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ORIGINAL ARTICLE

The influence of probiotic supplementation on gut permeability in patients with metabolic syndrome: an open label, randomized pilot study

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BACKGROUND/OBJECTIVES: Obesity and metabolic disorders are linked to inflammation via gut microbiota and/or gut permeability. Gut-derived endotoxin triggers inflammation leading to metabolic syndrome (MetS) and contributing to oxidative stress. We intended to investigate the effect of *Lactobacillus casei* Shirota on gut permeability, presence of endotoxin and neutrophil function in MetS.

SUBJECTS/METHODS: Patients with MetS were randomized to receive $3 \times 6.5 \times 10^9$ CFU *L. casei* Shirota (probiotic group) or not for 3 months. Gut permeability was assessed by a differential sugar absorption method and by determination of diaminooxidase serum levels, endotoxin by an adapted limulus amoebocyte lysate assay, neutrophil function and toll-like receptor (TLR) expression by flow cytometry and ELISA was used to detect lipopolysaccharide-binding protein (LBP) and soluble CD14 (sCD14) levels.

RESULTS: Twenty-eight patients and 10 healthy controls were included. Gut permeability was significantly increased in MetS compared with controls but did not differ between patient groups. None of the patients were positive for endotoxin. LBP and sCD14 levels were not significantly different from healthy controls. High-sensitive C-reactive protein and LBP levels slightly but significantly increased after 3 months within the probiotics group. Neutrophil function and TLR expression did not differ from healthy controls or within the patient groups.

CONCLUSIONS: Gut permeability of MetS patients was increased significantly compared with healthy controls. *L. casei* Shirota administration in the MetS patients did not have any influence on any parameter tested possibly due to too-short study duration or underdosing of *L. casei* Shirota.

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INTRODUCTION

The incidence of metabolic syndrome (MetS) steadily increases.[1] Metabolic disorders are tightly linked to inflammation.[2] However, the triggering factor linking inflammation to MetS has not been fully elucidated yet. It has been hypothesized that the gut microbiota and/or gut permeability is an important factor in this vicious cycle of obesity, MetS and inflammation.[1]

Metabolic activities of the gut microbiota facilitate the extraction of calories from ingested dietary substances and help to store these calories in host adipose tissue.[3] The gut microbiota of obese mice and humans has been shown to be different from that of their lean counterparts, suggesting differences in caloric extraction dependent on the composition of the gut microbiota.[4] The gut microbiota serves as reservoir for bacterial lipopolysaccharides that may trigger inflammation, linking it to high-fat diet-induced MetS. High-fat diet leads to low-grade (metabolic) endotoxemia in mice and infusion of endotoxin causes weight gain and insulin resistance.[5] In mice, treatment with antibiotics improved glucose tolerance by altering expression of genes involved in inflammation and metabolism.[6] Similar results were achieved in mice treated with probiotics that increase the number of *Bifidobacterium* spp., leading to improved glucose tolerance, insulin secretion and a decrease in inflammatory tone.[7] Treatment of mice with probiotics also decreased hepatic insulin resistance, supporting the concept that intestinal bacteria induce endogenous signals that have a pathogenic role in hepatic insulin resistance.[8] Furthermore, oral administration of *Lactobacillus casei* Shirota has been shown to improve insulin resistance and glucose intolerance in obese mice.[9]

MetS-induced insulin resistance and oxidative stress in mice is also associated with increased gut permeability for bacteria and their products.[10] Patients with fatty liver also revealed a susceptibility to increased gut permeability, possibly related to increased endotoxin levels.[11] Those increased endotoxin levels may further trigger inflammatory reactions and/or immune dysfunction.[12] This is also underlined by impaired cell-mediated immune responses *in vivo* and *in vitro* and a reduced intracellular killing by neutrophils.[13]

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Weight loss is the most effective therapy of obesity, which also influences gut microbiota composition[14] and improves monocyte function.[15] As weight loss is usually not easy to achieve, other therapeutic strategies are warranted. In rats and mice *L. casei* is able to improve gut permeability.[16,17]

Probiotics are a promising strategy to influence gut microbiota and gut permeability. Therefore, we aimed to investigate the effect of a probiotic (*L. casei* Shirota) on (i) gut permeability, (ii) presence of endotoxin and (iii) neutrophil function in subjects with MetS.

