of May 3rd 2021) and one month after the end of the 12 weeks' probiotic intervention (week of September 6th 2021). The average values of corporal weight, body fat content, and BMI of all subjects meeting the inclusion criteria at the start of the study are summarized in **Table-1**. BMI and body fat content higher than 30 kg/m^2 and 25 %, respectively, are indicative of an obese person [1,2]. As shown in **Table-**

1, the average BMI and % of body fat statistically exceeded the specified limit values, indicating that the entire population under study was obese (the percentage of obese participants in the study greatly exceeds the number of overweight participants, data not shown). During the 12 weeks of the study, the subjects of both sex incorporating the probiotic *B. subtilis* DG101 in their diets showed a statistically significant and more efficient decrease of corporal weight (kg) (intervention group) compared to the subjects of the control group (not incorporating the probiotic, placebo group (**Fig-1**).

The average weight loss per month of men in the placebo and intervention groups was 2.14 kg and 3.25 kg, respectively (yellow and white circles in **Fig-1A**). This result represents a 51.59 % more efficiency in weight decrease of men in the intervention group compared to men of the placebo group (**Fig-1A**). Accordingly, for the group of women, the average weight loss in patients of the placebo and intervention groups was of 1.74 kg and 2.49 kg, respectively (yellow and white circles in **Fig-1B**). Therefore, there is a 43.10 % more efficiency in weight decrease of women in the intervention group compared to women of the placebo group (**Fig-1B**). It is interesting to note that some participants (men and women) with morbid obesity (BMI higher than 40 kg/m^2) responded to the probiotic intervention with a high loss of corporal weight. For example, in the intervention group shown in **Fig-1A**, there is a patient that responded to the probiotic treatment with a weight decrease of ~7,10 kg/month (signalled by a red arrow in **Fig-1A**, middle panel). This extreme case corresponded to a 39-year-old man, with 1.80 m of height and 152.80 kg of corporal weight (BMI of 47.2 Kg/m^2). When analyzing the data from the women population consuming the probiotic, the highest value of corporal weight loss (~4.7 kg/month, signalled by a red arrow in **Fig-1B**, middle panel) corresponded to a morbidly obese patient of 57 years old woman, with 1.68 m of height and 117.10 kg of corporal weight (BMI of 41.50 kg/m^2).

When analyzing the percentage values of total body fat decrease/month both in men and women after the 12 weeks of study, we found interesting results (**Fig-2**).

