Lactobacillus plantarum PCS 26 kot probiotično prehransko dopolnilo pri slovenskih bolnikih s presnovnim sindromom: pilotna klinična študija

Lactobacillus plantarum PCS 26 as a probiotic dietary supplement in Slovenian patients with metabolic syndrome: a pilot clinical study

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Ključne besede:
presnovni sindrom, debelost, holesterol, probiotične bakterije.

Key words:
metabolic syndrome, obesity, cholesterol, probiotic bacteria.

Članek prispel / Received
23.03.2015
Članek sprejet / Accepted
13.08.2015

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Izvleček

Namen: *V pilotni klinični študiji smo želeli ugotoviti učinke probiotičnega prehranskega dopolnila na fizikalno-antropometrične in biokemijske markerje pri slovenskih bolnikih s presnovnim sindromom.*

Metode: *V prospektivno, randomizirano, dvojno slepo in s placebom kontrolirano pilotno klinično študijo je bilo vključenih 16 prostovoljcev, ki so prejemali probiotično prehransko dopolnilo ali placebo vsak dan tri mesece. Prostovoljci so bili klinično pregledani pred in ob koncu prejemanja prehranskega dopolnila.*

Rezultati: *Ugotovljeno je bilo, da je prehransko dopolnilo pri skupini, ki je prejemala Lactobacillus plantarum PCS 26 v koncentraciji 1 × 10^9 CFU/dan (N = 8), izražalo pozitivne učinke na zmanjšanje obsega pasu (p = 0,043), na znižanje serumskega holesterola (p = 0,028) in na vzdrževanje nivoja adiponektina v serumu*

Abstract

Purpose: *In this study, we aimed to determine the effect of a probiotic dietary supplement on physical-anthropometric and biochemical markers in Slovenian patients with metabolic syndrome.*

Methods: *This prospective, randomized, double-blind, placebo-controlled pilot clinical trial involved 16 volunteers who consumed a probiotic dietary supplement over a period of 3 months. The volunteers underwent clinical assessments before and at the end of the period of consuming the dietary supplement or placebo.*

Results: *At a concentration of 1 × 10^9 colony-forming units per day (N = 8), the Lactobacillus plantarum PCS 26 supplement decreased waist circumference (p = 0.043), lowered serum cholesterol (p = 0.028), and maintained serum adiponectin levels (p = 0.629). In the group that received the placebo (N = 8), no differences*

(p = 0,629). Pri skupini, ki je prejemala placebo (N = 8), razlik v obsegu pasu (p = 0,750) in serumskemu holesterolu nismo opazili, ugotovili pa smo statistično značilno znižanje adiponektina v serumu (p = 0,036).

Zaključek: *Čeprav je pilotna klinična študija temeljila na majhnem raziskovalnem vzorcu, smo lahko ugotovili določene vplive probiotičnega seva Lactobacillus plantarum PCS 26. Probiotično prehransko dopolnilo je ugodno vplivalo na nekatere značilnosti presnovnega sindroma, kar lahko pripomore k lajšanju tega sindroma in ogroženosti zaradi srčno-žilnih bolezni. Rezultati naše študije nedvomno predstavljajo temeljno izhodišče za načrtovanje in izvedbo dolgoročne in obsežnejše klinične študije, s katero bi pridobili natančnejši vpogled v mehanizme delovanja probiotičnih bakterij v preprečevanju ali lajšanju sestavin presnovnega sindroma.*

were found in the waist circumference (p = 0.750) or serum cholesterol levels (p = 0.225); however, we found a statistically significant reduction of the serum adiponectin levels (p = 0.036).

