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EFFICACY OF INULIN SUPPLEMENTATION IN METABOLIC CONTROL AND *AKKERMANSIA MUCINIPHILA* LEVELS IN SUBJECTS WITH TYPE 1 DIABETES: A PILOT STUDY

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ABSTRACT – *Objective:* Several factors, as genetics, diet, and gut microbiota, are associated with the development of type 1 diabetes (T1D). *Akkermansia muciniphila*, an abundant bacterium in human microbiota, has anti-inflammatory properties and can correct metabolic disorders. The effects of the administration of inulin, a prebiotic which increases *Akkermansia muciniphila* gut levels, are unknown in subjects with T1D.

Materials and Methods: 49 subjects with T1D, age 46 [37-53] years, 30 females (61%), duration of disease 20 [11-27] years, HbA1c 64 [59-72] mmol/mol, were randomized in group A (inulin 3 g twice daily for 3 months + insulin, n=24) and in group B (insulin alone, n=25). Body weight, glycated hemoglobin (HbA1c), daily insulin units, continuous glucose monitoring (CGM) metrics, and Bristol stool scale (BSS) score were collected at enrollment and after 3 months.

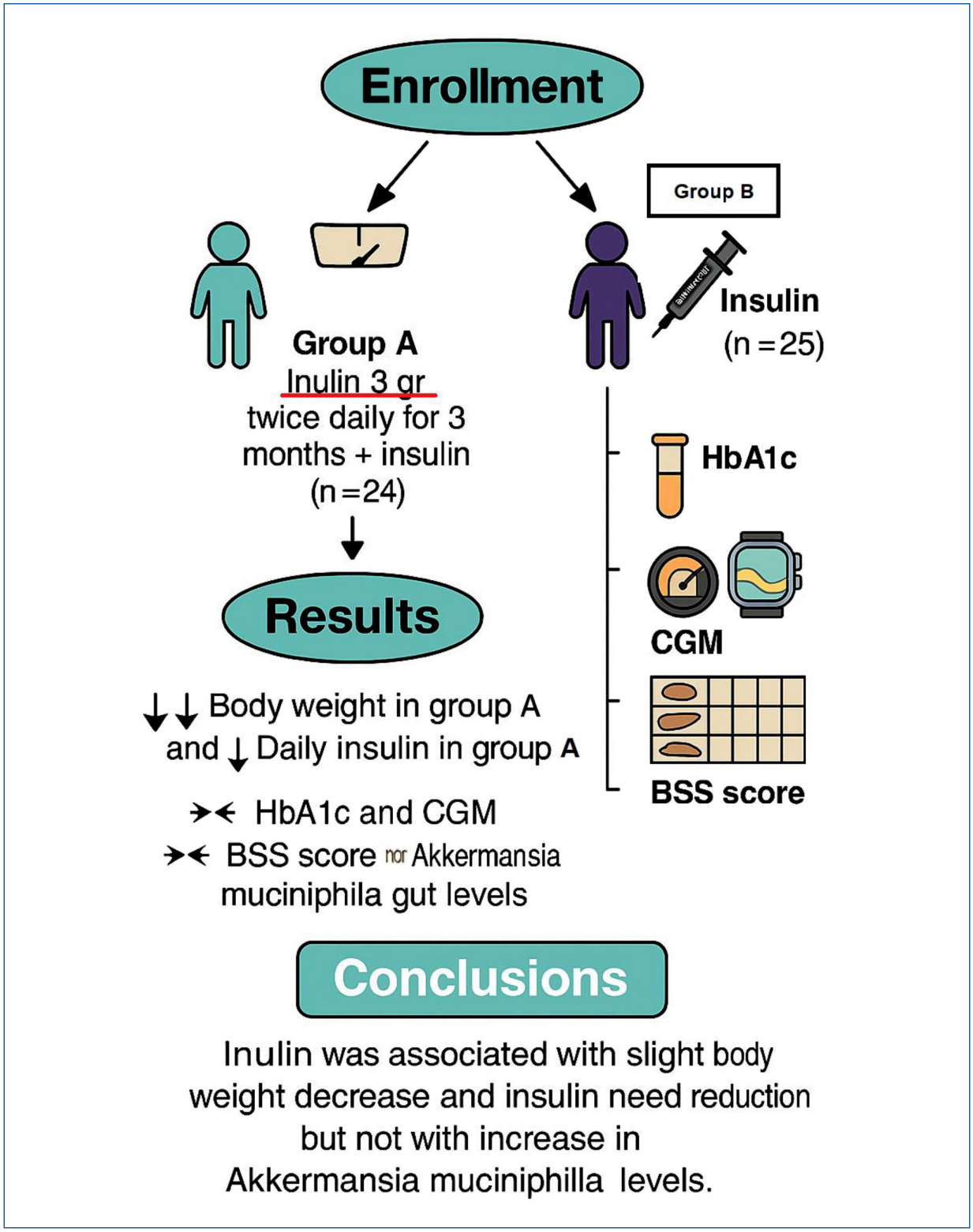
Results: After 3 months, subjects in group A showed a significant decrease in body weight [group A -2 (-3; 0) kg and group B 0 (-1; 1) kg, p=0.03] and daily insulin units [group A -1.5 UI (-3.1; 0) *vs.* group B 0.6 (0; 1.7), p=0.01]. After 3 months, changes in HbA1c and CGM were similar between groups. In both groups, there was no change in BSS score (p=0.39) nor in *Akkermansia muciniphila* gut levels.