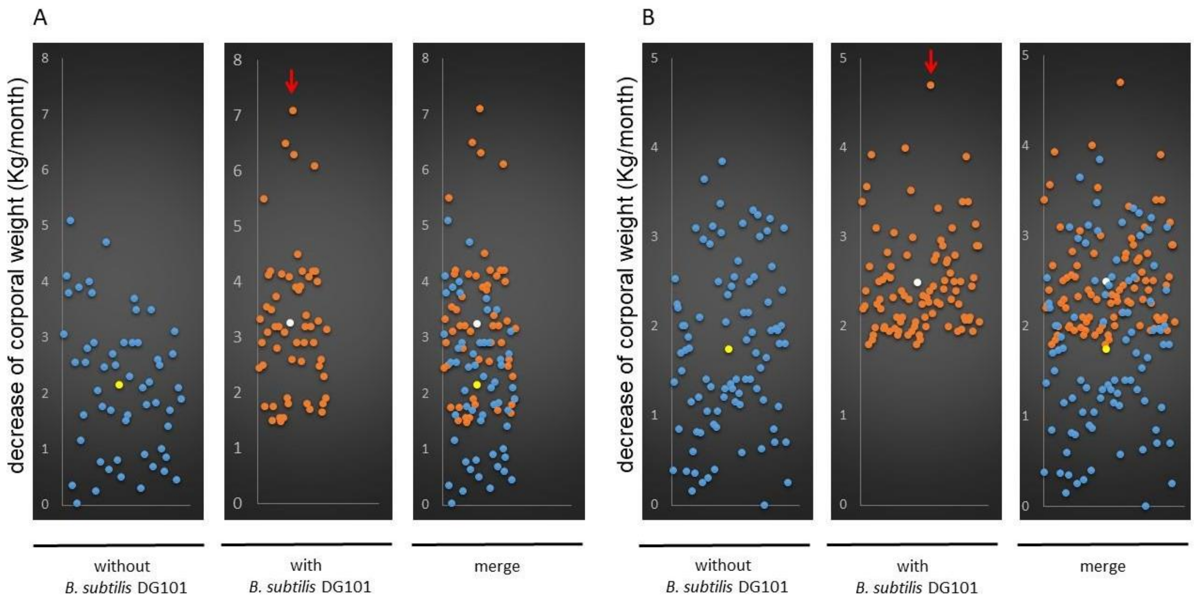

Fig-1:

Effect of B. subtilis DG101 on weight loss of overweight / obese patients. Decrease of corporal weight expressed as Kg/month in men (n=112) (A) and women (n=182) (B) with or without B. subtilis DG101 intervention. Yellow circles represent mean of decrease in corporal weight per month without B. subtilis DG101 intervention in men (A, left panel) and women (B, left panel). White circles represent mean of decrease in corporal weight per month after B. subtilis DG101 intervention in men (A, middle panel) and women (B, middle panel). Right panels in (A) and (B) represent the merge in the decrease of corporal weight per month, plus / minus B. subtilis DG101 intervention in men (A) and women (B). Red arrows correspond to a male (A, middle panel) and a female subject (B) showing the highest value of corporal weight loss after the probiotic B. subtilis DG101 intervention, respectively.

The average percentage of body fat loss per month in men of the control and intervention groups was 5.69 % and 8.39 %, respectively (yellow and white circles in **Fig-2A**). This represents a 47.45 % more efficiency in total body fat decrease of men in the intervention group compared to men of the control group. Accordingly, for the group of women, the average percentage of total body fat loss in patients of the control and intervention groups were 4.44 % and 5.65 %, respectively (yellow and white circles in **Fig-2B**). This represents a 27.25 % more efficiency in body fat decrease of women in the intervention group compared to women of the control group. When analyzing the percentage values of decrease of total body fat/month, two extreme and interesting values, highlighted by red arrows in **Fig-2A** and **Fig-2B** (middle panels), correspond to one man and one woman of the intervention groups. One case corresponded to a 56-year-old man of 1.73 m of height and 127.7 kg of weight (BMI of 42.7 kg/m^2) that showed a body fat reduction of ~18.50 % / month; and a 64-year-old woman of 1.51 m of height and 79.9 kg of weight (BMI 35.1 kg/m^2) that showed a decrease of body fat of ~16.50 % /month.

