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Linninge, Caroline; Ahrné, Siv; Molin, Göran; Berggren, Anna; Palmquist, Ingrid; Nordin Fredrikson, Gunilla; Jeppsson, Bengt

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PO Box 117
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ABSTRACT

Objective: This study aimed to clarify the microbial change in the intestinal microbiota in patients, with cardiovascular disease, consuming a drink with high numbers of live *Lactobacillus plantarum*.

Methods: Sixteen males, with atherosclerotic plaque on the carotid wall, were randomly selected from a larger cohort and included in this double blind, placebo controlled study. Colonic biopsies, taken before and after four weeks of probiotic treatment, were analysed with Terminal Restriction Fragment Length Polymorphism, including digestion with *MspI* and *HaeIII*. Microbial diversity was calculated, short-chain fatty acids in faeces, and blood markers were analysed.

Results: Consumption of one probiotic strain of *L. plantarum* (DSM 9843) increased intestinal microbial diversity. The probiotic group had an increased diversity after consumption of the probiotic drink compared to the change in the placebo group when Shannon and Weaner diversity index (*MspI* and *HaeIII*, $p=0.026$) and Simpson index of diversity (*MspI*, $p=0.044$ and *HaeIII*, $p=0.026$) were calculated. The fermentation pattern of short-chain fatty acids in faeces were unaffected for most acids, but the probiotic group had decreased concentration of isovaleric acid ($p=0.006$) and valeric acid ($p=0.029$). Viable count of lactobacilli increased in the probiotic group ($p=0.001$), but no significant changes in blood markers were observed.

Conclusion: Administration of a single-strain probiotic increases the bacterial diversity in the gut, and affects the concentration of some short-chain fatty acids. Consumption of the single strain *L. plantarum* DSM 9843 might be a strategy to favour a diverse intestinal microbiota, which is beneficial for the host.

Probiotic therapy to men with incipient arteriosclerosis initiates increased bacterial diversity in colon: a randomized controlled trial.

Caroline Karlsson¹⁾, Siv Ahrné¹⁾, Göran Molin¹⁾, Anna Berggren²⁾, Ingrid Palmquist³⁾, Gunilla Nordin Fredrikson⁴⁾, and Bengt Jeppsson³⁾*

¹⁾ Food Hygiene, Department of Food Technology, Engineering and Nutrition, Lund University, Lund, Sweden.

²⁾ Probi AB, Lund, Sweden.

³⁾ Department of Clinical Science, Surgery Research Unit, Lund University, Malmö University Hospital, Malmö, Sweden.

⁴⁾ Department of Clinical Sciences, Experimental Cardiovascular Research Unit, Lund University, Malmö University Hospital, Malmö, Sweden.

**Address for correspondence:*

Professor Bengt Jeppsson

Department of Clinical Sciences

University Hospital MAS, Entrance 42, Surgery Research Unit

SE-205 02 Malmö

Sweden

Telephone: +46 40 333760

Fax: +46 40 927877

E-mail: Bengt.Jeppsson@med.lu.se

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INTRODUCTION

In biology, it is a general consensus that a high diversity of organisms is beneficial for the ecosystem on both global and local levels. A high diversity indicates an ecosystem in healthy balance and it provides a higher resilience to ecological disturbances which also applies for microbial ones.¹ In contrast, an unbalanced, disturbed, or diseased ecosystem opens up for “overgrowth” of a few organisms, and contributes to further disturbances and new diseased conditions, a fact valid for the microbial ecosystem of the human gut.² For example, it has been shown that patients with Crohn’s disease and ulcerative colitis have low bacterial diversity in the gastro-intestinal (GI) tract.^{3, 4} Furthermore, infants with low bacterial diversity in faeces at one week of age more frequently develop atopic eczema after 18 months.⁵

The bacterial flora of the GI-tract makes up a complex ecosystem and the composition and activity of the different bacterial groups play important roles for the health status due to its contribution to, for example, nutrition, colonisation resistance, and development and tuning of the immune system.⁶ Pro-inflammatory components of the resident microbiota can increase the permeability of the mucosal barrier and cause subclinical inflammation.⁷