Conclusions: Inulin was associated with a slight body weight decrease and insulin need reduction, but not with an increase in *Akkermansia muciniphila* levels. More studies are required to explore this issue.

KEYWORDS: Inulin, Type 1 diabetes, *Akkermansia muciniphila*, Gut microbiota, Insulin.

INTRODUCTION

Type 1 (T1D) and type 2 diabetes (T2D) represent a major public health issue worldwide. Regarding the autoimmune pathogenesis of T1D, many factors are associated with its development, such as genetic features, diet, and intestinal microbiota composition[1]. Gut microbiota plays a crucial role in the improvement of metabolism and host homeostasis[1]. Several studies[2] have demonstrated alterations in gut microbiota composition and a crosstalk between



Graphical Abstract. *Akkermansia muciniphila*, an abundant bacterium in human microbiota, has anti-inflammatory properties and can correct metabolic disorders in patients with T1D. The effect of the administration of inulin, a prebiotic that increases *Akkermansia muciniphila* gut levels, is not known in subjects with T1D. In this study, patients with T1D were randomized into group A (inulin 3 g twice daily for 3 months + insulin, n=24) and group B (insulin alone, n=25). After 3 months, patients of group A showed a significant decrease in body weight and daily insulin units compared to group B. Inulin was associated with a slight body weight decrease and insulin need reduction, but not with an increase in *Akkermansia muciniphila* levels.

human gut microbiota and host in autoimmune and metabolic diseases such as T1D. Currently, the underlying molecular mechanisms are still unknown. Diabetes is characterized by chronic inflammation and oxidative stress. The modulation of gut microbiota by some dietary supplements, including probiotics and prebiotics, has been proven to act positively on glucose control and mechanisms of insulin-resistance in people affected by this condition[3]. In particular, studies[4] have shown that a specific bacterium known as *Akkermansia muciniphila*, which is an abundant constituent of human gut microbiota, can correct some metabolic disorders in obese insulin-resistant mice. *Akkermansia muciniphila*, belonging to the genera Verrucomicrobium, is a gram-negative, oval-shaped, anaerobic and mucus-layer-degrading bacterium[5]. It has anti-inflammatory properties and beneficial roles in both autoimmune and inflammatory chronic diseases. In mice, this bacterium, when added as a probiotic, slowed down the progression of diabetes and performed immunomodulatory activities[6]. Due to its ability to provide numerous positive beneficial effects on specific metabolic diseases, *Akkermansia muciniphila* has become one of the most promising biotherapeutic agents. Improvements in metabolic parameters, increased gut integrity and secretion of gut peptide hormones, and reduced metabolic inflammation are some of these outcomes. In particular, *Akkermansia muciniphila* modulates host metabolism and it was able to decrease intestinal permeability, thus improving leaky gut. Despite its well-known ability to degrade mucin, *Akkermansia muciniphila* can also enhance mucin production in mice fed a high-fat diet by increasing the quantity and the density of goblet cells, restoring the thickness of the mucus layer, and strengthening the intestinal barrier. *Akkermansia muciniphila* colonizes the human gut and produces short-chain fatty acids (SCFAs), such as propionate and acetate, which act as substrates for the host and for other bacteria. It may regulate body weight gain and perform anti-inflammatory and anorexic-metabolic effects[7]. The obese population shows a reduced concentration of *Akkermansia muciniphila* compared to healthy individuals. *Akkermansia muciniphila* negatively correlates with body mass index, weight-gain, adiposity and blood glucose levels after a meal[8]. Literature studies reported that this bacterium can control diabetes due to its beneficial effects on the inflammation of the liver and adipose tissue and on blood insulin levels. It also maintains the integrity of the gut barrier too[9]. Furthermore, *Akkermansia muciniphila* produces proteins, named postbiotics, that have been taken into account as promising targets against chronic autoimmune, inflammatory, and metabolic diseases[10]. T1D is an autoimmune disease characterized by progressive destruction of pancreatic β-cells that are engaged in insulin production[11]. Gut dysbiosis has been demonstrated to speed up and worsen these processes[12]. Studies[13] on animal models (mice) of 28-day life treated with antibiotics reported that treatment with *Akkermansia muciniphila* lowered the incidence of T1D development, suggesting a protective effect. Moreover, these mice showed reduced destruction of pancreatic β-cells and pancreatic inflammation[14]. *Akkermansia muciniphila* can decrease bacterial translocation, increase macrophage activity, and maintain gut homeostasis. Furthermore, it can increase insulin secretion and T-reg lymphocytes cells in the pancreas[15]. Some factors, such as diet, can influence the abundance of *Akkermansia muciniphila*[16]. The ingestion of fiber may increase the fecal content of *Akkermansia muciniphila,* as well as the administration of inulin[17]. Mechanisms involved in this pathway are not fully known. Several reports[18] showed that the abundance of *Akkermansia muciniphila*, compared to other species, could significantly increase after the administration of the prebiotic inulin. *Akkermansia muciniphila* significantly increases in the human gut microbiota after supplementation with inulin[19]. Inulin is a prebiotic fiber supplement in the diet, with beneficial health properties. It is able to improve lipid metabolism and modulate the composition of gut bacteria, increasing the Verrucomicrobia species[20]. Inulin is a soluble fiber, a reserve of polysaccharides found in many plant species. It acts as a prebiotic and has many beneficial properties on gut microbiota. It stimulates the growth of beneficial bacteria, regulates lipid metabolism, lowers blood sugar, and downregulates inflammatory factors[21]. Moreover, inulin and its metabolites regulate the pH of the human gut, maintain the homeostasis of the intestinal environment, and improve the systemic immune function. Sources of inulin are represented by artichoke, garlic, onion, and chicory, which are often used by the food industry to produce inulin supplements[21]. Inulin stimulates the growth of Lactobacilli and Bifidobacteria. Inulin is also the only prebiotic approved by the European Food Safety Authority due to its properties to improve gut function[22]. It is metabolized by beneficial bacteria, such as *Akkermansia* and *Ruminococcus*, into short-chain fatty acids (SCFAs), such as propionate, acetate, and butyrate, that affect various physiological processes and maintain gut homeostasis[22]. Furthermore, they mitigate the low-grade inflammatory re-