Conclusion: *Although the number of patients examined was too low to draw any firm conclusions, dietary supplementation with the probiotic strain Lactobacillus plantarum PCS 26 exerted some favorable effects that could alleviate the symptoms of metabolic syndrome and reduce the clinical manifestations and risks of cardiovascular diseases. These results represent a starting point to carry out an extensive long-term clinical trial that would provide insight into the mechanisms of action of probiotic bacteria in preventing or relieving metabolic syndrome or its components.*

INTRODUCTION

Metabolic syndrome, also known as "syndrome X" or "insulin resistance syndrome," has been documented since the late 1980s, and its occurrence has been increasing worldwide, including in developing countries (1–3). The International Diabetes Federation (IDF) defines metabolic syndrome as a common occurrence of central obesity that is prevalent in specific ethnic groups, and includes at least two of the following parameters: raised fasting glucose (≥5.6 mmol/L) or known diabetes, raised fasting triglycerides (≥1.7 mmol/L), lowered high-density lipoprotein (HDL) (<1 mmol/L in men; <1.3 mmol/L in women), and raised blood pressure (≥130/85 mmHg) or known arterial hypertension (4, 5). Metabolic syndrome and its clinical manifestations are strongly associated with cardiovascular diseases such as myocardial infarction and stroke (6–12). The National Heart, Lung, and Blood Institute and the American Heart Association have identified three etiologic categories: obesity and fatty tissue dysfunction, insulin resistance, and molecules of hepatic, immunologic, and vascular origin (13). In the state of obesity, the visceral adipose tissue has a well-developed vascular system and is composed of large insulin-resistant adipocytes with the presence of inflammatory infiltrate; therefore, it is recognized as a source of potential pathogenic cytokines, which participate in low-grade inflammation (13–21). Previous studies have shown that the secretion of cytokines such as resistin, leptin, tumor necrosis factor alpha (TNF-α), interleukin-6, C-reactive protein (CRP), fibrinogen, and plasminogen activator inhibitor-1 (PAI-1) is raised in obese individuals, in individuals with diabetes, and in individuals with metabolic syndrome, and contribute to the pathogenesis of the disease (22–28). On the contrary, the secretion of adiponectin, which has anti-inflammatory effects, is lowered in these individuals (23–25, 27, 28). Probiotic bacteria are known to exert positive effects on health, and can have beneficial effects on raised serum cholesterol, arterial hypertension, inflammation, and metabolic diseases, which in turn leads to reduced risk of atherosclerosis and coronary heart disease (29–35).

The aim of the present study was to evaluate if probiotic bacteria can contribute to the prevention or

amelioration of metabolic syndrome in Slovenian patients. For this pilot clinical trial, we used the probiotic strain Lactobacillus plantarum PCS 26, which was isolated from traditional Slovenian cheese during the "PathogenCombat project" supervised by Professor Dr. Avrelija Cencic. A previous in vitro study had already confirmed that probiotic Lactobacillus plantarum PCS 26 does not cause adverse effects on the host, and exerts promising effects for the relief of metabolic syndrome (35).

MATERIALS AND METHODS

This pilot clinical study was approved by the National Medical Ethics Committee of Slovenia (9.7.2012; 70/05/12), and is registered at ClinicalTrials.gov (NCT02288572). Written informed consent was obtained from all patients, and we used a prospective, randomized, double-blind, and placebo-controlled study design.

Patients and research sample

We enrolled 16 volunteers who matched the following inclusion criteria: presence of central obesity and at least two of the following: raised fasting plasma glucose or diagnosed diabetes type 2, raised fasting plasma triglycerides, raised fasting plasma cholesterol levels, lowered plasma HDL levels, and raised blood pressure or diagnosed arterial hypertension; in addition to the IDF definition, we also added raised plasma low density lipoprotein (LDL) (≥4.9 mmol/L) to the inclusion criteria. The exclusion criteria were: planned invasive procedure, immunosuppressive therapy, lactose intolerance, and inflammatory bowel disease. The volunteers were randomly and evenly divided into the probiotic dietary supplement group and the placebo group (N = 8 for both). They were specifically asked to consume the dietary supplement each day for 3 months and to not change any dietary habits. The volunteers underwent clinical assessment at the Department of Gastroenterology, University Clinical Centre Maribor, before and at the end of the period of consuming the dietary supplement. The 16 volunteers fasted for 12 hours before the clinical assessment, and strenuous physical activity and alcoholic, caffeinated, or energizing beverages were prohibited during these 12 hours. The probiotic dietary supplement group (eight patients) included six (75%) male and two (25%) female volunteers, and the placebo group (eight patients) included four (50%) male and four (50%) female volunteers. The mean age of the volunteers was 48.3 ± 16.9 years.

