OPEN ACCESS ISSN: 2834-5231 Rodriguez Ayala F, et al. (2024): 236
Doi: 10.47991/2834-5231/ACMCRR-118

Research Article | Annals of Clinical and Medical Case Reports & Reviews

A Double Blind, Placebo-Controlled Study to Evaluate the Efficacy of Probiotic *Bacillus Subtilis* DG101 In Maintaining Blood Sugar Homeostasis in Healthy Adults

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Citation: Rodriguez Ayala F, Leñini C, Cardinali N, Ricardo Grau R (2024) A Double Blind, Placebo-Controlled Study to Evaluate the Efficacy of Probiotic *Bacillus Subtilis* DG101 In Maintaining Blood Sugar Homeostasis in Healthy Adults. Ann Clin Med Cas Rep Rev: ACMCRR-118.

Received Date: 08 January, 2024; **Accepted Date:** 17 January, 2024; **Published Date:** 23 January, 2024

Abstract

The maintenance of blood glucose homeostasis is crucial for the prevention of several non-communicable diseases (NCDs) in healthy people. A good balance between diet and lifestyle is beneficial to maintain glucose homeostasis, but also a balanced gut microbiota is required. Probiotics (i.e., friendly gut bacteria) emerge as a plausible strategy that might contribute to the prevention of NCDs related to high blood sugar levels. In this work, we report the property of the probiotic *Bacillus subtilis* DG101 to maintain and improve blood glucose homeostasis in healthy individuals.

Keywords: *Blood glucose, NCDs, gut microbiota, probiotics, Bacillus subtilis DG101, diabetes, obesity, dementia.*

Introduction

A high level of sugars (i.e., glucose) in blood increases the risk for NCDs (e.g., obesity, diabetes, cardiovascular failure, dementia) in people. Therefore, the blood glucose concentration (i.e., glycemia) constitutes one of the most important biochemical parameters to be regulated in the body (glucose homeostasis) [1,2]. Blood glucose values vary during the day depending on the diet (nutrient intake), the physical activity and the genetic [3,4]. Brain activity and the levels of many hormones are related to glucose metabolism, including insulin and glucagon (both produced in the pancreas), adrenaline (from the adrenal gland), glucocorticoids, and steroids (secreted by the gonads and adrenal glands) [5]. A useful measure of glycemia is the fasting blood sugar value (i.e., the determination of the level of free blood sugar after fasting for at least 8 hours) [1,3]. Normal fasting blood glucose levels range between 70 and 100 mg/dl (3.9 to 5.6 mmol/L) [1,3]. Blood glucose values higher than 100 mg/dl are indicative of an unbalanced metabolism that could cause overweight, obesity, or pre-diabetes, and blood glucose values greater than 125 mg/dl (i.e., hyperglycemia) are indicators of diabetes risk [2,4]. Worsening the situation, hyperglycemia can lead to an elevated expression of angiotensin-converting enzyme 2 (ACE2), which contributes to increase the risk of coronavirus infection of pancreatic β-cells and COVID-19 severity [6,7].

In addition to glycemia, glycosylated hemoglobin (HbA1c) represents a useful tool to estimate the steady-state blood sugar levels. Glycosylated hemoglobin indicates the stable (covalent) binding of glucose (and other simple sugars) to hemoglobin (Hb). Because the Hb of the red blood cells have an average life of 120 days, HbA1c values indicate the average value of sugar bound to Hb for the last 2 to 4 months [3,4]. A high value of HbA1c is harmful *per se* because it causes an increase in the levels of free radicals that damage the red blood cell membrane, increasing the tendency of aggregation (atheroma formation), inflammation, and impaired blood flow caused by the increased blood viscosity [1,2,4]. HbA1c values less than 6-7% are considered physiologic and help to prevent neurodegenerative and cardiovascular diseases [1-4].

