Original Article

Probiotics Improve Chemerin and Metabolic Syndrome Parameters in Obese Rats

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Background: Chemerin is a recently discovered adipokine that plays a role in adipocyte metabolism. It is a novel chemotactic adipokine; expression and secretion are increased by adipogenesis.

Aims: We evaluated probiotic supplementation effects on chemerin, inflammation, and metabolic syndrome components in obese Wistar rats.

Study Design: Animal experiment

Materials and methods: We formed three groups of experimental animals, each consisting of 8 rats. Group 1 was the control group. Group 2 was the experimentally obese group using a high-fat diet. Group 3 was the obese intervention group with probiotic supplementation after obesity induction.

Results: At the end of the study, there was a statistically significant difference between groups in final weights, weight changes, and body mass index values (p<0.05). Weight gain was 34.12±3.70 g in Group 3 post-probiotic supplementation and 53.25±8.35 g in Group 2 (p<0.05). In obese rats, fasting plasma glucose (FPG), insulin, insulin resistance (HOMA-IR), total cholesterol, low-density lipoprotein cholesterol, inflammatory markers, and leptin levels increased compared to the control group. Chemerin levels were 14.31±13.34 ng/mL in Group 2 and 2.67±2.42 ng/mL in Group 3 (p<0.05).

Conclusion: In conclusion, probiotic supplementation (Group 3) reduced weight gain and there were positive effects on FPG, insulin, HOMA-IR, triglycerides, inflammatory markers, leptin, and chemerin levels.

Keywords: Chemerin adipokine; Metabolic syndrome; Obesity; Probiotic

According to the World Health Organization (WHO), obesity is abnormal or excessive fat accumulation caused by an imbalance between energy intake and energy expenditure (1). Worldwide, 390 million adult women and 281 million adult men were obese in 2016 (2). In recent years, intestinal microflora emerged as important components for prevention of obesity and related diseases as well as support of treatment. Regulation of intestinal microflora (such as antibiotic, prebiotic, and probiotic use) and the effectiveness of treatment for obesity and related diseases are being investigated using human studies following successful animal studies (3-5). Probiotics, viable microorganisms with beneficial health effects, taken in sufficient quantities have the potential to reduce obesity by decreasing fat storage and increasing satiety and energy expenditure (6).

The strong link between diet, intestinal microbiota and obesity has been an important research topic in recent years to better understand the etiology of obesity and to develop new treatment methods (7). Especially in recent metagenomics-based studies, intestinal microbiota; it affects not only the energy balance, but also the immune and intestinal barrier functions, as a factor affecting the whole body metabolism (8, 9).

Chemerin is a recently discovered adipokine shown to play a role in adipogenesis and adipocyte metabolism (10, 11). Chemerin expression and secretion are increased by adipogenesis (12). Therefore, it is important to determine and control circulating levels of chemerin, because it is associated with the increased risk of obesity, Type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), inflammation, metabolic syndrome (MetS), and many other diseases (13, 14).
In studies investigating the relationship between chemerin and obesity, individuals with various methods for weight loss have low serum chemerin levels compared to obese individuals without weight loss and the decrease in the serum concentrations of chemerin were found to be related to the improvement of weight loss and metabolic parameters (15-18).

MetS is a combination of several cardiovascular risk factors that are closely associated with abdominal adiposity and insulin resistance (18). Circulating chemerin increases in obesity and positively correlates with MetS markers including BMI, waist / hip ratio, systolic blood pressure and serum triglycerides (19).

In the current study, we evaluated the effects of probiotics on diabetes, insulin resistance, lipid profile, obesity, inflammation, and chemerin in obese rats.

MATERIALS AND METHODS

Experimental animals

The Ondokuz Mayis University Experimental Animal Application and Research Center supplied twenty-four 4-6 week-old male rats with an average weight of 270–290 g. All rats were housed in standard conditions (such as heat, humidity, light, and ventilation) and fed ad libitum with dry chow (20-25 g/day per rat) during the study. All rats were housed in plastic cages at 24±3 °C in 12 h light/12 h dark cycles with 40–60% humidity in the laboratories of the University of Ondokuz Mayis. Rats had free access to water and food. The Ondokuz Mayis University Ethical Committee of Animal Experiments gave ethical approval (IRB Number: 28.12.2016/12).

Experimental design and animal grouping

The experiment lasted 16 weeks (w) and was divided in two periods: 1) induction of obesity (diet-induced obesity) (0-8 w) and 2) intervention (9-16 w). Twenty-four rats were used for this study and randomized into three groups containing eight rats. Group 1 was the control group fed with a standard diet, Group 2 was a study group fed with a high-fat diet (HFD) (60% fat), and Group 3 was a study group fed with a HFD supplemented with probiotics (Figure 1).