Physical-anthropometric measurements

The physical-anthropometric measurements were performed using a Maltron BioScan 920-II body analyzer (Maltron International, Essex, UK), which measures the body composition based on impedance, resistance, and reactance. The measured parameters included body weight, body height, waist and hip circumference, body mass index (BMI), body mass without body fat, body fat, body volume, muscle mass, visceral fat surface, subcutaneous fat surface, and blood pressure.

Biochemical blood serum analyses

Venous blood was withdrawn from the cephalic, basilic, or median cubital veins and collected in 5 mL Vacuette tubes with separation gel and clotting factors (Greiner Bio-One, Kremsmünster, Austria). The blood serum analyses were performed at the Department of Laboratory Diagnostics, University Clinical Centre Maribor, and included serum glucose, serum cholesterol, serum triglycerides, serum LDL, serum HDL, high-sensitivity CRP (hsCRP), and TNF-α.

Serum adiponectin measurements

The serum adiponectin levels were measured using an enzyme-linked immunosorbent assay following the manufacturer's instructions (Invitrogen, Camarillo, CA, USA) at the Central Laboratory for Cell and Tissue Cultures, Faculty of Medicine, University of Maribor.

Dietary supplement

The dietary supplement was manufactured by the International Probiotic Company s. r. o. (Košice, Slovakia), and was in powder form. The vehicle consist-

ed of 70% (w/w) glucose, 15% (w/w) powder milk, 15% (w/w) whey, and Lactobacillus plantarum PCS 26. The placebo comprised the same ingredients, but without the probiotic bacteria. The daily dose was 1×10^9 colony-forming units per day, and was determined according to previously published studies (36–42). Based on the scientific literature, the daily dose in the present study corresponded to the lower quartile of previously tested daily doses. The manufacturer also provided product safety specifications and analyses. The supplement was taken every morning for the 3-month duration of the study, and was consumed in powder form or mixed with water, juice, or warm tea.

Statistical analyses

Data were analyzed with SPSS Statistics software (IBM Inc., Armonk, NY, USA) using paired t-tests and paired Wilcoxon signed-rank tests after the Shapiro–Wilk test of normality. P values ≤0.05 were considered statistically significant. Due to the pilot nature of the present study, Bonferroni corrections for multiple tests were not applied to any of the probability values.

RESULTS

Physical-anthropometric measurements

The physical-anthropometric measurements were performed before and at the end of the period of consuming the dietary supplement (Table 1). The measurements showed that the waist circumference was reduced by 3.57 cm on average (p = 0.043 at the beginning vs. end of the trial) in the probiotic treatment group. In the placebo group, the waist circumference did not change (p = 0.750). The other physical-anthropometric measurements did not show any statistically significant changes, but some tendencies towards positive effects of the probiotic dietary supplement were observed. In the probiotic group, the body weight was reduced by 0.5 kg on average (p = 0.993), and the same parameter increased by 0.92 kg (p = 0.345) in the placebo group. Similarly, the hip circumference measurements were reduced by 1.86 cm (p = 0.072) in the probiotic group and increased by 0.83 cm (p = 0.343) in the placebo group. The mean body fat mass was also reduced in the probiotic group and raised in the placebo group by 0.52 kg (p = 0.866) and 0.38 kg (p = 0.715), respectively. The total body volume was reduced by 0.53 L (p = 0.933) in the probiotic group and increased by 0.55 L (p = 0.465) in the placebo group. The BMI and muscle mass measurements showed no changes between the beginning and the end of the dietary supplement consumption in either groups. The subcutaneous fat measurements showed no changes in the probiotic group; conversely, this measure increased by 35 cm^2 on average (p = 0.225) in the placebo group. The visceral fat measurements also reduced by 24.74 cm^2 on average (p = 0.735) in the probiotic group and increased by 8 cm^2 (p = 0.593) in the placebo group.