Although formerly neglected, the gut microbiota (i.e., the trillions of microorganisms living in the human gut) emerges as an attractive and natural strategy against hyperglycemia [8-9]. The gut microbiota influences the efficiency of energy extraction from ingested foods, time of food intestinal residence, intestinal permeability, and is a key player in the communication between the gut and the brain (i.e., gut-microbiota-brain axis), all factors influencing sugar homeostasis and health [5,10,11]. Interestingly, administration of healthy bacteria (e.g., probiotics) has been reported as an approach to modulate the eubiosis of the gut microbiota [12,13]. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) defined probiotics as live microorganisms, which when administered in adequate amounts and arriving alive to their sites of action (i.e., the intestine) confer a health benefit on the host [12]. The probiotic bacterium *Bacillus subtilis* DG101[14] has been reported as an efficient intervention in overweight / obese people to decrease body weight, fat content and BMI; and the maintenance of these parameters within physiological levels in healthy adults [15,16] Here, we report the results of a double-blind, placebo-controlled trial demonstrating the effectiveness of *B. subtilis* DG101 for maintaining glycemia and HbA1c values in physiological ranges in healthy individuals.

Results

Healthy adults, 18 – 75 years of age, who periodically assist to the Instituto de Nutrición Grupo Cardinali (INGC) for maintenance checkups, where invited to participate in the study. The inclusion criteria were healthy individuals determined by body mass index (BMI) lower than 25.0 kg/m^2; glycemia and HbA1c lower than 100 mg/dl and 7 %, respectively; physical examination, clinical biochemistry, liver and kidney functions tests; no consumption of probiotics, postbiotics, prebiotics or fermented-foods for 2 months before the start of the study. The exclusion criteria were pregnant and breast-feeding females; a history of chronic diseases (i.e., intestinal disorders, diabetes, celiac disease, thyroid disorders, cancer) and any disease affecting the individual safety or ability to follow and complete the study. The work consisted of a double-blind, placebo-controlled study of 9 months of duration between January 2023 to September 2023. After confirming eligibility, the participants were randomized to receive *B. subtilis* DG101 (intervention group, 20 drops of probiotic, equivalent to 1 x 10^8 colony forming units, CFU) or a placebo (placebo group, 20 drops of distilled water) dissolved in ~ 100 – 200 ml of water. The probiotic and the placebo intake were separated by at least 2 h from main meals. The visits to the nutritional clinic (i.e., INGC) were scheduled every three months to collect data and compliance with the regime. The participants were instructed to maintain a low caloric and healthy diet (recommended by health professionals of INGC) and the habitual physical activity throughout the study. The probiotic *B. subtilis* DG101 (Kyojin® Probiotic) and the placebo (distilled water) were provided by Kyojin S.A. (www.kyojin.com.ar) in indistinguishable dropper cap bottles of 90 ml of content. The bottles (intervention and placebo) were labeled with a code (in accordance with the Good Clinical Practice Guidelines) by members of the staff of the INGC who were blinded in conducting any phase of the study. A total of 3 bottles were used by each person in the intervention or placebo group throughout the study. The initial sample size comprised 220 healthy participants which were randomized equally in 2 groups (probiotic intervention and placebo, n = 110 persons /group) in a double-blind manner. A total of 104 and 105 participants from the intervention and placebo groups, respectively, reached the end of the study successfully (Figure 1).

