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THE CLINICAL EFFECT OF PROBIOTIC-CONTAINING PRODUCT ON HEALTH INDICATORS AMONG A POPULATION OF CENTRAL ASIA

Almagul R. Kushugulova[1,2*], http://orcid.org/0000-0001-9479-0899
Sofia K. Forslund[3,4,5,6], https://orcid.org/0000-0003-4285-6993
Samat S. Kozhakhmetov[1,2], https://orcid.org/0000-0003-4358-7879
Ulykbek E. Kairov[1], http://orcid.org/0000-0001-8511-8064
Askhat B. Molkenov[1], http://orcid.org/0000-0003-2339-5204
Shynggys D. Sergazy[1,2], https://orcid.org/0000-0002-6030-620X
Saule A. Saduakhassova[2],
Gulzhanat S. Urazbayeva[7], https://orcid.org/0000-0001-6723-4598
Saltanat Ch. Beysembayeva[7],
Raushan Zh. Karabaeva[7],
Roza A. Bakenova[7],
Talgat S. Nurgozhin[8], https://orcid.org/0000-0002-8036-604X
Valery V. Benberin[7], https://orcid.org/0000-0002-7286-1593
Zhaxybay Sh. Zhumadilov[1, 9], https://orcid.org/0000-0003-3855-6429
Bork Peer[6], https://orcid.org/0000-0002-2627-833X

Institutes:
[1] National Laboratory Astana, Nazarbayev University, Nur-Sultan, Republic of Kazakhstan;
[2] Kazakhstan society of researchers of human microbiome", Nur-Sultan, Republic of Kazakhstan;
[3] Experimental and Clinical Research Center, a cooperation of Charité-Universitätsmedizin Berlin and Max Delbruck Center for Molecular Medicine, 13125 Berlin, Germany;
[4] Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, 10117 Berlin, Germany;
[5] Max Delbruck Center for Molecular Medicine in the Helmholtz Association, 13125 Berlin, Germany;
[6] The European Molecular Biology Laboratory (EMBL), Structural and Computational Biology Unit, Heidelberg, Germany;
[7] Medical Centre Hospital of President's Affairs Administration of the Republic of Kazakhstan, Astana, Republic of Kazakhstan;
[8] Asfendiyarov Kazakh National Medical University, Almaty, Republic of Kazakhstan;
[9] University Medical Center, Nazarbayev University, Nur-Sultan, Republic of Kazakhstan.

Abstract

Introduction. The main goal is to test the efficacy of a synbiotic product which consists of the selected probiotic strains (*Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum*), inulin, pectin, and fish collagen in adults diagnosed with metabolic syndrome.

Materials and methods. This double-blind placebo-controlled trial was conducted among 180 participants aged 25 to 75 years. Subjects with and without metabolic syndrome were randomly assigned to receive synbiotic or placebo daily for three months. 134 of 180 participants completed the study.

Results. Compared with the placebo group, the synbiotic group had significantly decreased total cholesterol (TC) from 5.16±0.95 to 4.86±0.86 mmol/L, due to lowering the levels of LDL and triglyceride ($p < 0.0005$). The present findings suggest positive influence of the tested synbiotic on controlling excess weight and in treating some metabolic disorders in adults.

Conclusion We conclude that the presented synbiotic formulation here has potential within efforts to reduce the risk and progression of the metabolic syndrome. This trial suggests beneficial effects of a synbiotic supplement for controlling excess weight, as well as the progression of some metabolic disorders, as determined from circulating levels of risk factor biomarkers among adults.

Key words: *synbiotic, metabolic syndrome, lipid profile.*

Резюме

КЛИНИЧЕСКИЙ ЭФФЕКТ ПРОБИОТИК-СОДЕРЖАЩЕГО ПРОДУКТА НА ПОКАЗАТЕЛИ ЗДОРОВЬЯ В ПОПУЛЯЦИИ ЦЕНТРАЛЬНОЙ АЗИИ

Алмагуль Р. Кушугулова[1,2*], http://orcid.org/0000-0001-9479-0899
София К. Форсланд[3,4,5,6], https://orcid.org/0000-0003-4285-6993
Самат С. Кожахметов[1,2], https://orcid.org/0000-0003-4358-7879
Улыкбек Е. Каиров[1], http://orcid.org/0000-0001-8511-8064
Асхат Б. Молкенов[1], http://orcid.org/0000-0003-2339-5204
Шынгыс Д. Сергазы[1,2], https://orcid.org/0000-0002-6030-620X
Сауле А. Садуахасова[2],
Гульжанат С. Уразбаева[8], https://orcid.org/0000-0001-6723-4598
Салтанат Ч. Бейсембаева[8],
Раушан Ж. Карбаева[8],
Роза А. Бакенова[8],
Талгат С. Нургожин[9], https://orcid.org/0000-0002-8036-604X
Валерий В. Бенберин[8], https://orcid.org/0000-0002-7286-1593
Жаксыбай Ш. Жумадилов[1, 10], https://orcid.org/0000-0003-3855-6429
Пир Борк[7], https://orcid.org/0000-0002-2627-833X

Institutes:
[1] National Laboratory Astana, Назарбаев Университет, Нур-Султан, Республика Казахстан;
[2] Казахстанское общество исследвоателей микробиома человека, Нур-Султан, Республика Казахстан;
[3] Центр экспериментальных и клинических исследований в кооперации с Шарите Университетская медицина Берлина и Центр молекулярной медицины Макс Дельбрука, 13125 Берлин, Германия;
[4] Шарите Университетская медицина Берлина, корпоративный член Свободного университета в Берлине, Гумбольдтского университета в Берлине и Берлинского института здоровья, 10117 Берлин, Германия;
[5] Центр молекулярной медицины Макса Дельбрюка при Ассоциации Гельмгольца, 13125 Берлин, Германия;
[6] Европейская лаборатория молекулярной биологии (EMBL), отдел структурной и вычислительной биологии, Гейдельберг, Германия;
[7] Больница Медицинского Центра Управления Делами Президента Республики Казахстан, Нур-Султан, Республика Казахстан;
[8] Казахский Национальный медицинский университет имени С. Д. Асфендиярова, Алматы, Республика Казахстан;
[9] University Medical Center, Назарбаев Университет, Нур-Султан, Республика Казахстан.

