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ORIGINAL PAPER

EFFECTS OF PROBIOTIC SUPPLEMENTATION ON METABOLIC SYNDROME COMPONENTS IN TYPE 2 DIABETES MELLITUS PATIENTS – A CASE-CONTROL STUDY

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Abstract

Introduction. Probiotics are well-known adjuvants, used as complementary therapeutic agents in health (e.g. metabolic or gastrointestinal) disorders, considering their beneficial role on gut microbiota, and their support in immunity.
The objective of the study. This research followed the impact of probiotic supplementation on some clinical parameters related to metabolic syndrome (MS) and type 2 diabetes mellitus (T2DM) (weight status, body mass index, carbohydrate/ lipid profiles).
Materials and methods. The comparative monitoring of the parameters was conducted over a 3- month period, on 41 subjects diagnosed with both MS and T2DM, who were separated into two groups, as follows: the study group (probiotics associated with allopathic treatment) and the control group (without probiotics).
Results. Administration of dietary probiotics had a major impact on body weight, weight loss being

Résumé

Effets de la supplémentation probiotique sur les composants du syndrome métabolique chez les patients avec diabète sucré de type 2 – une étude de contrôle de cas

Introduction. Les probiotiques sont des adjuvants connus, utilisés comme agents thérapeutiques complémentaires dans les déséquilibres de la santé (métaboliques ou gastro-intestinaux), compte tenu de leur rôle bénéfique sur le microbiote intestinal et de leur appui à l'immunité.
L'objectif de l'étude. Cette recherche a suivi l'impact de la supplémentation de probiotiques sur certains paramètres cliniques liés au syndrome métabolique (SM) et au diabète sucré de type 2 (T2DM) (statut pondéral, indice de masse corporelle, profils glucidiques/ lipidiques).
Matériels et méthodes. Le suivi comparatif des paramètres a été réalisé sur une période de 3 mois, sur

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significantly enhanced in the probiotic group than in the diet-only group (p=0.01). The effect of dietary probiotic administration on glucidic and lipidic profile was small (effect size (ES) 0.26 and 0.33, respectively), but better than in the control group, in whom the evolution was insignificant (ES 0.10 and 0.10, respectively). From a statistical point of view, the differences were insignificant (p>0.05).

Conclusions. In the metabolic profile management of patients suffering from both MS and T2DM, probiotics administration had beneficial results, as highlighted by the results of the present study.

Keywords: metabolic syndrome, type 2 diabetes, weight status, glucidic profile, lipidic profile.

List of abbreviations:
BMI – body mass index
BP – blood pressure
CVD – cardiovascular disease
ES – effect size
HbA1c – glycosylated hemoglobin A1c
HDLc – high-density lipoprotein cholesterol
LDLc –low-density lipoprotein cholesterol
MS – metabolic syndrome
T2DM – type 2 diabetes

41 sujets diagnostiqués à la fois avec SM et T2DM, qui ont été séparés en deux groupes, comme suit : le groupe d'étude (probiotiques associés au traitement allopathique) et le groupe témoin (sans probiotiques).

Résultats. L'administration de probiotiques alimentaires a eu un impact majeur sur le poids corporel, la perte de poids étant significativement améliorée dans le groupe aux probiotiques par rapport au groupe avec de la diète seule (p = 0,01). L'effet de l'administration de probiotiques alimentaires sur les profils glucidique et lipidique était faible (taille de l'effet (ES) 0,26 et 0,33, respectivement), mais meilleur que dans le groupe témoin, chez qui l'évolution était insignifiante (ES 0,10 et 0,10, respectivement). D'un point de vue statistique, les différences étaient insignifiantes (p> 0,05).

Conclusions. Dans la gestion du profil métabolique des patients souffrant à la fois de SM et de T2DM, l'administration de probiotiques a eu des résultats bénéfiques, comme le soulignent les résultats de la présente étude.

Mots-clés: syndrome métabolique, diabète de type 2, poids, profil glucidique, profil lipidique.

Introduction

The increasing prevalence of obesity is a wide-spread public health issue. Obesity enhances comorbidities, increasing mortality especially as result of cardiometabolic complications[1]. Specifically, the layer of visceral adipose tissue generates inflammatory cytokines that produce insulin resistance implicated in hypertension, type 2 diabetes mellitus (T2DM) and dyslipidemia, accentuating the risk of cardiovascular disease, the main cause of death[2].

In this frame, T2DM represents also an important health issue world-wide, being the main risk factor for several ordinary diseases like coronary heart disease, hypertension, retinopathy, stroke and kidney failure[3]. Data from International Diabetes Federation (IDF) show a global DM rate of 8.8% in 2015 and a prediction of growth to 10.4% in adults by the year 2040[4]. Growing rates of obesity generates conditions for the increasing ratio of metabolic syndrome (MS) and T2DM[5].

Insulin resistance and expended white adipose tissue are characteristics of MS, determining an increased risk of cardiovascular diseases (CVD)[6]. The IDF characterizes MS as resulting from central obesity together with two of the following factors: high-density lipoprotein cholesterol (HDLc) <50 mg/dL in females, <40 mg/dL in males or specific therapy for this condition; increased triacylglycerols (>150 mg/dL) or specific therapy for this condition; increased fasting plasma glucose >100 mg/dL or prediabetes; increased blood pressure (BP) (systolic >130 mmHg or diastolic >85 mmHg) or specific treatment in patients already diagnosed with hypertension[7]. Some studies present MS as a group of biochemical, metabolic and physiological risk factors usually connected to obesity, T2DM and cardiovascular disease[8,9].