sponses and improve glucose metabolism. In mice models, the treatment with inulin reduced the lipopolysaccharide (LPS) level and pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and TNF-α in the human gut[22]. The aim of this pilot study was to evaluate the efficacy of inulin supplementation on metabolic control in people with T1D on insulin treatment.

MATERIALS AND METHODS

The present study was designed as a single-center, open-label pilot study with 2 parallel groups. From 1st October 2022 to 31st December 2023, subjects with T1D aged ≥18 years, on insulin treatment for at least one year, all on continuous subcutaneous insulin infusion, were enrolled. Subjects with advanced complications related to T1D (diabetic chronic kidney disease, severe retinopathy and diabetic neuropathy) were excluded. All participants were identified among patients regularly followed at the Diabetes Care Unit of Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome. Participants were randomized with a standard randomization 1:1 into two groups: group A, patients treated with insulin plus supplementation with inulin 3 g twice a day for 3 months; group B, control group, i.e., patients treated with insulin alone. For each of the following outcomes, data were collected before randomization and 3 months after: body weight and body mass index (BMI), blood pressure, glycated hemoglobin (HbA1c), and lipid profile; standardized glucose metrics, obtained through continuous glucose monitoring (CGM), according to Advanced Technology and Treatment in Diabetes 2019 Consensus[23].

Specifically, data about the percentage of time spent in the recommended glucose interval [time in range (TIR), 70-180 mg/dl], the percentage of time spent in hyperglycemia [time above range (TAR) level 1, 181-250 mg/dl, and level 2, >250 mg/dl], the percentage of time spent in hypoglycemia [time below range (TBR) level 1, 54-69 mg/dl, level 2, <54 mg/dl], the coefficient of variation and the glucose management indicator (GMI) were collected.

Participants recorded the amount of insulin administered every day. They also recorded in a specific diary the number of evacuations per week, including stool consistency assessed by the Bristol Stool Scale (BSS) for the whole study period (3 months). In a subset of participants, levels of *Akkermansia muciniphila* in the stool were evaluated before randomization and after 3 months. The study was conducted according to the Declaration of Helsinki and was approved by the Ethical Committee of Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, with protocol number 3099 (16/September/2022).

Patients' enrolment and sample collection

Thirty-two fecal samples obtained from sixteen patients with T1D were tested for the detection of *Akkermansia muciniphila* by a real-time PCR. Stool samples were delivered to the microbiology laboratory of the same hospital and collected at -80°C. *Akkermansia muciniphila* detection in stool samples was performed at baseline (T0) and after three months (T1).

Nucleic acid extraction

Bacterial nucleic acid was extracted from the 32 fecal samples, starting from an amount range of 50-200 mg, using Microbiome Fecal DNA kit (Danagen-Bioted S.L., Barcelona, Spain). According to the manufacturer's protocol, stool samples were placed in 2.0 ml bead microtube with the addition of 1.0 ml of extraction buffer cetyltrimethylammonium bromide (CTAB) and 20 µl of internal control DNA (RIDA®GENE *Akkermansia muciniphila,* Darmstadt, Germany). The re-suspended samples were incubated at 70°C for 10 minutes (no vortex), and after the incubation time, were homogenized by MagNA Lyzer Rotor (La Roche SA, Basilea, Switzerland) for 180 seconds at 7,000 rpm. After the homogenization, microtubes were centrifuged at 14,000 rpm for 5 minutes. A volume of 600 µl of supernatant was transferred to a clean microcentrifuge tube with 200 µl of electrical conductivity (EC) buffer vortex. The samples were incubated at 4°C for 5 minutes, and then centrifuged at 14,000 rpm for 5 minutes. For each sample, 500 µl of supernatant was picked up (crossing the superficial fat layer) and treated with 25 µl of Proteinase K. The lysates were placed into combined microbial DNA column-collection tubes and were centrifuged at 10,000 rpm for 60 seconds. The collection tubes were removed, and the microbial DNA columns were transferred to new collection tubes, where 500 µl of disinhibition buffer was added. The samples were centrifuged at 12,000 rpm for 1 minute. After, the flow-through was discarded and the columns were allocated in new collection tubes with the addition of 700 µl of wash buffer. A washing phase was performed using a DNA column-collection tube. Lastly, 200 µL of elution buffer was added in order to obtain the same volume of purified DNA.

