High-fat-diet–induced obesity is associated with decreased anti-inflammatory Lactobacillus reuteri sensitive to oxidative stress in mouse Peyer’s patches

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Abstract

Objective: Diet-induced inflammation in the small intestine may represent an early event that precedes and predisposes to obesity and insulin resistance. This is related to decrease of lactobacilli in Peyer’s patches (PP) revealed in our previous study. The present study aimed to clarify specific changes of PP Lactobacillus on the strain level and related biological activity.

Methods: C57 BL/6 J male mice were fed with either low-fat diet (control [CT]; 10% calories from fat) or high-fat diet (HFD; 50% calories from fat) for 25 wk, and the HFD-fed mice were classified into obesity prone (OP) or obesity resistant (OR) on the basis of their body weight gain. Lactobacillus was isolated from PP using a selective medium. Oxidative resistance and cytokine-inducing effect were analyzed in vitro.

Results: We obtained 52, 18, and 22 isolates from CT, OP, and OR mice, respectively. They belonged to 13 different types according to enterobacterial repetitive intergenic consensus sequence-PCR analysis. Lactobacillus reuteri was the most abundant strain, but its abundance in OP mice was much lower than that in CT and OR mice. This strain includes eight subgroups according to genotyping. L. reuteri L3 and L. reuteri L8 were the specific strains found in CT and OP mice, respectively. Oxidative-resistant L. reuteri was much higher in HFD-fed mice. When co-cultured with PP cells, L8 induced higher production of proinflammatory cytokines such as interleukin (IL)-6, IL-12, and tumor necrosis factor-α, whereas L3 induced higher production of an anti-inflammatory cytokine (IL-10).

Conclusion: HFD may induce oxidative stress that drives strain selection of Lactobacillus strains, resulting in decreased anti-inflammatory response in PP.

Introduction

Obesity is associated with low-grade systemic inflammation, which is considered a major mechanism driving insulin resistance in obese individuals. Adipose tissue, particularly visceral adipose tissue, was found to be the source of inflammation and proinflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, and monocyte chemo-attractant protein–1 [1]. Recently, some evidence supported the hypothesis that diet-induced inflammation in the intestine, particularly the small intestine, may represent an early event that precedes and predisposes to obesity and insulin resistance. In the
obesogenic environment, that encourages overconsumption. Differences between susceptible (prone) and resistant human individuals can be seen. Outbred rodents, when subjected to high-fat diet (HFD), show marked differences in body weight response; some are obesity prone (OP), whereas others remain lean (obesity resistant, OR). Such heterogeneity is of particular significance for obesity research. De La Serre et al. [2] found that HFD upregulated myeloperoxidase activity, a marker of inflammation, in ileum of OP rather than OR Sprague Dawley rats. De Wit et al. [3] found that HFD induced expression of macrophage migration inhibitory factor in ileum of OP C57 Bl/6 mice. More recently, Ding et al. [4] found that TNF-α was upregulated by HFD, and this occurred specifically in the ileum and within 2 to 6 wk of HFD, preceding diet-induced weight gain and increased fat mass. They also found the activation of NFκB-driven transcription in ileum Peyer’s patches (PP). Change of bacterial composition might mediate inflammation in OP animals. Dramatic increases in Enterobacteriales, a member of the minor proteobacteria phylum, have been associated specifically with development of intestinal inflammation and obesity in OP but not OR rats [2]. This bacterium might disrupt the gut barrier and induce gut inflammation [5]. Therefore, it was proposed that microbiota is necessary for development of intestinal inflammation associated with HFD [4]. It is not yet known how HFD-induced bacterial shift directs inflammation in the gut.

PP serves as a gatekeeper at the physical interphase between body and diet, including small intestine microbiota. Immune cells in PP secrete signaling molecules such as gut hormones and pro- and anti-inflammatory cytokines, to which liver, muscle, and adipose tissue respond by modulating their metabolism to keep homeostatic control. The terminal ileum, which contains approximately 50% of the PP in the gut, plays a central role as a sensor of bacterial colonization and tolerance and is likely the primary inductive site for mucosal inflammation [6]. Commensal bacteria that breach the follicle-associated epithelium of PP are typically phagocytosed and cleared by macrophages. However, some commensal bacterial species could stably colonize the interior of PP [7]. Because of a direct reaction with intestinal immune cells, such as dendritic cells [8], these bacteria play an important role in balancing the intestinal immune homeostasis [9]. These are the least understood class of bacteria that colonize the gastrointestinal tract.

