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1 **ABSTRACT**

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

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

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

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

24 **Probiotic therapy to men with incipient arteriosclerosis initiates increased bacterial**

25 **diversity in colon: a randomized controlled trial.**

26

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51 **INTRODUCTION**

52 In biology, it is a general consensus that a high diversity of organisms is beneficial for the
53 ecosystem on both global and local levels. A high diversity indicates an ecosystem in healthy
54 balance and it provides a higher resilience to ecological disturbances which also applies for
55 microbial ones.[1] In contrast, an unbalanced, disturbed, or diseased ecosystem opens up for
56 "overgrowth" of a few organisms, and contributes to further disturbances and new diseased
57 conditions, a fact valid for the microbial ecosystem of the human gut.[2] For example, it has
58 been shown that patients with Crohn's disease and ulcerative colitis have low bacterial
59 diversity in the gastro-intestinal (GI) tract.[3, 4] Furthermore, infants with low bacterial diversity
60 in faeces at one week of age more frequently develop atopic eczema after 18 months.[5]
61
62 The bacterial flora of the GI-tract makes up a complex ecosystem and the composition and
63 activity of the different bacterial groups play important roles for the health status due to its
64 contribution to, for example, nutrition, colonisation resistance, and development and tuning of
65 the immune system.[6] Pro-inflammatory components of the resident microbiota can increase
66 the permeability of the mucosal barrier and cause subclinical inflammation.[7]
67
68 Cardiovascular diseases (CVD) attributes one-third of all global deaths.[8] The aetiology is not
69 fully understood but obesity, high blood pressure, physical inactivity and a diet high in
70 saturated fats, salt and refined carbohydrates have been shown to increase the risk to develop
71 CVD.[8] Oxidative stress and oxidation of low-density lipoprotein (LDL) are important factors
72 involved in the chronic inflammation of the arteries and atherosclerosis onset.[9, 10]
73 Atherosclerosis is the pathopsysiological process underlying CVD, but exactly what signals
74 starting the disease is not fully understood and microbial components signalling through toll-
75 like receptors (TLRs) may play a role.[10] Enhanced expression of TLRs has been shown in
76 human atherosclerotic plaques.[11] Previously, *Chlamydia pneumonie* has been associated with

77 increased risk of CVD[12] and it has been hypothesised that live bacteria colonise the artery
78 wall and initiate infection leading to atherosclerosis. But this has been hard to prove and a
79 refined version of the infection hypothesis focuses on pathogen-associated molecular patterns
80 (PAMPs). Bacterial molecules e.g. lipopolysaccharides, flagellin and bacterial lipopeptide are
81 examples of PAMPs that bind to TLRs and initiate signalling.[10] Disruption of the epithelial
82 tight junctions can be mediated by inflammatory mediators and the consequence will be
83 increased intestinal translocation during inflammatory conditions.[13] PAMPs from the GI-
84 microbiota can translocate through the intestinal epithelial cell layer into the circulation, even
85 in relatively healthy persons,[14] making this process to one of many possible explanation to the
86 origin of atherosclerosis onset. Furthermore, bacterial overgrowth in the intestine increases
87 the translocation,[15] highlighting the importance of a balanced microbiota.
88
89 The bacterial species *L. plantarum* frequently occurs spontaneously and in high numbers in
90 most lactic acid fermented foods, especially when based on plant material, for example, in
91 brined olives, capers, sauerkraut, salted gherkins and sourdough.[16] However, *L. plantarum* is
92 also present on human oral and intestinal mucosa.[17] The strain *L. plantarum* DSM 9843 has
93 been isolated from healthy human intestinal mucosa[17] and is by now a well studied probiotic
94 strain used in a number of human studies.[18, 19] Intake of this strain in a rosehip drink has
95 shown to affect the intestinal environment by effects on the short-chain fatty acid content
96 (SCFA).[19] *L. plantarum* DSM 9843 also attaches to human mucosa cells by a mannose-
97 dependent adherence mechanism,[20] which favour persistence and close interaction with the
98 host. The concept of probiotics implies that the balance between beneficial and harmful
99 bacteria in the GI-microbiota can be positively affected by eating the right type of living
100 microorganisms. This is in line with the present study where the aim was to clarify the
101 microbial change in the intestinal microbiota in subjects, with well-controlled CVD,
102 consuming an oat drink, with either *L. plantarum* DSM 9843 or without bacteria. Different