Previous published data have demonstrated the essential role of gut microbiota in influencing the functioning of gut metabolism like lipids, proteins and carbohydrates decomposition, energy and nutrients absorption and gut motility[10,11]. Recent research associates MS with particular disorders in gut microbiota[12-14]. It was also suggested that probiotic intake has a positive impact on some clinical elements of MS[14]. The bacteria generating beneficial effects for animals and for humans are usually known as probiotics. Probiotics definition considers that they are live microorganisms which have effect on the host's health when they are consumed in proper quantity[15]. In animal models, probiotics were shown to decrease the blood glucose, ameliorating inflammation and prevent β-cell destruction[16]. Human clinical trials on various probiotics obtained mixed outcomes, some of them found no effect[17], and other revealed a considerable glucose decreasing[18].

The objective of the study was to monitor the impact of probiotics in patients with T2DM and MS, regarding some of the clinical components of both disorders (weight status, body mass index (BMI), BP, glycemic and lipid profiles).

Materials and methods

A prospective 3-month comparative study was conducted between January – June 2020, in private medical offices of diabetes and nutritional diseases from Oradea, Romania, on 41 patients with T2DM and MS, of whom 19 patients were administered probiotics (study group) and 22 patients were not given probiotics (control group). Belonging to one group or another was decided by the patients' option to supplement with probiotics their treatment established by the diabetologist. According to the clinical characteristics and associated pathology, the allopathic therapy was individualized for each patient. The probiotic product used in this study is a spore-based supplement that contains *Bacillus licheniformis, Bacillus indicus, Bacillus subtilis, Bacillus clausii, Bacillus coagulans,* which produce antioxidants and thus manage to repair the imbalances of the intestinal flora. The administration was made according to the manufacturer's instructions (one capsule per day during the meal for 2 weeks, after which it was changed to 2 capsules per day for 3 months).

The patients' evaluation was performed at the beginning of the therapy and after 3 months. The evaluated parameters were as follows: lipid profile (total cholesterol, HDLc, low density lipoprotein cholesterol (LDLc), triglycerides), BP, glucose profile and weight status.

BP monitoring was carried out according to the 2018 European Society of Cardiology/ European Society of Hypertension Guidelines for the Management of Arterial Hypertension[19]. To evaluate the patients' weight status, the BMI (kg/m^2) was used.

The blood samples were taken in the morning (after 12-14 hours of fasting) and were used to determine the levels of HDLc, LDLc and total cholesterols, triglycerides, glycosylated hemoglobin A1c (HbA1c) and basal blood glucose. The first four parameters afore-mentioned were determined using Beckman Coulter reagents with the Beckman Coulter AU680 analyzer (provided by Beckman Coulter Inc, Ireland). The analyses methods comprise enzymatic glycerol-3-phosphate oxidase method to determine triglycerides, the oxidase-peroxidase method to determine cholesterol, and colorimetric direct method to determine HDLc and LDLc. Hexokinase method was used to evaluate basal blood glucose, the selection of subjects being performed taking into account the fasting values. Plasmatic concentration of <100 mg/dL was regarded as normal value of basal blood glucose. HbA1c represents the accurate value of glucose over a period of 90-120 days prior to determination and was evaluated only for diabetic patients. Venous blood collected on ethylenediamine tetra-acetic acid anticoagulant was analyzed by – high-performance liquid chromatography (gold standard) using BioRad D-10 reagents and equipment.

Agreed by the Ethics Commission of the Clinical County Emergency Hospital, Oradea, Romania (no. 6398/08.03.2019), this research was performed according to WMA Ethical Declaration of Helsinki. Before being included in the study, each patient signed an informed consent form.

Statistical analysis was completed using EPIINFO, version 11, an Atlanta Centre of Disease Control and Prevention program adjusted to the medical statistics processing. The Student's method (t test) and c^2 tests were used to assess average parameter values like standard deviations, frequency ranges and statistical significance. Using assumptions that include numerical data, a test distribution similar to the normal one was performed. The paired t-test was used. Bravais-Pearson's correlation coefficient was applied to achieve a marker separated from the two variables measurement units. Statistical significance was established for a p-value of <0.05. The effect size (ES) was determined to assess the parameters change magnitude at different times. According to literature, the index decoding was systematized: small ES=0.20, medium ES=0.50, large ES=0.80. ES is essential to show the results of a quantitative study, as p-value identifies the effect, but does not show its magnitude.

Results

Demographic data and clinical characteristics

In the study group, men predominated (57.89%), while women predominated (59.09%) in the control group. There are no significant differences between the two groups in terms of sex distribution (p=0.284). Most patients in both groups were aged between 51-60 years (52.63%, respectively 40.91%). The mean age was insignificantly higher in the study group compared to the control group (60.74 vs 58.14 years, p=0.348). From the point of view of the origin environment, in the study group over 63% of patients came from the urban environment, and in the control group the urban/ rural ratio is 1:1 (p=0.403) (Table 1).