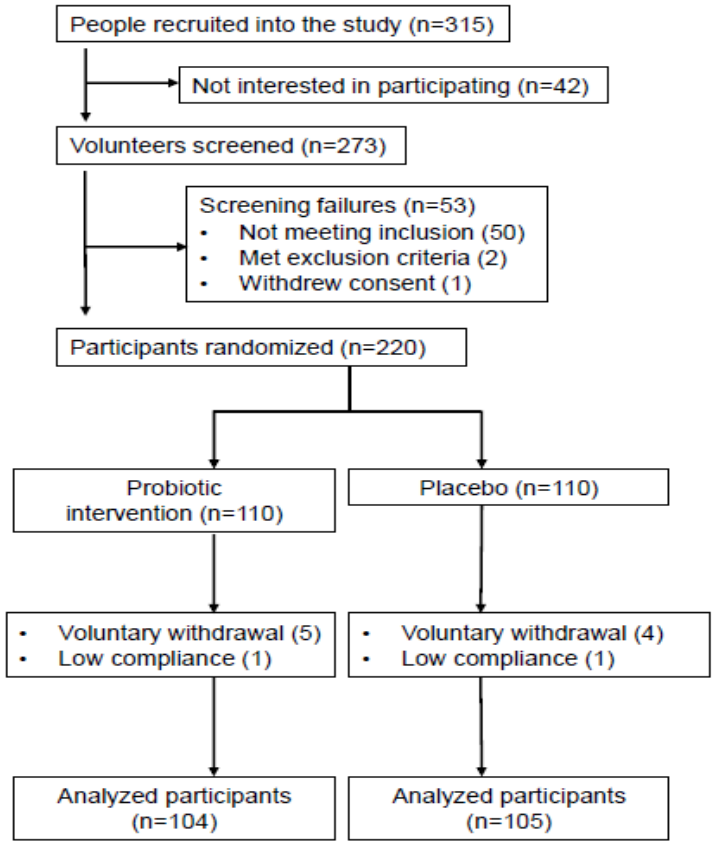

Figure 1: Study design.

At each tri-monthly visit to the INGC, the outcome measures were vital signs (resting heart rate and blood pressure), BMI, glycemia and HbA1c. No adverse effects attributed to the probiotic consumption were observed. Linear regression (ANOVA) was used to test differences in response to treatments (probiotic and placebo). The obtained values are presented as means ± S.D. unless otherwise specified. A *p*-value of < 0.05 was considered statistically significant. All analyzes were performed using the Statistical Analysis System (SAS 9.2; SAS Institute, Cary, NC, USA).

To evaluate the efficiency of the probiotic *B. subtilis* DG101 as an adjuvant intervention to maintain the homeostasis of blood glucose, we measured the glycemia and HbA1c of the participant every 3 months, and averaged these values over the 9 months of the study duration. The starting averaged values of glycemia, HbA1c, and BMI of the population under study are shown in Table 1.

Table 1: Participant features at the start of the study. Average age, corporal weight (kg), BMI (kg/m^2), glycemia (mg/dl), and HbA1c (%) at the start of the study. Data are means ± S.D.; $p < 0.005$.

Measure	*B. subtilis* DG101 (n=104)		Placebo (n=105)	
	FEMALE	MALE	FEMALE	MALE
Sex (n)	62	42	62	43
Age	41	46	40	47
Body weight (Kg)	62.5 ± 0.7	71.5 ± 0.6	61.6 ± 0.7	71.8 ± 0.8
BMI (Kg/m^2)	21.89 ± 0.5	24.31 ± 0.6	21.70 ± 0.4	24.0 ± 0.6
Glycemia (mg/dl)	90.5 ± 0.10	93.6 ± 0.13	92.0 ± 0.11	91.8 ± 0.12
HbA1c (%)	5.40 ± 0.08	5.35 ± 0.10	5.38 ± 0.09	5.30 ± 0.11

As shown in Figure 2, healthy subjects of both sexes who incorporated the probiotic *B. subtilis* DG101 into their diets (1 x 10^8 CFU/day) were able to maintain physiological values of glycemia, and in many cases improved (i.e., decreased) the glycemia levels (blue circles in Figure 2). By contrast, in the placebo group there is tendency to increase the glycemia values (Figure 2, orange circles). The change in glycemia of women in the intervention and placebo groups (Figure 2 A and B, respectively) after the 9 months of study was – 0.74 % and + 4.73 %, respectively (yellow circles in Figure 2 A-B, and Table 2). For men, the average change in glycemia in the intervention and placebo groups (Figure 2 C and D, respectively) was – 0.53 % and + 6.00 %, respectively (yellow circles in Figure 2 C-D, and Table 2).

Table 2: Summary of the variation of blood sugar-related parameters produced by *B. subtilis* DG101 consumption. Average variation in % of glycemia and HbA1c after the 9 months of study, in men and women with or without probiotic intervention. Data are means ± S.D.; $p < 0.005$.

