Microbial Host Interactions

Intestinal *Lactobacillus* sp. is associated with some cellular and metabolic characteristics of blood in elderly people

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1. Introduction

Gut microbiota forms an essential part of the complex ecosystem of the host and is involved in nutrition and health. Sequencing 16S rRNA genes of human stool samples accompanied with biochemical analyses have shown that some dominant bacterial divisions are associated with host nutrient uptake, metabolism, harvesting of energy and increased body weight. In obese people, a higher proportion of gram-positive *Firmicutes* as compared to gram-negative *Bacteroidetes* was found in gut [1,2]. However, which particular groups of microbes from the *Firmicutes* division found in the gut could mainly exert the collective metabolic influence on human health has not been fully explored among different age groups.

Elderly persons (>65 years) are the fastest growing subpopulation in the world [3]. During aging, mainly degenerative alterations in gut morphology and physiology occur. The disturbances in some metabolic pathways and decreased energy expenditure commonly result in excessive weight and obesity, driving elderly people to several medical complications – such as cardiovascular disease (CVD), hypertension, diabetes and osteoarthritis [4,5].

Keywords: Intestinal lactobacilli, Elderly, Blood WBC count, Blood Glucose, Blood ox-LDL.
A wide variety of host, microbiological, dietary and environmental factors affect bacterial colonization of the intestinal tract. In the elderly, the reduction in number and species diversity of anaerobes like bacteroides and bifidobacteria, and the increased abundance of aerobes as enterobacteria has been shown [6–8]. Nevertheless, there have been contradictory data concerning prevalence and numbers of Lactobacillus sp. during aging, accentuated with high inter-individual variation in species composition [9–11]. It has not been assessed if the higher counts or particular species of lactobacilli are related to host metabolic and defence reactions, performing a balanced human ecosystem.

The purpose of the pilot study was to evaluate the relations between molecularly assessed intestinal Lactobacillus sp. composition, the counts of fecal cultivable lactobacilli and some clinical (BMI and blood pressure) and laboratory blood indices (cellular-erythrocytes, white blood cells, platelets; metabolic-glucose, cholesterol; oxidative stress-related markers – ox-LDL and BDC-LDL) in healthy elderly people.

2. Materials and methods

2.1. Subjects

A total of 42 elderly persons from an area in Southern Estonia were recruited into the pilot study. The healthy elderly were selected from the registry of family doctors and by orthopedists of Tartu University Hospital scheduling the subjects for elective orthopedic surgery. The inclusion criteria for volunteers were: the age 65 years or above, considered themselves generally healthy and wished to participate in the study (confirmed by written informed consent). Subjects following special dietary routines, having an unstable cardiopulmonary system, with a history of diabetes and malignancy, chronic renal or hepatic failure, gut surgery, the presence of an acute illness four weeks prior to the study, on anti-malignancy, chronic renal or hepatic failure, gut surgery, the presence of an acute illness four weeks prior to the study, on anti-inflammatory drugs, antibiotics during the last 6 months was 4.5%.

Blood samples were obtained in the early morning after 8 h of fasting. Samples (6 ml) were drawn from antecubital vein with vacutainer into heparinised tubes and immediately stored (on ice) at 4 °C. The plasma was separated from the cells by centrifugation at 3000 rpm for 10 min and the plasma samples were stored at −80 °C until analysis.

Hematological-clinical indices (hemoglobin, red blood cells, white blood cell count and platelets) as well as routine biochemical-clinical indices like plasma glucose and serum total cholesterol were determined by standard laboratory methods using certified assays in the local clinical laboratory of Tartu University Hospital.