We recently found that there was a significant decrease in bacterial diversity in the PP of OP mice [10]. Lactobacillus was significantly decreased in OP mice compared with control (CT) and OR mice, as indicated by sequencing of 16 S ribosomal RNA (rRNA) gene amplicons using universally conserved primers. However, because of the high similarity of 16 S rRNA gene sequences among different microorganisms, this approach only can confidently identify microorganisms at high taxonomic levels (e.g., genus and family) but not at the species or strain level [11].

The reason for the change of PP microbiota is yet to be resolved. According to our previous study, gut microbiota dysbiosis in OP mice was caused, at least in part, by the increased levels of oxidative stress [12]. HFD induced increase of aerobic bacteria in mouse PP, including Streptococcus, Pseudomonas, Comamonas, and Flavobacterium [10]. The decreased genera Allobaculum and Lactobacillus are anaerobic and facultatively anaerobic bacteria, respectively [10]. Because the two groups have significantly different oxidative stress resistance, oxidative stress probably drives the shifting of PP microbiota. Lactobacillus encounters oxidative stress during gastrointestinal tract colonization. Bacteria deploy specialized mechanisms to deal with oxidative stress, which include enzymes like superoxide dismutase (SOD), thioredoxin reductase system, glutathione-glutaredoxin system, and NADH oxidase–NADH peroxidase system [13]. Differences in expression of these genes might determine oxidative resistance and survival of Lactobacillus in the intestine of obese mice.

In the present study, we aimed to isolate Lactobacillus from CT, OP, and OR mice to analyze taxonomic differences in the strain level and to explore their differences in immune-modulating and oxidative-resistant properties.

Materials and methods

Animal experiments

Animal experiments were performed in compliance with the fundamental guidelines for proper conduct of animal experiments and related activities in academic research institutions under the jurisdiction of the Ministry of Education of China and approved by the Jiangnan University Institutional Animal Care and Use Committee (Approval No. 20130401). Thirty-eight 4-wk-old C57 Bl/6 J male mice were obtained from Shanghai Slac Laboratory Animal, Co., Ltd. (Shanghai, China) and were fed either a CT diet (total calories 3.6 kcal/g, 10% calories in fat) or an HFD (total calories 4.7 kcal/g, 50% calories in fat).

Mice were kept in an environmentally controlled breeding room (temperature, 23 ± 2 °C; humidity, 60 ± 5%; 12 h light–dark cycle) and had free access to food and water throughout the study. After 25 wk, 30 mice in the HFD group were classified into OP and OR by body weight gain (range, 28.1–42.3 g), according to the method used previously [14,15]. Eight mice with the highest body weight gain (40.8 ± 1.5 g; P < 0.05 compared with that of CT mice) were designated OP mice, and eight mice with the lowest body weight gain (29.3 ± 1.2 g; P < 0.05 compared with that of CT mice) were designated OR mice. Other 14 mice that did not meet the standard were not used in the subsequent study.

Food intake and body weight of each animal were measured every 2 wk. Groups of eight mice were killed during deep anesthesia after a 4-h fast. The PP were aseptically removed, and tissue specimens were placed in Betadine antibiotic solution (Seton Healthcare Group plc, Oldham, UK) for 3 min to disinfect the surface. Subsequently, tissues were vortexed in multiple 500-μl aliquots of phosphate-buffered saline to encourage the removal of any bacteria on the tissue surface. Final washes were retained and analyzed by both culture-dependent and culture-independent techniques to determine whether surface decontamination was successful [16].