Cardiovascular diseases (CVD) attributes one-third of all global deaths.⁸ The aetiology is not fully understood but obesity, high blood pressure, physical inactivity and a diet high in saturated fats, salt and refined carbohydrates have been shown to increase the risk to develop CVD.⁸ Oxidative stress and oxidation of low-density lipoprotein (LDL) are important factors involved in the chronic inflammation of the arteries and atherosclerosis onset.^{9, 10} Atherosclerosis is the pathophysiological process underlying CVD, but exactly what signals starting the disease is not fully understood and microbial components signalling through toll-like receptors (TLRs) may play a role.¹⁰ Enhanced expression of TLRs has been shown in human atherosclerotic plaques.¹¹ Previously, *Chlamydia pneumoniae* has been associated with

increased risk of CVD¹² and it has been hypothesised that live bacteria colonise the artery wall and initiate infection leading to atherosclerosis. But this has been hard to prove and a refined version of the infection hypothesis focuses on pathogen-associated molecular patterns (PAMPs). Bacterial molecules e.g. lipopolysaccharides, flagellin and bacterial lipopeptide are examples of PAMPs that bind to TLRs and initiate signalling.¹⁰ Disruption of the epithelial tight junctions can be mediated by inflammatory mediators and the consequence will be increased intestinal translocation during inflammatory conditions.¹³ PAMPs from the GI-microbiota can translocate through the intestinal epithelial cell layer into the circulation, even in relatively healthy persons,¹⁴ making this process to one of many possible explanation to the origin of atherosclerosis onset. Furthermore, bacterial overgrowth in the intestine increases the translocation,¹⁵ highlighting the importance of a balanced microbiota.

The bacterial species *L. plantarum* frequently occurs spontaneously and in high numbers in most lactic acid fermented foods, especially when based on plant material, for example, in brined olives, capers, sauerkraut, salted gherkins and sourdough.¹⁶ However, *L. plantarum* is also present on human oral and intestinal mucosa.¹⁷ The strain *L. plantarum* DSM 9843 has been isolated from healthy human intestinal mucosa¹⁷ and is by now a well studied probiotic strain used in a number of human studies.^{18, 19} Intake of this strain in a rosehip drink has shown to affect the intestinal environment by effects on the short-chain fatty acid content (SCFA).¹⁹ *L. plantarum* DSM 9843 also attaches to human mucosa cells by a mannose-dependent adherence mechanism,²⁰ which favour persistence and close interaction with the host. The concept of probiotics implies that the balance between beneficial and harmful bacteria in the GI-microbiota can be positively affected by eating the right type of living microorganisms. This is in line with the present study where the aim was to clarify the microbial change in the intestinal microbiota in subjects, with well-controlled CVD, consuming an oat drink, with either *L. plantarum* DSM 9843 or without bacteria. Different

blood parameters, markers for systemic inflammation included, and the profile of SCFA in faeces were measured. To our knowledge, this is the first time it has been shown that consumption of a single probiotic strain can increase the diversity of the resident, dominating, intestinal microbiota.

MATERIAL AND METHODS

Patients

Male subjects included in the study were generally in good physical condition but with evidence of atherosclerotic plaque on the carotid wall. Sixteen patients were randomly selected from a larger cohort of subjects included in a randomized, double blind, placebo controlled study, and included in the present study. Subjects were patients at Department of Clinical Medicine at Malmö University Hospital (Malmö, Sweden), and enrolled into the study between April 2001 and May 2002. Participation was voluntary and patients gave written informed consent. Randomization was done by an external partner and the key was closed until the end of the study. The study was approved by the Committee of Ethics at Lund University. Men on antibiotic therapy or presence of infections at the time of study onset, or within four weeks prior to inclusion, were not included in the study. The subjects had neither ongoing intestinal disease, autoimmune disease, nor highly increased plasma concentration of inflammatory blood markers, but values for C-reactive protein varied highly among the patients. Medications and diagnoses are described in Table 1. The median age of subjects receiving active therapy was 70 (range 60-75), and 67 (range 58-74) years in the placebo group. Nine of the subjects consumed oat drink fermented with *L. plantarum* DSM 9843 (= 299v; Probi AB, Lund, Sweden). The active growth of the bacteria in the product (fermentation) granted high numbers of active bacteria. Seven subjects consumed the placebo