2.4. Blood samples

Oxidized low-density lipoprotein (ox-LDL) was measured in plasma, which had been stored at −70 °C as previously described [15]. Ox-LDL levels were measured using an enzyme-linked immunosorbent assay kit (Mercodia, AB, Uppsala, Sweden). This kit uses monoclonal antibody 4E6 [16] directed against a conformational epitope in the apo B-100 moiety of LDL that is generated as a consequence of substitution of at least 60 lysine residues of apo B-100 with aldehydes. The number of substituted lysines corresponds to the minimum number required for scavenger-mediated uptake of oxidized LDL (or apo B-100 aldehyde-modified form of ox-LDL). Substituting aldehydes can be produced by peroxidation of lipids of LDL, resulting in the generation of ox-LDL. Additionally, aldehydes that are released by endothelial cells under oxidative stress or by activated platelets may also induce the oxidative modification of apo B-100 in the absence of lipid peroxidation of LDL. For ox-LDL, within-assay variation coefficient (CV) was 6.3% and between-assay CV 4.7% [17].

For the measurement of baseline diene conjugates in low-density lipoproteins (BDC-LDL) the serum was separated from blood cells by centrifugation at 3000 × g for 15 min [18]. To precipitate the LDL, 1 mg/l of EDTA was added and precipitation reagents were allowed to equilibrate to room temperature. The blood sample (0.5 ml) was added to 3.5 ml of the heparin-citrate buffer. The precipitation buffer consisted of 0.064 mol/l trisodium citrate adjusted to pH 5.05 with 5 mol/l HCl, and contained 50.000 IU/l heparin. After mixing with a Vortex mixer, the suspension was allowed to stand for 10 min at room temperature. The insoluble lipoproteins were sedimented by centrifugation at 1000 × g for 10 min. The pellet was resuspended in 0.5 ml of 0.1 mol/l Na-phosphate buffer, pH 8.0, and containing 0.9% of NaCl. Lipids were extracted from LDL samples (100 μl) by chloroform–methanol (2:1), dried under nitrogen, then re-dissolved in cyclohexane, and analysed spectrophotometrically at 234 nm for diene conjugates. Absorbance units (difference A334–A300) were converted to molar units using the molar extinction coefficient 2.95 × 10² M⁻¹ cm⁻¹. For BDC-LDL, the CV for within-assay precise determinations of samples precipitated from the same serum was 4.4%, and the CV for the between-assay precision over a period of 3 months was 4.5%.

2.5. Oxidative stress indices of blood

The different epitopes of LDL particles were measured by testing both the level of oxidation of apolipoprotein B (ox-LDL assay) and baseline diene conjugates in LDL lipids as a direct measure of in vivo LDL particle oxidation (BDC-LDL assay).

Lipids were extracted from LDL samples (100 μl) by chloroform–methanol (2:1), dried under nitrogen, then re-dissolved in cyclohexane, and analysed spectrophotometrically at 234 nm for diene conjugates. Absorbance units (difference A334–A300) were converted to molar units using the molar extinction coefficient 2.95 × 10² M⁻¹ cm⁻¹. For BDC-LDL, the CV for within-assay precise determinations of samples precipitated from the same serum was 4.4%, and the CV for the between-assay precision over a period of 3 months was 4.5%.

2.6. Molecular investigation of intestinal Lactobacillus sp in fecal samples

Approximately 2 g of fresh stool samples were obtained and put into sterile plastic cups. The samples were collected and delivered
within 1 h after defecation to the laboratory where the samples were stored at –20 °C before processing.

Bacterial DNA from fecal samples was extracted using a QIAamp DNA stool mini kit (QIAGen, Hilden, Germany) with some modifications. The amount of 0.22 g of feces were resuspended in 200 µl of TE buffer (10 mM Tris, 10 mM EDTA pH 8, 20 mg/ml lysozyme, 200 µ/ml mutanolysin) and incubated for 1 h at 37 °C. A total of 0.3 g of 0.1 mm zirconia/silica beads and 1.4 ml of ASL solution from the stool mini kit was added to fecal samples. The tubes were then agitated for 3 min at a speed of 5000 rpm in a mini-beadbeater (BiospecProducts). The protocol was then continued as described by the manufacturer. The amount of DNA was determined visually after electrophoresis on a 1.2% agarose gel containing ethidium bromide.