Isolation of Lactobacillus from PP

PP were homogenized in phosphate-buffered saline using a sterile glass homogenizer. Afterward, serial dilutions were spread on plates of Lactobacillus anaerobic Man-Rogosa-Sharpe (MRS) media with vancomycin and bromocresol green (LAMVAB), an elective and selective medium for Lactobacillus spp [17], and incubated at 37°C for 96 h in an anaerobic jar. Because of the presence of bromocresol in LAMVAB, lactobacilli colonies were differentiated by their blue or green color. Colonies were selected either randomly or, if there were fewer than 10 colonies, per each plate. Purity of the isolates was checked again by streaking them onto fresh agar plates of the isolation media, followed by microscopic examinations. Identified strains of lactobacilli were kept in MRS broth with 15% (v/v) glycerol at −20°C.

DNA extraction and ribosomal DNA analysis

Genomic DNA was extracted from each sample individually using a bead beating method followed by phenol-chloroform-isooamyl alcohol extraction as described previously [18]. Two universal primers (5′-AGAGTTT-GATCCTGGCTCAG-3′ [19], and 805 R, 5′-GATCACCAGGTTCAATCT-3′ [20]) for 16 S ribosomal DNA were used for amplifications. Polymerase chain reaction (PCR) products were directly sequenced using the forward and the reverse primer on the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the user’s manual for electrophoresis procedures.

Enterobacterial repetitive intergenic consensus sequence-PCR analysis

PCR was performed using the forward primer 5′-ATGTAATGTCGTTCTGGGATT-CAC-3′ and the reverse primer 5′-AAGTAA GTGACCTGGTTACG-3′ [21]. The cycling program was 95°C for 7 min of predenaturation, and 90°C for 60s, 52°C for 60s, and 65°C for 8 min for the next 30 cycles and one cycle at 65°C for 16 min. All PCR amplicons (400 ng) were electrophoresed on 1.5%(mass per volume) agarose
gels at a constant voltage of 80 V/cm. PCR patterns were stained with ethidium bromide (0.5 mg/mL) and observed under ultraviolet (UV) light at 254 nm.

**Quantification of Lactobacillus reuteri by quantitative real-time PCR**

Quantitative real-time PCR (qPCR) was performed on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems) using the sg-Lreu-F primer 5’-GAAGATCGATCCGAYTGCCCAA-3’ and the sg-Lreu-R primer 5’-TCCATTGTGGCCGATCAG-3’ [22]. Primers for universal bacteria were UB338, 5’-ACTCTTACGGAGGAGCAGAC-3’, and EUB581, 5’-ATTACCGCGGTGTCG-3’. qPCR assays were performed in each 10-μL reaction mixture using AccuPower 2X GreenStar qPCR Master Mix (Bioneer, Seoul, Korea) with the following parameters: 1 cycle of 95°C for 5 min; 45 cycles of 95°C for 20 sec, 60°C for 20 sec for L. reuteri or 53°C for 10 sec for universal bacteria, and 72°C for 20 sec; and 1 cycle of 72°C for 5 min. A melting curve analysis was performed after amplification. All samples were analyzed in duplicate. Results of qPCR are presented as the ratio of the copy number of L. reuteri to the copy number obtained in the universal bacterial assay.

**Resistance of L. reuteri to hydrogen peroxide**

Strains were suspended in phosphate-buffered saline at a concentration of 10^9 colony-forming units/mL and incubated with 4 mM hydrogen peroxide (H₂O₂; 30 wt% solution in water; Sigma-Aldrich Chemicals) at 37°C. At 2-h intervals, 100 μL of aliquots was placed in a 96-well plate, and added with 20 μL of 5 mg/mL thiazolyl blue tetrazolium bromide. The mixture was vortexed and incubated at 37°C for 2 h, and added with 100 μL of dimethyl sulfoxide (DMSO), vortexed, and read at 570 nm.

**Inhibition rate determination of lipid peroxide formation induced by Fe²⁺-vitamin C system by lactobacilli**

Inhibition of lipid peroxide formation was determined according to our previous study [23]. Briefly, liver homogenate from 8-wk-old male Kunming (KM) mice was incubated with a Lactobacillus suspension at 37°C for 5 min, and then FeSO₄ (100 mM) and vitamin C (100 mM) were added before 1 h of incubation. The mixture was boiled for 20 min after addition of 20% trichloroacetic acid. After centrifugation, thiobarbituric acid reactive substances (TBARS) in supernatant were determined at 532 nm. Inhibition rate of lipid peroxidation was calculated as follows: [(C − T)/(C − B)] × 100, where C is TBARS of a test sample incubated without the bacterial cells, T is TBARS of a test sample incubated with the bacterial cells, and B is TBARS of distillated water.