product, which was an unfermented oat drink (without any *L. plantarum*) where pH had been adjusted, by addition of lactic acid, according to that of the fermented one. The products were produced and packed by Skane Dairy (Malmö, Sweden). Products were consumed daily, 100 ml per day for four weeks. For the probiotic group the daily intake of *L. plantarum* DSM 9843 was 10^{11} colony forming units (CFU). The volunteers were not allowed to ingest any foods containing lactobacilli with known probiotic effects two weeks before start of consumption of test products nor during the study. Patients were asked to not ingest olives, sauerkraut, pickled raw salmon or gherkin, salami or German sausage, but no other dietary restrictions were applied. Rigid rectoscopy were performed and all biopsies were taken with a sterile forceps at 20 cm from anal verge before and after ingestion of test product for four weeks. Biopsies were stored in TE buffer at -80°C until analysis. Blood samples were taken from one blood vessel in an arm, and blood pressure and heart rate were measured by the research nurse. Subjects reported number of faeces and bowel function in a diary.

DNA extraction

Mucosal biopsies were treated in an ultra sonic bath for 5 minutes, vortexed for 2 minutes and transferred to sterile UV treated tubes and centrifuged at 9 000 rpm for 7 min. Buffer G2 (380 µl) and 30 µl Proteinase K (Qiagen, Hilden, Germany) were added to the pellet and the samples were treated in water bath at 56°C until totally dissolved. The suspension were further disintegrated by shaking together with 12-15 glass beads (2 mm in diameter) for 45 minutes at 4°C in an Eppendorf Mixer (model 5432, Eppendorf, Hamburg, Germany). After centrifugation at 5 000 rpm for 1 min, the supernatant was transferred to two different tubes (200 µl in each tube). Further purification was done in BioRobot® EZ1 with EZ1 DNA Tissue Card and EZ1 DNA Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The DNA was eluted in 200 µl.

PCR amplification, purification, and measurement of DNA concentration

The 16S rRNA genes were amplified with the universal primers Cy5-ENV1 (fluorescently labelled with Cy5 at the 5'-end), and ENV2.⁵ The PCR reaction mixture contained 0.2 µM of each primer, 0.2 mM of each deoxyribonucleotide triphosphate (Roche Diagnostics, Indianapolis, IN), 5 µl of 10x PCR reaction buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3), 2.5 U/µl Taq polymerase (Roche Diagnostics, Mannheim, Germany) and 0.2-10 µl of template, in a final volume of 50 µl. Amplification was made for 32 cycles in an Eppendorf Mastercycler (Hamburg, Germany) using the following program: one cycle at 94°C for 3 minutes, followed by 32 cycles of 94°C for 1 min, 50°C for 45 sec and 72°C for 2 min, with an additional extension at 72°C for 7 min. PCR products were verified on agarose gel after staining with ethidium bromide. Products from three PCR reactions were pooled, purified and concentrated by MinElute PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The DNA was eluted in 30 µl of sterile distilled water.

The concentration of the purified DNA was measured spectrofluorometric by FlouoroMax-2 with DataMax for Windows™ (ISA Jobin Yvon – Spex Instruments S.A., Inc., New Jersey), using Quant-iT™ PicoGreen® (Invitrogen, Eugen, OR, USA) according to the manufacturer's instruction. Excitation was performed at 480 nm.

Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis

Aliquots of 200 ng purified PCR products were digested for 16 h at 37°C by 15 U of restriction endonucleases *MspI* and *HaeIII* (Sigma-Aldrich, St Louis, USA), separately, in a total volume of 10 µl. The enzymes were inactivated at 65°C for 15 min and digestion products were treated as previously described,⁵ except for the internal size standards that contained Cy5-ENV1 primer, 20 basepairs (bp), and 697 bp PCR product amplified from *E. coli* ATCC 11775 by using primer 685r (5'-TCT ACG CAT TTC ACC GCT AC-3';

E. coli numbering 705-685) and Cy5-ENV1. External size standards were used as previously described.⁵ The labelled fragments were separated and detected with an ALFexpress II DNA sequencer with a 7 % ReproGel Long Read gel (GE Healthcare, Uppsala, Sweden) for 700 min under the following conditions: 1500 V, 60 mA, and 55°C.

Diversity analysis

The peak areas of fluorescently labelled T-RFs were estimated by using ALFwin™ Fragment Analyser 1.03 program (Amersham Biosciences, Uppsala, Sweden). The relative abundance of each T-RF within a given T-RFLP pattern was calculated as the peak area of the respective T-RF divided by the total peak area, in the given T-RFLP pattern, within a fragment length of 20 to 697 bp. Simpson's (D) and Shannon and Weaner (Shannon, H') indices were calculated by using the equations: $1-D$ where $D = \sum p_i^2$ and $H' = - \sum p_i \ln p_i$, where p_i is the relative abundance of i th peak in the community.²¹ For each individual, indices were calculated before and after treatment. The difference in diversity was obtained by the following calculation: diversity index after treatment – diversity index before treatment = change in bacterial diversity.

Lactobacilli

For viable count of lactobacilli, faeces were cultured on Rogosa plates (Oxoid) using conventional dilution procedure and anaerobic incubation for three days at 37°C.

SCFA

The amounts of SCFA in faeces were analysed by capillary gas-liquid chromatography after silylation.²² Samples were homogenized with 2-ethylbutyric acid (internal standard), hydrochloric acid was added to protonise the acids and then the SCFA were extracted with

diethylether and silylated. The samples were allowed to stand for 48 h to complete the derivatization before injection.

Biochemical markers

Highly sensitive methods for analysing CRP, tumor necrosis factor alpha (TNF α) and interleukin-6 (IL-6) have been developed at the Department of Clinical Sciences University Hospital Malmö, Lund University. Plasma CRP was measured using rabbit anti-human CRP (Dako A0073) as capture antibody, rabbit anti-human CRP (peroxidase conjugated, DAKO P0227) as detection antibody, human CRP high control (Dako x0926) as standard and TMB one substrate (Dako S1600) as substrate. Detection limit was 0.1 μ g/l. Inter-CV =8%. Plasma TNF α were measured using mouse anti-human TNF α (R&D systems MAB 610) as capture antibody, rabbit anti-human TNF α (Biotin conjugated, R&D BAF210) as detection antibody and streptavidin conjugated ALP (AMPAK Dako K6200) as substrate. Detection limit was 0.5 pg/ml. Inter-CV = 18%. IL-6 was measured in EDTA-plasma using mouse anti-human IL-6 (R&D systems MAB206) as capture, goat anti-human IL-6 (Biotin conjugated, R&D systems BAF 206-IL) as detection and substrate as for TNF α . Detection limit was 0.2 pg/ml.

Fibrinogen, plasminogen activator inhibitor (PAI), triglycerides, total cholesterol, high-density lipoprotein (HDL), and LDL were analysed at Central Laboratory of Clinical Chemistry at Malmö University Hospital, Sweden, using standard laboratory methods.

Statistical analysis

Statistical evaluation was performed using Mann-Whitney Rank Sum Test (SigmaStat 3.1, Systat Software, Point Richmond, USA). The individual changes in the probiotic group were

compared with the changes within the placebo group. A *p*value of <0.05 was considered statistically significant.

RESULTS

Consumption of *L. plantarum* DSM 9843 increased the intestinal bacterial diversity (Table 2). For the probiotic group the individual intestinal diversity was higher compared to the diversity before the start of consumption. The change in diversity was significantly different between the probiotic group and the placebo group, both when Shannon and Simpson indices were calculated (Table 2).