After electrophoresis on a 1.2% agarose gel containing ethidium bromide, the stool mini kit was added to fecal samples. The tubes were then agitated for 3 min at a speed of 5000 rpm in a mini-beadbeater (BiospecProducts). The protocol was then continued as described by the manufacturer. The amount of DNA was determined visually after electrophoresis on a 1.2% agarose gel containing ethidium bromide.

The Lactobacillus species-specific qualitative PCR was carried out by primers listed in Table 1 targeted on the 16S-23S rRNA intergenic spacer region [19–21]. A reaction mixture (50 µl) consisted of 10X reaction buffer, a 200 µM concentration of each deoxyribose triphosphate, 10 pmol of each primers, 5 µl of bacterial DNA (extracted from fecal samples) and 1.5 U of HotStar Taq Plus DNA polymerase (QIAGen, Hilden, Germany). The amplification program was 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 30 s at the appropriate annealing temperature (Table 1), and 72 °C for 30 s. A cycle of 72 °C for 10 min concluded the program. Amplification products were detected by agarose gel electrophoresis on 2% agarose gel, ethidium bromide staining, and UV transillumination.

The size of PCR products was compared with the aforementioned Lactobacillus reference strains (Lactobacillus acidophilus ATCC 4356, Lactobacillus delbrueckii spp. delbrueckii ATCC 9649, Lactobacillus helveticus ATCC 8018, Lactobacillus paracasei DSM 20020, Lactobacillus casei spp. casei NCDC150, Lactobacillus rhamnosus ATCC 53103, Lactobacillus plantarum DSM 9843, Lactobacillus fermentum ATCC 14931, L. reuteri DSM 20016). The number of different Lactobacillus species colonizing the individual and their prevalence in elderly was assessed.

2.7. Estimation of cultivable lactobacilli

For bacteriological analyses the weighed fecal samples were serially diluted (10⁻²–10⁻⁸) in pre-reduced phosphate buffer (pH 7.2) inside the anaerobic glove box (Sheldon Manufacturing Inc., USA, with a gas mixture: 5% CO₂; 5% H₂, 90% N₂). A quantitative analysis of the gut bacteria was performed using duplicate samples (abdominal pain, flatulence, bloating, and stool frequency; p > 0.05; data not shown). Only three current smokers were registered and 16 persons were habitual consumers of probiotic functional food. Differences were considered statistically significant if the value was p < 0.05.

3. Results

3.1. Questionnaire data

According to self-reported questionnaires, the participants did not complain about expressed habitual gastrointestinal symptoms (abdominal pain, flatulence, bloating, and stool frequency; p > 0.05; data not shown). Only three current smokers were registered and 16 persons were habitual consumers of probiotic functional food. The female elderly were more frequent consumers of probiotic food than male (OR = 5.2 CI95 1.15–23.54; p = 0.0324).

2.8. Statistical analysis

Statistical analysis was performed by using the computer program Sigma Stat for Windows 2.0 (Jandel Corporation, USA). The Student’s t-test or Mann–Whitney U test (nonparametric distribution) was applied to compare the differences in the clinical and microbiological indices of the probiotic consumers and non-consumers. The χ²-test or Fisher exact test was used to determine the between-group differences in categorical variables. Spearman correlations were used to determine the relationships between variables. Linear multiple regression analysis with an additive model adjusted for age, sex and BMI was used to detect the association between variables 1 and 2 of Table 3. Logistic regression analyses were performed to compare the binary variables and/or continuous variables. Results are given with odds ratios (OR) with 95% CI. The models were adjusted for BMI, sex and age. Differences were considered statistically significant if the value was p < 0.05.

