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Research Article

Lactobacillus plantarum reduces insulin resistance and yacon or symbiotic reduces oxidative stress in rats with metabolic syndrome

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Abstract

There is an increasing interest in exploring the effects of probiotics, prebiotics and symbiotic on metabolic syndrome (MetS) and oxidative and nitro sative stress has been implicated in its physiopathology. The aim of this study was to evaluate metabolic parameters and oxidative and nitro satives tress in metabolic syndrome treated with prebiotic, probiotic or symbiotic. MetSwas induced by diet supplemented with 66% fructose in male wistar rats. The animals were divided into five groups (n=10 each): G1received a standard diet without inducing MetS. Animal's fromG2, G3, G4 e G5 were fed with 66% fructose supplement. G2 had no therapeutic interventions; G3 received treatment with probiotic Lactobacillus plantarumLp 115 (109 CFU); G4 received prebiotic yacon powder (1011 CFU) and G5 (symbiotic group) was treated with a beverage containing L. plantarumLp 115 and yacon (1011 CFU). All diets were administered for eight weeks. In relation to G1, rats fed high-fructose diet (G2) showed laboratorial features compatible with MetS; G2 showed reduced nitric oxide metabolites (NOx) levels ($p = 0.012$) and increased levels of sulfhydryl (SH) ($p < 0.0001$) and total radical-trapping antioxidant parameter/uric acid (TRAP/UA) ($p = 0.044$). In relation to G2, probiotic decreased insulin and HOMA-IR ($p = 0.015$ and $p = 0.004$, respectively), whereas prebiotic reduced hydroperoxides levels ($p = 0.002$), and increased SH ($p = 0.049$) and TRAP/UA ($p = 0.034$). Symbiotic resulted in increased HOMA-IR ($p = 0.034$), reduced hydroperoxides ($p = 0.015$) and Increased levels of NOx ($p = 0.002$) and SH ($p = 0.031$) compared to G3. In conclusion, Lactobacillus plantarumLp 115 reduces insulin resistance and yacon or symbiotic reduces lipid Oxidation and increases antioxidant defenses in male wistar rats with high-fructose diet-induced MetS.

Key words: metabolic parameters; probiotic; prebiotic; symbiotic; oxidative stress.

Introduction

Metabolic syndrome (MetS) is a cluster of risk factors for cardiovascular disease and type 2 diabetes, such as abdominal obesity, insulin resistance, dyslipidemia, hypertension and endothelial dysfunction.Oxidative stress, which occurs due to an imbalance between production and inactivation of reactive oxygen and nitrogen species and antioxidant defenses, has been implicated in the physiopathology of obesity, hypertension, endothelial dysfunction, and MetS [1].

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There is an increasing interest in exploring the effects of probiotics, prebiotics and symbiotic on MetS and its individual components. Probiotics are defined as live microorganisms that when administered in adequate amounts confer health benefits to the host. Probiotics, such as strains of Lactobacillus improve metabolic parameters such as obesity, hypertension, glucose homeostasis disorders, abnormal plasma lipid levels and oxidative stress [2,3]. In turn, Prebiotics, which are selectively fermented dietary fibers that are naturally found in plants,also appears to be effective in the modulation of the components of the MetS. Prebiotics, such as Yacon roots, the most abundant source of fructooligosaccharides (FOS), have been associated to hypoglycemic, hypolipidemic, and antioxidant effects [4,5].

Symbiotic is defined as a mixture of probiotics and prebiotics and articles on symbiotic in MetS are still scarce. Therefore, the aim of the present study was to evaluate the effect of Lactobacillus plantarum Lp115, yacon (Smallanthus sonchifolius) isolated and in symbiosis on metabolic parameters and oxidative stress status in rats with MetS.