**Measurement of superoxide dismutase and glutathione peroxidase activity in intracellular cell-free extract**

Cell-free extracts of different Lactobacillus strains were made according to the method we previously used [23]. Total SOD and glutathione peroxidase activity was assayed using kits provided by the Nanjing Jiancheng Bioengineering Institute. The protein content in cell lysates was measured by Bradford method. The protein content in cell lysate was measured by Bradford’s method using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, USA).

**PP cell isolation and co-culture with lactobacilli**

Bacteria were killed under UV light, and the death was confirmed via colony counts. Cells were lyophilized according to the method of Chiba et al. [24].

**Results**

**High-fat-induced change in Lactobacillus of PP**

LAMVAB medium was chosen for the isolation and enumeration of lactobacilli. Growth was determined as colonies >1 mm in diameter and a yellow discoloration of the medium, caused by acid production. Most colonies were green because of the uptake of bromocresol green into the cell and either circular or irregularly circular in shape. Microscopically, they were Gram positive, rod-shaped motile, and catalase negative, and lacked endospores. We obtained 52, 18, and 22 isolates from PP of CT, OP, and OR mice, respectively. Enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR) generated 13 typical fingerprints of Lactobacillus reuteri by quantitative real-time PCR.

**Statistical analysis**

All values are expressed as mean ± standard deviation (SD). Each value is the mean of at least three separate experiments. Statistical comparisons were carried out by ANOVA, followed by Tukey’s test. P < 0.05 was considered significantly different.

**Table 1**

<table>
<thead>
<tr>
<th>ERIC-PCR fingerprint</th>
<th>Isolate amount</th>
<th>Strain</th>
<th>Similarity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>14</td>
<td>Lactobacillus reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L2</td>
<td>12</td>
<td>L. reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L3</td>
<td>10</td>
<td>L. reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L4</td>
<td>6</td>
<td>L. reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L5</td>
<td>12</td>
<td>L. reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L6</td>
<td>6</td>
<td>L. reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L7</td>
<td>7</td>
<td>L. reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L8</td>
<td>12</td>
<td>L. reuteri</td>
<td>99</td>
</tr>
<tr>
<td>L9</td>
<td>2</td>
<td>Lactobacillus buchneri</td>
<td>97</td>
</tr>
<tr>
<td>L10</td>
<td>1</td>
<td>Lactobacillus plantarum</td>
<td>98</td>
</tr>
<tr>
<td>L11</td>
<td>3</td>
<td>Lactobacillus pentosus</td>
<td>97</td>
</tr>
<tr>
<td>L12</td>
<td>5</td>
<td>Lactobacillus rhamnosus</td>
<td>99</td>
</tr>
<tr>
<td>L13</td>
<td>2</td>
<td>L. rhamnosus</td>
<td>98</td>
</tr>
</tbody>
</table>

L1 to L13 refer to ERIC-PCR profiles of all lactobacillus isolates of Peyer’s patches
profiles that varied within and among species. Isolate amount of each phenotype was listed in Table 1. A typical isolate of each phenotype was identified by 16S rRNA gene sequencing. The sequencing revealed that *L. reuteri* was the most predominant *Lactobacillus* strain, consisting of 82% of isolates. They belong to eight phenotypes according to the ERIC-PCR analysis (Fig. 1). PP *L. reuteri* decreased significantly in OP mice when determined using qPCR (*P* < 0.05) (Fig. 2). The phenotype distribution of *L. reuteri* varied greatly between different mouse groups (Fig. 3). The phenotype of L3 was found only in the CT group. L8, however, was the typical phenotype of HFD-treated mice, which was absent in CT mice and specially enriched in OP mice.