Biochemical blood serum measurements

Venous blood was withdrawn at the beginning and at the end of the period of consuming the dietary supplement for biochemical analyses, and the results of these are shown in Table 2. Consumption of the probiotic dietary supplement decreased the total serum cholesterol by 0.3 mmol/L (p = 0.028). In the placebo group, the total cholesterol levels also decreased (0.61 mmol/L), but the difference did not reach statistical significance (p = 0.225). The serum HDL levels also decreased by 0.1 mmol/L (p = 0.043) and 0.04 mmol/L (p = 0.500) in the probiotic and placebo groups, respectively. This trend was also observed for the serum LDL and triglyceride levels. The serum LDL levels were reduced by 0.17 mmol/L (p = 0.248) and 0.32 mmol/L (p = 0.223) in the probiotic and placebo groups, respectively; further, the triglycerides diminished by 0.04 mmol/L (p = 0.798) and 0.6 mmol/L (p = 0.104) in the probiotic and placebo groups, respectively. No effect was observed on the glucose levels in either group. The hsCRP levels increased in both groups: by 0.17 mg/L (p = 0.866) and 0.30 mg/L (p = 0.102) in the probiotic and placebo groups, respectively. On the other hand, the adiponectin levels significantly decreased (2.21 µg/mL, p = 0.036) in the placebo group and remained stable in the probiotic group.

Table 1. *Physical-anthropometric measurements of body composition.*

Parameter	Dietary supplement	At the beginning Day 0	At the end Day 90	↓↑N
Body mass (kg)	Probiotic PCS 26	99.7; 73; 103; 116.2	99.2; 74.7; 103; 109	N
	Placebo	92.75; 60; 95.75; 109.2	93.7; 62; 95.4; 113.2	N
Waist c (cm)	Probiotic PCS 26	105; 94; 106; 113	**101.4; 94; 102*; 107**	↓
	Placebo	113; 95; 115; 125	112.7; 96; 113.5; 125	N
Hip c (cm)	Probiotic PCS 26	110.4; 97; 112; 116	108.6; 98; 111; 113	N
	Placebo	108.7; 95; 107.5; 127	109.5; 96; 109; 124	N
Body fat-free mass (kg)	Probiotic PCS 26	69.8; 56.23; 74.71; 81.8	69.8; 56.1; 73.1; 80.4	N
	Placebo	56.5; 39.93; 59.145; 68.2	56.7; 39.9; 57.7; 69	N
Body fat mass (kg)	Probiotic PCS 26	29.8; 16.04; 28.9; 40.5	29.3; 18.5; 31.5; 35.8	N
	Placebo	36.3; 20.07; 36.46; 52.49	36.6; 20.1; 36.9; 53.3	N
Body volume (L)	Probiotic PCS 26	96.6; 69.6; 99.5; 113.8	96.1; 71.7; 99.1; 105.8	N
	Placebo	91.6; 58.6; 93.2; 107.6	92.2; 58.6; 93.6; 111.8	N
BMI (kg/m^2)	Probiotic PCS 26	30.3; 23.8; 30.9; 34.7	30.2; 24.3; 31.5; 32.5	N
	Placebo	32.9; 23.7; 32.5; 38.8	33.1; 23.7; 32.95; 40.1	N
Muscle mass (kg)	Probiotic PCS 26	34.3; 24.54; 36.9; 41.	34.4; 24.8; 36.3; 40.4	N
	Placebo	26.5; 16.89; 27.6; 33.9	26.6; 16.9; 26.9; 34.44	N
Subcutaneous fat (cm^2)	Probiotic PCS 26	136.7; 80.2; 129.5; 207.7	134.2; 91.1; 147.4;157.9	N
	Placebo	133.3; 100.8; 135.6; 178	167.7; 102.6; 176.4; 234	N
Visceral fat (cm2)	Probiotic PCS 26	241.5; 161; 203.1; 452.7	216.7; 157; 202.5; 290.8	N
	Placebo	340.9; 198; 362.8; 455.5	348.7; 198.2; 362; 496.2	N
Mean blood p (mmHg)	Probiotic PCS 26	109.5; 98; 111; 120.6	106.9; 99.7; 105.7; 120	N
	Placebo	109.5; 91.3; 112.8; 121.7	106.9; 90; 106; 129	N