Введение. Основная цель - проверить эффективность синбиотического продукта, который состоит из отобранных пробиотических штаммов (*Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum*), инулин, пектин и рыбный коллаген среди взрослого населения с диагностированным метаболическим синдромом.

Материалы и методы. Это двойное слепое плацебо-контролируемое исследование было проведено среди 180 участников в возрасте от 25 до 75 лет. Субъекты с метаболическим синдромом и без были случайным образом распределены на прием синбиотика или плацебо. Продукт принимали ежедневно в течение трех месяцев. 134 из 180 участников завершили исследование.

Результаты. По сравнению с плацебо, в группе, принимавшей синбиотики, общий уровень холестерина (ТС) значительно снизился с 5,16 ± 0,95 до 4,86 ± 0,86 ммоль / л за счет снижения уровня ЛПНП и триглицеридов (p <0,0005). Полученные данные свидетельствуют о положительном влиянии тестируемого синбиотика на контроль избыточного веса и лечение некоторых нарушений обмена веществ у взрослых.

Заключение. Мы пришли к выводу, что исследуемый синбиотик имеет потенциал по снижению риска и прогрессирования метаболического синдрома. Настоящее исследование определило позитивный эффект применениясинбиотика в качестве вспомогательной терапии для контроля избыточного веса, а также прогрессирование некоторых метаболических нарушений.

Ключевые слова: *синбиотик, метаболический синдром, липидный профиль.*

Түйіндеме

ОРТА АЗИЯ ТҰРҒЫНДАРЫНЫҢ ДЕНСАУЛЫҚ КӨРСЕТКІШТЕРІНЕ ПРОБИОТИКАЛЫҚ ДӘРІЛІК ЗАТТАРДЫҢ КЛИНИКАЛЫҚ ӘСЕРІ

Алмагуль Р. Кушугулова[1,2*], http://orcid.org/0000-0001-9479-0899
София К. Форсланд[3,4,5,6], https://orcid.org/0000-0003-4285-6993
Самат С. Кожахметов[1,2], https://orcid.org/0000-0003-4358-7879
Улыкбек Е. Каиров[1], http://orcid.org/0000-0001-8511-8064
Асхат Б. Молкенов[1], http://orcid.org/0000-0003-2339-5204
Шынгыс Д. Сергазы[1,2], https://orcid.org/0000-0002-6030-620X
Сауле А. Садуахасова[2],
Гульжанат С. Уразбаева[8], https://orcid.org/0000-0001-6723-4598
Салтанат Ш. Бейсембаева[8],
Раушан Ж. Карбаева[8],
Роза А. Бакенова[8],
Талгат С. Нургожин[9], https://orcid.org/0000-0002-8036-604X
Валерий В. Бенберин[8], https://orcid.org/0000-0002-7286-1593
Жаксыбай Ш. Жумадилов[1, 10], https://orcid.org/0000-0003-3855-6429
Пир Борк[7], https://orcid.org/0000-0002-2627-833X

Institutes:
[1] **National Laboratory Astana Назарбаев Университеті, Нұр-Сұлтан, Қазақстан Республикасы;**
[2] **Адам микробиомасын зерттеушілердің қазақстандық қоғамы, Нұр-Сұлтан, Қазақстан Республикасы;**
[3] **Берлиннің Charite University Medicine және Макс Делбрук молекулалық медицина орталығымен бірлесе отырып эксперименттік және клиникалық зерттеулер орталығы, 13125 Берлин, Германия;**
[4] **Берлиннің Charite University Medicine, Берлиннің еркін университетінің, Берлиннің Гумбольдт университетінің және Берлин денсаулық сақтау институтының, 10117 Берлин, Германия;**
[5] **Макс Делбрюк Молекулярлық медицина орталығы, Гельмгольц қауымдастығы, 13125 Берлин, Германия;**
[6] **Молекулалық биологияның Еуропалық зертханасы (EMBL), Құрылымдық және есептеу биологиясы бөлімі, Гейдельберг, Германия;**
[7] **Қазақстан Республикасы Президенті Іс басқармасы Медициналық орталығының Ауруханасы, Нұр-Сұлтан, Қазақстан Республикасы;**
[8] **С.Ж. Асфендияров атындағы Қазақ ұлттық медицина университеті, Almaty, Қазақстан Республикасы;**
[9] **University Medical Center, Назарбаев Университет, Нур-Султан, Казахстан Республикасы.**

Кіріспе. Негізгі мақсат - таңдалған пробиотикалық штаммдардан (*Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum*), инулин, пектин және балық коллагенінен тұратын синбиотикалық өнімнің тиімділігін метаболикалық синдром диагнозы қойылған ересек тұрғындар арасында тексеру.

Материалдар мен әдістер. Бұл қосарлы жасырын плацебо-бақыланатын зерттеу 25-тен 75 жасқа дейінгі 180 қатысушылардың арасында жүргізілді. Метаболикалық синдромы бар және жоқ субъектілер синбиотик немесе плацебо қабылдауға кездейсоқ бөлінген. Өнім күн сайын үш ай ішінде қабылданды. 180 қатысушының 134-і зерттеуді аяқтады.

Нәтижелер. Плацебомен салыстырғанда, синбиотиктерді қабылдаған топта ТТЛП мен үшацилглицеридтер деңгейінің төмендеуі есебінен холестериннің (ЖХ) жалпы деңгейі 5,16 ± 0,95-тен 4,86 ± 0,86 ммоль/л-ге дейін (p <0,0005) айтарлықтай төмендеді. Алынған мәліметтер сыналатын синбиотиктің ересектерде артық салмақты бақылауға және зат алмасудың кейбір бұзылыстарын емдеуге оң әсерін дәлелдейді.

Қорытынды. Біз, зерттелетін синбиотиктің метаболикалық синдромның қатерін және үдеуін азайту әлеуеті бар деген қорытындыға келдік. Осы зерттеу артық салмақты бақылауда, сондай-ақ кейбір метаболикалық бұзылыстардың үдеуінде қосымша терапия ретінде синбиотикті қолданудың оң әсерін анықтады.