PATIENTS AND METHODS

Patient recruitment and randomization

Adult patients with MetS were identified from the outpatient clinic at the Division of Endocrinology and Metabolism at the Medical University of Graz. All patients gave written informed consent; the study protocol was approved by the Ethics Committee of the Medical University of Graz (20-037 ex 08/09), registered at clinicaltrials.gov (NCT01182844) and performed according to the Declaration of Helsinki. MetS was defined using the modified National Cholesterol Education Program-Adult Treatment Panel-III (NCEP-ATP-III)-Guidelines.[18] For further details, concerning inclusion/exclusion criteria and sample size calculation, see Supplementary Data.

Patients were randomized into two groups (Randomizer, https://www.randomizer.at, Institute for Medical Informatics, Statistics and Documentation of the Medical University of Graz, Graz, Austira). One group received three bottles containing 65 ml of YAKULT light (containing *L. casei* Shirota at a concentration of 10^8/ml, Yakult Austria, Vienna) per day for 3 months (probiotic) and the other group did not receive YAKULT light and served as a control group (standard). Treatment adherence was assessed fortnightly. Patients were advised not to change their medical therapy and lifestyle without informing the study team. Patients did not take any antibiotics during the study and gave written consent not to consume any other probiotic during these 3 months. Compliance was tested by patient interviews. Furthermore, patients got supply of probiotics for 14 days at once and were asked to return to the clinic for every further package of probiotics. Every patient collected their new packages on time.

Data for neutrophil function, endotoxin, lipopolysaccharide-binding protein (LBP), soluble CD14 (sCD14) and gut permeability were compared with 10 healthy controls (4 female, 6 male; age: median 35 years, range 24–66 years).

All parameters were determined once at the day of inclusion in the study (baseline) and a second time at the end of the study (3 months).

Determination of gut permeability

After overnight fasting, patients drank 100 ml of a solution containing 10 g lactulose (LAC), 5 g mannitol (MAN) and 20 g saccharose in the morning (between 0700 and 0800 h). Urine was collected over 5 h while fasting was continued. Furthermore, patients were not allowed to drink within the first 2 h after ingestion of the sugar solution. The urine volume collected within 5 h was measured and 1 ml aliquots were frozen at $-80\,^{\circ}C$ after addition of 100 μl of Thimerosal (10 mg/ml; Sigma-Aldrich Handels GmbH, Vienna, Austria) for subsequent analysis by high-performance liquid chromatography. For further details see Supplementary Data.

Furthermore, diaminooxidase (DAO) levels were determined by a commercially available ELISA Kit (DAO ELISA, Immundiagnostik, Bensheim, Germany) according to the manufacturer's instructions.

Determination of endotoxin

To detect endotoxin in serum samples, an adapted *Limulus Amoebocyte* lysate based assay was used (all *Limulus Amoebocyte* lysate related products are purchased from Charles River Laboratories, Kissleg, Germany) as previously described.[19] For further details see Supplementary Data.

Determination of sCD14 and LBP

A ready-to-use solid-phase sandwich ELISA (Hycult biotechnology, Uden, The Netherlands) was used to detect LBP and sCD14 levels in patients' plasma according to the manufacturer's instructions.

Oxidative burst

The Phagoburst kit (Glycotope, Heidelberg, Germany) was used to determine the percentage of neutrophils that produce reactive oxidants with or without stimulation by flow cytometric analysis according to the manufacturer's instructions.

Phagocytosis

The Phagotest (Glycotope) was used to measure phagocytosis by flow cytometric analysis using fluorescein isothiocyanate-labeled opsonized *E. coli* bacteria according to the manufacturers' protocol.

Toll-like receptor (TLR) expression

TLR expression of neutrophils is measured by flow cytometric analysis. Heparinized whole blood was used for the staining procedure. For surface staining, TLR2-APC, TLR4-PE-Cy7 (eBioscience, Vienna, Austria), CD16-fluorescein isothiocyanate (Immunotools, Friesoythe, Germany) and CD11b-PacBlue (Beckton Dickinison, Heidelberg, Germany) were used. Cells were fixed with Fixation solution (eBioscience). For intracellular staining, cells were kept in permeabilization buffer (eBioscience) and incubated with TLR9-PE (US Biological, Swampscott, MA, USA).