Materials and Methods

Fifty male Wistar rats, seven weeks of age (195,7 g of body weight), were housed in polypropylene cages (one animal per cage). Room temperature was maintained at 22 ± 2ºC with a 12-h light/12-h dark cycle.MetS was induced by diet supplemented with 66% fructose.The Animal Ethics Committee of Londrina University approved the study.

Study Design

Animals were randomly assigned to five groups: a negative control (G1; n=10) that consumed a standard diet and 0.9% saline;a positive control with metabolic syndrome (G2; n=10) that consumed 66% w/w high-fructose diet and received saline solution similar to control; probiotic (G3; n=10) that consumed 66% w/w high-fructose diet supplemented with fermented milk containing Lactobacillus plantarum Lp115 (109 CFU/ml per day); prebiotic (G4; n=10) that consumed 66% w/w high-fructose diet supplemented with Giroil® yacon powder (10%) reconstituted in water, providing 0,041g/FOS/ml(1011 CFU/ml day), and symbiotic (G5; n=10) that consumed 66% w/w high-fructose diet supplemented with symbiotic drink containing Lactobacillus plantarum (Lp115) and yacon (10%). All diets were administeredby oral gavage for eight weeks. Animals had free access to water and food with or without fructose. All variables were measured at baseline and after 8 weeks.

Biochemical Measurements

Before sacrifice, rats were fasted for 8 h and anesthetized. Blood samples were taken from exsanguination by cardiac puncture; the animals underwent the following laboratory blood analysis: glucose, high density lipoprotein cholesterol (HDL-C), triacylglycerol and uric acid, which were evaluated by a biochemical auto-analyzer (Dimension Dade AR, Dade Behring, Deerfield, IL, USA), using Dade Behring® kits. Plasma insulin was determined with a rat enzyme-linked immunosorbent assay kit (Spio bio, USA). Insulin resistance was assessedbyhomeostasis model of assessment insulin resistance(HOMA-IR). Body weight was measured once a week.

Oxidative Stress Measurements

Samples for evaluating oxidative stress and total antioxidant capacity were performed with EDTA as anticoagulant and antioxidant. All samples were centrifuged at 3,000 rpm for 15 minutes and plasma aliquots stored at -70ºC until assayed.

Tert-butyl hydroperoxide-initiated chemiluminescence (CL-LOOH): CL-LOOH in plasma was determined as described by Gonzalez et al. [6]CL-LOOH is considered much more sensitive and specific than the thiobarbituric acid reactive substances (TBARS) method [7], the usual method to determine lipid oxidation. For CL measurement, reaction mixtures were placed in 20-mL scintillation vials (low-potassium glass) containing final concentrations of plasma (250 uL), 30 mMKH2PO4/K2HPO4 buffer (pH 7.4), and 120 mMKCl with 3 mM of LOOH in a final volume of 2 mL. CL-LOOH was measured in a Beckman LS 6000 liquid scintillation counter set to the out-of-coincidence mode, with a response of 300 to 620 nm. The vials were kept in the dark up to the moment of assay, and determination was carried out in a dark room at 30°C. The results were expressed in counts per minute.

Evaluation of nitric oxide metabolites (NOx): Serum NO levels were assessed on the basis of nitrite (NO2-) and nitrate (NO3-) concentration according to the Griess reaction supplemented by the reduction of itrate to nitrite with cadmium [8].

Total radical-trapping antioxidant parameter (TRAP): TRAP was determined as reported by Repetto et al. [9]and valueswere expressed in μmol/l of Trolox. This method detects hydrosoluble and/or liposoluble plasma antioxidants by measuring the chemiluminescence inhibition time induced by 2,2-azobis (2-amidinopropane). The system was calibrated with the vitamin E analog TROLOX, and the values of TRAP were expressed in equivalent of μMTrolox/mg UA. TRAP measurements in conditions associated with hyperuricemia, such as MetS, maybe inaccurate because uric acid concentration accounts for 60% of total plasma antioxidant capacity. Some reports have verified an unexpected increase in TRAP in MetS subjects [10,11]. Thus, a correction of TRAP based on uric acid concentration was performed [10].