For the duration of the experiment, body weights were recorded weekly and mean body weight and weight gain were calculated. For the evaluation of obesity, body mass index (BMI=body weight (g)/height (cm²)), which was calculated by measuring the body weight and the naso-anal height length, was used. Rats with a BMI>0.68 g/cm² were considered obese (20).

Diet-induced obesity (DIO) rats and diets of animals

The rats in Group 1 were fed with standard rat chow (16% carbohydrate, 54% protein, 30% fat) throughout the study (16 w). The rats in Group 2 and Group 3 were fed with a HFD (8.9% carbohydrate, 30.8% protein, 60.3% fat) for 8 w to stimulate DIO. After 8 w, DIO rats in Group 2 continued eating a HFD. However, the DIO rats in Group 3 received probiotic supplementation via oral gavage in addition to the HFD.

Probiotic administration protocol

A pool of probiotics that included *Lactobacillus acidophilus*, *Bacillus lactis*, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus* (Solgar®, Advanced Multi Billion Dophilus™, Turkey) was given for 8 w daily (6×10^8 of each strain; final concentration 2.4×10^9 cfu bacteria). Prior to gavage, the probiotics were diluted in sterile water (21).

Blood Samples

The animals were decapitated under anesthesia and the maximum amount of blood that could be taken directly was used for biochemical analysis. Blood samples were collected in anticoagulant-free biochemical tubes, centrifuged at 3000 rpm for 10 minutes, and the resulting sera were stored at −80 °C until analysis.

Biochemical analyses of serum

We analyzed insulin level and fasting plasma glucose (FPG) as an indicator of diabetes; the cytokines interleukin-6 (IL-6), interleukin-10 (IL-10), C-reactive protein (CRP), and tumor necrosis factor-α (TNF-α) levels as indicators of inflammation; leptin level as an indicator of obesity; triglyceride (TG), total cholesterol (TC), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) levels to determine the lipid profile; and serum levels of chemerin in serum samples. For this analysis, the ELISA (enzyme-linked immunosorbent assay) technique was applied using a commercially available kit (Rel Assay Diagnostics®, Turkey).

Insulin resistance was measured using the homeostasis model assessment of insulin resistance (HOMA-IR)=(fasting insulin (μU/mL)×fasting glucose (mmol/L))/22.5). An indirect measure of insulin sensitivity was calculated using the quantitative insulin sensitivity check index (QUICKI) as follows: 1/[log(fasting insulin μU/mL)+log(fasting glucose mg/dL)].

Statistical analysis

The data was tested for normal distribution with the Shapiro-Wilk test. When the criteria for normal distribution were not achieved, the non-parametric Kruskal-Wallis and Mann-Whitney U pair-wise comparison tests were used. Mann-Whitney U test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. An overall %5 type-I error level was used to inter statistical significance. The results are presented as mean ± standard deviations. Values were considered significant when p<0.05; the different superscript letters (a, b) indicate significant variations at p<0.05 in the tables. Considering leptin values, the minimum number of samples for 95% confidence, a significant difference of 0.55, a standard
deviation of 0.35, and a test power of 0.80 was eight rats (per group) (22, 23). Statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences) 21 application. A minimum difference of 0.55, a standard deviation of 0.35, and a test power of 0.80, for 95% confidence, taking into account the minimum number of samples, the leptin value, were eight rats.

RESULTS

Evaluation of Morphometric Measurements

DIO rats were fed a HFD with or without probiotic supplementation for 16 w. Table 1 shows initial and final weights, BMI values, and weight changes of the rats. At baseline, there was no significant difference in body weight between groups (p>0.05). After the first 8 w, the average weight gain was 63.37±9.69 g in Group 2 and 90.50±29.07 g in Group 3. Between weeks 8 and 16, the average weight gain was 53.25±23.62 g in Group 2 and 34.12±10.46 g in Group 3. Weight changes and BMI values were significantly increased with a HFD (p<0.05) at 8 and 16 w. The changes in weight and weight gain during the study are shown in Figure 2. However, despite having a higher BMI than the other groups at week 8, group 3 significantly decreased the rate of BMI increase after receiving probiotics. Figure 3 shows the BMI changes during the study.

Evaluation of Biochemical Parameters

To evaluate the effects of HFD and probiotic supplementation on biochemical parameters in DIO rats, we examined FPG, insulin, lipid profile, inflammatory markers, chemerin, and leptin levels. Biochemical parameters of rats are given in Table 2. Insulin levels, insulin resistance measured with HOMA-IR and insulin sensitivity measured with QUICKI, significantly decreased after probiotic supplementation compared to HFD without supplementation. Chemerin significantly increased in response to a HFD (Group 2), but significantly recovered after introduction of probiotic supplementation (Group 3). There was no statistically significant difference in the remaining parameters (p>0.05).