Since more than one bacterial group can present T-RFs of the same length when cut with one enzyme, two restriction endonucleases were used when analysing the intestinal ecosystem. In the current study *MspI* and *HaeIII* were used, both showing higher diversity after probiotic treatment but lower after placebo treatment when compared to the individual diversity before study onset (Table 2).

The probiotic group had significantly lowered faecal concentration of isovaleric acid (*p*=0.006) and valeric acid (*p*=0.029) compared to the placebo group. No other statistically significant changes in concentration of carboxylic acids were observed (Table 3).

The probiotic group had significantly higher viable count of lactobacilli at the end of the study than the placebo group (*p*=0.001). The probiotic group also reported decreased numbers of defecations, compared to the change in the placebo group (*p*=0.034). No significant differences were observed in the biochemical markers (Table 4).

DISCUSSION

The present study included 16 males in good physical condition but with a defined well-controlled cardiovascular disease. Before and after ingestion of test products, the patients underwent rigid rectoscopy. Biopsies were analysed with T-RFLP, which is a powerful molecular genetic method,^{5, 23} generating a fingerprint of the dominating intestinal bacterial groups in a culture-independent way. Only about 20% of the organisms in the GI-tract are cultivable²⁴ and to obtain a more complete picture, genetic methods have been widely used when analysing anaerobic ecosystems.^{5, 23, 25}

It has generally been assumed that the composition of the GI-microbiota is stable over time. However, when using molecular genetic methods with high sensitivity it is possible to detect minor microbial changes, for example, changes induced by diet.²⁵ Increased bacterial load and diversity have been shown in pouchitis patients after administration of a multi-strain probiotic (VSL#3), using culture-independent techniques.²⁶ However, in the present study administration of a single bacterial strain increased the bacterial diversity of the gut, and to our knowledge this is the first time such a thing has been shown.

Since each individual gut can be regarded as an individual ecosystem²⁴ this study accesses the individual change in diversity after consumption of test product. The indices of Shannon and Simpson are well established and are also frequently used to estimate bacterial diversity.^{5, 21} These indices take into account both the richness and evenness of the flora, i.e. the number of bacterial groups and the abundance of each bacterial group. But, Shannon index is more

affected by change in abundance of rare groups while Simpson index is more sensitive to changes in the abundance of the dominating groups.²¹

The present results show that individuals consuming *L. plantarum* DSM 9843 got significantly increased richness and evenness in their intestinal microbiota compared to the change in individuals consuming the placebo product (Table 2). The reason for this effect is unknown but can give rise to several speculations. One explanation may be that *L. plantarum* DSM 9843 has a relatively large genome and a well developed ability to ferment not only many different carbohydrates but also the ability to split and catabolise polyphenols, which might lead to production of metabolites that affect the living conditions favourably of other groups of bacteria. Another suggested explanation may be that the probiotics improve the condition of the GI-mucosa and a healthy mucosa opens up for a more diverse bacterial flora. Mack *et al.*²⁷ have shown increased mucin production by colonic cells when exposed to *L. plantarum* DSM 9843, a fact that perhaps could influence diversity of the intestinal microbiota.

Oral consumption of *L. plantarum* DSM 9843 in a fruit drink has been shown previously to affect the profile of SCFA in faeces of healthy human subjects.¹⁹ This indirectly gives a hint that the microbiota in some way is affected by the probiotic administration. In the present study the change in concentration of SCFA varied between individuals (data not shown) which made it difficult to obtain statistically significant differences between the probiotic group and the placebo group. But still, the probiotic group showed a significantly decrease in the concentrations of isovaleric acid and valeric acid compared to the change in the placebo group (Table 3). Accumulation of isovaleric acid in plasma is observed in the metabolic disease isovaleric acidemia. Since both isovaleric acid and valeric acid are cytotoxic even at physiological concentrations,²⁸ it is preferred to have them kept at low levels. Furthermore,

patients with celiac disease, an inflammatory disorder, have been reported to have increased levels of both isovaleric acid and valeric acid compared to healthy controls.²⁹ Thus to a certain extent, these carboxylic acids may be seen as markers for disorders and the decreased levels in persons consuming *L. plantarum* DSM 9843 might be regarded as beneficial. Moreover, Finley *et al.*³⁰ recently reported a link between decreased concentration of isovaleric acid and increased amount of *Bifidobacterium longum*, indicating more favourable microbiota in individuals with lower amount of isovaleric acid. In accordance with previous results,¹⁹ subjects consuming *L. plantarum* DSM 9843 had increased numbers of lactobacilli in the faeces in the end of the study (Table 4). This further indicates a beneficial microbiota after consumption of *L. plantarum* DSM 9843. On the other hand, the probiotic administration in this case, did not lead to overgrowth of lactobacilli.