Measure	*B. subtilis* DG101		Placebo	
	FEMALE (n=62)	MALE (n=42)	FEMALE (n=62)	MALE (n=43)
Glycemia (mg/dl)	89.83 ± 0.08	92.80 ± 0.05	96.35 ± 0.06	95.86 ± 0.07
Glycemia variation (%)	- 0.74	- 0.53	+ 4.73	+ 6.00
HbA1c (%)	5.36 ± 0.7	5.30 ± 0.5	5.55 ± 0.8	5.53 ± 0.4
HbA1c variation (%)	- 0.72	- 0.85	+ 3.19	+ 4.42

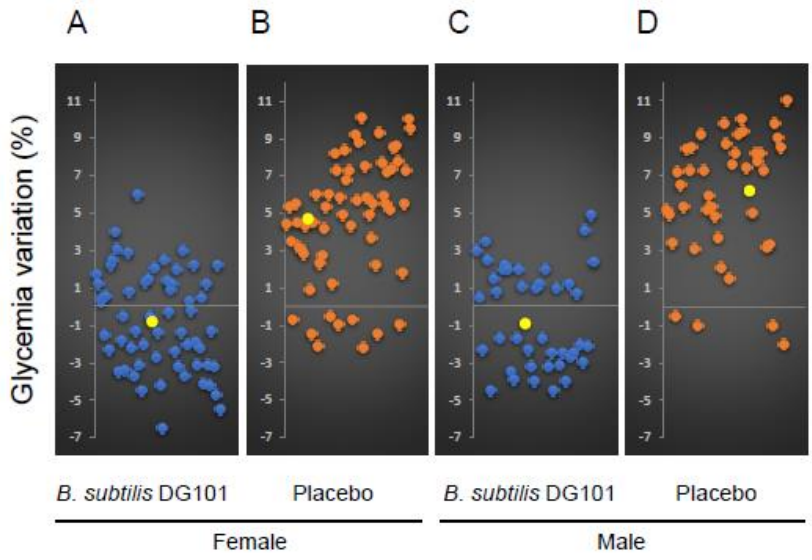

Figure 2: Effect of *B. subtilis* DG101 on the maintenance of glycemia in healthy individuals. Percentage variation (%) in blood glucose levels in women (A-B) and men (C-D) with or without intervention with the probiotic *B. subtilis* DG101. The blue and orange circles (participants who consumed the probiotic and participants who did not, respectively) indicate the average variation (%) in glycemia of each participant throughout the study. The yellow circles represent the mean variation in glycemia (%) of the participants in each group (placebo and intervention).

The determination of HbA1c in men and women that consumed the probiotic, or the placebo points out to similar conclusions to the ones obtained from the analysis of glycemia maintenance (Figure 3). The percentage change of HbA1c in participants of the probiotic groups (blue circles, Figure 3) was much smaller than the percentage change of HbA1c values in the placebo groups that showed a tendency to increase the level of HbA1c (orange circles, Figure 3). The change in HbA1c in healthy subjects of both sexes who incorporated the probiotic *B. subtilis* DG101 into their diets (1 x 10^8 CFU/day, blue circles in Figure 3) was - 0.72 % and - 0.85 % for women and men, respectively (yellow circles in Figure 3 A and C, and Table 2). On the contrary, for women and men in the placebo groups (Figure 3 B and D, respectively), a significant increase in HbA1c of + 3.19 % and + 4.42 %, respectively, was noted for each sex (Table 2 and Figure 3 B and D, yellow circles).