Негізгі сөздер: *синбиотик, метаболикалық синдром, липидтер бейіні.*

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Introduction

A recent meta-analysis performed by Shoumeng Yan and collegues demonstrated that the use of probiotics improves lipid metabolism [26]. However, among the populations of Asia, high heterogeneity of lipid concentrations was noted, which requires additional research. This study shows for the first time the effect of probiotic products on health indicators in metabolic syndrome among a population of Central Asia. The most active area of research in the market of functional products is probiotic fermented milk products. Such probiotic products are able to restore digestive functions, improve the tolerance of lactose, and increase the resistance to infectious agents by modulating the host microflora [7]. Majority of the studies indicate that the effectiveness of probiotics depends on properties of the specific strain used, furthermore, other recent advances suggest that efficacy will vary depending on the microbiome of the recipient as well as on the nature of disorders[10]. Since ancient times nomadic people consume probiotic functional foods, such as saumal (mare's milk), koumiss, etc. Koumiss is a fermented dairy product made from mare's milk, common among the people of Central Asia. Medicinal properties of koumiss have been claimed with regards to tuberculosis, malnutrition, diseases of the pulmonary and cardiovascular systems as well as the gastrointestinal tract, also for anemia, allergies, immunodeficiency and cancer[5]. A new synbiotic yoghurt named NAR developed in our laboratory, contains microbial strains isolated from the koumiss as a basis for probiotic action, and supplemented by prebiotic components was used to investigate possible properties on metabolic syndrome patients [15].

The present study aimed to evaluate the effects of the intake of this synbiotic yoghurt as an auxiliary in the treatment of metabolic disorders such as obesity, insulin resistance, diabetes mellitus and their comorbidities.

The metabolic syndrome can be characterized as a pandemic of the 21st century. It consists of four main components: obesity, high blood pressure, high blood glucose and high levels of hard digestible fatty acids in the blood. These components interplay and reinforce each other, and eventually lead to functional shifts in organs and systems of the human organism. Moreover, over time, it drives the progression of various other metabolic and cardiovascular diseases, with sudden, premature death as a possible endpoint. Patients diagnosed with metabolic syndrome have 3-6 times higher risk of coronary heart disease, myocardial infarction, stroke, and diabetes [3, 11].

According to the Kazakh Statistics Committee for 2017, the incidence of type 2 diabetes per 100 000 inhabitants in urban Kazakh districts was 204,2, compared with 188.4 in 2013[1]. In rural areas these figures were only 225.1 and 133.3 respectively, suggesting that factors of the urban lifestyle (diet, stress, etc.) strongly increase this risk. Epidemiological studies from 2016 showed, the overall prevalence of type 2 diabetes in Kazakhstan is 12.5% [23]. According to the data obtained in cross-sectional study [21] 17,9% (95% confidence interval (CI)) 14.7–21.1) and 15,3% (95% CI 10.7–19.9) women and men, respectively, are suffering from the metabolic syndrome in South Kazakhstan [9]. Previous studies reviewed in[4 , 25] have demonstrated that probiotics have beneficial effects in the prevention and treatment of metabolic disorders, through a variety of different mechanisms. These include modulation of host responses, inhibition of pathogens and beneficial interaction with the intestinal microbiota [6, 13].

Materials and methods

Study design and participant recruitment

We performed a randomized, double-blind, placebo-controlled trial in Nur-Sultan, Kazakhstan. The study was carried out following the principles of the Helsinki Declaration and good clinical practice guidelines. All participants signed the consent documents. The study protocol and consent documents were approved by the Ethics Committee of the Center for Life Sciences National Laboratory Astana Nazarbayev University with ethical approval number 311/2537 (IORG0006963) and registered in http://www.isrctn.com with number ISRCTN37346212.

Using the database of the Medical Centre Hospital of President's Affairs Administration of the Republic of Kazakhstan, Nur-Sultan, divisions of registered patients with diagnosed metabolic syndrome and patients without information on metabolic syndrome, the randomization was conducted by computer with two subsets of possible participants' results. As a result, 180 patients accepted the invitation. Eligible patients

were ranged between 25-75 years of age. Adults with an acute illness or fever at the time of recruitment, who tested positive for HIV, hepatitis B or C, or for human papillomavirus (HPV), or who had anamnesis for surgery of the gastrointestinal tract, including any bowel resection, or who were pregnant or breastfeeding, were not included in the study. In addition to the above, participants were excluded who had used the following medications during the last 6 months: antibiotics, antifungal, antiviral or antiparasitic drugs; corticosteroids; cytokines; commercial probiotics; or vaccines. The clinical trial duration was 90 days, and the participants of both groups received similar counselling for lifestyle modification regarding dietary habits. Enrolled patients (either with metabolic syndrome or healthy with regards to it) were randomly allocated by computer to the synbiotic group or the placebo group, respectively. The flowchart in Figure 1 shows the flow of the study from recruitment until its end. Among 162 participants who took part in the study, 134 individuals completed it whereas 13 volunteers stopped consuming the synbiotic during the study. 15 volunteers participated as a control group to exclude seasonal factors. All participants who completed the study exhibited good compliance with the synbiotic consumption and no adverse effects were reported.