Moreover, in the study group, insulin-dependent patients represented 63.16%, an insignificantly higher percentage than in the control group (63.64%) (p=0.975) (Figure 1). Complications of diabetes had a prevalence of 100.00% in the study group, slightly

Table 1. Distribution by demographic characteristics

Demographic characteristics	*Study group*		*Control group*	
	No.	%	*No.*	%
Gender				
Male	11	57.89	9	40.91
Female	8	42.11	13	59.09
Total	**19**	**100.00**	**22**	**100.00**
Age (years)				
	1	5.26	5	22.73
	10	52.63	9	40.91
	7	36.84	5	22.73
	1	5.26	3	13.64
Min-Max	48-76		43-72	
Average age	60.74±5.84		58.14±11.17	
Environment				
Rural	7	36.84	11	50.00
Urban	12	63.16	11	50.00

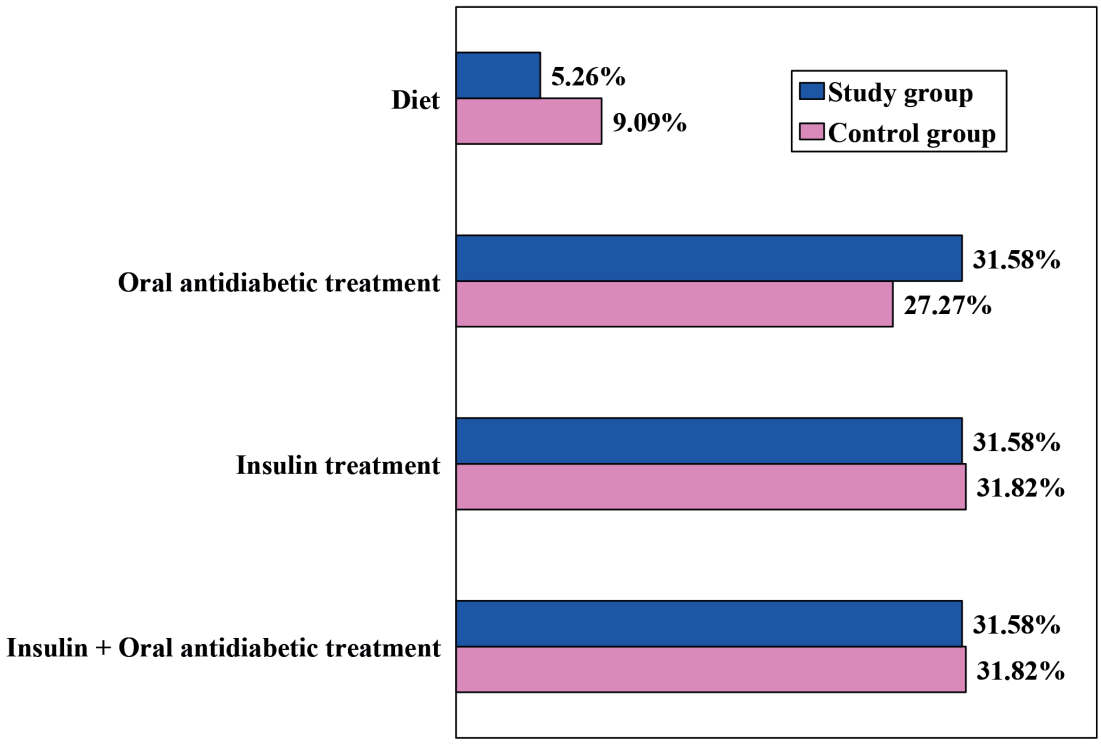

Figure 1. Distribution by antidiabetic treatment

higher than in the control group (81.82%) (p=0.053). In both groups the most frequent complication was kidney disease (73.68 vs 40.91%, p=0.037), stages 3-4 having an insignificantly higher prevalence in the control group (27.27 vs 15.79%, p=0.382). Both polyneuropathy and retinopathy were more common in the study group, but without significant differences (61.70 vs 45.28%, p=0.102, respectively 14.89 vs 9.43%, p=0.404) (Table 2).

In both groups, most cases with MS met all 5 criteria (47.37 vs. 54.55%, p=0.651) (Figure 2). Obesity had a prevalence of 94.74% in the study group, insignificantly lower than in the control group (95.45%, p=0.917). The mean value of BMI was 34.17 kg/m^2 in the study group, insignificantly lower than in the control group (35.51 kg/m^2, p=0.337) (Table 2). Also, the associated diseases were identified in all patients, the most common being heart

Table 2. Distribution by complications of diabetes and weight status

Complications	*Study group*		*Control group*	
	No.	%	*No.*	%
Diabetes				
With complications	**19**	**100.00**	**18**	**81.82**
Diabetic polyneuropathy	16	84.21	12	54.55
Diabetic retinopathy	3	15.79	3	13.64
Chronic kidney disease	17	89.47	15	68.18
Stage 1	0	0.00	0	0.00
Stage 2	14	73.68	9	40.91
Stage 3	2	10.53	6	27.27
Stage 4	1	5.26	0	0.00
Weight status				
Normal weight	0	0.00	0	0.00
Overweight	1	5.26	1	4.55
Obesity	18	94.74	21	95.45
Obese grade 1	13	68.42	10	45.45
Obese grade 2	3	15.79	8	36.36
Obese grade 3	2	10.53	3	13.64
Min/Max	29.75/41.66		25.15/50.39	
Average BMI (kg/m^2)	34.17±3.58		35.51±5.22	