Real-time PCR for *Akkermansia Muciniphila*

Qualitative and quantitative detection of 16S-rRNA was performed by a real-time PCR RIDA®GENE *Akkermansia muciniphila* (R-Bio-

pharm AG, Darmstadt, Germany). Results of quantitative detection of *Akkermansia Muciniphila* performed by real-time PCR have been shown in Figure 1. The thirty-two nucleic acid samples were analyzed in duplicate using the CFX96 TM platform (Bio-Rad, Hercules, USA). The preparation of the reagent solution was performed by calculating the total number of PCR reactions needed (sixty-four samples, two controls, and three standards). The components (19.3 µl of reaction mix and 0.7 µl of Taq-polymerase) were thawed before use and stored at 2-8°C during the PCR preparation protocol. Twenty microliters of PCR mix were inoculated in each reaction vial, and 5 µl of each nucleic acid sample was added in the pre-planned position. In two vials, we added 5 µl of negative control and 5 µl of positive control to a pre-pipetted master mix. The three standards (A=10 2 copies/reaction, B=10 4 copies/reaction, C=10 6 copies/reaction) were added in order to include a standard curve in the run. The amplification protocol was settled as follows: 1 min at 95°C for initial denaturation, 15 sec at 95°C for PCR denaturation, 30 sec at 60°C for annealing/extension, for 45 cycles. Data obtained from the amplification were then converted in order to obtain cells/g stool values using the formula: C[-cells/g] = c[copies/reaction]x K (correction factor provided by the manufacturer). The c-value was the result of the average of the two measurements for each sample.

Statistical analysis

Qualitative variables were summarized as absolute and percentage frequency tables. Quantitative variables were summarized as mean and standard deviation, and as median and interquartile ranges. Continuous variables were compared between groups with parametric and non-parametric tests for paired samples as appropriate. Categorical variables were compared with the Chi-squared test. The primary endpoint was the change in body weight after three months. The main secondary endpoints were the change in total daily insulin dose, the change in HbA1c, and the change in time in range (TIR 70-180 mg/dL). Other secondary endpoints were the change in time below and time above range, the change in coefficient of variation, and the change in glucose management indicator. The comparison was performed using a Chi-squared test. Statistical significance was set for *p*-values <0.05. Statistical analysis was performed using the R language (R v. 4.3.1, Vienna, Austria).

RESULTS

Forty-nine subjects were enrolled in the study, with an age of 46 [37-53] years, 30 females (61%), a duration of disease of 20 [11-27] years, and an HbA1c of 64 [59-72] mmol/mol. 24 subjects were randomized in group A and

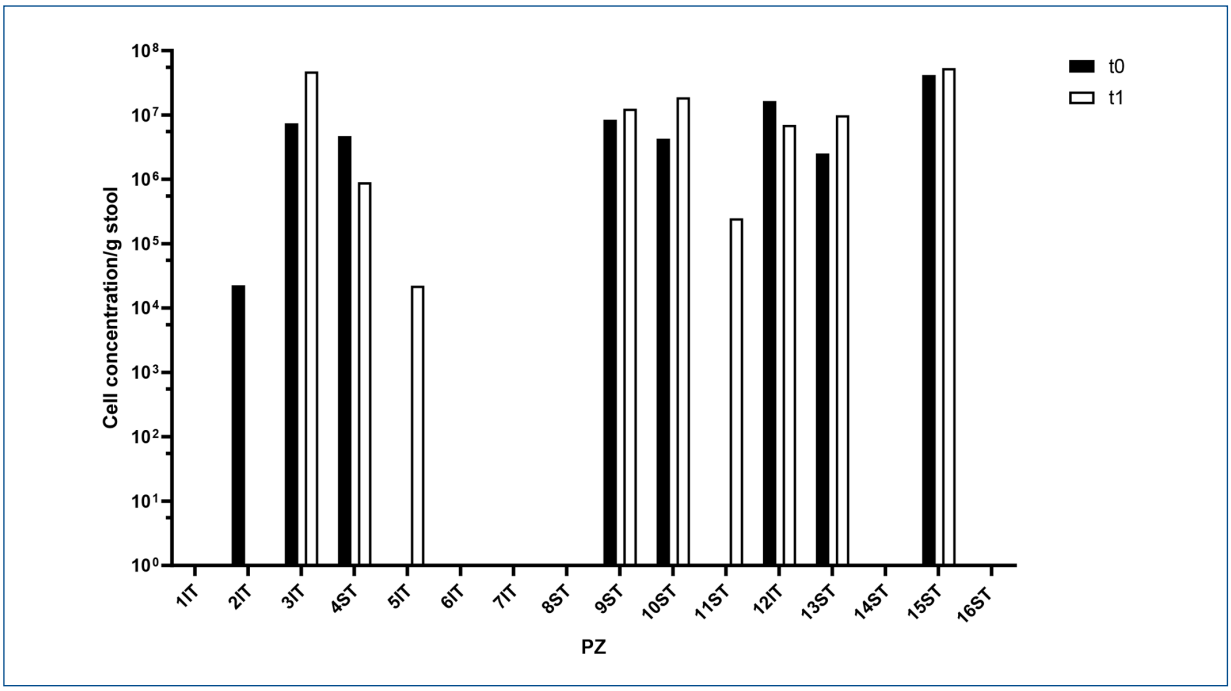


Figure 1. Results of quantitative detection of *Akkermansia Muciniphila* performed by real-time PCR. For each patient, two time points (T0 = baseline, T1 after 3 months) were considered.