Statistical analysis

Data are expressed as mean ± s.d. For comparison of independent, parametric data student *t*-test and for non-parametric data Mann–Whitney-*U*-test were used. For dependent, parametric data paired *t*-test and for non-parametric data Wilcoxon-Test was used. For statistical analyses, PASW 18.0 program (SPSS GmbH Software, an IBM Company, Munich, Germany) was used. Graphs were created by means of GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). A two-sided $P<0.05$ was considered as statistically significant.

RESULTS

Patients and controls

Thirty-five subjects were screened for the study between January and August 2010; 30 patients were finally included, whereof 28 finished the study (2 dropped out due to withdrawal of informed consent). Five patients did not fulfill the inclusion criterion of fasting glucose above 100 mg/dl at the day of screening any more. Thirteen patients were randomized to the probiotic group and 15 to the standard therapy group. Baseline characteristics of patients with MetS and a healthy control group are shown in Table 1. Subjects in the probiotic group had a significantly higher BMI ($35.4 \pm 5.3\,kg/m^2$) than those in the standard therapy group ($31.6 \pm 3.6\,kg/m^2$, $P=0.027$) at baseline.

Standard laboratory tests

Clinical and biochemical markers of MetS, such as blood pressure, BMI, waist circumference, triglyceride, total cholesterol or fasting glucose levels, did not change over 3 months, neither in the probiotic nor in the standard therapy group (Table 1). However, we found a statistically significant decrease in systolic blood pressure after 3 months (141 ± 14 mm Hg) vs baseline (149 ± 17 mm Hg) without a change in antihypertensive medication when analyzing the total study population ($P=0.026$) (Table 1).

Bilirubin levels were significantly higher, but still well within the normal range (0.1–1.2 mg/dl), in the probiotic group (0.73 ± 0.18 mg/dl) after 3 months compared with the standard therapy group (0.53 ± 0.27 mg/dl, $P=0.030$) but did not reveal different levels when compared with baseline neither for the probiotic nor for the standard therapy group or all patients or to controls. Alanine aminotransferase (ALT) levels were slightly elevated in 35.7% of the patients. None of the patients had ALT levels above two times the upper limit of normal. ALT levels did not change after 3 months compared with baseline neither for the probiotic nor for the standard therapy group or for the whole cohort (Table 1).

Smoking had no statistically significant impact on any standard laboratory parameter tested.

Gut permeability

Gut permeability was significantly higher in subjects with MetS compared with healthy controls (saccharose: $P<0.001$; LAC/MAN ratio: $P=0.010$, DAO: $P<0.001$, Figure 1 and Table 2).

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Table 1. Patient characteristics regarding standard laboratory parameters