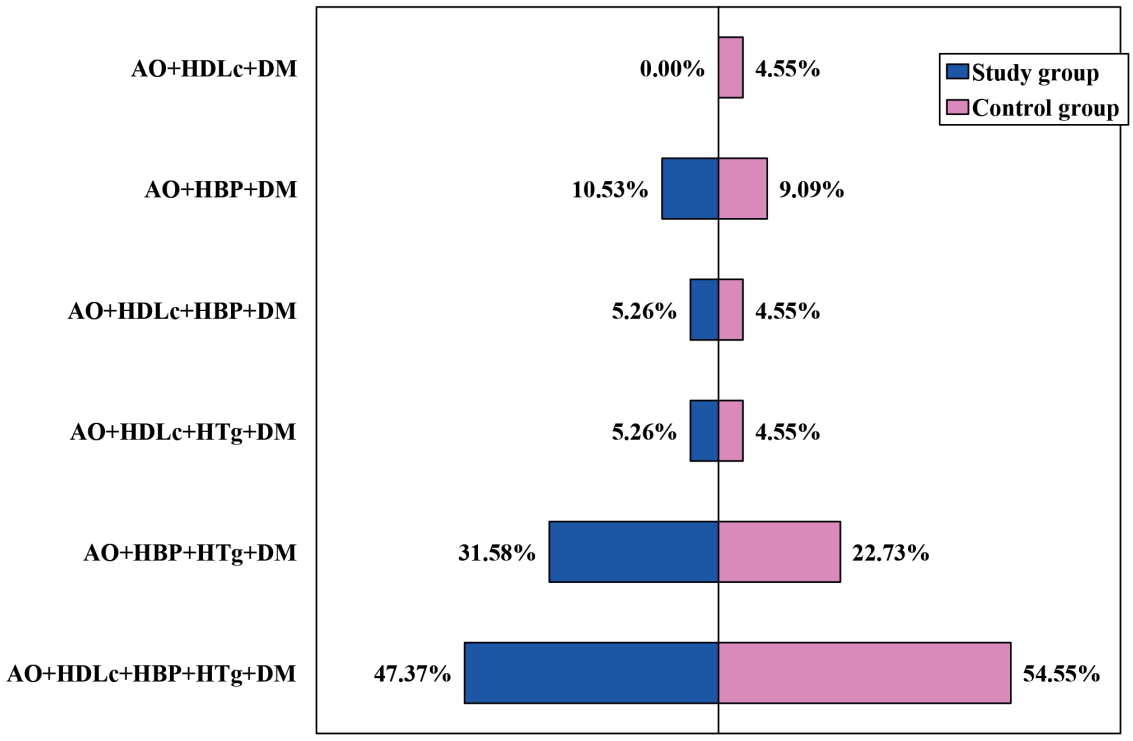

Figure 2. MS criteria

disease (94.74 vs. 95.45%, p=0.917), liver disease (73.68 vs. 86.36%, p=0.313) and dyslipidemia (57.89 vs 81.82%, p=0.097) (Table 3). The history of major events was registered in 15.79% of the patients of the study group and in 9.09% of the patients of the control group (p=0.518) (Figure 3). The most used drugs in both groups were diuretics (63.16 vs 54.55%, p=0.582), beta-blockers and statins (36.84 vs 22.73%, p=0.328), and calcium channel blockers (31.58 vs. 22.73%, p=0.529) (Table 3).