**Resistance to H$_2$O$_2$ of PP *L. reuteri* strains**

As shown in Figure 4, eight *L. reuteri* strains showed varied ability of surviving in H$_2$O$_2$. LGG, a typical probiotic, presented in 50% of viable cells after 6.2-h incubation with H$_2$O$_2$. Compared with LGG, L8, L7, L5, and L1 showed higher H$_2$O$_2$ resistance (*P* < 0.05). L8 was the most H$_2$O$_2$-resistant strain and L3 was the most H$_2$O$_2$-sensitive strain. Their 50% survival rates were 8.1 and 4.1 h, respectively.

**Inhibition of mouse cell lipid peroxide formation by PP *L. reuteri***

Incubation of the liver homogenates with vitamin C and FeSO$_4$ caused an enhancement in malondialdehyde formation, because of membrane lipid peroxidation. As shown in Figure 5, *L. reuteri* inhibited more than 10% of malondialdehyde formation. L2, L7, and L8 showed significantly higher malondialdehyde inhibition ability than LGG (*P* < 0.05). L3 showed the lowest malondialdehyde inhibition ability, without statistical difference with LGG.

**Antioxidative enzyme activities of cell-free extracts from PP *L. reuteri***

As shown in Figure 6, L3 and L8 had the highest SOD activity, more than 8 U/mg protein, among eight strains determined. When LGG served as a positive CT, L1, L3, L5, L7, and L8 showed higher SOD activity (*P* < 0.05).

**Distribution of oxidative-resistant and oxidative-sensitive *L. reuteri* isolates in different mice**

An *L. reuteri* isolate was defined as oxidative-resistant strain if it showed three oxidative indexes not less than that of LGG. According to this criterion, isolates of L1, L5, L7, and L8 phenotypes belonged to strong oxidative strains. As shown in Table 2, oxidative-sensitive isolates were markedly decreased in mice receiving HFD. These mice were enriched with strong antioxidative isolates, especially OP mice, which had more of the most oxidative-resistant strain, L8.

**Cytokines released by PP immune cells co-cultured with *L. reuteri***

PP cells were cultured with *L. reuteri* of different phenotypes, and the concentrations of IL-12 p70, TNF-α, IL-6, and IL-10 in the supernatants were measured. As shown in Figure 7, all strains...
showed strong dose-dependent cytokine-inducing ability. Except L3, all L. reuteri strains induced significantly more IL-6 than did LGG (P < 0.05), especially L. reuteri L7 and L8. L. reuteri L8 (10 μg/mL) even induced IL-6 to 60% of lipopolysaccharide (1 μg/mL). These two phenotypes also induced significantly higher TNF-α than LGG did (P < 0.05) and IL-12 p70 comparing with L2 (P < 0.05). All strains showed IL-10-inducing ability compared with lipopolysaccharide. L3 and LGG showed markedly higher IL-10 induction than other L. reuteri phenotypes (P < 0.05). Therefore, less anti-inflammatory L. reuteri strains were isolated from OP mice. The balance of TNF-α/IL-10 and IL-12/IL-10 production induced by these L. reuteri strains was highly strain specific (Fig. 7). Except L3 and L6 (at 10 μg/μL), all strains induced more TNF-α and IL-12 than IL-10. Ratio of IL12p70 to IL10 is of one- to threefold of LGG. This suggests their Th1-type proinflammatory response-stimulating potential.

Discussion

The present study confirmed that L. reuteri was the predominant Lactobacillus strain colonizing PP. In rodents, L. reuteri is one of the most abundant species present in the gut [26], especially at proximal digestive tracts [27], from which they might reach and colonize PP. L. reuteri plays a great role in inhibiting the growth of other potentially pathogenic microorganisms by secreting antibiotic substances such as reuterin [28]. Decrease of L. reuteri levels in small intestine might favor the growth of opportunistic pathogens. In humans, the prevalence of L. reuteri is much lower [29], but L. reuteri has been considered autochthonous to the digestive tract [30]. The largest case-control study reported higher levels of L. reuteri in fecal microbiota of obese [31], and fecal L. reuteri of humans was positively correlated with body mass index although a relatively low prevalence of Lactobacillus species was found in individuals with high body mass index values [32]. Decreased colonizing of Lactobacillus and L. reuteri strain in PP of OP mice in our study revealed a decrease of their concentration in small intestine. Changes in the gut environment might drive the selective increase of certain L. reuteri phenotypes in the PP of OP mice.