DISCUSSION

In recent years, it has been suggested that regulation of microbiota by various mechanisms using probiotics may play a role in preventing obesity and related diseases (6). It has been determined that the diets where 45%-60% of the diet energy come from fats provide 20-40% weight gain (24, 25). Our results were similar to the literature and mean body weight and weight gain (weight increase of 21% at 8 w and 39% at 16 w), due to increased adipogenesis, in DIO rats were higher than in the control group. (p<0.05). In addition, rats in Group 3 appear to have gained more weight than rats in Group 2, but the result is not significant.

Probiotics can affect MetS, T2DM, and obesity by regulating intestinal microbiota, favoring insulin signaling, and decreasing cholesterol. According to evidence from animal studies, probiotic supplementation partially reduces weight gain and positively affects parameters related to obesity and HFD-related diseases (26, 27). In our study, body weight gain decreased in DIO rats with probiotic supplementation (Group 3) compared to DIO rats (Group 2). After probiotic supplementation in our study FPG, insulin, and HOMA-IR levels were consistent with the literature. A HFD causes adverse changes in the lipid profile, which can be normalized after probiotic supplementation (28, 29). Some studies observed no effect after probiotic supplementation on TC, HDL-C, LDL-C, and TG levels (30, 31). According to the results obtained from our study, although there was no significant difference in TC, LDL-C, and HDL-C levels after probiotic supplementation, the decrease in triglyceride levels is similar to the variable results of the lipid profile in the literature.

Studies show that supplementation of probiotics to a HFD reduces leptin levels compared to standard diets (32). In a similar study, there was no difference in leptin levels between groups after 12 weeks of probiotic supplementation (33). According to our study, we found leptin levels decreased in DIO rats with probiotic supplementation (Group 3) compared to DIO rats (Group 2), although this difference was not significant. The supplementation of probiotics to rats fed a HFD showed an anti-inflammatory response demonstrated by the reduction of pro-inflammatory cytokines such as IL-6, IL-17, and TNF-α. Results from experimental animal studies suggest that supplementation of probiotics to a HFD stimulates the anti-inflammatory response (29, 32). Another study reported probiotic supplements to a HFD did not alter TNF-α and IL-6 levels (33). In our study, levels of IL-6, IL-10, TNF-α, and CRP decreased in DIO rats with probiotic supplementation (Group 3) compared to DIO rats (Group 2), although this difference did not reach statistical significance.

Expression and secretion of the chemerin adipokine is increased by adipogenesis (10, 12). Studies show that rats fed with a HFD gain more weight and have higher levels of chemerin than those on a standard diet (34, 35). Similarly, the weight increase in rats fed a HFD was associated with an increase in chemerin levels in our study. The rats fed with a HFD had higher levels of chemerin compared to the control group (p<0.05). Chemerin levels are associated with MetS markers (17, 36). In our study, increased levels of chemerin were associated with elevated levels of FPG, insulin, HOMA-IR, TC, HDL-C, and LDL-C and reduced levels of TG in DIO rats. Chemerin levels decreased in DIO rats after probiotic supplementation (p<0.05).
CONCLUSION
In conclusion, although we show a relationship between chemerin and MetS components, the effect of probiotic supplementation on serum levels of chemerin, and hence obesity and related diseases, is unknown. No studies investigate the effect of probiotics on chemerin, although probiotics are related to the diseases and are current topics under investigation. For this reason, our study contributes to the literature as the first study to evaluate these parameters together.

For this purpose, it is important to show that chemerin is a new modifiable factor of obesity affected by probiotic supplementation. These results obtained from obese rats will provide both weight control and reduction risk of developing obesity-related diseases by increasing the chemerin levels to the desired levels with probiotic supplementation in the diet of obese individuals.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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REFERENCES
35. Lloyd J, Zerfass K, Heckstall E, Evans K. Diet-induced increases in chemerin are attenuated by exercise and mediate the effect of diet on insulin and HOMA-IR. Ther Adv Endocrinol Metab 2015;6(5):189-98.
Table 1. Effects of normal diet and HFD on body weight and BMI in rats