Stool consistency and frequency

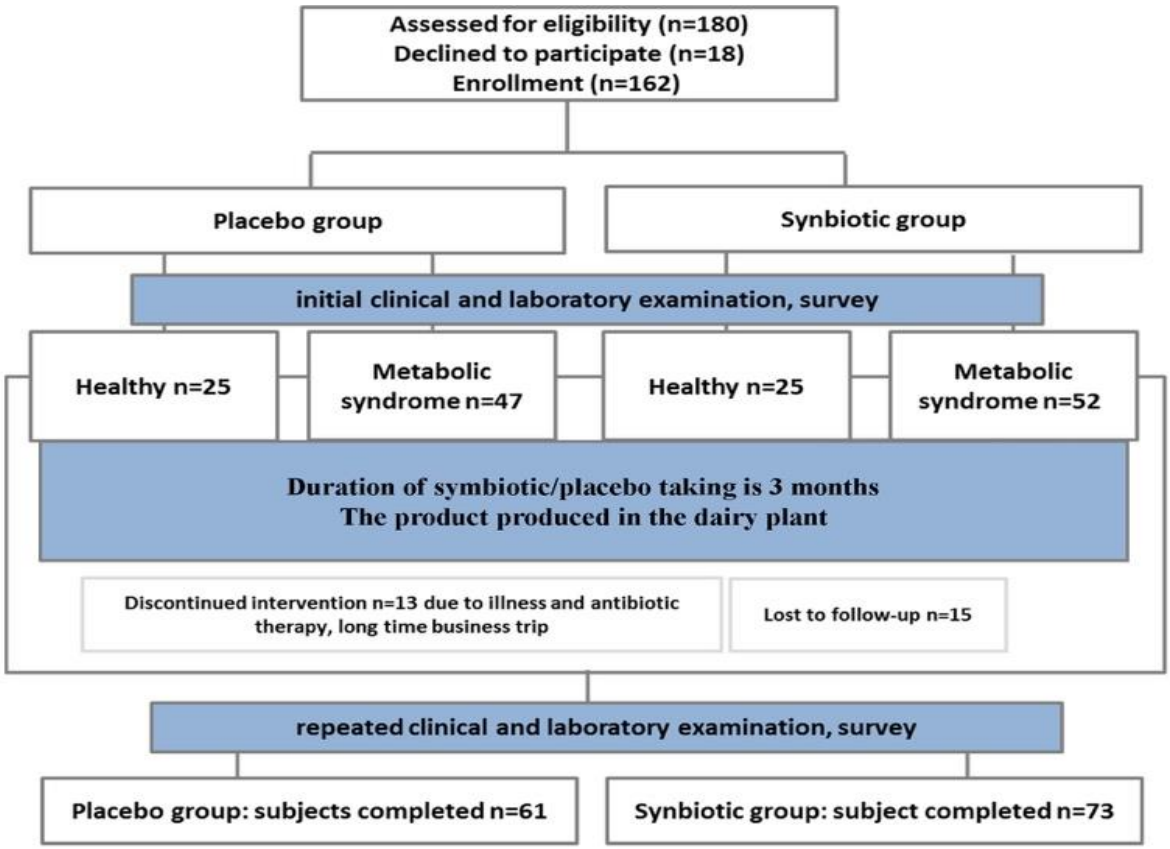

Figure 1. Overview of the design of the study.

Stool consistency was assessed according to the Bristol Stool Form Scale (BSS), classified into seven consistency categories with the highest scores corresponding to loose stools and fast transit, while lower scores represent hard stools and longer colon transit times. Stool frequency was assessed on a 5-point scale, where each point corresponds to the following: 1 - two-three times a day; 2 - once daily; 3 - every other day; 4 - 1 time per week; 5 - at least 1 time per week [8, 25].

Physical examination

All anthropometric measurements were made by the same trained person and under the supervision of the same physicians. Physical examination was conducted under standard protocols through calibrated instruments at the beginning and end of the trial. Bodyweight was measured with a digital floor scale to 100g accuracy, without shoes, and with minimum clothing. Height was measured to 1 mm accuracy, with a non-elastic tape. Waist and hip circumferences were measured with a non-elastic tape. Waist circumference was measured at a point midway between the lower border of the rib cage and the iliac crest at the end of normal expiration. Hip circumference was measured at the maximum girth of the buttocks, and the waist-to-hip ratio was calculated.

Biochemical measurements

Blood samples were taken from the antecubital vein. Blood glucose, glycosylated hemoglobin, TC, LDL, HDL, triglycerides, C-reactive protein were measured by Abbott Architect c8000 analyzer. Hemoglobin, erythrocytes, leukocytes, platelets were

analyzed by Sysmex XN3000. ESR was measured by Abbott Alifax test 1 in the Medical Center under the Office of the Kazakh President, Nur-Sultan, Kazakhstan.

Subpopulation composition of lymphocytes and its quantitative ratio was studied by flow cytometry:
CD3+CD19- (T-lymphocytes),
CD3-CD19+ (B-lymphocytes),
CD3+CD4+ (T-helper),
CD3+CD8+ (T-cytotoxic lymphocytes),
CD3-CD(16+56+) (NK-natural killer),
CD3+CD(16+56+) (TNK-lymphocytes),
CD3+HLA-DR+ (activated T-lymphocytes),
CD3-HLA-DR+ (activated B-lymphocytes),
CD4+/CD8+ ratio (immunoregulatory index).

Participant questionnaire

The participant questionnaire developed based on Food frequency questionnaire (FFQ) and included more than 200 questions, including issues related to nutrition, general health, past illnesses, as well as marital status, parenthood and education. The questions related to nutrition included a comprehensive list of different kinds of food and meals adapted according to common Kazakh dietary habits. These data were converted to macro- and micronutrient quantifications.

Synbiotic administration

Synbiotic and placebo were manufactured in the same dairy plant, packaged and labelled identically. Synbiotic containing bacteria to a concentration of 10^8 CFU/daily dose. It contains a combination *of S. thermophilus, L. lactis, L. plantarum, L. fermentum, L. acidophilus, B. longum*, and *B. bifidum*, derived from koumiss starter cultures and human isolates from donors (healthy individuals according to WHO definitions), with additional prebiotics added, namely inulin (2%), pectin (0.2%), and fish collagen (0.3%). It further contains vitamin C, A, D, E, B3, B12, B2, B1, as well as a complex of essential trace elements and amino acids. The placebo contained S. thermophilus only, with taste and smell identical to that of NAR. The participants were instructed to take two cups (200 gram) a day for three months of either synbiotic or placebo. Packaging the label of the synbiotic and placebo product was identical. Whereas patients and physicians allocated to the intervention group were not aware of the allocated arm, outcome assessors and data analysts were kept blinded to the allocation.