Determination of sulfhydryl (SH) groups of proteins: SH groups of proteins were evaluated in plasma samples by a spectrophotometric assay based on 2,2-dithiobisnitrobenzoic acid (DTNB), as reported by Hu [12] and the results are expressed in μM.

Statistical analysis

Continuous variables were analyzed using the nonparametric Kruskal-Wallis test with Dunn's post and the results were expressed as median and interquartile range (IQR) 25%-75%.The results were considered significant when p <0, 05.

Results

Rodents fed diet supplemented with fructose (G2) developed laboratorial characteristics of MetS in relation to the control group, such as increased plasma levels of fasting glucose (p< 0.001), insulin (p< 0.005), HOMA-IR (p<0.0003), triacylglycerols (p< 0.005) and reduced HDL-cholesterol levels (p<0,005). However, there was a reduction in weight (p<0.0001) and no significant change was verifiedin uric acid levels (Table 1).

Regarding treatment, probiotic (G3) reduced plasma insulin levels (p=0.0145) and HOMA-IR (p=0.0037) compared to G2 and increased the weight (p=0.0194) of the animals.Treatment with prebiotic (G4) decreased levels of uric acid (p=0.0129) when compared to G2. On the other hand, administration of symbiotic (G5) resulted in decreasing of HOMA-IR (p = 0.0337) when compared to G2 and increased HOMA-IR (p = 0.0148) when compared to group G3 (Table 1).

Oxidative stress assessments are shown in Figures 1-4.Treatment with prebiotic (G4) decreased (p=0.0021) lipid peroxidation (CL-LOOH) compared to G2, whereas symbiotic treatment (G5) showed reduction in relation to G2 (p=0.0147) and G3(probiotic) (p=0.0443) (Figure 1). Regarding SH groups, G2 had increased (p=<0.0001) levels compared to G1 (negative control), whereas G4 (prebiotic) (p=0.0488) and symbiotic (p=0.0306) treatments showed enhanced levels compared to G2 (Figure 2). In relation to NOx levels, G2 had decreased (p=0.0115) levels compared to G1, whereas symbiotic (G5) increased NOx levels compared to G2 (p=0.0021), G3 (p=0.0263) and G4 (p=0.0183) (Figure 3). Total antioxidant capacity (TRAP) demonstrated an increase

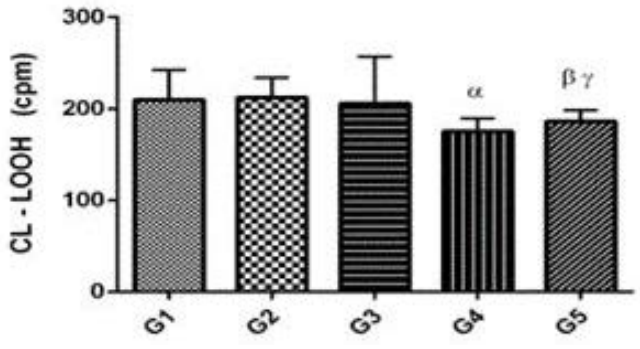

Figure 1. Evaluation of chemiluminescence (CL-LOOH) in rates with metabolic syndrome induced by fructose, submitted to treatments with probiotic, prebiotic or symbiotic.

Group 1: negative control, Group 2: metabolic syndrome (MetS) positive control, Group 3: MetS and probiotic, Group 4: MetS and prebiotic, Group 5: MetS and symbiotic.