103 blood parameters, markers for systemic inflammation included, and the profile of SCFA in
104 faeces were measured. To our knowledge, this is the first time it has been shown that
105 consumption of a single probiotic strain can increase the diversity of the resident, dominating,
106 intestinal microbiota.
107
108
109 **MATERIAL AND METHODS**
110
111 **Patients**
112 Male subjects included in the study were generally in good physical condition but with
113 evidence of atherosclerotic plaque on the carotid wall. Sixteen patients were randomly
114 selected from a larger cohort of subjects included in a randomized, double blind, placebo
115 controlled study, and included in the present study. Subjects were patients at Department of
116 Clinical Medicine at Malmö University Hospital (Malmö, Sweden), and enrolled into the
117 study between April 2001 and May 2002. Participation was voluntary and patients gave
118 written inform consent. Randomization was done by an external partner and the key was
119 closed until the end of the study. The study was approved by the Committee of Ethics at Lund
120 University. Men on antibiotic therapy or presence of infections at the time of study onset, or
121 within four weeks prior to inclusion, were not included in the study. The subjects had neither
122 ongoing intestinal disease, autoimmune disease, nor highly increased plasma concentration of
123 inflammatory blood markers, but values for C-reactive protein varied highly among the
124 patients. Medications and diagnoses are described in Table 1. The median age of subjects
125 receiving active therapy was 70 (range 60-75), and 67 (range 58-74) years in the placebo
126 group. Nine of the subjects consumed oat drink fermented with *L. plantarum* DSM 9843 (=
127 299v; Probi AB, Lund, Sweden). The active growth of the bacteria in the product
128 (fermentation) granted high numbers of active bacteria. Seven subjects consumed the placebo

129 product, which was an unfermented oat drink (without any *L. plantarum*) where pH had been
130 adjusted, by addition of lactic acid, according to that of the fermented one. The products were
131 produced and packed by Skane Dairy (Malmö, Sweden). Products were consumed daily,
132 100 ml per day for four weeks. For the probiotic group the daily intake of *L. plantarum*
133 DSM 9843 was 10^{11} colony forming units (CFU). The volunteers were not allowed to ingest
134 any foods containing lactobacilli with known probiotic effects two weeks before start of
135 consumption of test products nor during the study. Patients were asked to not ingest olives,
136 sauerkraut, pickled raw salmon or gherkin, salami or German sausage, but no other dietary
137 restrictions were applied. Rigid rectoscopy were performed and all biopsies were taken with a
138 sterile forceps at 20 cm from anal verge before and after ingestion of test product for four
139 weeks. Biopsies were stored in TE buffer at -80°C until analysis. Blood samples were taken
140 from one blood vessel in an arm, and blood pressure and heart rate were measured by the
141 research nurse. Subjects reported number of faeces and bowel function in a diary.

142

143 **DNA extraction**

144 Mucosal biopsies were treated in an ultra sonic bath for 5 minutes, vortexed for 2 minutes and
145 transferred to sterile UV treated tubes and centrifuged at 9 000 rpm for 7 min. Buffer G2
146 (380 µl) and 30 µl Proteinas K (Qiagen, Hilden, Germany) were added to the pellet and the
147 samples were treated in water bath at 56ºC until totally dissolved. The suspension were
148 further disintegrated by shaking together with 12-15 glass beads (2 mm in diameter) for
149 45 minutes at 4ºC in an Eppendorf Mixer (model 5432, Eppendorf, Hamburg, Germany).
150 After centrifugation at 5 000 rpm for 1 min, the supernatant was transferred to two different
151 tubes (200 µl in each tube). Further purification was done in BioRobot® EZ1 with EZ1 DNA
152 Tissue Card and EZ1 DNA Tissue Kit (Qiagen, Hilden, Germany) according to the
153 manufacturer's instruction. The DNA was eluted in 200 µl.

154

155 **PCR amplification, purification, and measurement of DNA concentration**

156 The 16S rRNA genes were amplified with the universal primers Cy5-ENV1 (fluorescently
157 labelled with Cy5 at the 5´-end), and ENV2.[5] The PCR reaction mixture contained 0.2 µM of
158 each primer, 0.2 mM of each deoxyribonucleotide triphosphate (Roche Diagnostics,
159 Indianapolis, IN), 5 µl of 10x PCR reaction buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3),
160 2.5 U/µl Taq polymerase (Roche Diagnostics, Mannheim, Germany) and 0.2-10 µl of
161 template, in a final volume of 50 µl. Amplification was made for 32 cycles in an Eppendorf
162 Mastercycler (Hamburg, Germany) using the following program: one cycle at 94ºC for 3
163 minutes, followed by 32 cycles of 94ºC for 1 min, 50ºC for 45 sec and 72ºC for 2 min, with an
164 additional extension at 72ºC for 7 min. PCR products were verified on agarose gel after
165 staining with ethidium bromide. Products from three PCR reactions were pooled, purified and
166 concentrated by MinElute PCR Purification Kit (Qiagen, Hilden, Germany) according to the
167 manufacturer's protocol. The DNA was eluted in 30 µl of sterile distilled water.