	Baseline			*3 months*			*Δ*			*Controls/*
	Probiotics (n = 13)	*Standard therapy (n = 15)*	P	*Probiotics (n = 13)*	*Standard therapy (n = 15)*	P	*Probiotics (n = 13)*	*Standard therapy (n = 15)*	P	*Normal value*
Age (years)	51.5 ± 11.4	54.5 ± 8.9	0.430	51.5 ± 11.4	54.5 ± 8.9	0.430	—	—	—	40.6 ± 15.2[a]
Sex (m/f)	9/4	9/6	—	9/4	9/6	—	—	—	—	6/4
Weight (kg)	108.3 ± 15.9	90.9 ± 14.3	*0.004*	107.8 ± 16.6	90.8 ± 15.1	*0.010*	− 0.58 ± 2.54	− 0.13 ± 1.68	0.586	76.8 ± 8.5[b]
BMI (kg/m^2)	35.4 ± 5.3	31.6 ± 3.6	*0.027*	35.3 ± 5.5	31.6 ± 4.0	0.054	− 0.18 ± 0.78	− 0.05 ± 0.60	0.642	25.2 ± 2.6[c]
Statin therapy (%)	39.3	60.7	—	39.3	60.7	—	—	—	—	0
Smoking (%)	39.3	60.7	—	39.3	60.7	—	—	—	—	0
RR_sys (mm Hg)	150 ± 19	149 ± 16	0.917	142 ± 17	139 ± 11	0.642	7.45 ± 18.1	10.2 ± 18.3	0.725	< 130[d]
RR_dia (mm Hg)	94 ± 12	96 ± 18	0.950	93 ± 12	89 ± 9	0.498	1.45 ± 3.59	6.17 ± 16.3	0.360	< 90[d]
Chol	220 ± 69	209 ± 43	0.628	219 ± 59	211 ± 34	0.765	0.46 ± 21.41	1.27 ± 38.19	0.982	212 ± 38
TG	215 ± 169	170 ± 106	0.662	202 ± 123	159 ± 66	0.549	—	—	0.712	144 ± 129
Albumin (mg/dl)	4.58 ± 0.25	4.68 ± 0.28	0.358	4.50 ± 0.32	4.70 ± 0.25	0.079	− 0.08 ± 0.29	0.02 ± 0.23	0.301	4.53 ± 0.26
Bilirubin (mg/dl)	0.67 ± 0.36	0.47 ± 0.14	0.078	0.73 ± 0.27	0.53 ± 0.18	*0.030*	0.06 ± 0.17	0.06 ± 0.15	0.993	0.57 ± 0.24
ALT (U/l)	47.2 ± 25.1	34.5 ± 17.8	0.170	47.3 ± 33.5	37.2 ± 21.6	0.294	0.08 ± 22.85	2.67 ± 13.19	0.637	< 50 (m), < 35 (f)
hsCRP (mg/l)	3.66 ± 4.03	4.86 ± 4.38	0.499	5.47 ± 5.67[e]	3.10 ± 3.45	0.229	1.86 ± 2.48	− 1.60 ± 5.24	*0.016*	1.86 ± 1.93

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; Chol, total cholesterol; hsCRP, high-sensitive C-reactive protein; RR_sys, systolic blood pressure; RR_dia, diastolic blood pressure; TG, triglycerides. *P* values < 0.05 for group comparisons are highlighted in italics. [a]*P* = 0.038 vs probiotics group and *P* = 0.020 vs standard therapy group. [b]*P* < 0.001 vs probiotics (baseline and 3 months), *P* = 0.010 vs standard therapy group at baseline and *P* = 0.014 vs standard therapy group after 3 months. [c]*P* < 0.001 vs probiotics and standard therapy groups. [d]Normal values according to WHO. [e]*P* = 0.037 vs probiotics group at baseline.

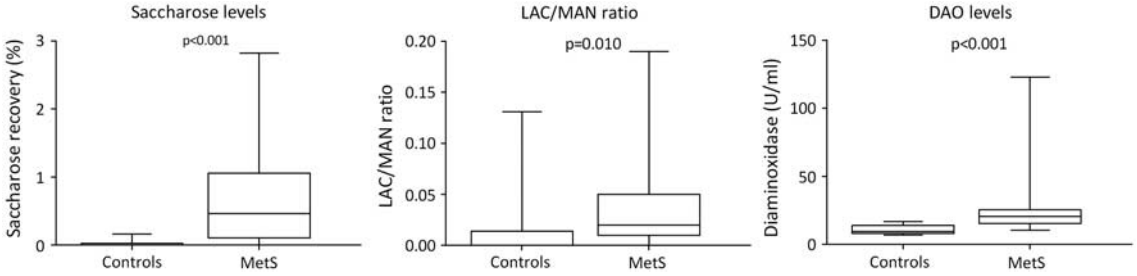

Figure 1. Gut permeability in patients with MetS and controls. Recovery of saccharose (gastroduodenal permeability), LAC/MAN ratio (small intestinal permeability) and diaminoxidase (DAO) levels are significantly increased in patients with MetS compared with healthy controls.

There were no differences in gastroduodenal (saccharose) or small bowel (LAC, MAN) permeability or in DAO levels between the two patient groups at any timepoint. Furthermore, no changes of gut permeability could be observed when comparing baseline levels to levels detected after 3 months in any of the patient groups. We did not observe statistically significant correlation between the age of patients and gut permeability. BMI, hyperlipidemia or smoking did not influence gut permeability.