Table 1

<table>
<thead>
<tr>
<th>Target</th>
<th>Primers</th>
<th>Sequence (5'-3')</th>
<th>Annealing temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. acidophilus</td>
<td>F_acid_IS</td>
<td>GAAACGACCCCAAACCAAGTATT</td>
<td>59</td>
</tr>
<tr>
<td>L. casei</td>
<td>R_casei_IS</td>
<td>CTTCCAGATAATCCAACTACGTTA</td>
<td>59</td>
</tr>
<tr>
<td>L. delbrueckii</td>
<td>R_delb_IS</td>
<td>CTTCTGGCGGTACTGAGATCT</td>
<td>59</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>R_ferm_IS</td>
<td>CGAATCTTCCTCGCTT</td>
<td>58</td>
</tr>
<tr>
<td>L. paracasei</td>
<td>R_paca_IS</td>
<td>TAATCGACTTGAGATGTCCTA</td>
<td>58</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>R_plan_IS</td>
<td>AACCCGAGAACCCCGTATT</td>
<td>58</td>
</tr>
<tr>
<td>L. reuteri</td>
<td>R_reut_IS</td>
<td>ACATCGTAGTATGTCAGCTAAT</td>
<td>60</td>
</tr>
<tr>
<td>L. rhamnosus</td>
<td>F_rham_IS</td>
<td>CCGCCCTGACCTCCTTTTCTTTCT</td>
<td>59</td>
</tr>
<tr>
<td>L. helveticus</td>
<td>R_helv_IS</td>
<td>CTTCCTCCTCGCTT</td>
<td>56</td>
</tr>
</tbody>
</table>

Table 1

List of primers used in this study for detection of Lactobacillus sp according to literature [19–21].

F_acid_IS: GAAACGACCCCAAACCAAGTATT
R_acid_IS: CTTCCAGATAATCCAACTACGTTA
F_casei_IS: CTTCTGGCGGTACTGAGATCT
R_casei_IS: CGAATCTTCCTCGCTT
F_delb_IS: TAATCGACTTGAGATGTCCTA
R_delb_IS: AACCCGAGAACCCCGTATT
F_paca_IS: ACATCGTAGTATGTCAGCTAAT
R_paca_IS: CCGCCCTGACCTCCTTTTCTTTCT
F_plan_IS: CTTCCTCCTCGCTT
R_plan_IS: CTTCCTCCTCGCTT
F_helv_IS: GAATGATGCGAGATGACATT
R_helv_IS: CTTCCTCCTCGCTT
3.2. Clinical and blood indices

No substantial shifts were found in the functioning of cardiovascular system and the state of skin and mucosa (data not shown). The values of fasting plasma glucose, Hgb and the counts of blood cells (erythrocytes, white blood cells and platelets) responded to the reference values (Table 2). As compared to reference data the increased values of systolic (34%) and diastolic (16%) blood pressure, overweight (37%) and obesity (24%) were registered in several subjects while increased cholesterol values were found in 76% of the elderly persons.

3.3. Lactobacillus species composition of elderly

The overall prevalence of the 8 species out of 9 in the fecal samples of the elderly is depicted in Fig. 1. We could not detect the specific banding pattern of L. rhamnosus in any elderly person. The facultative heterofermentative lactobacilli L. casei and L. paracasei were the most frequent (89% and 97%, respectively) species found, while L. acidophilus, L. plantarum and L. reuteri were present in almost half of the elderly persons (47%, 58% and 53%, respectively). According to PCR products the number of species simultaneously colonizing the individuals was 1–7 (median 4).

The colonization by L. acidophilus was negatively related ($r = -0.367, p = 0.0275$) to L. reuteri adjusted for age, sex and BMI (OR = 0.16, CI95 0.04–0.73; $p = 0.018$) applying logistic regression analysis.

3.4. Quantitative composition of fecal microbiota

Lactobacilli were present in all investigated elderly persons, their median count was 6.8 (range 3.3–10.8 log cfu/g). The counts of lactobacilli were similar (median 7.3, range 3.7–9.7 vs. median 6.8, range 3.3–10.8 log cfu/g, $p > 0.05$) if the persons were divided according to the values of BMI (<24.9 and ≥25 kg/m²) and no correlations between these indices were found. Also, the counts of lactobacilli were not predicted from the Lactobacillus species composition and the number of species simultaneously colonizing the individuals (data not shown).