25 subjects were randomized in group B. At enrolment, groups were comparable for HbA1c, total cholesterol, and creatinine (Table I). Both groups were matched for baseline diet and physical activity. At randomization, total daily insulin dosages were comparable between the two groups. Between groups, there were no differences in baseline TIR, TBR level 1, and level 2. Coefficient of variation and GMI were also comparable between groups at randomization (Table I). BSS scores were comparable between groups at enrolment (Table I). After 3 months, group A showed a slight but significant reduction in body weight [median (IQR) body weight change group A -2 (-3; 0) kg and group B 0 (-1; 1) kg, p=0.029] (Table II, Figure 2). A small but significant difference in total daily insulin dose reduction was also observed in subjects randomized to inulin + diet compared to diet alone [median (IQR) change in total daily insulin dose group A -1.5 (-3.1; 0) UI *vs.* group B 0.6 (0; 1.7) UI (p=0.010)] (Table II, Figure 3). Although not significant, a slight difference in change of HbA1c after 3 months was detected [median (IQR) change of HbA1c after 3 months group A -4.5 (-7; -3) mmol/mol *vs.* group B -2 (-3.5; 0) (p=0.077)]. After 3 months, the change in TIR was comparable between groups [median (IQR) change of TIR after 3 months: group A, 7 (1.25; 12.75), and group B, 5.5 (-1; 6), (p= 0.089)]. Consistently, change in CV (p=0.310) and GMI (p=0.865) were comparable between the two groups. In both groups, there was no change in BSS score (p=0.396). After 3 months, there was no difference in the distribution of each BSS class between the two groups (p=0.591). As regards the levels of *Akkermansia Muciniphila*, we obtained complete results from a subset of 16 patients; 7 subjects were enrolled into group A, and 9 in group B. In group A, two patients showed an increase in the *Akkermansia muciniphila* abundance; in two patients, a decrease in the amount of *Akkermansia muciniphila* was observed, and three patients showed no detectable levels both at enrollment and after 3 months. In group B, the analysis of stool samples revealed in four patients an increase of *Akkermansia muciniphila* after three months; two patients showed a decrease in terms of relative abundance, and three patients showed no detectable levels of *Akkermansia muciniphila* (Figure 1 and Table III).

DISCUSSION

This is the first *in vivo* study conducted on people with T1D that analyzed the use of 12 g of inulin/day for three months on the production of *Akkermansia muciniphila* and metabolic control.

Table I. Baseline characteristics of subjects randomized to inulin + insulin (group A) and to insulin alone (group B).

	Group A (n=24) Median [25th-75th percentile]	Group B (n=25) Median [25th-75th percentile]	*p*-value
Age (years)	45.5 [34.7-51.5]	46.0 [37.0-56.0]	0.53
Diabetes duration (years)	20.5 [12.0-25.2]	20.0 [10.0-27.0]	0.90
Weight (Kg)	65.5 [61.7-75.2]	71.0 [62.5-77.2]	0.46
BMI (Kg/m^2)	23.7 [22.5-25.3]	24.4 [23.7-25.7]	0.37
Insulin total daily dose (UI)	37.0 [31.2-42.5]	33.2 [29.0-45.3]	0.58
Daily insulin need (UI/Kg)	0.5 [0.4-0.6]	0.5 [0.4-0.6]	0.68
HbA1c (mmol/mol)	67.0 [60.0-72.0]	62.0 [58.0-69.0]	0.13
Total cholesterol (mg/dL)	183.0 [165.5-208.0]	181.0 [163.0-187.0]	0.17
HDL cholesterol (mg/dL)	60.0 [48.5-100.5]	66.5 [52.5-73.75]	0.64
Triglycerides (mg/dL)	74.5 [31.2-104.0]	73.0 [50.7-115.0]	0.60
Creatinine (mg/dL)	0.7 [0.7-0.9]	0.7 [0.6-0.8]	0.50
TIR (70-180 mg/dL) (%)	50.0 [46.0-58.7]	58.0 [50.5-65.0]	0.12
TBR level 1 (54-69 mg/dL) (%)	2.0 [1.0-3.0]	3.0 [1.0-5.0]	0.11
TBR level 2 (<54 mg/dL) (%)	0.0 [0.0-0.7]	0.0 [0.0-1.0]	0.80
TAR level 1 (181-250 mg/dL) (%)	28.5 [21.2-35.0]	27.0 [24.0-29.0]	0.53
TAR level 2 (>250 mg/dL) (%)	13.5 [9.5-18.7]	8.5 [7.0-16.7]	0.19
CV (%)	36.7 [33.5-42.6]	40.0 [37.0-45.5]	0.18
GMI (%)	8.1 [7.8-8.4]	7.8 [7.5-8.3]	0.06
Bristol Stool Chart Score	3.0 [2.0-4.0]	2.5 [2-3.5]	0.54

Data are summarized as median [25th-75th percentiles]. Statistical significance set at p<0.05. CV: Coefficient of variation; GMI: Glucose Management Indicator; HbA1c: glycated hemoglobin; TAR: time above range; TBR: time below range; TIR: time in range.

Table II. Anthropometric parameters, glycated hemoglobin and continuous glucose monitoring metrics at enrolment in subjects randomized to inulin + insulin (group A) and to insulin alone (group B).