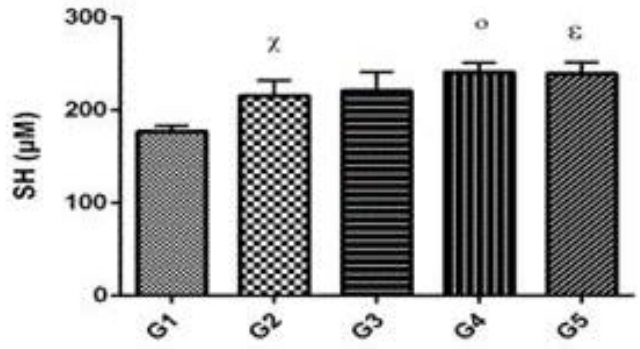

Figure 2. Evaluation of sulphydryl group (SH) in rats with metabolic syndrome induced by frucyose. Submitted to treatm.

Group 1: negative control, Group 2: metabolic syndrome (MetS) positive control, Group 3: MetS and probiotic, Group 4: MetS and prebiotic, Group 5: MetS and symbiotic. ents with probiotic, prebiotic or symbiotic.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	G1xG2	G2XG3	G2XG4	G2XG5	G3XG5	G4XG5
Weight (gr)*	192.0 167.8 – 202.8	122.5 109.8-131.3	135.0 131.0 – 165.8	129.5 119.3 – 145.3	134.0 128.0 – 140.0	<0.0001	0.0194	NS	NS	NS	NS
Glucose (mg/dl)	157.5 149.3 – 164.8	195.5 178.3 – 206.3	200.5 193.0 – 206.5	192.0 180.5 – 196.8	188.0 177.0 – 213.0	<0.001	NS	NS	NS	NS	NS
Insulin (pg/ml)	1.34 1.0 – 1.71	2.47 2.02- 2.70	1.70 1.27 – 2.28	2.04 1.55 – 2.65	1.95 1.54 – 2.41	<0.005	0.0145	NS	NS	NS	NS
HOMA-IR	2.205 1.603 – 2.933	5.300 4.520 – 5.520	3.330 2.885 – 3.855	4.000 2.885 – 3.855	4.625 3.668 – 5.025	<0.0003	0.0037	NS	0.0337	0.0148	NS
TRI (mg/dl)	61.0 37.0 – 75.5	133.0 114.3 – 157.3	179.5 117.3 – 257.0	205.0 111.0 – 244.0	137.0 111.0 – 186.0	<0.005	NS	NS	NS	NS	NS
HDL (mg/dl)	61.0 55.0 – 74.3	53.5 46.3 – 60.8	50.0 43.8 – 57.3	49.5 44.5 – 56.8	51.0 44.0 – 55.0	<0.005	NS	NS	NS	NS	NS
Uric Acid(mg/dl)	2.07 1.77 – 2.37	1.99 1.82 – 2.35	2.10 1.96 – 2.25	1.74 1.56 – 1.84	1.97 1.77 – 2.25	NS	NS	0.0129	NS	NS	NS

Table 1. Metabolic parameters in rats with metabolic syndrome induced by fructose, submitted to treatments with probiotic, prebiotic or symbiotic.

Group 1: negative control, **Group 2:** metabolic syndrome (MetS) positive control, **Group 3:** MetS and probiotic, **Group 4:** MetS and prebiotic, **Group 5:** MetS and symbiotic.

* Differences in weight: (final weight - initial weight); NS: not significant; HOMA-IR: homeostasis model of assessment – insulin resistance; TRI: triacylglycerol; HDL: high-density lipoprotein.

The data are presented as the median (25%–75%).

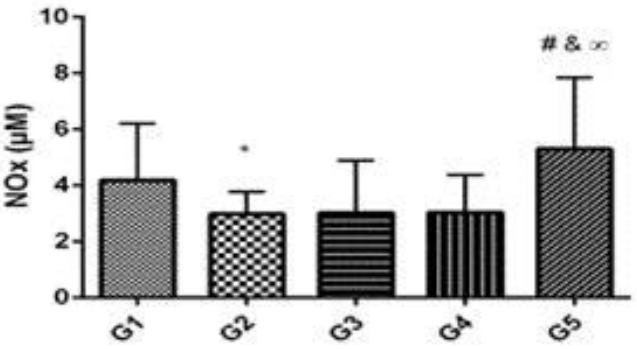

Figure 3. Evaluation of nitric oxide metabolites (NOx)in rats with metabolic syndrome inducted by fructose. Submitted to treatments with probiotic, prebiotic or symbiotic.