It is generally believed that the lifestyle in developed countries causes adverse conditions that induce subclinical inflammation, including cardiovascular diseases,⁸ and it has been shown that people with such conditions have a low bacterial diversity in the gastrointestinal tract.^{3, 23} It remains to be elucidated, if treatment of this lower diversity also is a means by which to overcome the increased incidence of inflammation. However, in the present cohort of patients no significant changes in blood markers were observed. The patients showed highly individual CRP concentrations, but other inflammatory markers were not remarkable different neither before nor after the study period.

In conclusion, the present study showed increased diversity of the dominating intestinal microbiota after consumption of a single bacterial strain with probiotic effects. These results should be verified in larger trials. However, the results suggest that administration of *L. plantarum* DSM 9843 might be a strategy to favour a diverse intestinal microbiota, which

presumably is favourable for the condition of the mucosa, and a healthy mucosa decrease the risk of translocating PAMPs that negatively affects atherosclerosis.

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443

Table 1 Medications and diagnosis of the 16 patients included in the study

	<i>Age</i>	<i>Diagnosis</i>	<i>Medication</i>
<i>Probiotic group</i>			
	60		No
	71	BPH, osteoporosis	Finasterid, vitamin D
	70	Diabetes, gout, dyslipidemia	Glibenclamid,allopurinol, salicylic acid, beta blocker, statin
	70		No
	72	Hypertension, dyslipidemia	Beta-blocker, dipyridamol, calcium antagonist, glyceryl nitrate
	63		No
	65	Hypertension	Beta blocker, ticlopidin
	72		No
	75		No
<i>Placebo group</i>			
	67	Gout	Allopurinol
	58		No
	74		No

62		No
62		No
66	Hypertension, dyslipidemia, depression	Beta-blocker, statin, venlafaxin
68	Dyslipidemia	Statin, salicylic acid
74		No

445

446 **Table 2** Change in colonic microbial diversity indices after four weeks daily administration of
 447 100 ml oat drink fermented by *L. plantarum* DSM 9843 (probiotic group) or unfermented oat
 448 drink (placebo group). Diversity was calculated with Shannon and Simpson diversity indices,
 449 after analysis with T-RFLP using *MspI* or *HaeIII* for DNA cleavage.

	<u><i>Probiotic group</i></u>	<u><i>Placebo group</i></u>	<u><i>p-value</i></u>
<i>Shannon</i>			
<i>MspI</i>	0.0325	-0.450	p=0.026
	(-0.201 – 0.315)	(-0.758 - 0.368)	
<i>HaeIII</i>	0.355	-0.0901	p=0.026
	(0.0632 – 0.601)	(-0.0621 - 0.0814)	
<i>Simpson</i>			
<i>MspI</i>	0.0203	-0.0254	p=0.044
	(-0.028 – 0.0299)	(-0.0577 - 0.0199)	
<i>HaeIII</i>	0.0307	0.0073	p=0.026
	(0.00629 – 0.105)	(-0.0464 - 0.00435)	

450 Values are expressed as group medians (interquartile range)

451 **Table 3** Change in concentration of carboxylic acids ($\mu\text{mol/g}$ faeces) after four weeks
 452 daily administration of 100 ml oat drink fermented by *L. plantarum* DSM 9843 (probiotic
 453 group) or unfermented oat drink (placebo group).