168

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

173

174 **Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis**

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

181 *E. coli* numbering 705-685) and Cy5-ENV1. External size standards were used as previously

182 described.[5] The labelled fragments were separated and detected with an ALFexpress II DNA

183 sequencer with a 7 % ReproGel Long Read gel (GE Healthcare, Uppsala, Sweden) for

184 700 min under the following conditions: 1500 V, 60 mA, and 55ºC.

185

186 **Diversity analysis**

187 The peak areas of fluorescently labelled T-RFs were estimated by using ALFwin™ Fragment

188 Analyser 1.03 program (Amersham Biosciences, Uppsala, Sweden). The relative abundance

189 of each T-RF within a given T-RFLP pattern was calculated as the peak area of the respective

190 T-RF divided by the total peak area, in the given T-RFLP pattern, within a fragment length of

191 20 to 697 bp. Simpson's (D) and Shannon and Weaner (Shannon, H´) indices were calculated

192 by using the equations: 1-D where $D = \Sigma\, p_i^2$ and $H' = -\Sigma\, p_i \ln p_i$, where p_i is the relative

193 abundance of *i*th peak in the community.[21] For each individual, indices were calculated before

194 and after treatment. The difference in diversity was obtained by the following calculation:

195 diversity index after treatment – diversity index before treatment = change in bacterial

196 diversity.

197

198 **Lactobacilli**

199 For viable count of lactobacilli, faeces were cultured on Rogosa plates (Oxoid) using

200 conventional dilution procedure and anaerobic incubation for three days at 37°C.

201

202 **SCFA**

203 The amounts of SCFA in faeces were analysed by capillary gas-liquid chromatography after

204 silylation.[22] Samples were homogenized with 2-ethylbutyric acid (internal standard),

205 hydrochloric acid was added to protonise the acids and then the SCFA were extracted with

206 diethylether and silylated. The samples were allowed to stand for 48 h to complete the
207 derivatization before injection.
208
209 **Biochemical markers**
210 Highly sensitive methods for analysing CRP, tumor necrosis factor alpha (TNFα)
211 and interleukin-6 (IL-6) have been developed at the Department of Clinical Sciences
212 University Hospital Malmö, Lund University. Plasma CRP was measured using rabbit anti-
213 human CRP (Dako A0073) as capture antibody, rabbit anti-human CRP (peroxidase
214 conjugated, DAKO P0227) as detection antibody, human CRP high control (Dako x0926) as
215 standard and TMB one substrate (Dako S1600) as substrate. Detection limit was 0.1 μg/l.
216 Inter-CV =8%. Plasma TNFα were measured using mouse anti-human TNFα (R&D systems
217 MAB 610) as capture antibody, rabbit anti-human TNFα (Biotin conjugated, R&D BAF210)
218 as detection antibody and streptavidin conjugated ALP (AMPAK Dako K6200) as substrate.
219 Detection limit was 0.5 pg/ml. Inter-CV = 18%. IL-6 was measured in EDTA-plasma using
220 mouse anti-human IL-6 (R&D systems MAB206) as capture, goat anti-human IL-6 (Biotin
221 conjugated, R&D systems BAF 206-IL) as detection and substrate as for TNFα. Detection
222 limit was 0.2 pg/ml.
223
224 Fibrinogen, plasminogen activator inhibitor (PAI), triglycerides, total cholesterol, high-
225 density lipoprotein (HDL), and LDL were analysed at Central Laboratory of Clinical
226 Chemistry at Malmö University Hospital, Sweden, using standard laboratory methods.
227
228 **Statistical analysis**
229 Statistical evaluation was performed using Mann-Whitney Rank Sum Test (SigmaStat 3.1,
230 Systat Software, Point Richmond, USA). The individual changes in the probiotic group were

231 compared with the changes within the placebo group. A *p*value of <0.05 was considered
232 statistically significant.
233
234
235 **RESULTS**
236
237 Consumption of *L. plantarum* DSM 9843 increased the intestinal bacterial diversity (Table 2).
238 For the probiotic group the individual intestinal diversity was higher compared to the diversity
239 before the start of consumption. The change in diversity was significantly different between
240 the probiotic group and the placebo group, both when Shannon and Simpson indices were
241 calculated (Table 2).
242
243 Since more than one bacterial group can present T-RFs of the same length when cut with one
244 enzyme, two restriction endonucleases were used when analysing the intestinal ecosystem. In
245 the current study *Msp*I and *Hae*III were used, both showing higher diversity after probiotic
246 treatment but lower after placebo treatment when compared to the individual diversity before
247 study onset (Table 2).
248
249 The probiotic group had significantly lowered faecal concentration of isovaleric acid
250 (p=0.006) and valeric acid (p=0.029) compared to the placebo group. No other statistically
251 significant changes in concentration of carboxylic acids were observed (Table 3).
252
253 The probiotic group had significantly higher viable count of lactobacilli at the end of the study
254 than the placebo group (p=0.001). The probiotic group also reported decreased numbers of
255 defecations, compared to the change in the placebo group (p=0.034). No significant
256 differences were observed in the biochemical markers (Table 4).

