OFFICIAL TITLE:

THE EFFECTS OF PROBIOTICS ON METABOLIC BIOMARKERS, INFLAMMATION, AND THE ANTIOXIDANT SYSTEM IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background and Objectives

Type 2 diabetes mellitus (T2DM) prevalence has increased alarmingly due to obesity and sedentary lifestyle trends [1, 2]. T2DM causes end-stage renal failure, blindness, and lower limb amputations through progressive atherosclerosis, leading to 3-fold increased cardiovascular events and 2-fold increased cerebrovascular events [3, 4].

Recent data suggest that the gut microbiota plays a key role in development and progression of T2DM. This complex ecosystem of at least 10^{14} different bacteria affects energy homeostasis through the microorganism-gut-brain axis [5, 6]. Gut microbiota changes can alter enteroendocrine signals and gut hormones that regulate β-cell function, insulin secretion, and energy homeostasis [7]. The gut microbiota can affect the host's inflammation response and energy metabolism, in other words, alteration of the gut microbiota can affect glucose and lipid metabolism and insulin action. One of the most effective methods of maintaining the balance of the gut microbiota is the use of probiotics, defined as live microorganisms that, when given in adequate amounts, show host-specific benefit [8]. Recently, a growing number of studies have found that probiotics can alter gut flora, improve total cholesterol (Total-C) and Low-Density Lipoprotein Cholesterol (LDL-C) levels [9–11], and reduce blood glucose levels and insulin resistance [12, 13]. Nowadays, oxidative stress is also suggested to be a mechanism underlying diabetes and its complications. Antioxidant mechanisms of probiotics include scavenging of reactive oxygen species, metal ion chelation, enzyme inhibition, reduction activity, and inhibition of ascorbate autoxidation [14].

Modification of gut microflora with probiotics may be seen as a novel tool for the regulation of glucose metabolism and improvement of oxidative stress in T2DM. Increased severity of beta cell dysfunction was positively correlated with increased high-sensitivity C-Reactive Protein (hs-CRP) concentrations [15].

Although T2DM is recognized as a chronic inflammatory disease, the role of probiotics and prebiotics in its management remains insufficiently explored, and existing evidence regarding their overall efficacy in health and disease is inconsistent [16].

Regulation of gut microbiota through dietary interventions (e.g., probiotic intake) may be beneficial in reducing inflammation [hs-CRP and Ceruloplasmin (Cp)] and oxidative stress [Glutathione (GSH) and Malondialdehyde (MDA)], as well as regulating glucose and lipid metabolism in T2DM. The aim of this study was to investigate the effects of probiotics on glucose and lipid metabolism, inflammation and antioxidant system in patients with T2DM.

Methods

Trial design

This was a single center, prospective controlled study conducted at the Internal Medicine Outpatient Clinic of Ege University Faculty of Medicine between July 2020 and June 2023. The study included a screening visit, a baseline visit (D0) and one follow-up visit during a 60-days supplementation period. The primary outcomes of the study were defined as the effects of the intervention on inflammatory markers, specifically focusing on a significant decrease in hsCRP and Cp levels, and oxidative stress markers, including a significant reduction in MDA levels and a significant increase in reduced GSH levels. Secondary outcomes included assessing the metabolic and lipid profile changes, which specifically targeted a significant decrease in Fasting Blood Glucose (FBG), Postprandial Blood Glucose (PPBG), and Glycated Hemoglobin (HbA1c), alongside a reduction in LDL-C and Triglyceride (TG) levels, and an increase in High-Density Lipoprotein Cholesterol (HDL-C) levels.

Trial participants

Power analysis was performed and the sample size was calculated as 79 with a statistical power of 80% and an effect size of 0.35. Since two groups were to be studied, 80 patients were planned to participate in the study to equalize the number of participants.

This study included adults aged 35-65 years who were diagnosed with T2DM according to American Diabetes Association guidelines, who volunteered to participate in this study. Exclusion criteria were as follows: Use of any systemic antibiotics, multivitamins, minerals, herbal medicines, prebiotic, probiotic and postbiotic supplements in the last 3-6 months, any inflammatory bowel disease, severe renal dysfunction or hepatic dysfunction, immunodeficiency diseases, acute infection, rheumatoid arthritis, cancer history, history of alcohol abuse or drug dependence, pregnant or lactating women. Dietary habits, physical activity, and glucose-lowering therapy of the participants were not intervened during the study.