Outcome	Group A (n=24)			Group B (n=25)			*p*-value
	Baseline	Follow-up	Median change	Baseline	Follow-up	Median change	
Weight (Kg)	65.5 [61.7; 75.2]	65.0 [60.5; 72.5]	-2.0 [-3.0; 0.0]	71.0 [62.5; 77.2]	70.0 [63.0; 76.7]	0.0 [-1.0; 1.0]	0.03
Total daily dose (UI)	37.0 [31.2; 42.5]	34.8 [29.4; 40.3]	-1.5 [-3.1; 0.0]	33.2 [29.0; 45.3]	33.6 [29.8; 47.9]	0.6 [0.0; 1.7]	0.01
HbA1c (mmol/mol)	67.0 [60.0; 72.0]	63.5 [58.7; 67.5]	-4.5 [-7.0; -3.0]	62.0 [58.0; 69.0]	60.0 [56.0; 65.0]	-2.0 [-3.5; 0.0]	0.08
TIR (70-180 mg/dL) (%)	50.0 [46.0; 58.7]	58.0 [50.2; 70.0]	7.0 [1.2; 12.7]	58.0 [50.5; 65.0]	62.5 [55.2; 66.7]	5.5 [-1.0; 6.0]	0.09
TBR Level 1 (<70 mg/dL) (%)	2.0 [1.0; 3.0]	2.0 [0.2; 3.7]	0.0 [-0.7; 1.0]	3.0 [1.0; 5.0]	3.0 [1.2; 4.7]	0.0 [-1.0; 1.0]	0.63
TBR Level 2 (<54 mg/dL) (%)	0.0 [0.0; 0.7]	0.0 [0.0; 1.0]	0.0 [0.0; 0.0]	0.0 [0.0; 1.0]	0.0 [0.0; 1.0]	0.0 [-1.0; 1.0]	0.95
TAR Level 1 (>180 mg/dL) (%)	28.5 [21.2; 35.0]	26.5 [19.2; 32.0]	-2.0 [-6.7; -0.2]	27.0 [24.0; 29.0]	25.0 [23.0; 27.7]	-1.5 [-3.7; 0.7]	0.52
TAR Level 2 (>250 mg/dL) (%)	13.5 [9.5; 18.7]	9.0 [8.0; 14.0]	-10.0 [1.0; 20.0]	8.5 [7.0; 16.7]	7.0 [6.0; 14.0]	-2.0 [-6.0; 0.0]	0.33
CV (%)	36.7 [33.5; 42.6]	34.0 [31.5; 37.9]	-4.0 [-4.8; -1.8]	40.0 [37.0; 45.5]	36.0 [31.8; 43.2]	-5.0 [-9.7; -2.7]	0.31
GMI (%)	8.1 [7.8; 8.4]	7.8 [7.6; 8.3]	-0.6 [-0.9; -0.4]	7.8 [7.5; 8.3]	7.7 [7.4; 7.8]	-0.6 [-1.0; -0.4]	0.86

Data are summarized as median [25th-75th percentiles]. Statistical significance set at $p<0.05$ for comparison of the change in Group A *vs*. change in Group B. CV: Coefficient of variation; GMI: Glucose Management Indicator; HbA1c: glycated hemoglobin; TAR: time above range; TBR: time below range; TIR: time in range.

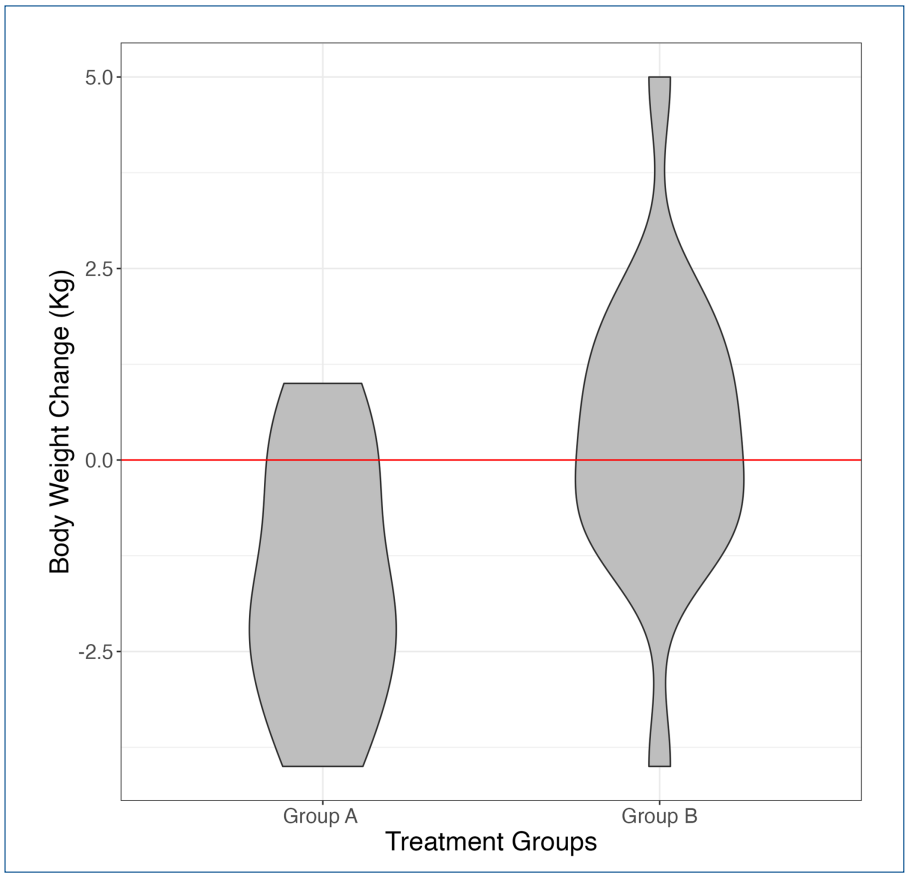


Figure 2. Body weight change at the end of the study period compared to baseline in group A (insulin with supplementation of inulin) and group B (insulin alone).