257

258

259 **DISCUSSION**

260

261 The present study included 16 males in good physical condition but with a defined well-
262 controlled cardiovascular disease. Before and after ingestion of test products, the patients
263 underwent rigid rectoscopy. Biopsies were analysed with T-RFLP, which is a powerful
264 molecular genetic method,[5, 23] generating a fingerprint of the dominating intestinal bacterial
265 groups in a culture-independent way. Only about 20% of the organisms in the GI-tract are
266 cultivable[24] and to obtain a more complete picture, genetic methods have been widely used
267 when analysing anaerobic ecosystems.[5, 23, 25]

268

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

276

277 Since each individual gut can be regarded as an individual ecosystem[24] this study accesses the
278 individual change in diversity after consumption of test product. The indices of Shannon and
279 Simpson are well established and are also frequently used to estimate bacterial diversity.[5, 21]
280 These indices take into account both the richness and evenness of the flora, i.e. the number of
281 bacterial groups and the abundance of each bacterial group. But, Shannon index is more

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

284

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

297

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

308 patients with celiac disease, an inflammatory disorder, have been reported to have increased
309 levels of both isovaleric acid and valeric acid compared to healthy controls.[29] Thus to a
310 certain extent, these carboxylic acids may be seen as markers for disorders and the decreased
311 levels in persons consuming *L. plantarum* DSM 9843 might be regarded as beneficial.
312 Moreover, Finley *et al.*[30] recently reported a link between decreased concentration of
313 isovaleric acid and increased amount of *Bifidobacterium longum*, indicating more favourable
314 microbiota in individuals with lower amount of isovaleric acid. In accordance with previous
315 results,[19] subjects consuming *L. plantarum* DSM 9843 had increased numbers of lactobacilli
316 in the faeces in the end of the study (Table 4). This further indicates a beneficial microbiota
317 after consumption of *L. plantarum* DSM 9843. On the other hand, the probiotic administration
318 in this case, did not lead to overgrowth of lactobacilli.
319
320 It is generally believed that the lifestyle in developed countries causes adverse conditions that
321 induce subclinical inflammation, including cardiovascular diseases,[8] and it has been shown
322 that people with such conditions have a low bacterial diversity in the gastrointestinal tract.[3, 23]
323 It remains to be elucidated, if treatment of this lower diversity also is a means by which to
324 overcome the increased incidence of inflammation. However, in the present cohort of patients
325 no significant changes in blood markers were observed. The patients showed highly
326 individual CRP concentrations, but other inflammatory markers were not remarkable different
327 neither before nor after the study period.
328
329 In conclusion, the present study showed increased diversity of the dominating intestinal
330 microbiota after consumption of a single bacterial strain with probiotic effects. These results
331 should be verified in larger trials. However, the results suggest that administration of
332 *L. plantarum* DSM 9843 might be a strategy to favour a diverse intestinal microbiota, which

333 presumably is favourable for the condition of the mucosa, and a healthy mucosa decrease the
334 risk of translocating PAMPs that negatively affects atherosclerosis.
335

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340

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344 *Lactobacillus plantarum* DSM 9843. Probi AB was involved in analysis of SCFA, and
345 culturing lactobacilli. Probi AB did not take part in any other analysis, collection of patients,
346 interpretation of data, nor statistical evaluation. CK, SA, GM and BJ, are minority
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443

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

	Age	Diagnosis	Medication
Probiotic group			
	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
Placebo group			
	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 *Msp*I or *Hae*III for DNA cleavage.

	Probiotic group	***Placebo group***	***p-value***
Shannon			
*Msp*I	0.0325	-0.450	p=0.026
	(-0.201 – 0.315)	(-0.758 - 0.368)	
*Hae*III	0.355	-0.0901	p=0.026
	(0.0632 – 0.601)	(-0.0621 - 0.0814)	
Simpson			
*Msp*I	0.0203	-0.0254	p=0.044
	(-0.028 – 0.0299)	(-0.0577 - 0.0199)	
*Hae*III	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 (μmol/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).

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

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