Participants were allocated sequentially according to order of presentation (alternate allocation). According to the order of presentation to the outpatient clinic, the first patient who met the inclusion criteria was included in the probiotic group and the second patient who presented to the outpatient clinic was included in the control group. Patients in the intervention group were given a probiotic supplement containing *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis* and *Lactobacillus paracasei*, each containing 1.25 billion live microorganisms, without vitamins and minerals, twice a day in addition to their current treatment for 12 weeks (Probiotic group). Patients in the control group were not given any

additional treatment and were allowed to continue their antidiabetic treatment for 12 weeks (Control group). Participants taking probiotics were instructed to discontinue probiotic intake if they experienced any serious adverse events requiring intervention. The probiotic product was supplied without placebo equivalent, and due to COVID-19-related supply limitations no identical-appearing placebo powder could be obtained. Sociodemographic data and medical data were recorded for all participants.

Anthropometric Measurements

Body weight, height, waist (WC) and hip circumferences (HC) were collected following the standardized procedures recommended in the World Health Organization Expert Committee Guidelines for physical examination at baseline and after 12 weeks [17]. Body mass index (BMI) (kg/m^2) and waist-to-hip ratio (WHR) (WC/HC) were calculated.

A Food Frequency Questionnaire (FFQ)

A Food Frequency Questionnaire was employed to determine how often specific food groups or individual food items were consumed. The frequency of consumption was recorded on a daily, weekly, biweekly, or monthly basis, providing insight into participants' overall dietary patterns. The FFQ is a commonly used tool to assess the relationship between dietary habits and health outcomes and can be adapted in various ways depending on the researcher's objectives. Food items can be listed individually or grouped according to categories such as full-fat, low-fat, or fat-free options [18]. The FFQ consisted of 68 individual food items clustered into five main food groups. In this study, the FFQ included five main food groups: dairy products, meat-egg-legumes, fruits and vegetables, cereals, fats and sweets, and beverages. Participants were asked to indicate how often they consumed each item (e.g., 1-2 times daily, 1-3 times weekly, 4-6 times weekly, once every two weeks, once monthly, or never), which enabled the calculation of their food consumption frequency over the past month.

The International Physical Activity Questionnaire (IPAQ)

When applying the IPAQ, participants were asked about their vigorous physical activity, moderate physical activity, and walking duration in the last seven days. Additionally, the number of days per week they performed these activities and time spent sitting were determined [19]. The Turkish validity and reliability study of the questionnaire was conducted by Öztürk [20]. Scoring was calculated as Metabolic Equivalent of Task (MET) scores from the sum of duration and frequency of moderate activity, vigorous activity, and walking [21]. Categories:

• Category I: Inactive individuals (<600 MET-min/week)

• Category II: Minimum active individuals (600-3000 MET-min/week)

• Category III: Very active individuals (>3000 MET-min/week)

Ferrans and Powers Quality of Life Index© Diabetes Version- III

The scale was developed by Ferrans and Powers to measure quality of life in diabetic patients, consisting of 34 satisfaction and 34 importance items. It includes 5 subscales: Total quality of life, Health and Functioning, Social and Economic, Psychological/Spiritual, and Family scores [22]. Higher scores indicate better quality of life. The Turkish validity study was conducted by Ozer [23].

Eating Attitudes Test (EAT-26)

The EAT-26 was developed for Garner et al.[24]. The test consists of 26 items, and the total score is between 0-53. A score of 20 and above is defined as "abnormal eating behavior" and a score less than 20 is defined as "normal eating behavior". The Turkish validity and reliability study was conducted by Erguney-Okumus and Sertel-Berk [25].

Biochemical measurements

Fasting blood samples were collected from the participants at baseline and week 12, following overnight fasting and after 2 hours of satiety. FBG, PPBG, HbA1c, Total-C, LDL-C, HDL-C, TG and hs-CRP were measured using an autoanalyzer. Serum MDA levels were determined by the thiobarbituric acid method [26], GSH levels by enzymatic recycling method [27] and Cp levels by colorimetric method [27] using a microplate reader.

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