Group 1: negative control, Group 2: metabolic syndrome (MetS) positive control, Group 3: MetS and probiotic, Group 4: MetS and prebiotic, Group 5: MetS and symbiotic.

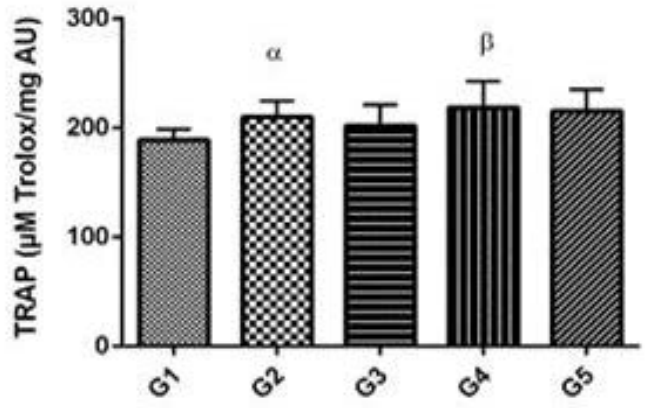

Figure 4. Evaluation of total radical-trapping antioxidant parameter (TRAP) in rats with metabolic syndrome induced by fructose, submitted to treatments with probiotic, prebiotic or symbiotic.

Group 1: negative control, Group 2: metabolic syndrome (MetS) positive control, Group 3: MetS and probiotic, Group 4: MetS and prebiotic, Group 5: MetS and symbiotic.

(p=0.0443) in G2 compared to G1 and an increase (p=0.0337) in prebiotic (G4) compared to G2 (Figure 4).

Discussion

The present study verified that Lactobacillus plantarum Lp 115 reduces insulin resistance and yacon or symbiotic reduced lipid oxidation and increased antioxidant defenses.

Insulin resistance is a condition in which insulin is unable to produce its responses. Fructose is readily absorbed and rapidly metabolized by humansorrodents liver. The exposure of the liver to such large quantities of fructose leads to rapid stimulation of lipogenesis and triacylglycerols accumulation, which contributes to reduced insulin sensitivity and hepatic insulin resistance/glucose intolerance [13]. Other studies have also shown improvement in insulin resistance with the intake of probiotics, and the mechanism by which probiotics may improve insulin resistance in mice was attributed to increased hepatic natural killer receptors and T-cell receptors and to reduced inflammatory signaling [14].L plantarum has been considered the strain, which has shown more favorable and significant improvements for most components of MetS [15]. The current study is in agreement with Park`s et al [16] in which L.plantarum KY1032 (109 CFU)administration significantly lowered plasma insulin and reduced insulin resistance measured by HOMA-IR in high-fructose diet-induced MetS.

Unexpectedly, in the present study, diet with high fructose content did not increase uric acid levels; however, administration of prebiotic reduced the uric acid amount. In fructose-fed rats, increased plasma uric acid levels have been reported. Nakagawa et al. [17] hypothesized a causal role of uric acid in fructose-induced MetS by showing that uric acid dose dependently, blocked acetylcholine-mediated arterial dilatation, suggesting that uric acid can impair endothelial function. An increased concentration of uric acid may also contribute to the reduced NO and increased oxidative stress, leading to increased risk factors for the development of hypertension, and cardiovascular disease [10,18].