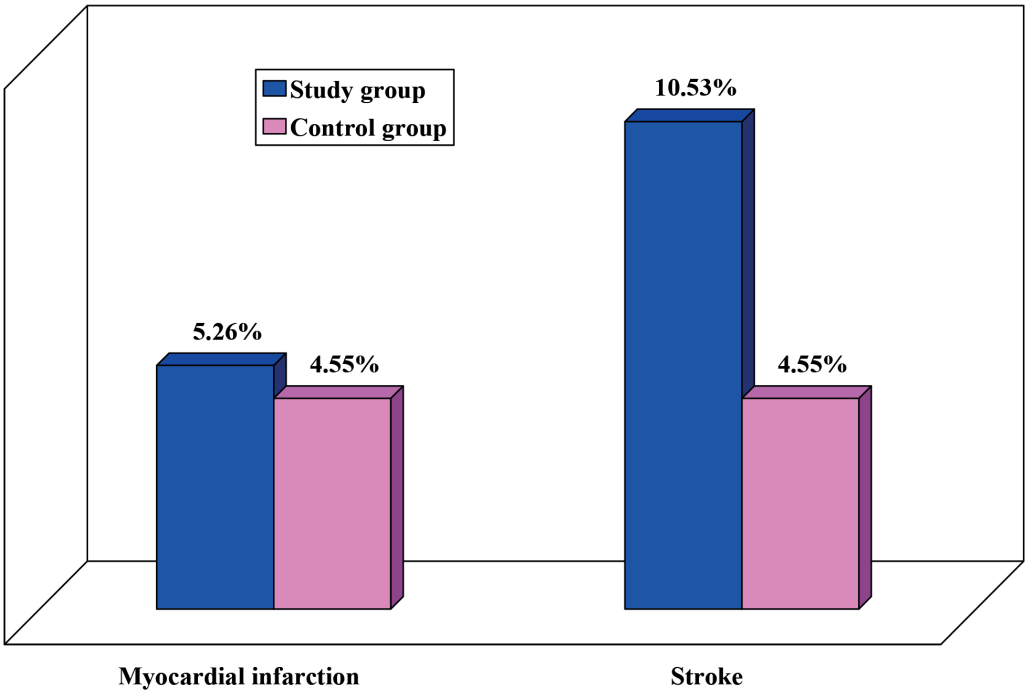

Figure 3. Distribution by major events

Table 3. Distribution by associated diseases and treatment

Associated diseases	*Study group*		*Control group*	
	No.	%	*No.*	%
Total	**19**	**100.00**	**22**	**100.00**
Heart diseases	18	94.74	21	95.45
Hypertension	18	94.74	21	95.45
Ischemic heart disease	14	73.68	12	54.55
Heart failure	5	26.32	3	13.64
Dyslipidemia	11	57.89	18	81.82
Liver disease	14	73.68	19	86.36
Lung disease	3	15.79	1	4.55
Thyroid damage	3	15.79	1	4.55
Depression	1	5.26	2	9.09
Other comorbidities	8	17.02	7	13.21
Treatment				
Angiotensin receptor blocker	4	21.05	3	13.64
Calcium channels blocker	6	31.58	5	22.73
Beta blocker	7	36.84	4	18.18
Diuretic	12	63.16	12	54.55
Statin	7	36.84	5	22.73
Fibrate	4	21.05	3	13.64
Antiplatelet drug	2	10.53	5	22.73

Evolution at 3 months

The mean BMI values decreased insignificantly in both groups (from 34.17 to 33.43 kg/m^2, p=0.519, respectively from 35.51 to 35.07 kg/m^2, p=0.786). Compared to the control group, the BMI value was insignificantly lower in the study group, both at the initial and at the final evaluation (34.17 vs 35.51 kg/m^2, p=0.337, respectively (33.43 vs 35.07 kg/m^2, p=0.244).The effect of dietary probiotic administration on BMI was small in the study group (ES=0.21) and insignificant in the control group (ES=0.08) (Figure 4).

Dietary follow-up and/ or probiotic administration resulted in weight loss in 73.68% of patients in the study group and in 68.18% of patients in the control group. The weight loss was significantly higher in the study group compared to the control group (2.05 vs 1.18 kg, p=0.011) (Table 4).

The mean values of systolic/diastolic BP decreased insignificantly, both in the study group (from 140.21 to 137.42 mmHg, p=0.618, respectively from 85.32 to 84.42 mmHg, p=0.861), and in the control group (from 142.23 to 140.45 mmHg, p=0.752, respectively from 84.95 to 84.50 mmHg, p=0.866). At both evaluations, initial and at 3 months, there were no significant differences between the two groups, in terms of both BP (systolic and diastolic); moreover, the effect on systolic and diastolic BP was insignificant (ES=0.16 vs ES=0.09, respectively ES=0.06 vs ES=0.04) (Figure 5).