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=8)</th>
<th>Group 2 (n=8)</th>
<th>Group 3 (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>275.25±11.02</td>
<td>294.63±10.15</td>
<td>292.25±8.31</td>
<td>0.650</td>
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<tr>
<td>8 w</td>
<td>288.63±7.18</td>
<td>358.00±18.47</td>
<td>382.75±11.11</td>
<td>0.000*</td>
</tr>
<tr>
<td>Final (16 w)</td>
<td>336.13±10.02</td>
<td>411.25±29.6a</td>
<td>416.88±18.94</td>
<td>0.000*</td>
</tr>
<tr>
<td>Weight Gain (0-8 w)</td>
<td>53.37±10.75</td>
<td>63.37±9.69</td>
<td>90.50±29.07</td>
<td>0.021*</td>
</tr>
<tr>
<td>Weight Gain (8-16 w)</td>
<td>47.50±6.80</td>
<td>53.25±23.62</td>
<td>34.12±10.46</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>BMI (g/cm^2)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>0.44±0.02</td>
<td>0.55±0.01a</td>
<td>0.55±0.34</td>
<td>0.000*</td>
</tr>
<tr>
<td>8 w</td>
<td>0.54±0.01</td>
<td>0.68±0.03b</td>
<td>0.72±0.02b</td>
<td>0.000*</td>
</tr>
<tr>
<td>Final (16 w)</td>
<td>0.63±0.01</td>
<td>0.78±0.56a</td>
<td>0.78±0.03</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**BMI: Body Mass Index; Group 1: Control Group, Group 2: Diet-Induced Obesity (DIO) Group, Group 3: DIO with Probiotic Supplementation Group**

*p<0.05 (compared to Group 1); *p<0.05 (compared to Group 2); *p<0.05

Table 2. Effects of HFD and probiotic supplementation on biochemical parameters in DIO rats

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=8)</th>
<th>Group 2 (n=8)</th>
<th>Group 3 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FPG (mg/dL)</strong></td>
<td>194.32±10.99</td>
<td>212.13±45.11</td>
<td>199.23±18.64</td>
</tr>
<tr>
<td><strong>Insulin (mIU/L)</strong></td>
<td>25.28±10.26</td>
<td>27.97±10.61</td>
<td>14.44±1.30</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>11.06±4.29</td>
<td>7.30±0.89b</td>
<td>7.30±0.89b</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.27±0.01</td>
<td>0.28±0.00</td>
<td>0.28±0.00</td>
</tr>
<tr>
<td><strong>TC (mmol/L)</strong></td>
<td>2.64±0.71</td>
<td>2.60±1.13</td>
<td>4.69±0.64</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>2.20±0.57</td>
<td>1.81±0.80</td>
<td>1.50±0.26</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dL)</strong></td>
<td>71.40±33.44</td>
<td>74.59±39.72</td>
<td>48.43±10.89</td>
</tr>
<tr>
<td><strong>LDL-C (µg/mL)</strong></td>
<td>6.25±0.25</td>
<td>7.12±0.70</td>
<td>6.91±0.17</td>
</tr>
<tr>
<td><strong>IL-6 (pg/mL)</strong></td>
<td>215.41±55.66</td>
<td>215.62±72.27</td>
<td>205.00±26.33</td>
</tr>
<tr>
<td><strong>IL-10 (pg/mL)</strong></td>
<td>383.93±151.77</td>
<td>286.56±120.87</td>
<td>348.36±136.80</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>2.04±1.73</td>
<td>4.15±1.12</td>
<td>4.55±1.10</td>
</tr>
<tr>
<td><strong>TNF-α (ng/L)</strong></td>
<td>283.22±196.08</td>
<td>485.77±271.99</td>
<td>336.52±142.42</td>
</tr>
<tr>
<td><strong>Leptin (ng/L)</strong></td>
<td>434.34±167.81</td>
<td>311.79±123.62</td>
<td>331.72±24.16</td>
</tr>
<tr>
<td><strong>Chemerin (ng/mL)</strong></td>
<td>0.78±0.39</td>
<td>14.31±13.3a</td>
<td>2.67±2.43</td>
</tr>
</tbody>
</table>

**FPG, Fasting Plasma Glucose; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; QUICKI, Quantitative Insulin Sensitivity Check Index; TC, Total cholesterol; TG, Triglyceride; LDL-C, Low Density Lipoprotein Cholesterol; HDL-C, High Density Lipoprotein Cholesterol; IL-6, Interleukin-6; IL-10, Interleukin-10; CRP, C-Reactive Protein; TNF-α, Tumor Necrosis Factor.**

*p<0.05 (compared to Group 1); *p<0.05 (compared to Group 2)
Figure 1. Flow diagram outlining the experimental design.

Figure 2. Changes in body weight and weight gain
Group 1: Control Group, Group 2: Diet-Induced Obesity (DIO) Group, Group 3: DIO with Probiotic Supplementation Group
Figure 3. BMI changes of groups (0-16 w)
Group 1: Control Group, Group 2: Diet-Induced Obesity (DIO) Group, Group 3: DIO with Probiotic Supplementation Group

Figure 4. Effect of probiotic supplementation on chemerin levels in DIO rats
Group 1: Control Group, Group 2: Diet-Induced Obesity (DIO) Group, Group 3: DIO with Probiotic Supplementation Group