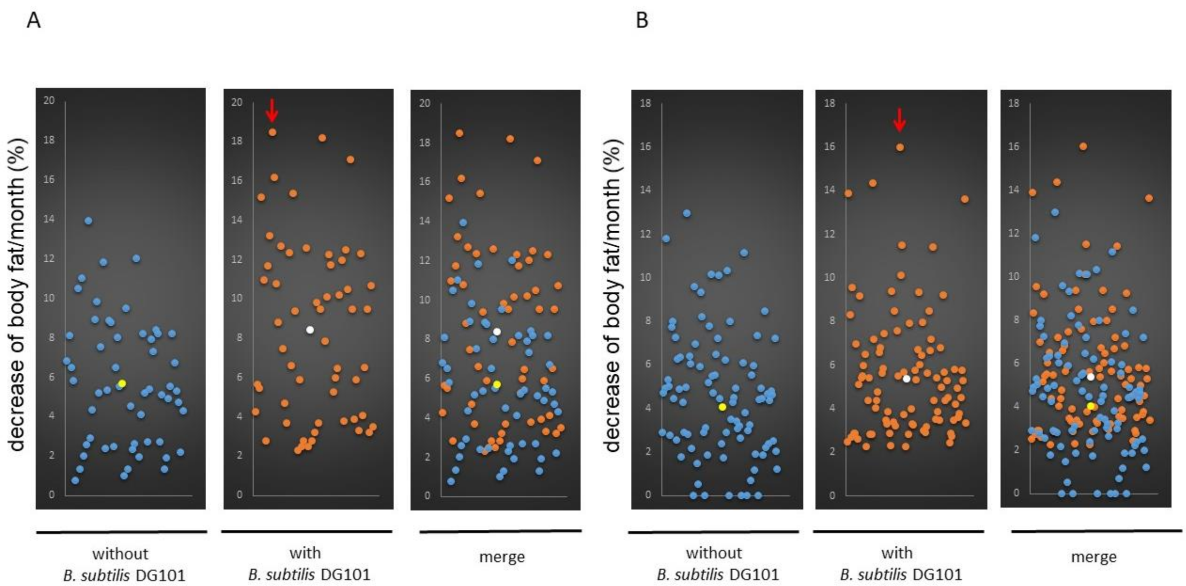

Fig-2:

Effect of B. subtilis DG101 on fat loss of overweight / obese patients. Decrease of body fat per month expressed as % in men (n=112) (A) and women (n=182) (B) with or without B. subtilis DG101 intervention. Yellow circles represent mean of decrease in body fat per month without B. subtilis DG101 intervention in men (A, left panel) and women (B, left panel). White circles represent mean of decrease in body fat per month, with B. subtilis DG101 intervention in men (A, middle panel) and women (B, middle panel). Right panels in (A) and (B) represent the merge in the decrease of body fat per month, plus / minus B. subtilis DG101 intervention in men (A) and women (B). Red arrows correspond to a male (A, middle panel) and a female subject (B, middle panel) showing the highest value of body fat reduction after the probiotic B. subtilis DG101 intervention, respectively.

When we studied the average percent of BMI decrease, after the 12 weeks of the study in men of the control and intervention groups, we found that the BMI reduction in men of the control and intervention groups was 5.99 % and 9.19 %, respectively (yellow and white circles in **Fig-3A**). This represents a 53.42 % more efficiency in total BMI decrease of men in the intervention group compared to men of the control group. Accordingly, for the group of women, the average % of BMI decrease in patients of the control and intervention groups was 6.65 % and 8.22 %, respectively (yellow and white circles in **Fig-3B**). This represents a 23.61 % more efficiency in BMI loss for women in the intervention group compared to the women of the control group. In **Table-2**, we summarized the results related to BMI, body fat, and weight reductions in the control and intervention groups divided by sex.

In our study, ~ 90 % of the participants were prediabetic with average glycaemia values of 113.90 mg/dl and 114.17 mg/dl for women and men,