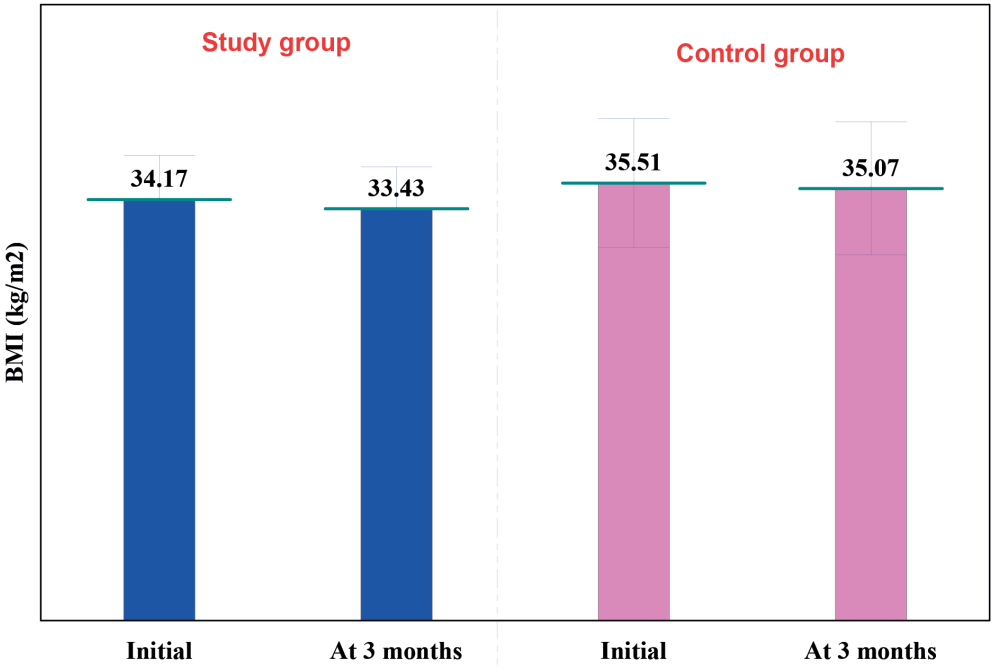

Figure 4. BMI evolution

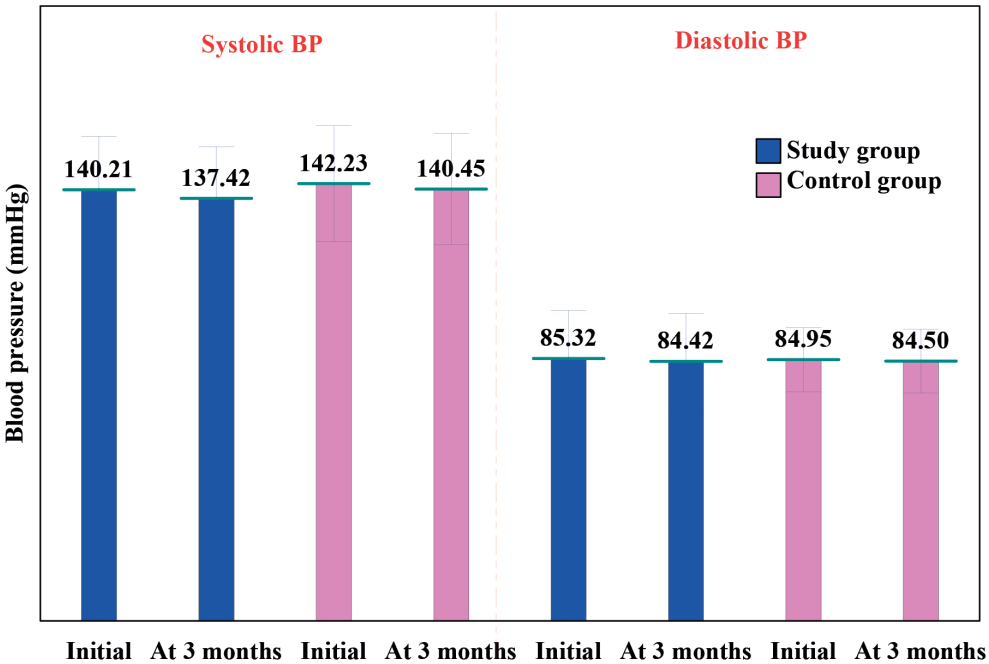

Figure 5. Blood pressure evolution

Table 4. Evolution of body weight (p=0.11)

Weight status	*Study group*		*Control group*	
	No.	%	*No.*	%
Increased weight	3	15.79	5	22.73
Unchanged weight	2	10.53	2	9.09
Decreased weight	14	73.68	15	68.18
Weight loss at 3 months	2.05±1.06		1.18±1.02	

Mean blood glucose values decreased insignificantly in both groups (from 143.21 to 135.32 mg/dL, p=0.414, respectively from 148.36 to 145.32 mg/dL, p=0.706). In both evaluations the mean blood glucose value was insignificantly lower in the study group compared to the control group (143.21 vs 148.36 mg/dL, p=0.577 and 135.32 vs 145 mg/dL, p=0.245). The effect of dietary probiotic administration on blood glucose was small in the study group (ES=0.26) and insignificant in the control group (ES=0.10). The mean values of HbA1c decreased insignificantly in both groups (from 7.65 to 7.30 %, p=0.428, respectively from 8.35 to 8.10 %, p=0.695). In both evaluations, the mean value of HbA1c was insignificantly lower in the study group compared to the control group (7.65 vs 8.35 %, p=0.220, respectively 7.30 vs 8.10 %, p=0.160). The effect of dietary probiotic administration on HbA1c was small in the study group (ES=0.26) and insignificant in the control group (ES=0.12) (Figure 6).