Stool sample collection

Stool samples were collected from both the synbiotic and placebo groups at baseline and on day 90 of the trial. Samples were kept refrigerated before they were transferred to the laboratory, where all samples were maintained at -80°C until they were used for metagenomic studies.

Statistical analysis

Statistical analysis was performed using the computer software Statistica 8.0. and RStudio 3.1.3. The difference between study groups was considered significant when $p<0.05$. Also, 95% confidence intervals (CI) were calculated for the outcome measures wherever indicated. Statistical analysis of the FFQ was performed using the FETA software[18]. Agglomerative hierarchical clustering has been performed by the cluster library using Ward's method.

Results

The characteristics of new synbiotic NAR

The synbiotic product is a complex of probiotic bacteria, and also contains inulin as a prebiotic component, pectin as a plant fiber, and fish amino peptide collagen as a source of essential amino acids and minerals. Previously probiotic strains were isolated from traditional functional products of koumiss, shubat, airan: *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum* [16]. In vitro and preclinical in vivo laboratory tests were performed. As a result, it was found that the synbiotic drink obtained according to this formula has antioxidant, immunomodulatory properties and reduces cholesterol due to the mechanisms of cholesterol degradation [17].

Independent control tests performed by the Kazakh Academy of Nutrition by the order of the manufacturer's plant, the agrocompany "Rodina", demonstrated the content of the following vitamins in mg /100 gram: B1- 0.061; B2 – 0.237; B3 – 0.134; B5 – 0.424; B6 – 0.043; C – 1.615; E – 0.163; as well as folic acid – 3.7 mcg; retinol – 16 mcg; Calciferol – 2.1 mcg. Along with vitamins, the synbiotic NAR contains calcium 64.5±12.9 mg/100g, magnesium 19.5±3.9 mg/100g, iron 4.35±0.87 mg/100g, zinc 1.96±0.14 mg/100g, copper 0.241±0.0007 mg/100g. The product also contains the full range of essential and non-essential amino acids.

Baseline

The demographic and clinical characteristics of the participants in the placebo and synbiotic groups were similar at baseline (Table 1).

Table 1.

An overview of the demographic data of participants.

	Synbiotic		Placebo	
	Metabolic syndrome n=52	Healthy n=25	Metabolic syndrome n=47	Healthy n=25
Age (y)	30-73	29-75	30-75	25-71
Gender (M/F)	10/42	5/20	8/39	8/17
BMI	30.70	22.91	30.42	22.46
Smoking habit (y/n)	5/47	0/25	7/36	7/18
Alcoholic drinks (y/n)	26/26	17/8	15/32	6/19
Coffee (y/n)	18/34	19/6	24/23	18/7

Converting the dietary information available for these samples into a uniform format, we calculated macro- and micronutrient intake for each participant. As Table 2 shows, the energy and nutrient consumption was likewise similar between the synbiotic and control groups at baseline.

Table 2.

An overview of energy and nutrient consumption in study participants.

	synbiotic	SE	placebo	SE	P (synbiotic/ placebo)
Data for patients with metabolic syndrome					
Energy, kcal	4388.11	444.53	5081.54	427.87	0.77
Protein, g	177.58	16.83	216.38	20.02	0.42
Fat - total, g	183.22	18.80	221.99	21.94	0.48
Carbohydrate - total, g	539.57	58.96	591.64	52.65	0.51
Cholesterol, mg	887.90	88.30	933.50	100.71	0.57
Data for healthy patients					
	synbiotic	SE	placebo	SE	P
Energy, kcal	4310.78	558.02	6309.67	423.52	0.59
Protein, g	164.35	21.52	206.64	16.53	0.67
Fat - total, g	198.26	37.59	294.09	25.29	0.37
Carbohydrate - total, g	497.90	54.03	756.77	53.67	0.80
Cholesterol, mg	711.59	77.21	1120.52	113.42	0.18

Shown are mean values and standard errors, as well as P-values of t-tests for the difference between synbiotic and placebo groups. No macronutrient differs significantly with regards to intake in the two study groups at baseline.

Clinical and laboratory data include anthropometric measurements, cardiovascular status (systolic and diastolic blood pressure, heart rate), lipid profile, levels of inflammatory markers, immunologic status, general analysis of feces (e.g. coprogram), and reports on stool consistency and frequency. The baseline of clinical and laboratory data of the 134 participants completing the study are shown in Table 3. The findings suggest significant differences in the main parameters among patients with metabolic syndrome and healthy, and clinically confirm that patients with metabolic syndrome are overweight and hypertension, as well as the majority of patients in the biochemical blood test, have elevated glucose and characteristic signs of a lipid disorder.

Table 3.

An overview of clinical and laboratory measurements for the 134 study participants. Shown are mean and standard errors, as well as P-values for t-tests of significant differences between healthy and metabolic syndrome subcohorts.

Variables	Total	Metabolic syndrome	Healthy	P(Metabolic syndrome/Healthy)
BMI	27.21±0.43	29.11±0.39	22.99±0.41	2.292e-16
Waist, cm	88.81±1.74	94.20±1.35	76.78±3.88	0.0001
Hip, cm	81.13±1.74	82.9±3.25	77.11±5.64	
Stool frequency	1.84±0.07	1.75±0.08	2.04±0.12	0.07901
Stool consistency (BSS)	2.70±0.19	2.68±0.21	2.73±0.42	
Hemoglobin, g/L	129.58±0.12	131.93±3.28	124.34±5.80	
Blood glucose, mmol/L	5.13±0.86	5.22±0.14	4.93±0.23	
Triglyceride, mmol/L	1.38±0.08	1.56±0.10	0.995±0.08	6.375e-05
TC, mmol/L	4.87±0.12	5.03±0.12	4.52±0.28	
HDL, mmol/L	1.20±0.05	1.15±0.05	1.30±0.11	
LDL, mmol/L	2.88±0.09	3.01±0.10	2.60±0.19	0.0778
Systolic BP	118.86±2.4	124.9±2.24	105.38±5.12	0.001315
Diastolic BP	76.6±1.4	80.08±1.21	68.84±3.38	0.00381
CRP	2.86±0.34	3.13±0.44	2.26±0.48	
Immuno-regulatory Index	1.19±0.04	1.19±0.05	1.17±0.08	

BMI: Body mass index **BSS**: Bristol Stool Form Scale **TC:** Total cholesterol
HDL: High density lipoprotein **LDL**: Low density lipoprotein **CRP**: C-reactive protein

From the initial results, the differences in the history of patients with metabolic syndrome and healthy volunteers were noted. Cluster analysis was performed according to personal and familial disease anamnesis, including 22 features such as personal history of pathologies of the digestive and circulatory systems, as well as cancer, and also familial history of cardiovascular and metabolic diseases (Fig 2).