*Data are presented as the mean; minimum; median; maximum. *Statistical significance at $p \leq 0.05$; BMI: body mass index; c: circumference; p: pressure; ↓: significant reduction after treatment; ↑: significant increase after treatment; N: no change.*

DISCUSSION

The physical-anthropometric measurements showed that the waist circumference significantly decreased in the probiotic group. According to the IDF definition, waist circumference is one of the fundamental criteria for the diagnosis of metabolic syndrome (4, 5). We also observed a trend of decreasing visceral and subcutaneous fat surface in the probiotic group, which is in accordance with the waist circumference reduction; however, no statistical significance was confirmed for this measure. In keeping with a previous in vitro study (35), the results of the present pilot clinical study indicated that dietary supplementation with Lactobacillus plantarum PCS 26 reduces serum cholesterol levels. Hypercholesterolemia is a known risk factor for atherosclerosis and coronary heart disease. Studies involving animals have shown

Table 2. *Biochemical tests of peripheral venous blood.*

Parameter	Dietary supplement	At the beginning Day 0	At the end Day 90	↓↑N
Glucose (mmol/L)	Probiotic PCS 26	5.2; 4.6; 5.1; 6.1	5.2; 4.6; 5.1; 6	N
	Placebo	6.8; 4.7; 5.5; 11.2	6.5; 4.1; 4.9; 11.7	N
Cholesterol (mmol/L)	Probiotic PCS 26	5.7; 4.38; 5.46; 6.9	**5.4; 4.23; 5.22*; 6.48**	↓
	Placebo	5.05; 3.2; 4.7; 6.5	4.4; 3.12; 4.22; 5.56	N
Triglycerides (mmol/L)	Probiotic PCS 26	1.7; 0.7; 1.6; 2.8	1.7; 0.6; 1.8; 2.9	N
	Placebo	2.16; 0.8; 2.1; 3.4	1.6; 0.7; 1; 3.2	N
HDL (mmol/L)	Probiotic PCS 26	1.2; 0.9; 1.2; 1.55	1.1; 0.86; 1.19*; 1.3	↓
	Placebo	1.6; 1.05; 1.64; 1.98	1.5; 1.1; 1.5; 2.09	N
LDL (mmol/L)	Probiotic PCS 26	3.8; 3.1; 3.6; 4.6	3.6; 3; 3.6; 4.6	N
	Placebo	2.8; 1.3; 2.6; 3.9	2.4; 1.2; 2.4; 3.4	N
hsCRP (mg/L)	Probiotic PCS 26	1.2; 0.4; 1.1; 2.44	1.4; 0.47; 1.16; 3.08	N
	Placebo	2.2; 1.36; 1.8; 3.4	2.5; 1.4; 2.62; 3.4	N
Adiponectin (µg/mL)	Probiotic PCS 26	7.32; 3.92; 7.44; 9.44	7.23; 3.71; 7.55; 9.76	N
	Placebo	11.34; 5.15; 11.28; 17.64	**9.13*; 4.3; 9.07; 14.06**	↓