The MetS induction did not reduce the levels of SH and TRAP/AU, probably due to an increased mobilization of antioxidant defenses in the group with MetS, without diet supplementation. However, the use of prebiotic and symbiotic increased the antioxidant capacity of rodents. The antioxidant capacity in yacon has a strong correlation with the phenolic compounds such as caffeic acid and chlorogenic acid, being an indication that these compounds are mainly responsible for this high antioxidant capacity of the yacon tuber [19].

Also, the induction of MetS did not change CL-LOOH levels. However, the administration of prebiotic and symbiotic reduced the hydroperoxides amount. CL-LOOH is considered much more sensitive and specific than TBARS and suffers less interference in the evaluation of oxidative stress in patients with MetS [7]. Phenolic compounds present in yacon exhibit cytoprotective effects in rat hepatocytes with oxidative damage caused by tert-butyl hydroperoxide [20]. The beneficial symbiotic effect in the reduction of lipid oxidation and in the increase of antioxidant defenses is due to a synergistic effect of the phenolic compounds present in yacon and antioxidant peptides mainly derived from αs- casein present in fermented milks [20, 21]. Lee et al. [22] evaluated the antioxidant capacity in vitro of L.plantarum KCTC 3099 and observed that this species has high antioxidant activity by inhibiting lipid peroxidation and has the ability to eliminate reactive oxygen species (ROS) (mainly superoxide and hydrogen peroxide) and increase antioxidant enzymes.

In the pathophysiology of MetS, endothelial dysfunction is considered a hallmark associated with hypertension, and can be evaluated by several means, including the assessment of NOx levels. NO is synthesized in endothelial cells by endothelial nitric oxide synthase (eNOS) activity, and is responsible for vasodilation and for the maintenance of endothelial function [23]. In the present study, animals with MetS and no therapeutic interventions had reduced levels of NO. Results of studies on serum NOx levels in patients with MetS have been contradictory. Sun et al. [24] showed that NOx levels were reduced in MetS. However, Asl et al. [25] showed higher NOx concentration in subjects with MetS and type 2 diabetes. Simao et al. [26] has reported that MetS patients had significantly lower serum NOx levels and were inversely correlated with BMI, waist circumference, inflammatory status, and insulin resistance evaluated by HOMA-IR. On the other hand, NOx was positively correlated with lipid hydroperoxide levels measured by chemiluminescence. These findings were attributed to the fact that NO is consumed in a reaction with superoxide anion yielding a strong oxidant specie, peroxynitrite($ONOO^-$), which in turn accelerates the lipid peroxidation reaction [27, 28, 29]. Therefore, although oxidative stress may induce NO production, NO decrease found in the present study is probably related to NO higher consumption by oxidative stress, reducing NO bioavailability in MetS rodents.With the administration of symbiotic beverage, it was observed an increase in NO levels, indicating a positive response.

In conclusion, Lactobacillus plantarumLp 115 reduces insulin resistance and yacon or symbiotic reduces lipid oxidation and increases antioxidant defenses in male Wistar rats with high-fructose diet-induced MetS. Human and animal studies are needed to confirm the present data as well as in-depth research on the mechanisms involved.

Abbreviations

BMI –Body Mass Index, CFU- Colony Forming Units, CL-LOOH -Hydroperoxide concentrations by tert-butyl hydroperoxide-initiated chemiluminescence, DTNB - 2,2-dithiobisnitrobenzoic acid, eNOS - Nitric Oxide Synthase, FOS – Fructooligosaccharides, HDL-c - High Density Lipoprotein Cholesterol, HOMA-IR - Homeostasis Model of Assessment – Insulin Resistance, IQR - Median and Interquartile Range, MetS - Metabolic Syndrome, NO – Nitric Oxide, NOx -Nitric Oxide Metabolites, ONOO -Peroxynitrite, ROS - Reactive Oxygen Species, SH - Grouping Sulfhydryl, TBARS -Thiobarbituric Acid Reactive Substances, TRAP - Total Radical-Trapping Antioxidant Parameter, UA - Uric Acid,

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