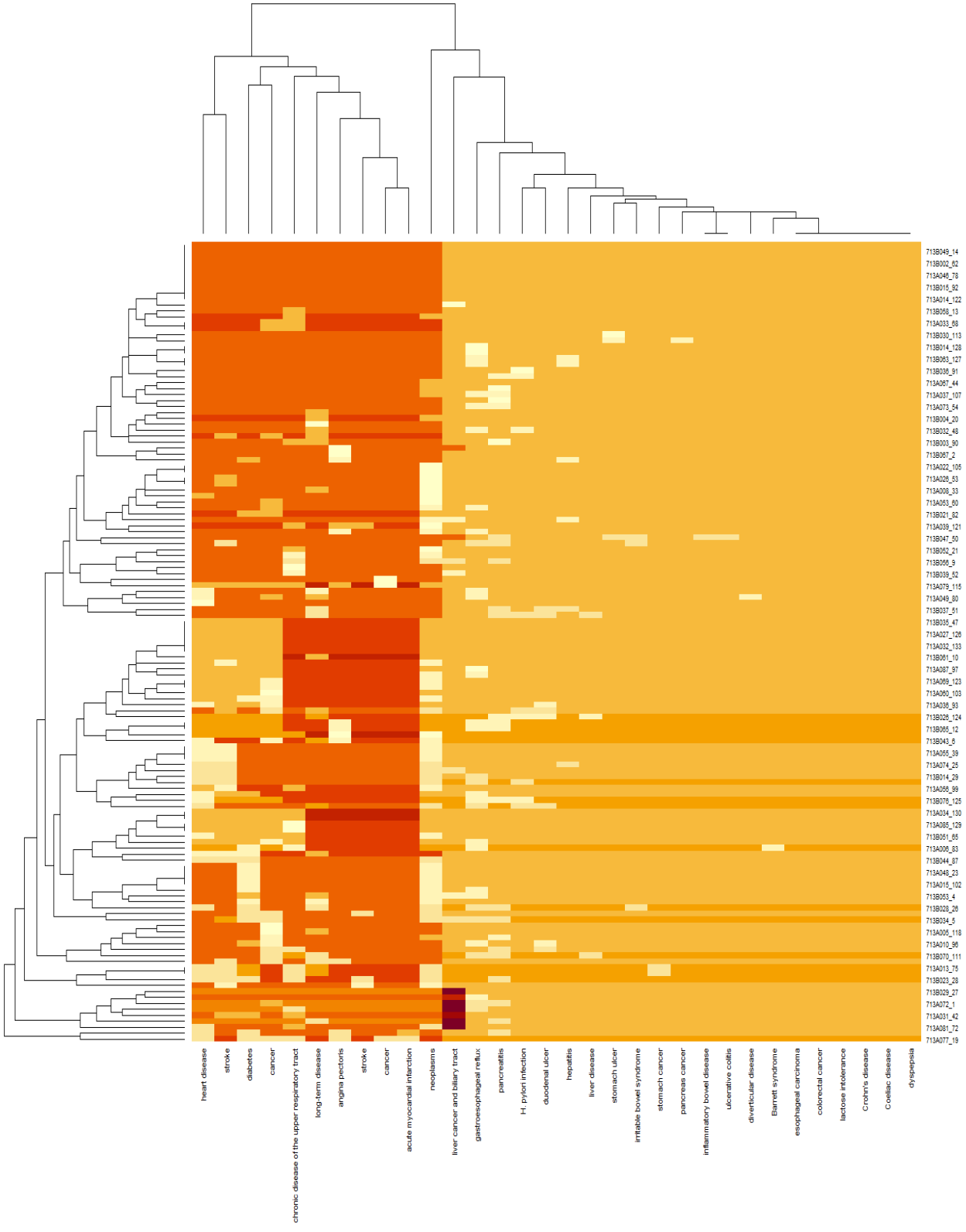

Figure 2. Cluster analysis.

As Figure 2 shows, samples shape two clusters, the first containing participants with a history of diseases of the digestive system, and the second containing individuals with a history of cancer, or who have a family history for diabetes or cardiovascular disease. It should be noted that the majority of the project participants have become residents of urban Nur-Sultan merely within the last two decades, due to the transfer of the capital and the recruitment of experts from all regions of Kazakhstan to this administrative centre.

Clinical aspects correlated to synbiotic administration

The use of synbiotics in the group with metabolic

syndrome improves health outcomes. One main indicator is body mass index which decreased from 29.11±0.39 to 27.97±2.55 (p<0,05). Similar results were obtained for change of waist circumstances down to 93.55±1.35 cm from 94.20±1.35 (p<1e-7), whereas in the placebo group neither feature changed significantly. On average, participants receiving synbiotics had lower systolic blood pressure (metabolic syndrome patients; 123.25±13.23 mm Hg; p<0.05) and lower diastolic blood pressure (77.50±8.16 mm Hg), compared to the placebo group.

Interesting results were obtained in the analysis of the frequency and characteristics of the stool. At the baseline evaluation, there was no significant difference between the mean stool frequency in the metabolic syndrome and healthy groups (table 3), the same held between the synbiotic and placebo groups (mean differences of 0.14 points). However, mean stool frequency increased significantly during the study in the synbiotic group (metabolic syndrome patients) compared with the placebo group (1.85±0.36, p = 0.005). Compared with the placebo group, the synbiotic group thus had improved stool frequency. Prior to the study 35.2%, participants of the synbiotic group with metabolic syndrome and 23.8% of the healthy had some stool disorders, such as constipation or diarrhoea. After applying the synbiotic the percentage of participants with normal stool increased from 64.8% to 88.2% in the metabolic syndrome group, and in the healthy group from 76.2% to 90.4%, while the rate in the placebo group increased from 81.5% to 84.2% in metabolic syndrome and from 61.1% to 72.2% in the healthy group.