In elderly consuming probiotics the lactobacilli counts were significantly higher than in those not consuming (median 7.8, range 4.2–10.8 vs. median 6.3, range 3.3–9.7 log cfu/g, $p = 0.005$). This difference stayed significant if adjusted for sex and BMI (OR = 1.71, CI95 1.04–2.82; $p = 0.035$).

3.5. Comparisons of cellular and metabolic markers with Lactobacillus species composition and counts in the elderly

A positive correlation ($r = 0.402; p = 0.014$) Table 3 was found between BMI and fasting blood glucose content adjusted for age and sex applying linear regression analysis (Adj. $R^2 = 0.084$, $p = 0.022$).

Also the values of ox-LDL were positively correlated to cholesterol values ($r = 0.414, p = 0.011$) adjusted for age, sex and BMI (Adj. $R^2 = 0.217, p = 0.018$). The counts and colonization with different species of lactobacilli were not directly associated with BMI of elderly. However, the blood glucose concentration showed a tendency for a negative correlation for colonization with L. fermentum ($r = -0.309, p = 0.062$) adjusted for BMI (Adj. $R^2 = 0.181, p = 0.013$) yet the sex and age were the confounding factors.

The increased WBC count was related ($r = 0.434, p = 0.007$) to presence of L. reuteri (Adj. $R^2 = 0.193, p = 0.027$) adjusted for age, sex and BMI. The values of ox-LDL were negatively related to lactobacilli counts ($r = -0.389, p = 0.016$) which association stayed significant according to linear regression analysis adjusted for age, sex and BMI (Adj. $R^2 = 0.184, p = 0.029$).

4. Discussion

In our study, the association between some principal cellular (WBC count), metabolic (concentration of plasma glucose) and oxidative stress (ox-LDL) indices of blood with intestinal Lactobacillus sp. was assessed, demonstrating the systemic defense potential of Lactobacillus sp. in host microbial ecology.

The excessive weight, increased values of blood glucose and WBC counts together with oxidative stress indices are used widely as predictors for inflammation, metabolic syndrome and cardiovascular disease (CVD) [4,25,26]. At the same time it is well-known

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**Table 2**

Clinical and biochemical data of healthy elderly persons.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total $n = 38$</th>
<th>Reference values$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.1 ± 4.0</td>
<td></td>
</tr>
<tr>
<td>Normal (N%)</td>
<td>15 (39%)</td>
<td>Normal 18.5–24.9 kg/m²</td>
</tr>
<tr>
<td>Overweight (N%)</td>
<td>14 (37%)</td>
<td>Overweight &gt; 25</td>
</tr>
<tr>
<td>Obesity (N%)</td>
<td>9 (24%)</td>
<td>Obese &gt; 30</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134 ± 16.1</td>
<td>&lt; 130 mmHg</td>
</tr>
<tr>
<td>Over normal value 130 (N%)</td>
<td>13 (34%)</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>79 ± 8.0</td>
<td>&lt; 85 mmHg</td>
</tr>
<tr>
<td>Over normal value 85 (N%)</td>
<td>61 (16%)</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.2 ± 0.6</td>
<td>3.1–6.4 mmol/l</td>
</tr>
<tr>
<td>Erythrocytes ($\times 10^{12}$/l)</td>
<td>4.6 ± 0.4</td>
<td>Men 4.5–5.5; women 3.8–4.8</td>
</tr>
<tr>
<td>Hgb (g/l)</td>
<td>139 ± 11</td>
<td>Men 130–175; women 120–160</td>
</tr>
<tr>
<td>WBC (\times 10^{9}/l)</td>
<td>5.7 ± 1.4</td>
<td>4–10\times 10^{9}/l</td>
</tr>
<tr>
<td>Platelets ($\times 10^{12}$/l)</td>
<td>227.4 ± 41</td>
<td>150–400 \times 10^{9}/l</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.8 ± 1.1</td>
<td>&lt; 5.0 mmol/l</td>
</tr>
<tr>
<td>Over normal value 5.0 (N%)</td>
<td>29 (76%)</td>
<td></td>
</tr>
<tr>
<td>Ox-LDL (U/l)</td>
<td>168.3 ± 45.4</td>
<td>145 ± 54.2 U/l</td>
</tr>
<tr>
<td>BDC-LDL (µmol/l)</td>
<td>48.2 ± 12.0</td>
<td>45.8 ± 12.1 µmol/l</td>
</tr>
</tbody>
</table>