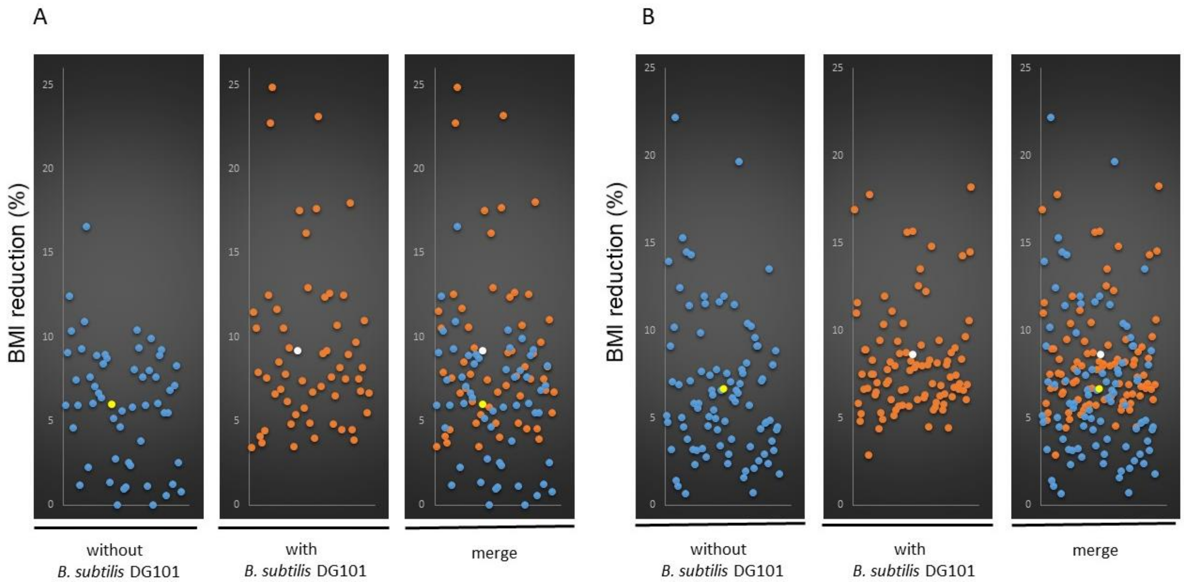

Fig-3:

Effect of B. subtilis DG101 on BMI reduction of overweight / obese patients. BMI reduction expressed as % in men (n=112) (A) and women (n=182) (B) without or with B. subtilis DG101 intervention. Yellow circles represent mean of decrease in BMI without B. subtilis DG101 intervention in men (A, left panel) and women (B, left panel). White circles represent mean of decrease in BMI with B. subtilis DG101 intervention in men (A, middle panel) and women (B, middle panel). Right panels in (A) and (B) represent the merge in the BMI reduction, plus / minus B. subtilis DG101 intervention in men (A) and women (B).

Table-2: Summary of Weight Control Effects Produced by *B. subtilis* DG101

Gender	Probiotic Intervention	Decrease of weight (Kg/month)	Decrease of body fat (%)	Decrease of BMI (Kg/m²)
Male	No	2.14±0.11	5.69±0.28	5.99±0.30
	Yes	3.25±0.16	8.31±0.41	9.19±0.46
Female	No	1.74±0.09	4.44±0.22	6.65±0.33
	Yes	2.49±0.12	5.65±0.28	8.22±0.41

Summary of weight control effects produced by B. subtilis DG101. Average decrease of weight expressed as kg / month, decrease of body fat content expressed as % / month and total decrease of BMI after the 12 weeks of study, expressed as kg / m2, in both men and women with or without probiotic intervention. Data are means ± S.D.; p<0.005

respectively. The remaining ~ 10 % of participants were diabetic with average glycemic values of 151.6 mg/dl and 187 mg/dl for women and men. respectively. Therefore, it was interesting to investigate if the observed anti-obesity effects of the probiotic *B. subtilis* DG101 (**Table-2**) would produce better management of prediabetic and diabetic patients. Therefore, after the 12 weeks of the study (May 3rd to August 3rd), patients of the intervention group ceased the consumption of the probiotic, and together with the patients of the control group continued during one more month (August 3rd to September 3rd) with the low calorie / low fat diet. After this fourth month, we measured the percent of glycosylated hemoglobin (HbA1c) and insulin (expressed as µIU/ml) and compared them with the values of both parameters before the participants started the study. As shown in **Table-3**, the beneficial anti-obesity effect of the probiotic was nicely reflected in better management of the prediabetic and diabetic conditions of patients of the intervention group, compared with the prediabetic and diabetic management of patients of the placebo group, no matter their sexes. For the group of men, the intervention group showed a 35.88 % and 58.40 % of reduction in the levels of HbA1c and insulin, respectively, compared with their initial levels. By contrast, for the men control group, even though the low-calorie/low-fat diet rendered a positive effect on the management of both parameters related to blood sugar concentration, its efficiency was significantly lower than the observed output in the intervention group. The reduction of HbA1c and insulin levels in men of the control group was 30.39 % and 17.08 %, respectively (**Table-3**). For the group of women, the response was similar although the results were lower compared to the ones obtained for the group of men (**Table-3**). The reduction of HbA1c and insulin levels in women of the control group was of 20.54 % and 24.20 %, respectively (**Table-3**). These results, although positive, are lower than the values obtained in the group of women of the intervention group. Here, the percentage of reduction of HbA1c and insulin values were 31.47 % and 50.00 %, respectively, (**Table-3**).