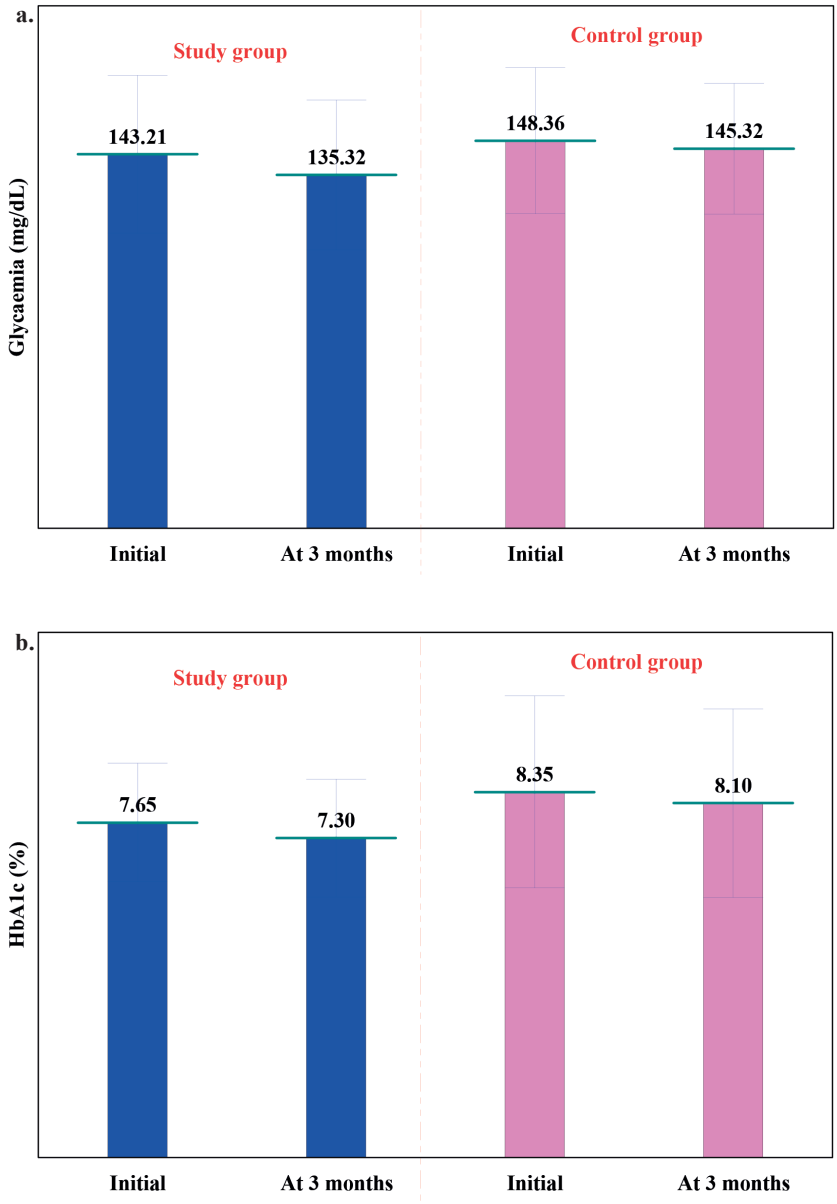

Figure 6. a. Glycaemia and **b.** HbA1c evolution

The mean cholesterol values decreased insignificantly in both groups (from 176.79 to 165.47 mg/dL, p=0.309, respectively from 183.18 to 179.09 mg/dL, p=0.753), being insignificantly lower in the study group compared to the control group, in both evaluations (176.79 vs 183.18 mg/dL, p=0.600, respectively 165.47 vs 179.09 mg/dL, p=0.258). The effect of dietary probiotic administration on cholesterol was small in the study group (ES=0.33) and insignificant in the control group (ES=0.10). Mean LDLc values decreased insignificantly in both groups (from 105.00 to 99.26 mg/dL, p=0.563, and from 117.86 to 111.23 mg/dL respectively, p=0.476). In both evaluations, the mean LDLc value was

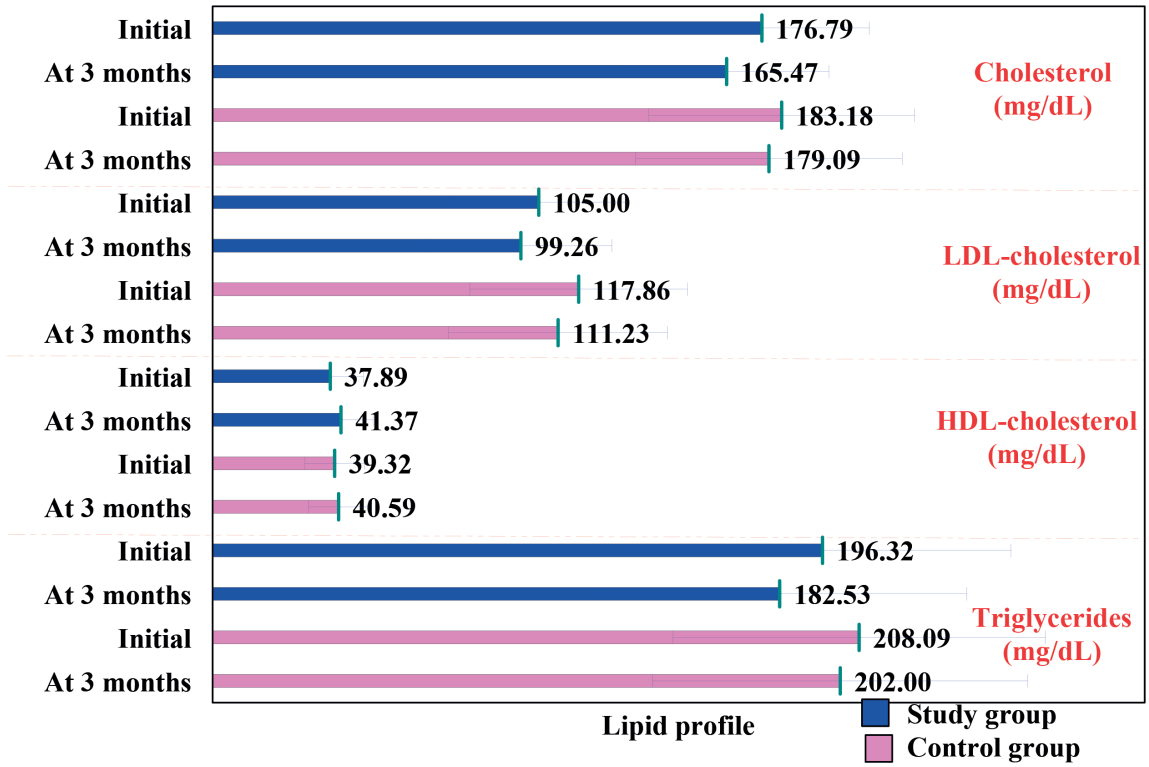

Figure 7. Lipid profile evolution

insignificantly lower in the study group compared to the control group (105.00 vs 117.86 mg/dL, p=0.160, respectively 99.26 vs 111.23 mg/dL, p=0.242). The effect of dietary probiotic administration on LDLc was small in the study group (ES=0.20) and insignificant in the control group (ES=0.19). The mean values of HDLc increased insignificantly in both groups (from 37.89 to 41.37 mg/dL, p=0.316, respectively from 39.32 to 40.59 mg/dL, p=0.663). At the initial evaluation, the mean value of HDLc was insignificantly lower in the study group compared to the control group (37.89 vs 39.32 mg/dL, p=0.660), and at the evaluation at 3 months it was insignificantly higher (41.37 vs 40.59 mg/dL, p=0.805). The effect of dietary probiotic administration on HDLc was small in the study group (ES=0.32) and insignificant in the control group (ES=0.13). Mean triglyceride values decreased insignificantly in both groups (from 196.32 to 182.53 mg/dL, p=0.487, respectively from 208.09 to 202.00 mg/dL, p=0.739).