To further address the distinct phenotype profile differences between CT and OP mice, we analyzed the distribution of oxidative stress-resistant phenotypes between them because of the possible occurrence of oxidative stress in colonizing the gastrointestinal tract [33]. We found that there was a much higher oxidative-resistant phenotype in HFD mice, especially in OP mice. HFD induced production of higher reactive oxygen species in intestine [34] and higher markers of oxidative stress [35]. This induces a shift in the microbial composition toward an increase of bacteria that are tolerant to oxidative conditions, such as Escherichia coli and Enterococcus [12]. Reactive oxygen species play a role in the establishment of the extended bacterial phenotype [36]. Increased production of reactive oxygen species might drive the composition change of L. reuteri in OP mice. For another reason, HFD increases bile acid release in small intestine lumen [37], a host factor that controls the gut microbiota change [38]. It can impose oxidative stress on bacteria, because of the production of reactive oxygen/nitrogen species [39]. This may induce dysbiosis and thereby facilitate an increased inflammatory activity [40]. Protection against oxidative damages is a common consequence of bile exposure for L. reuteri, which might be employed to counteract some of the cellular damages caused by bile [41]. Antioxidative capacity is important for Lactobacillus to survive in the intestine when bile acid concentration is increasing [42].

Furthermore, the abnormal activation of resident intestinal immune cells by commensal bacteria might facilitate the development or persistence of intestinal inflammation. It was found that HFD caused a significant barrier decrease in rat PP [43] and induced translocation of pathogenic bacteria [10]. The HFD changes not only the PP bacteria from a taxonomic viewpoint, but also the phenotype of different immune modulating potentials. The present study provides evidence for the decrease of anti-inflammatory L. reuteri in OP mice. We also found that these mice were enriched with inflammatory L. reuteri strains. Unfavorable bacterial phenotype changes might drive inflammation in PP. In the present study, we used BALB/c mice derived

**Table 2**

Amount of Lactobacillus reuteri isolates with high and low Antioxidant capacity in different mouse groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Low-antioxidant isolates</th>
<th>High-antioxidant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L2</td>
<td>L3</td>
</tr>
<tr>
<td>CT</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>OP</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>OR</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

CT, normal chow diet–fed mice; OP, high-fat diet–induced obesity–prone mice; OR, high-fat diet–induced obesity–resistant mice

L1 to L8 refer to specific Lactobacillus reuteri phenotypes

**Fig. 6.** Superoxide dismutase (SOD, (A)) and glutathione peroxidase (GPx, (B)) activity of intracellular cell-free extracts of Lactobacillus reuteri strains. Enzyme activity was expressed in units per milligram protein. *P < 0.05 compared with Lactobacillus rhamnosus GG (LGG). Data are presented as mean ± SD.
PP cells for studying cytokine stimulation of *L. reuteri*. However, a recent research reported differences in the response of BALB/c and C57 BL/6 to certain strains of *Lactobacillus* [44]. A C57 BL/6 mouse-based study should be conducted to confirm the cytokine stimulation of PP *L. reuteri* more accurately.

In the present study, L3 showed notable immune-modulating properties compared with other strains. This type of strain might be a potential fine-targeted treatment effective for down-regulating production of IL-12 and TNF-α while inducing the anti-inflammatory IL-10, thus representing an alternative therapeutic approach to counterbalance the proinflammatory intestinal cytokine milieu. We recently found that oral administration of L3 prevented HFD-induced obesity and low-grade chronic inflammation in mice [45], demonstrating the importance of this specific commensal phenotype in maintaining intestine immune homeostasis. Similar strains should be isolated and identified by comparing the composition of *L. reuteri* in obese and lean humans or in gut microbiota-humanized animals. They might share the probiotic potential to prevent obesity.

In summary, susceptibility to obesity is characterized by an unfavorable microbiome predisposing the host to peripheral and central inflammation and promoting weight gain. Results of the present study reveal that HFD led to the misbalance of *L. reuteri*, with anti- or inflammatory function. This might be related to increased oxidative stress in the intestinal habitat because the two phenotypes of anti- or inflammatory potential share weaker and stronger oxidative resistance, respectively.

References


[2] De La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with...