References: [13,14,17,39]. Abbreviations: Hgb – hemoglobin; WBC – white blood cells; ox-LDL – oxidized low-density lipoproteins; BDC-LDL – baseline diene conjugates in low-density lipoproteins.
Table 3

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Correlation coefficients r and p-values</th>
<th>Linear multiple regression analysis adjusted for age, sex and/or BMI (Adj. R^2, p-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m²)</td>
<td>Plasma glucose (mmol/l)</td>
<td>r = -0.402, p = 0.014</td>
<td>Adj. R^2 = 0.084, p = 0.022</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>Colonization with L. fermentum</td>
<td>r = -0.305, p = 0.062</td>
<td>Adj. R^2 = 0.181, p = 0.013</td>
</tr>
<tr>
<td>Ox-LDL (U/l)</td>
<td>Total cholesterol (mmol/l)</td>
<td>r = -0.414, p = 0.011</td>
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</tr>
<tr>
<td>Count of lactobacilli (log cfu/g)</td>
<td>Ox-LDL (U/l)</td>
<td>r = -0.389, p = 0.016</td>
<td>Adj. R^2 = 0.184, p = 0.029</td>
</tr>
<tr>
<td>WBC (x 10³/l)</td>
<td>Colonization with L. reuteri</td>
<td>r = -0.434, p = 0.007</td>
<td>Adj. R^2 = 0.193, p = 0.027</td>
</tr>
</tbody>
</table>

that over 1000 species of bacteria reside in the colons of healthy adults, actively involved in human metabolism [27,28].

We found by the species-specific PCR that the number of Lactobacillus species, colonizing the gastrointestinal tract of elderly persons varied largely (from 1 to 7), and it was expressed with various individual combinations. The facultative heterofermentative species like L. casei/L. paracasei prevailed, followed by L. plantarum, similar to some other reports [10,29]. Homofermentative L. acidophilus and obligately heterofermentative L. fermentum were found in nearly half of the elderly, resembling the Italian and Greece culture-based approach [30,31]. The limitation of our study could be the set of applied primers not allowing to detect Lactobacillus ruminis [11].

In our elderly subjects the general health was assured using strict exclusion clinical criteria confirmed by the laboratory blood indices that generally responded to the widely accepted reference values. However, 61% of the subjects had excessive weight and respectively a half suffered from expressed osteoarthritis, scheduled for corrective operation. It has been shown that the prevalence of osteoarthritis is 68% in women and 58% in men >65 years of age and the high BMI is clearly associated with an increased risk of osteoarthritis [4]. The etiology and pathogenesis of osteoarthritis (whether of inflammatory origin or due to the degeneration of cartilage) are still an issue of debate [32,33]. According to normal WBC values there was found no systemic inflammation in the elderly. However, surprisingly, in elderly persons the prevalence of intestinal L. reuteri was positively related to white blood cell counts and the former species negatively to the L. acidophilus. Though no fermented food with probiotic L. reuteri has been marketed in Estonia, L. reuteri was found in 53% of investigated fecal samples. It is a warning sign to individuals with excessive weight and predisposition for inflammation, as the introduction of a probiotic L. reuteri can increase the inflammatory state and the risk for CVD. Recently the leukocyte count has been used as a predictor of cardiovascular events and mortality [26].