In both evaluations, the mean triglyceride value was insignificantly lower in the study group than in the control group (196.32 vs 208.09 mg/dL, p=0.537, respectively 182.53 vs 202.00 mg/dL, p=0.309). The effect of dietary probiotic administration on triglycerides was small in the study group (ES=0.23) and insignificant in the control group (ES=0.10) (Figure 7).

Discussion

MS has a multifactorial etiology, with diverse associations between factors such as genetic predisposition, behaviours, diet and environment. Physiological risk factors (including excessive adipose tissue, lipo-toxicity, increased cortisol, systemic inflammation, increased oxidative stress) are linked to pathogenesis and production of metabolic disorders (i.e. T2DM, dyslipidemia, hypertension, non-alcoholic fatty liver disease, etc)[20]. These scenarios also result in MS pathophysiology, increasing the risk of CVDs[21].

Increasing evidence underlines the gut dysbiosis impact on the pathogenesis of metabolic disorders like MS, T2DM and obesity. Studies highlighted the connection between gut microbiome diversity and richness decrease and increased risk for developing insulin resistance-based illnesses in obese subjects, therefore gut microbiome became important in improving metabolic disorders determined by lifestyle[22,23]. Clinical results sustain the idea that improving gut microbiota with probiotics is efficient in preventing and managing diabetes, having favorable influence on the T2DM patients' metabolic control[23,24].

Probiotics administered orally were demonstrated to lower serum glucose levels and improve lipid metabolism in animal models[25]. The probiotics

action on serum glucose and lipid profiles in human patients were also studied but with inconsistent findings[26]. The differences may result from the use of various probiotic strains. Multispecies probiotics seem to be more efficient on metabolic disorders[27].

Probiotics intake in MS patients have shown improvements in their BMI, BP, lipid profile and glucose metabolism, as Tenorio-Jiménez et al. mentioned in their meta-analysis. Although there is a diversity of results obtained in the published data that were studied, the administration of probiotics to patients with MS improves some clinical parameters, leading to a decrease in the inflammatory biomarkers (interleukin-6, soluble vascular cell adhesion molecule 1, vascular endothelial growth factor, tumour necrosis factor-α, and thrombomodulin)[28].

Significant decrease of at least one of the components generally determines reversal of the MS[29]. In the present study, the administration of dietary probiotics to diabetic patients had a major impact on body weight, with weight loss being significantly greater in the probiotic group than in the diet-only group. The effect of dietary probiotic administration on blood glucose and HbA1c was small (ES=0.26) but better than in the control group (ES=0.10). The effect of probiotics on glucose metabolism in various mice models and suitable human epithelial cell lines was assessed by several studies[30,31] all highlighting a decrease in fasting or postprandial glucose and HbA1c after probiotics intake. Probiotic yogurt intake was demonstrated to considerably enhance glucose metabolism in some studies on probiotics, while other studies did not present any improvements[14,32].

The main risk factors for CVDs are increased BP, T2DM and increased levels of total cholesterol[33]. To decrease the risk of severe cardiovascular events it is important to lower the LDLc/ total cholesterol increased values[34]. The risk of cardiovascular illnesses increases by 20% in women and 24% in men, with every 1 mmol/L increase in total cholesterol levels[5]. In this study, the effect of dietary probiotic administration on cholesterol was small in the study group (ES=0.33) and insignificant in the control group (ES=0.10). Positive changes of various parameters determine a decrease in the intensity of complications generated by T2DM, and implicitly lower mortality, though the reduced changes are not clinically significant.

The severity of some MS components was decreased in the period when probiotics were administered. Though the differences in the components' values were not statistically significant, using greater number of subjects the significance may increase.

The small number of patients and the short period of patients' monitoring are the main limitations of this study, which presents intermediate results of a complex ongoing research that evaluates the effects of manipulating the intestinal microbiome with probiotics in patients with T2DM and MS. Nevertheless, further research is needed to demonstrate whether probiotics can be used as prevention or therapeutic agents in patients with MS or diabetes.

Conclusions

According to the results of this study, the probiotics intake is efficient in managing the metabolic profile in patients with SM and T2DM. Data analysis indicated a significant effect only on body weight. The response to treatment of both systolic and diastolic blood pressure values, cholesterol, triglyceride, HDLc, LDLc levels, HbA1c and serum glucose values was better in the probiotics group, the differences being insignificant in the control group. The associated effects of various antidiabetic treatments and probiotic strains need further investigation.

Authors Contribution:

Conceptualization, R.A.C.A., D.M.T., C.M.V. and S.B.; methodology, D.M.T., A.L.P. and S.B.; software, R.A.C.A., T.C.G., D.M.T. and S.B.; validation, D.M.T and S.B..; formal analysis, R.A.C.A., T.C.G., A.A., G.A. and S.B.; investigation, R.A.C.A., T.C.G., D.M.T and S.B.; resources, R.A.C.A., A.A and G.A..; data curation, R.A.C.A; writing–original draft preparation, R.A.C.A., D.M.T., A.L.P, C.M.V. and S.B.; writing–review and editing, R.A.C.A., D.M.T., A.L.P, C.M.V. and S.B.; visualization, S.B.; supervision, S.B.; project administration, S.B. All authors have read and agreed with the final version of this article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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None

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