There was no significant difference between the synbiotic and placebo groups in stool consistency at baseline (mean difference of 0.10 (95% CI: –0.18–0.39), p = 0.47). However, a significant difference (p = 0.08) was found in metabolic syndrome patients after 3 months of synbiotic consumption (3.79±1.03)[9], which meant that stools were becoming less hard/more soft.

The frequency and consistency of the stool reflect a very important indicator of a Bowel Transit Time. This indicator is important for the architecture of intestinal microbiome and microbiome dependent health indicators. Additionally performed quantification of the gut metagenomes of the participants by means of Illumina shotgun sequencing are demonstrated that the Kazakh population mostly characterized by Enterotype II. Full results of this analysis are reported elsewhere [5].

In brief, however, no significant changes in gut taxonomic composition were observed as a result of the synbiotic treatment. This suggests that considerable alteration of the gut community is not the main driver of the positive health effects of the treatment, though it is possible that subtle shifts in strain composition may underlie some of the results. Such shifts would not be possible to detect since the specific bacterial strains of the synbiotic as yet have not had their genomes sequenced. It is also possible that effects occur through effects on the host either directly or by modulating changes in diet e.g. through an effect on perceived satiety.

Also, an important point of the study was to carry out primary and re-survey of participants in the different seasons (September and January, respectively). This allows testing effects of climate and seasonal diet, which are important factors in Kazakhstan. As a general rule, winter is characterized by a sharp decline in temperature to -25-30 ºC, while in September it is warm with an average temperature of 15-20 ºC. Diet in winter is characterized by high-calorie food items, compared to the summer. Thus, our study also involved an additional control group of 15 participants who did not use any yoghurt, either synbiotic or placebo. Comparative analysis of repeated clinical and laboratory parameters for all groups are significantly different, with the main features in Table 4. It is clear that the NAR synbiotic improves anthropometric and biochemical parameters. On the other hand, the non-yoghurt control group have increased BMI, waist and thigh measurements in the wintertime as well as a worsened lipid profile, likely as an effect of seasonal diet.

Comparison of the effects of synbiotic and placebo consumption on serum biochemical factors are presented in Table 4. Compared with the placebo group, the synbiotic group after adjusting for weight reduction had significant improvements in lipid profile and blood glucose. This improvement was characterized by decreased TG (p < 2e-5) and elevated HDL-C (p<0.001) levels.

Table 4.

Effects of synbiotic consumption on serum biochemical factors. Shown are measurements before and after the study, and a test for significant change.

mmol/l	Synbiotic (before/after)	Placebo (before/after)
TC	5.03±0.12 /4.86±0.86	5.03±0.12/5.23±1.15
HDL-C	1.15±0.05 /1.22±0.28	1.15±0.05/*1.10±0.30
LDL-C	3.01±0.10 /1.12±0.28	3.01±0.10/3.30±0.59
Glucose	5.22±0.14 /4.74±0.69	5.22±0.14/5.27±0.87
HBA1C	4.43±0.97/ 3.89±0.75	4.43±0.97/*5.26±0.97

TC - Total cholesterol
HDL-C - high density lipoprotein
LDL-C - Low density lipoprotein
HBA1C - The hemoglobin A1c, glycated hemoglobin
HDL - High density lipoprotein

Consumption of synbiotic twice daily over 3 months significantly improved lipoprotein profiles for patients with metabolic syndrome and also helped them with weight management.

Values of TC after 3 months of the synbiotic consumption decreased from 5.03±0.12 to 4.86±0.86 mmol/L, due to lowering the levels of LDL-C 1.12±0.28 mmol/l, and triglyceride levels were reduced by up to 1.45±0.67.

Regarding indices of blood glucose and Hba1C (Glycated Haemoglobin) after treatment, these were reduced in synbiotic patients whereas no change was observed in the placebo group.

CRP is a non-specific marker, which of high levels are associated with risk factors for dyslipidemia, hypertension, diabetes mellitus, and obesity. Most participants with metabolic syndrome had elevated CRP levels prior to the study. 43 of the 52 synbiotic recipients had increased baseline CRP (mean 3.87, SD 3.64, max 20.30, min 0.40), and 46 of the 47 in the placebo group (mean 3.39, SD 2.48, max 10.00, min 0.60). In comparison with metabolic syndrome patients, only 20 of the 50 healthy participants had elevated CRP (mean 1.21, SD 0.77, p<1e-7). After treatment, the synbiotic group showed a significant reduction in CRP (mean 2.62, SD 1.69, max 8.10, min 0.60, p = 2e-7). Only 3 of the placebo participants had elevated circulating levels of CRP (mean 3.80, SD 3.26, max 12.10, min 0.60, p<0.09).

In our study, it was shown that CRP is positively correlated with body mass index by waist circumference and negatively correlated in HDL. At the beginning of the study, only 38 synbiotic recipients fall within the normal range of immunoregulatory index, which reflects the ratio of T-helper to T- cytotoxic lymphocytes (CD3+CD4+/CD3+CD8+) cells. In patients with metabolic syndrome, the risks of complications are associated with chronic inflammatory processes.

The study of the population and subpopulation composition of lymphocytes in recipients did not show statistically significant changes in the groups before and after taking the synbiotic. This demonstrates an immunoregulatory index; the ratio of T-helpers to T-cytotoxic lymphocytes (CD4 + / CD8 +) is maintained in all groups in the same range by the end of the study (1.1-1.24).

The CD3-(CD16+56+) cell counts (natural killer cells) in moda values show the multidirectional effect of the synbiotic on individuals with metabolic syndrome and healthy people. In the first group, these indicators by the end of the study were increased 2 times (before -11.20, after-24.80), in the second group they were reduced 2 times (before-9.50, after-4.0). When calculating average values, this indicator CD3-(CD16+56+) is aligned with the original figures.