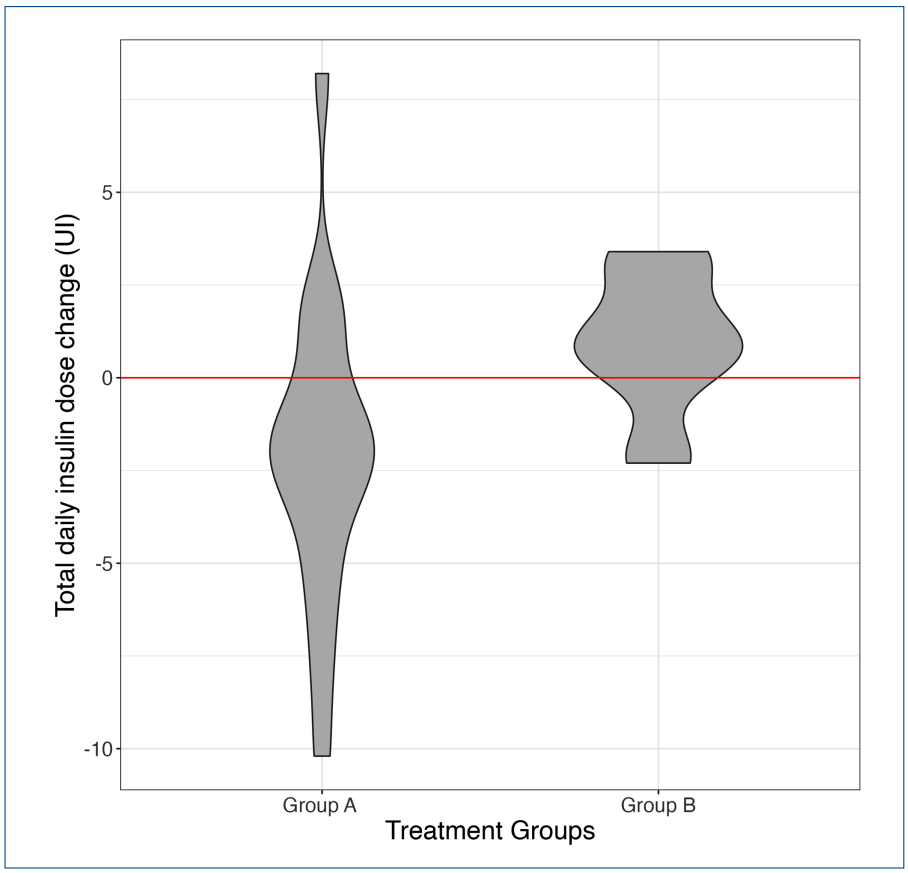


Figure 3. Total daily insulin dose change at the end of the study period compared to baseline in group A (insulin with supplementation of inulin) and group B (insulin alone).

Table III. Real-time PCR values. The c-value was the result of the average of two measurements for each sample.

Patient	Arm	Time Point	*Akkermansia muciniphila* (concentration/g stool)
#1	Treatment	T0	nd
		T1	nd
#2	Treatment	T0	2.26×10^4
		T1	nd
#3	Treatment	T0	7.42×10^6
		T1	
#4	Control	T0	4.69×10^6
		T1	9.05×10^5
#5	Treatment	T0	nd
		T1	2.23×10^4
#6	Treatment	T0	nd
		T1	nd
#7	Treatment	T0	nd
		T1	nd
#8	Control	T0	nd
		T1	nd
#9	Control	T0	8.42×10^6
		T1	1.25×10^7
#10	Control	T0	4.31×10^6
		T1	1.88×10^7
#11	Control	T0	nd
		T1	2.48×10^5
#12	Treatment	T0	1.64×10^7
		T1	7.05×10^6
#13	Control	T0	2.52×10^6
		T1	9.92×10^6
#14	Control	T0	nd
		T1	nd
#15	Control	T0	4.19×10^7
		T1	5.37×10^7
#16	Control	T0	nd
		T1	nd

Nd: not detected.

Several studies[24-28] agree that specific members of the gut microbiota, such as *Akkermansia muciniphila*, have shown antidiabetic effects when administered in mouse models. A study by Yan et al[29] found that *Akkermansia muciniphila* was able to reduce inflammation by repairing the intestinal gut barrier in rats and improve pancreatic β-cells function. In addition, this bacterium controls the essential regulatory system of glucose metabolism and can strengthen gut integrity, modulate insulin resistance, and protect the host from metabolic inflammation. In our patients, we observed a significant reduction in body weight and in insulin requirements in the group treated with inulin compared to controls. *Akkermansia muciniphila* increases the production of endogenous lipids with anti-inflammatory effects and regulates the endogenous production of gut peptides involved in the regulation of blood glucose levels and gut barrier, as glucagon-like peptides 1 and 2 (GLP-1 and GLP-2)[28]. A study by Cani et al[5] showed that *Akkermansia muciniphila* supports GLP-1 release *via* a newly discovered protein (P9), considered a "next-generation beneficial microbe"[5] in mice. The administration of *Akkermansia muciniphila* reduces body weight gain after a fatty diet, fat mass gain, and glucose intolerance. Literature studies[11,24,25] agree that *Akkermansia muciniphila* is able to strengthen gut integrity, modulate insulin resistance, and protect the host from metabolic inflammation. In addition, the increased secretion of GLP-1 (induced by *Akkermansia municiphila*) stimulates brown adipose tissue (BAT) thermogenesis *via* activation of the AMP-activated protein kinase (AMPK) pathway, thereby increasing energy expenditure. It reduces the expression of genes codifying for fatty acid and for the synthesis and transport of insulin in muscle and liver, leading to an increase in insulin-signaling activation[27-30]. Other effects of *Akkermansia muciniphila* include the downregulation of systemic and intes-