Endotoxin and markers of inflammation

None of the patients enrolled within this study was positive for endotoxin at any timepoint (Table 2). LBP and sCD14 levels were not different from healthy controls in patients with MetS. BMI or hyperlipidemia did not influence these parameters. At baseline, sCD14 levels were higher in patients randomized to the standard therapy group (probiotic: 1374 ± 190 ng/ml, standard therapy: 1636 ± 380 ng/ml; *P* = 0.028). After 3 months this difference has abolished.

However, high-sensitive CRP (hsCRP) levels were detectable (above 0.6 mg/dl) in 82% of the patients at baseline and were above the normal range (above 5 mg/dl) in 18% of the patients. There was no difference between the two groups at baseline. In the probiotic group hsCRP (*P* = 0.037) and LBP (*P* = 0.014) significantly increased over 3 months when compared with baseline (Figure 2). The difference in LBP levels between 3 months of treatment and baseline (delta LBP) showed a decrease in the standard therapy group but a significant increase in the probiotic group (standard therapy: − 1510 ± 8604 ng/ml, probiotic: 5827 ± 7312 ng/ml; *P* = 0.023). While mean delta hsCRP levels increased in the probiotic group (1.86 ± 2.48 mg/dl) they decreased in the standard therapy group (− 1.60 ± 5.24 mg/dl, *P* = 0.016). Furthermore, LBP and hsCRP levels correlated significantly at baseline (*r* = 0.608, *P* = 0.047) and showed borderline correlation after 3 months (*r* = 0.412, *P* = 0.045) for all patients.

Cigarette smoking did not have any impact on inflammation markers or endotoxin levels.

Neutrophil function

Neutrophil function (phagocytosis, burst and priming) and TLR 2, 4 and 9 expressions in subjects with MetS was not significantly different compared with healthy controls (Table 2). We also did not observe any statistically significant differences in neutrophil function and TLR 2, 4 and 9 expressions between probiotic and standard therapy groups at any timepoint or when baseline levels

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Table 2. Patient characteristics regarding special parameters

	Baseline			*3 months*			*Δ*			*Controls/*
	Probiotics (n = 13)	*Standard therapy (n = 15)*	P	*Probiotics (n = 13)*	*Standard therapy (n = 15)*	P	*Probiotics (n = 13)*	*Standard therapy (n = 15)*	P	*Normal range*
Markers of gut permeability										
Saccharose (%)	0.462 ± 0.550	0.842 ± 0.855	0.182	0.439 ± 0.357	0.737 ± 0.345	0.646	− 0.02 ± 0.57	− 0.47 ± 0.86	0.127	0.023 ± 0.055[a]
LAC/MAN ratio	0.035 ± 0.052	0.043 ± 0.040	0.139	0.030 ± 0.016	0.037 ± 0.029	0.522	− 0.01 ± 0.06	− 0.01 ± 0.05	0.998	0.019 ± 0.043[b]
DAO (U/ml)	20.85 ± 7.41	27.37 ± 27.04	0.751	18.04 ± 6.97	26.50 ± 31.35	0.586	− 2.81 ± 8.28	− 0.87 ± 6.12	0.683	10.62 ± 3.58
Markers of neutrophil function										
Phagocytosis (%)	62.4 ± 16.9	56.2 ± 16.6	0.340	65.9 ± 9.3	56.2 ± 20.6	0.112	3.48 ± 18.83	0.06 ± 23.98	0.685	55.9 ± 17.6
Burst (%)	67.5 ± 21.6	69.3 ± 21.4	0.825	58.8 ± 20.8	56.6 ± 28.2	0.675	− 12.69 ± 32.96	− 8.68 ± 31.40	0.749	71.6 ± 23.0
Priming (%)	6.62 ± 3.86	13.1 ± 12.2	0.079	8.91 ± 3.57	7.81 ± 3.46	0.174	2.30 ± 5.40	− 5.8 ± 14.3	0.064	5.56 ± 4.07
TLR2 GMFI	576 ± 245	431 ± 82	0.060	453 ± 114	463 ± 101	0.811	− 122.8 ± 322.2	32.4 ± 150	0.130	404 ± 91
TLR4 GMFI	681 ± 546	471 ± 177	0.171	523 ± 127	459 ± 55	0.113	− 158 ± 524	− 11.5 ± 181	0.320	415 ± 81
TLR9 GMFI	1393 ± 536	1545 ± 671	0.518	1421 ± 737	1419 ± 648	0.992	29 ± 558	− 126 ± 737	0.542	1482 ± 604
Markers of inflammation										
LBP (ng/ml)	18438 ± 5991	20860 ± 8678	0.406	24265 ± 8943[c]	19350 ± 6688	0.109	5827 ± 7312	− 1510 ± 8604	*0.023*	20310 ± 16415
sCD14 (ng/ml)	1374 ± 190	1636 ± 380	*0.028*	1438 ± 307	1517 ± 398	0.567	65 ± 271	− 119 ± 313	0.112	1426 ± 484