Conclusions

There is a significant amount of scientific evidence supporting the existence of a physiological relationship between intestinal microbial flora and the predisposition of a person to being overweight or obese [2-4]. This association places probiotics as an interesting tool for the prevention or treatment of obesity together with existing anti-obesity therapies (i.e., low caloric diets, physical exercises, pharmacotherapy) [3,4]. It has been estimated that a weight loss between 5 % and 10 % is sufficient to have a positive impact on health. Weight loss treatments can help delay the onset of type 2 diabetes mellitus or slow its progression [2,10]. Most of the studies carried out with probiotics for better weight management have been carried out on healthy individuals, and there are even a few cases in which the person has committed to follow a low-calorie diet in addition to the consumption of the probiotic [3,4,11,12]. On the contrary, our study worked with a universe of overweight and obese people with comorbidities associated with type 2 diabetes mellitus. The intervention with the probiotic *B. subtilis* DG101 was designed as adjuvant or complementary therapy of patients that follow a hypocaloric diet, and medication (i.e., metformin) when necessary (i.e., diabetic patients with insulin resistance). The obtained results show the potential of sporulated probiotics [6], in particular, *B. subtilis* DG101 [5,10] as a promising adjuvant therapy in the treatment of obese or overweight people with a prediabetic or diabetic condition. Interestingly, in obese patients with insulin resistance (IR), we have noticed that those that incorporated the probiotic *B. subtilis* DG101 into their daily diet needed an average of 4 months to normalize their blood glucose and insulin levels and stop the consumption of the medication (i.e., metformin). By contrast, those patients with IR who did not consume the probiotic but followed a low-calorie/low-fat diet, needed an average of 8 to 10 months to normalize their blood glucose and insulin levels (data not shown, unpublished result). Overall, the present results point out *B. subtilis* DG101 as an efficient probiotic strain for weight management in overweight and obese individuals with prediabetes or diabetic comorbidities.

Table-3: Diabetes Control Produced by the Consumption of the Probiotic *B. subtilis* DG101

Gender Treatment	Treatment	HbA1c (%)		Insulin (µIU/ml)	
		Before	After	Before	After
Male	Low caloric diet	10.2±0.51	7.1±0.36	12.3±0.62	10.2±0.51
	Low caloric diet + *B. subtilis* DG101	9.67±0.43	6.2±0.32	12.5±0.63	5.2±0.26
Female	Low caloric diet	8.47±0.42	6.73±0.34	13.1±0.66	9.93±0.50
	Low caloric diet + *B. subtilis* DG101	8.42±0.37	5.77±0.29	13.6±0.41	6.8±0.34

Control of prediabetes or type II diabetes mellitus produced by the consumption of the probiotic B. subtilis DG101. Average HbA1c expressed as % and insulin expressed as µIU/ml in both men and women before and after the intake of a low caloric diet supplemented or not with the probiotic B. subtilis DG101. Data are means ± S.D.; $p<0.005$

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Authors Contributions

All authors contributed to the study design, data analysis and critical review of the manuscript. FRA and RG wrote the manuscript. NC and personnel of Instituto Cardinali de Nutrición, were in charge of data recompilation and measurement of medical parameters related to the study.

Ethical Disclaimer

The present study followed the Helsinki Declaration and Good Clinical Practice Guidelines of the International Conference of Harmonization. All enrolled patients gave written and oral informed consent, and Agencia Santafesina de Seguridad Alimentaria (ASSAL) of Argentina approved probiotic *B. subtilis* DG101 use for human beings (RNPA: 21-119482). RG is an ad-hoc research consultant of Kyojin S.A. NC and FRA declare no conflict of interests.

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