To assess the presence of a chronic inflammatory process and the degree of its activity, the number of activated T-lymphocytes with the CD3+HLA-DR+ phenotype, TNK-cells with the phenotype CD3+(CD16+56+), and the number of activated B-lymphocytes with the CD3-HLA-DR+phenotype are included.

As is known, HLA-DR cells are a marker of late and prolonged activation of cells; they remain in the blood for a long time [20]. A decrease in this indicator in the peripheral blood indicates a decrease in the inflammatory reaction, an increase indicates the activation of the inflammatory process.

The results of our studies showed a decrease in CD3 + HLA-DR+ and CD3-HLA-DR + (activated T and B lymphocytes) by 6-8% in groups with metabolic syndrome treated with synbiotic. In patients with metabolic syndrome, there was a tendency to decrease TNK cells with the CD3 + (CD16 + 56 +) phenotype by 18% in comparison with the initial data. These results are based on statistical values of the mean and moda. But these changes are not significant, therefore, we can only talk about a tendency to decrease the number of markers of late activation of HLA-DR +, CD3 + (CD16 + 56 +) in people with metabolic syndrome when taking a synbiotic, that is, a tendency to reduce the chronic inflammatory process under the effect of taking a synbiotic. This is confirmed by the comparative data of the placebo group, where these figures did not change at the end of the study.

Discussion

One of the important points in the treatment of metabolic disorders is proper balanced nutrition and consumption the therapeutic food products as an adjunct therapy. Synbiotic is an affordable, easy to digest and delicious food that provides the body with important nutrients, which is particularly important for older people with maldigestion. Recent studies have shown that probiotic yoghurt consumption may have a positive role in the regulation of body weight as well as act to prevent cardiovascular diseases [14].

Our study is the first large clinical trial of probiotics in Kazakhstan and the first study on the new synbiotic yoghurt NAR. The synbiotic provides a complex effect due to the content of probiotic and prebiotic components. The uniqueness of this product lies in the fact that it consists of probiotic component strains isolated from a traditional Kazakh koumiss product. The prebiotic component contains long-chain and short-chain inulin polymers, which reduce intestinal transit time and also promote the growth of beneficial microflora. Primarily, the product is aimed at people with digestive problems. Therefore, it is rich in easily digestible trace elements and amino acids, essential for the gut microbiota to flourish. We adhered to the best practices for clinical trials to ensure that both investigators and patients were blinded to the study and that the data analysis was carried out by independent statisticians.

The potential of synbiotic in the treatment of metabolic disorders has not fully disclosed [12]. There are a small number of manuscripts confirming the effect of synbiotics and probiotics on atherosclerosis [22], obesity [14], diabetes [24] and cardiovascular diseases [2]. Our studies show a positive effect of a new synbiotic product on the human body by reducing weight, lowering TC, TG, LDL-C and normalization of digestion.

According to our data, the improvement of metabolic syndrome occurred after the use of synbiotic for 3 months. This is fully consistent with the data [12] claiming that this time is enough to improve the performance in metabolic syndrome. So in the study group, there was a decrease in BMI, systolic and diastolic pressure, there was an improvement in the lipid profile, blood glucose level. These results also agree with the data of other authors [19] investigating synbiotics and supplements in the treatment of the metabolic syndrome.

A metaanalysis of 23 randomized clinical trials [9] showed that the use of synbiotics helps to reduce body weight, however due to the limited number of participants in most studies (4-38 respondents), in some cases the short duration of the synbiotic consumption (4-8 weeks), a clear effect on the index bodyweight not detected. In present work, respondents used the synbiotic for 90 days, and we demonstrated a reliable effect not only on weight loss but also on body mass index (decreased from 28.36±2.34 to 27.97±2.55 ($p<1e-7$)).

The demonstrated effect of the synbiotic on a significant decrease in total cholesterol and low-density lipoproteins among respondents from the Central Asian population complements the meta-analysis, which included 12 clinical studies from Japan, India, Iran, Turkey, Denmark, Poland, Great Britain, Canada and Australia. Additionally, this study has a significant decrease in triglycerides. The improvement in the lipid profile is explained by the cholesterol-binding property of probiotics and as a consequence the decreased absorption of cholesterol in the intestine, as well as the ability of probiotics to reduce the intestinal-hepatic circulation of bile acids, promoting the induction by the liver of the re-synthesis of bile salts, thereby lowering cholesterol. Thus, the consumption of synbiotics can reduce the risk of complications from the cardiovascular system.

The present study has a few limitations that deserve discussion. First, during the study period, we did not control for the consumption of any other probiotic/synbiotic products or fermented dairy products. Second, we did not evaluate whether the diet of participants changed during the study period. A third limitation is that we have not conducted a genetic analysis of probiotic strains in the NAR synbiotic yoghurt; if genomes had been available we might have been able to more clearly control for effective compliance leading to presence of the probiotic strains in the gut, and thereby control the product consumption, whereas now we must rely on self-reported compliance regarding probiotic consumption. A fourth limitation of the present study is that most of the participants are females. While we have no reason to doubt the results generalize to males, we cannot explicitly show this using the current data making extrapolation to males less certain.

Conclusions

We conclude that the presented synbiotic formulation here has potential within efforts to reduce the risk and progression of the metabolic syndrome. This trial suggests beneficial effects of a synbiotic supplement for controlling excess weight, as well as the progression of some metabolic disorders, as determined from circulating levels of risk factor biomarkers among adults.

Conflicts of interest

There are no conflicts to declare.

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***Correspondence:**
Kushugulova Almagul Rakhimberliyevna - MD, D.M.Sc., Head of Human Microbiome Laboratory, Center for Life Sciences, National Laboratory Astana, Nazarbayev University, Nur-Sultan, Republic of Kazakhstan.
Mailing address: Republic of Kazakhstan, Nur-Sultan city, Kabanbay batyr ave, 53, block S1, 303.
E-mail: akushugulova@nu.edu.kz
Phone: +7 777 772 7813