<u><i>Fatty acids</i></u>	<u><i>Probiotic group</i></u>	<u><i>Placebo group</i></u>	<u><i>p-value</i></u>
Acetic acid	1.720 (-10.428 – 21.190)	20.490 (-12.070 – 32.780)	p=0.377
Propionic acid	-0.570 (-3.375 – 6.193)	0.785 (-1.690 – 4.770)	p=0.916
Isobutyric acid	0.080 (-0.158 – 0.575)	1.845 (0.000 – 2.990)	p=0.175
Butyric acid	0.400 (-4.878 – 6.713)	3.725 (-1.110 – 4.480)	p=0.086
Isovaleric acid	-0.910 (-1.405 - 0.645)	0.265 (-0.060 – 1.20)	p=0.006
Valeric acid	-0.760 (-2.015 – 0.165)	0.755 (0.040 – 1.090)	p=0.029
Caproic acid	-0.290 (-1.373 – 0.000)	0.000 (-0.600 – 0.130)	p=0.216
Hepatonoic acid	0.000	0.000	p=0.309

	(-0.618 – 0.000)	(0.000-0.000)	
Lactic acid	0.000	0.000	p=0.859
	(-4.107 – 1.028)	(0.000 – 0.000)	
Succinic acid	0.600	0.235	p=0.316
	(0.0725 – 3.398)	(-0.890 – 1.180)	
SCFA	15.070	26.690	p=0.377
	(-22.040 – 32.432)	(-3.160 – 46.810)	
Total carboxylic acids	-5.450	25.400	p=0.596
	(-24.695 – 34.930)	(-19.800 – 48.950)	

454 Values are expressed as group medians (interquartile range)

455 **Table 4** Change in concentration of biochemical and physiological markers after four weeks
 456 daily administration of 100 ml oat drink fermented by *L. plantarum* DSM 9843 (probiotic
 457 group) or unfermented oat drink (placebo group).

	<u><i>Probiotic group</i></u>	<u><i>Placebo group</i></u>	<u><i>p-value</i></u>
CRP (pg/ml)	93.093 (-135.2 - 4076.5)	79.200 (-81.50 - 405.20)	p=0.916
TNF- α (pg/ml)	0.140 (-0.205 – 0.805)	-0.070 (-0.208 – 0.515)	p=0.560
IL-6 (pg/ml)	0.310 (-1.138 – 2.703)	0.500 (-1.775 – 2.023)	p=0.832
Fibrinogen (g/L)	0.000 (-0.0375 – 0.145)	-0.01 (-0.117 – 0.0425)	p=0.525
PAI (kIE/L)	-1.000 (-4.000 – 3.750)	12 (-1.250 – 18.750)	p=0.125
Triglycerides (mmol/L)	0.120 (-0.075 – 0.427)	-0.010 (-0.398 – 0.665)	p=0.751
Total Cholesterol (mmol/L)	0.150 (-0.337 – 0.323)	0.190 (-0.575 – 0.563)	p=1.000
HDL (mmol/L)	0.000	-0.030	p=0.874

	(-0.110 – 0.125)	(-0.105 – 0.320)	
LDL (mmol/L)	0.100	0.000	p=0.874
	(-0.325 – 0.275)	(-0.375 – 0.450)	
LDL/HDL-cholesterol factor	0.000	0.300	p=0.560
	(-0.300 – 0.275)	(-0.375 – 0.650)	
Lactobacilli (CFU/g faeces)	3.600	0.000	p=0.001
	(3.400 – 4.750)	(0.000 – 0.000)	
Blood pressure, diastolic (mmHg)	8.000	-3.000	p=0.081
	(1.000 – 10.000)	(-7.250 – 0.000)	
Blood pressure, systolic (mmHg)	-2.00	-5	p=0.751
	(-12.75 – 12.50)	(-16 – 7.50)	
Heart rate	-2	-1	p=0.672
	(-2.5 - 0.00)	(-3.5 - 3.75)	
No of faeces / week	0	2	p=0.034
	(-2.25 – 0.75)	(1.25 – 4.50)	
Bowel function	0	-6	p=0.672
	(-6.75 – 1.75)	(-25 – 2.5)	

458 Values are expressed as group medians (interquartile range)