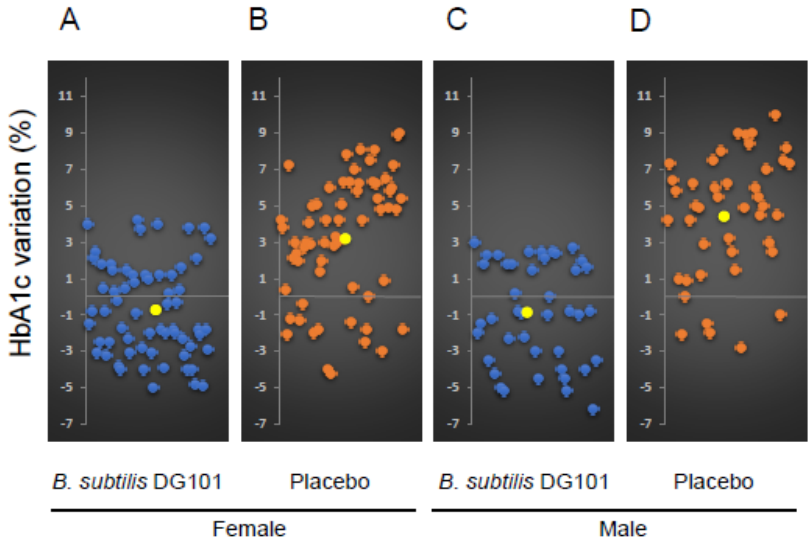

Figure 3: Effect of *B. subtilis* DG101 on the maintenance of HbA1c in healthy individuals. Percentage variation (%) in HbA1c levels in women (A-B) and men (C-D) with or without intervention with the probiotic *B. subtilis* DG101. The blue and orange circles (participants who consumed the probiotic and participants who did not, respectively) indicate the average variation (%) in HbA1c of each participant throughout the study. The yellow circles represent the mean variation in HbA1c (%) of the participants in each group (placebo and intervention).

Discussion

The spore-forming probiotic *B. subtilis* DG101[14] have been proven to be an effective adjuvant intervention for the treatment of different human illness such as insulin-resistant type 2 diabetes mellitus, SARS-CoV2 triggered diabetes, and obesity [15,17,18]. Also *B. subtilis* DG101 represents an efficient tool to improve physical fitness (body weight, fat content and BMI) in healthy people [16]. Here, we were intrigued to unveil if *B. subtilis* DG101 would collaborate in the maintenance (or improvement) of the blood glucose levels in healthy adults. The maintenance of physiological levels of glycemia (and HbAc1) are considered important for preventing several sugar-related metabolic and neurodegenerative diseases (i.e., diabetes, obesity, non-alcoholic fatty liver, dyslipidemia, cardiovascular failure, Parkinson and Alzheimer's disease) [1-5].

Table 2 summarizes the results related to the changes in glycemia and HbA1c in healthy participants of both sexes divided into the placebo and intervention groups after 9 months of study. These results demonstrate the effectiveness of the probiotic *B. subtilis* DG101 in maintaining, and improving, parameters linked to blood glucose (Table 2). In previous works, we propose that the *B subtilis* DG101 property to control the level of blood sugar in diabetic people would not be due to an action at pancreatic level (e.g., regulating insulin production) because the control of sugar blood levels in these patients was not mediated by an increase in insulin production [15,17]. Our hypothesis is that the probiotic *B. subtilis* DG101 could work, directly or indirectly, influencing the cellular glucose transporters and transforming them into more sensitive or better responders to the blood circulating levels of hormones such as insulin and/or adiponectin to internalize the sugar into the cell. Although this hypothesis needs to be further investigated, the present results argue for the beneficial effect that the probiotic *B. subtilis* DG101 possesses in the maintenance / improvement of blood glucose homeostasis (glycemia and HbA1c) in healthy adults which would result in the prevention of NCDs and a better quality of life [19-22].

Author Contributions

NC: conceptualization, methodology, data collection, and interpretation of the results.
FRA and CL: methodology, data collection and statistical analysis.
RG: conceptualization, methodology, interpretation and writing of the manuscript.
All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank the staff (secretaries, technicians, biochemists, nutritionists, doctors) of Instituto de Nutrición Grupo Cardinali and Kyojin S.A. for their technical assistance.

Conflict of Interest Statement

NC and RG declare that they have no conflict of interest regarding the publication of this article. FRA and CL are employees of Kyojin S.A.

Funding Information

This study was funded by Kyojin S.A.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

This study was conducted in accordance with the ethical principles indicated in the Declaration of Helsinki, its subsequent amendments, and the ethical recommended guidelines of the National University of Rosario and the Hospital Provincial del Centenario, Rosario - Santa Fe, Argentina. Agencia Santafesina de Seguridad Alimentaria (ASSAL) approved probiotic *Bacillus subtilis* DG101 use for human beings (RNPA 21-1194829.

Consent

Written informed consent was obtained from each participant of the present study in accordance with the journal's patient consent policy.

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