*Data are presented as the mean; minimum; median; maximum. *Statistical significance at $p \leq 0.05$. HDL: high-density lipoprotein; LDL: low-density lipoprotein; hsCRP: high-sensitivity C-reactive protein; ↓: significant reduction after treatment; ↑: significant increase after treatment; N: no change.*

that high cholesterol diets induce the development of cholesterol-loaded atherosclerotic plaques (43–47). Cholesterol levels are not included in the IDF definition; however, in accordance with the findings of the above studies, we assumed that lowering of cholesterol contributes to the amelioration of metabolic syndrome complications. A similar study with Lactobacillus plantarum also showed a significant reduction in serum cholesterol levels (48). In addition to the cholesterol reduction, Barreto and colleagues also reported a significant reduction in glucose and LDL serum levels, which is contrary to our findings. This discrepancy could be due to the smaller number of enrolled subjects in our study; we only observed a trend of LDL and triglyceride reduction in both groups, without statistical significance. The only negative effect of the probiotic dietary supplement was a reduction of the serum HDL levels. Although we did not specifically address the mechanism of action of this reduction, it could be a consequence of bile salt hydrolase activity and the subsequent decreased reabsorption of conjugated bile salts in the intestines, resulting in higher cholesterol consumption for de novo bile salt synthesis (49). This hypothesis is partially supported by the observed, but not significant, decrease of the serum LDL levels at the end of the dietary supplementation. On the other hand, a modest HDL decrease can also be beneficial because of the cholesterylester transfer protein (CETP), which is mainly bound to HDL in the blood serum. The activity of CETP is induced via high serum triglyceride levels, and leads to increased production of small, dense LDL (sd-LDL) and small, dense HDL (sd-HDL) (14, 50, 51). The latter could present a new point of research on the benefits of decreasing HDL, since sd-LDL particles are easily oxidized and recognized by macrophage scavenger receptors, which in turn, leads to atherogenic effects; additionally, sd-LDL particles also exert cytotoxic effects and can damage the arterial endothelium (52, 53). Further, sd-HDL particles are up to three-fold less effective at reverse cholesterol transfer in comparison with normal HDL, and have inferior anti-inflammatory and antioxidative properties (54). Adiponectin is a hormone that stimulates glucose consumption, oxidation of fatty acids in muscles, and insulin sensitiv-

ity in the liver; it also reduces glucose production in the liver, improves overall insulin sensitivity, and inhibits foam cell formation in arterial walls (23–25). In the present study, the adiponectin levels significantly decreased in the placebo group, but not in the probiotic group, which could lead to the worsening of metabolic syndrome and its related conditions.

CONCLUSION

The first limitation of the present pilot clinical study is the small sample size. However, we were still able to discern some effects of the probiotic strain Lactobacillus plantarum PCS 26. The probiotic dietary supplement exerted some favorable effects, including waist circumference reduction, serum cholesterol reduction, and maintenance of serum adiponectin levels. The second limitation was the pharmacological treatment of the enrolled patients that was administered to prevent cardiovascular complications. Although both the probiotic and placebo groups were treated in the same way, we cannot rule out the influence of the drugs on the outcome of the probiotic dietary supplementation. The authors of the present study agree that these results undoubtedly warrant and represent a starting point for the implementation of an extensive long-term clinical trial on supplementation with Lactobacillus plantarum PCS 26, which would provide a more accurate insight into the mechanisms of action of the probiotic bacteria in the prevention or relief of metabolic syndrome or its components.

ACKNOWLEDGEMENTS

The authors would like to thank Professor Dr. Avrelija Cencic and the Department for Gastroenterology, Clinic for Internal Medicine, University Clinical Centre Maribor. The authors have no conflicts of interest to declare. This study was supported by the Slovenian Research Agency, and Faculty of Agriculture and Life Sciences, University of Maribor, Research Group for Food Safety and Health Assurance (Grant Number: P1-0164).

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