tinal pro-inflammatory cytokines such as TNF-α, IL-6, IL-1α, and IL-12 with beneficial gut-health effects[15-17]. This bacterium can be influenced by various nutritional and genetic factors. The condition of dysbiosis contributes to a change in immune regulation, and the lack of *Akkermansia muciniphila* is potentially responsible for mucus loss and, on the other hand, this could lead to an increase in other Gram-negative bacteria to stimulate receptors, such as Toll-like receptor 4 (TLR-4), involved in diabetes incidence[27]. In mice models, the supplementation with prebiotics significantly improved glycemic control[29-31] and increased *Akkermansia muciniphila* by producing SCFA and facilitating mucin growth to feed the bacterium. A study by Pérez-Monter et al[24] observed that the prebiotic inulin reshaped the intestinal microbiota at the phylum level, increasing the levels of *Akkermansia muciniphila*. Studies in the literature[17,18] indicated that *Akkermansia muciniphila* intestinal abundance is promoted by the ingestion of the prebiotic fiber inulin in mammals. The treatment with inulin modifies the gut bacterial communities, reshaping the intestinal gut microbiota and significantly increasing the Verrucomicrobia phylum (and *Akkermansia muciniphila* increased by 5-fold) in the fat-diet group with inulin supplementation[25-27]. Some studies[28,30] concluded that inulin supplementation can promote a remodeling in the gut microbiota composition and regulate lipid metabolism, improving metabolic profile. In our study, the fecal samples of T1D patients did not show an increase in *Akkermansia muciniphila*, so we did not observe the expected effects with 12 g of inulin for 3 months, even though about 40% of enrolled patients lack *Akkermansia muciniphila* in their intestines. Therefore, we hypothesize that if this bacterium is not present in the gut, prebiotic supplementation alone may not be effective in increasing its levels, or perhaps the dosage of inulin used could be insufficient. This is a very interesting point to focus on: T1D patients have a different composition of gut microbiota. Patients in whom *Akkermansia muciniphila* has been detected and increased by inulin supplementation have achieved better results in glucose metabolism.

Furthermore, literature studies[31] reported that inulin, a type of polysaccharide often supplemented through diet, can interact with the gut microbiota of the host, affecting its composition and functionality. Host intestinal microbes fermented it in the lower gastrointestinal tract, but both the specific dynamics of this mutual interaction and the subsequent effects are not fully known. Although most researches[30,31] underline that inulin can overall improve metabolic function and regulate gut immunity, there are some limitations. A study by An et al[31] provides evidence that the effects on gut microbiota depend on the supplementation dosage and the time of administration. In fact, the intestinal microbial communities need time to achieve homeostasis after inulin supplementation. Furthermore, inulin treatment does not promote the growth of all gut bacteria, but only of certain bacteria, such as *Bifidobacterium* spp[32,33]. In fact, the inulin intake significantly reduces the ratio of Firmicutes and Bacteroidetes (and of some, not all, bacteria associated with a pro-inflammatory state)[34]. In addition, inulin acts on the mucosal immune system, regulating the differentiation and proliferation of T-reg cells and promoting the secretion of IL-C2s that stimulates eosinophilic cells (it could be a limitation in case of allergic responses in the gastrointestinal tract)[35,36]. As regards the impact on glucose metabolism, in patients affected by diabetes, a study by Perraudeau et al[37] showed that inulin, not alone, but in combination with probiotics, was able to improve postprandial glucose control; and the effects on it were more suitable in obese/overweight people (treated with inulin) than in normal weight secondary to an individual variability[37]. The individual variability can also determine different symptoms, such as nausea, bloating due to slower fermentation of inulin supplementation, and subsequent gas production[32]. In conclusion, most studies[13,21,22] have recognized the beneficial effects of inulin on human gut microbiota modulation and function, but the overall outcomes can depend on many factors, such as individual profile. More studies, involving a larger number of patients and a prolonged administration duration of inulin, are needed to gain a deeper understanding of this topic. A limitation of our study was also the small number of T1D patients enrolled.

CONCLUSIONS

Inulin is a prebiotic fiber supplement in the diet with beneficial health properties. It resulted in effective improvement of glycemic and metabolic control in T1D patients after three months of treatment. We observed a slight but significant decrease in body weight and insulin dose. Although subjects with T1D are traditionally described as lean, it has been shown that the prevalence of overweight and/or obesity is also rising in this group. The insulin requirement appears to play a pivotal role, due to the anabolic effect of this hormone, which leads to fat accumulation and insulin resistance. Weight management is therefore essential for people living with T1D[33,34].

Regarding the effects on gut microbiota composition, no significant differences emerged in the increase of *Akkermansia muciniphila*. Further studies, involving a larger number of patients, are needed to investigate this issue.

Conflict of Interest
The authors declare no conflicts of interest.

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Authors' Contributions
Conceptualization, D.P. and V.O.; methodology, A.R.; software, G.Q.; validation, A.S., M. Di L. and L.T.; formal analysis, L.M.; investigation, A.S.; resources, D.P.; data curation, A.R.; writing–original draft preparation, V.O. and A.S. and A.R. and D.P.; writing–review and editing, A.S. and V.O.; visualization, A.S.; supervision, D.P. and L.M.; personal funding, D.P. All authors read and approved the final version of the manuscript.

Data Availability
All data are available on request to the corresponding author.

Informed Consent
All patients signed the informed consent to participate in the study.

Ethics Approval
The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, with protocol number 3099 (16/September/2022).

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