Beside molecular studies we have applied bacteriological methods for the quantitative determination of Lactobacillus sp. The reasons were as follows: First, in enumeration of Lactobacillus sp. we aimed to avoid the registration of DNA of dead bacteria, seemingly not involved in the host and microbiota integrated metabolism. Second, the Lab158 probe hybridizes both to Lactobacillus and Enterococcus sp and therefore did not afford their clear distinction [11].

The consumption of probiotic functional food revealed by a questionnaire was correlated with higher counts of cultivable lactobacilli. This is in agreement with some other interventional probiotic studies [15,29,34]. Though there was no association between the counts of lactobacilli and the increased BMI, still, the tendency for lower content of blood glucose was predicted by colonization with L. fermentum adjusted for BMI but not for age and sex. In turn, the positive correlation, as anticipated, between BMI and fasting blood glucose was found. In elderly, due to large inter-individual differences in physical fitness, the BMI have not been considered as full predictor of health status or adverse outcomes [35].

Obligately heterofermentative lactobacilli, incl. L. fermentum play an important role in carbohydrate breakdown and fermentation in the intestine: beside lactic and acetic acids they are also the producers of succinate [36]. It can be speculated that colonization by L. fermentum decreases the uptake of both glucose (hexoses) and fructose (pentoses) in the intestine due to its most effective phosphoketolase pathway of carbohydrate fermentation [37].

Further, to the best of our knowledge, this is the first study showing the inverse association between the counts of cultivable intestinal lactobacilli and oxidized LDL particles in elderly individuals. The effect of probiotic L. rhamnosus GG intervention on global serum lipidomic profiles of healthy adults was recently published [38]. Besides, in our study, as anticipated, a close connection between total cholesterol and ox-LDL was revealed. The increased level of ox-LDL (oxidized apolipoprotein B-100) is suggested for an important marker of systemic inflammation and oxidative stress (oxS) driven responses [25,39]. According to a large population-based study the ox-LDL is correlated with an increased incidence of metabolic syndromes overall, as well as obesity, hyperglycemia and hyper-triglyceridemia already in people who are currently young and healthy [40]. Also the relative risk of death and coronary events increase with higher levels of ox-LDL affecting the endothelium of blood vessels as well as arterial elasticity [41,42].

How can the high count of metabolically active lactobacilli operate against oxidative stress–caused damage in the host? First, some lactic acid bacteria, particularly L. fermentum are involved in cholesterol metabolism [43]. In our study, more than a third (39%) of the elderly was colonized with this species. Second, lactobacilli possess several anti-oxidative mechanisms: catalase, glutathione-system-related compounds, and Mn-SOD [44–46]. Recently, the role of extra-cellular antioxidant enzymes for the attenuation of proliferation caused by reactive oxygen species in human smooth muscle cells was shown [47]. In addition, different species and particular strains of lactobacilli can create anti-oxidative peptides [48]. It is also possible that lactobacilli can influence host metabolic pathways to modulate the metabolism of lipoproteins, leading to decreased amounts of ox-LDL. Previously, we have shown an improvement of blood lipoprotein metabolism (ox-LDL, lag phase of LDL and BDC-LDL) after consumption of the antimicrobial and anti-oxidative probiotic L. fermentum ME-3 [15]. Nowadays, the role of antioxidants as a possible “third great wave” in cardiovascular secondary prevention has been widely discussed [49].

In conclusion, the pilot study of elderly persons showed for the first time that the intestinal lactobacilli are tightly bound to WBC count, blood glucose and content of oxidized lipoprotein which all serve as risk markers in pathogenesis of inflammation, metabolic syndrome and CVD (Fig. 2). The application of particular species of probiotic lactobacilli could be a challenge in terms of the health care of elderly people concerning the complex control of their
metabolic and systemic defence reactions, including oxidative stress-related ones.

Acknowledgments

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References