Abbreviations: DAO, diaminoxidase; LAC, lactulose; LBP, lipopolysaccharide-binding protein; MAC, mannitol; sCD14, soluble CD14; TLR, toll-like receptor. [a]$P < 0.001$ vs MetS at baseline. [b]$P = 0.010$ vs MetS at baseline. [c]$P = 0.014$ vs probiotics group at baseline. *P* values < 0.05 for group comparisons are highlighted in italics.

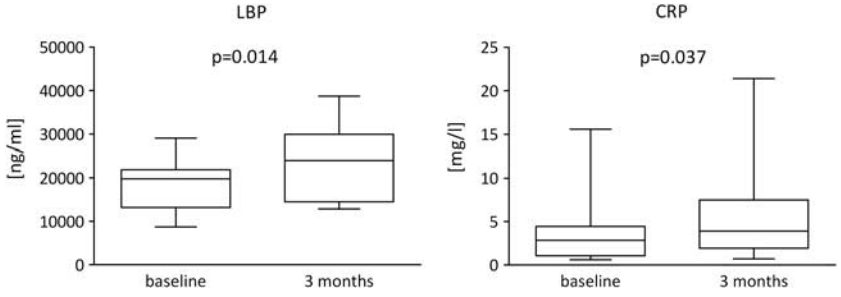

Figure 2. Significant increases in LBP and high-sensitive C-reactive protein (hsCRP) levels within the probiotic group over the study period of 3 months.

were compared with levels after 3 months of treatment in neither of these two patient groups. Neutrophil function and TLR expression was not influenced by BMI or hyperlipidemia. Phagocytosis correlated significantly with LBP levels in the probiotic group after 3 months ($r = 0.701$, $P = 0.007$) but did not correlate to LBP within the standard group or all patients. There was no difference between the patients receiving statin therapy and those without statin therapy at any timepoint. Also, smoking was not associated with any differences in neutrophil function or TLR expression.

DISCUSSION

We aimed to investigate the effect of supplementation with a probiotic containing *L. casei* Shirota on gut permeability, serum endotoxin and neutrophil function in MetS. The most important finding of this study is that the intestinal permeability in MetS patients is significantly increased compared with healthy controls. Increased gut permeability has been hypothesized to be the link between gut microbiota changes and inflammation in MetS. Patients with fatty liver are more susceptible to increased gut permeability upon stimulation.[11] We could show that subjects with MetS have increased gastroduodenal and small intestinal permeability compared with normal controls by using a differential sugar absorption method and by determination of DAO levels in serum. Saccharose (a disaccharide) is an accepted marker for gastroduodenal permeability because normally it is not able to cross the intestinal wall and is rapidly hydrolyzed in the upper part of the small intestine.[20] LAC (an oligosaccharide) and MAN (a monosaccharide) excretions were used to assess small intestinal permeability. MAN passage occurs trans- and paracellularly while LAC is absorbed across the pores between the crypt cells.[21] Calculating the LAC/MAN ratio gives us the possibility to exclude intestinal factors (for example, gut motility) that could probably affect both pathways. DAO is an active intracellular molecule in the cells of the intestinal mucosa and reaches circulation when the barrier function of the gut is impaired.[22] Thus, increased DAO levels represent increased gut permeability.

It could be limiting that the controls in our study are younger than the patients but as we did not observe significant correlations between age and gut permeability this effect might have no major impact on our data. Gut permeability data of controls within this study are in accordance with data of controls of similar age or older, published previously.[23] However, we have to keep in mind that the small number of patients may bias this result.

In contrast to animal studies in colitis or cow milk-induced increased permeability[16,17] we did not observe any changes in the gastroduodenal or small intestinal gut permeability after 3 months of food supplementation with *L. casei* Shirota. This could be due to several reasons. As there were no data available on gut permeability in MetS our study could be underpowered. As there is not even a trend in favor of the *L. casei* Shirota group this seems unlikely. The used dose of probiotics was shown in previous studies with alcoholic liver cirrhosis to be effective on neutrophil function and cytokine response,[24] but may be inadequately low in

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subjects with MetS. As the majority of the patients in this study were obese, a weight-based dosage regimen could be beneficial in future studies. Furthermore, the duration of intervention could have been too short to influence a low-grade inflammatory process that has developed slowly over years.

In obesity, patients with type 2 diabetes LBP and endotoxin levels have been shown to be elevated.[25] In animal models low-grade 'metabolic' endotoxemia has been described as a key factor in weight gain, insulin resistance and low-grade inflammation.[26] However, in our study we could not detect endotoxin in peripheral blood of any of the patients with MetS although we used an optimized endotoxin test.[27] The reason why we could not detect endotoxin in the serum of our subjects could be that the metabolic and inflammatory processes of MetS were not advanced enough in our patient cohort to cause detectable endotoxin levels in the circulation. Of note, only 4 out of 28 patients fulfilled the criteria of morbid obesity (BMI $\geqslant$ 40 kg/m^2). Owing to the fact that we did not stratify for BMI all four patients with a BMI above 40 kg/m^2 were randomized to the probiotics group. However, since all other baseline characteristics did not differ between the two groups and none of the investigated parameters did significantly differ between these morbidly obese and the non-morbidly obese subjects we are confident that this issue does not have a major impact on the study results.

In order to assess low-grade inflammation induced by endotoxin levels beyond the detection limit of the assay, we analyzed LBP and sCD14 levels. We did not find elevated LBP and sCD14 levels in patients with MetS, but we found that in a high proportion of our patients hsCRP, which is a well-defined biomarker for low-grade inflammation, was elevated. CRP was found to be elevated in patients with morbid obesity but did not improve after moderate weight loss.[28] We found no association between BMI and hsCRP levels in our study.

Unexpectedly, we found a slight increase of LBP and hsCRP after food supplementation with *L. casei* Shirota for 3 months, which is difficult to explain as we did not see any changes in gut permeability and endotoxin was not detectable in any patient at any timepoint. This finding has to be interpreted with caution, as the patient number is small and the observed difference could be due to a statistical type I error but may also be a side effect of probiotic supplementation. However, although none of our patients reported any symptoms of infection or inflammation, this finding has to be considered for future studies in patients with MetS and probiotic treatment as a safety measure.

Probiotics were well tolerated in numerous preclinical and clinical studies. The only recorded side effects were a mild laxative effect and flatulence. In our study we also did not observe any severe adverse events with the study medication. Only two patients reported minor flatulence during the first few days of probiotic food supplementation. Compliance with the study medication was excellent and there were only two drop outs during the study period.

Under chronic inflammatory conditions, such as liver disease, hypertension, smoking and renal failure[29] neutrophil granulocytes can be primed, which makes them more ready to full activation upon a second stimulus. In our study cohort neutrophil priming, oxidative burst upon stimulation and phagocytosis was not different to normal controls and hyperlipidemia was not associated with changes in neutrophil function. We further assessed the expression of TLR 2, 4 and 9 but could also not detect any differences in patients with MetS compared with healthy controls. As neutrophil function was normal at the onset of the study and we did not find any changes after 3 months, neither in the probiotic group nor in the standard therapy group. As statins improve neutrophil priming,[30] one reason for the observed normal neutrophil function in our study could be the high rate of statin-treated patients (11 out of 28). In our study, however, statin therapy did not have an impact on neutrophil function at baseline.

In conclusion we could show that gut permeability in patients with MetS is clearly increased, even without major changes in endotoxin levels, endotoxin related markers or neutrophil function. *L. casei* Shirota did not have any statistically detectable influence on the tested parameters. Therefore, further studies are necessary to study the effect of probiotics on gut permeability, gut microbiota composition and immune function in MetS.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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