Sex differences in energy metabolism and gut microbiota composition in diet-induced obesity

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Introduction

- Obesity is caused by an imbalance in the energy metabolism, and in particular by sex, shows a distinct difference in phenotype and metabolic regulations. Sex differences in obesity have been mostly attributed to sex hormones. However, recent development in genomic analysis tools indicated that genes which are located on the sex chromosomes may play a role.

- In addition, a large number of global statistics have indicated that significantly higher proportions of female population experience overweight and obesity. Thus far, this phenomenon has been mostly explained by socioeconomic circumstances and sex hormones. However, recent studies have suggested that X chromosome dosage is an important factor to determine body weight gain and co-morbidities.

Aims & Objectives

- We aimed to determine estrogen-dependent and estrogen-independent sex differences in energy metabolism and gut microbiota composition and to identify molecules responsible for these differences.

Materials and Methods

- Seven-week-old male and female C57BL/6J mice were divided into 3 groups: males, females, ovarioctomized females. All groups were fed with high fat diets for 10 weeks.

- Adipocytes size, the number of crown-like structure adipocytes (CLS) and the degree of macrophages infiltration were measured with hematoxylin-eosin (H&E) staining.

- Fasting blood glucose was measured using the blood glucose monitoring device (LifeScan, Inc., Milpitas, CA, USA) and circulating concentrations of insulin, leptin and adiponectin were measured with commercially available ELISA kits; insulin (Merck Milpore, Damstadt, Germany), leptin and adiponectin (R&D Systems, Inc., Minneapolis, MN, USA).

- Lipid metabolism-related genes (AMPK, ACC, FAS, HSL, LPL) and hormone homeostasis-related genes (P38K, Akt, GLUT4) were quantified by western blot analysis. Briefly, protein was extracted from the white adipose tissue and 50μg of protein was loaded on 4-20% SDS-PAGE and transferred to the PVDF membrane.

- The membranes were blocked with the blocking buffer and washed using PBST solution. After two hours of incubation at room temperature with primary and secondary antibody, respectively in a consecutive manner, the membrane was washed and the protein expression of target genes were detected.

- Fecal gut microbiota taxonomic profiling analysis were conducted by 16S rRNA sequencing using Illumina MiSeq Sequencing system (Illumina, San Diego, CA, USA).

- Statistical analysis was performed with SAS package (release 9.4, SAS Institute Inc., NC, USA). One-way ANOVA and the Duncan’s multiple test was used to determine the statistical differences between the groups. P-value of 0.05 was considered to be significant.

Summary

- Male showed higher gain fat and expression of lipogenesis-related genes, which is very similar in ovarioctomized females. Insulin sensitivity were higher in females compared to male irrespective of estrogen depletion.

Results

Figure 1. Changes in body weight change

- Body weight of M + Sham group was significantly higher than that of the female groups. The weight of the F + OVX group was significantly higher than that of the F + Sham group. The rate of increase in body weight of F + OVX group was 160% which is similar to that of the M + sham group.

- Fasting blood glucose and insulin concentrations of M + Sham group were significantly higher than those of female groups, and no significant differences among female groups.

Figure 2. The rate of increase in body weight (%) at sacrifice

Table 1. Circulating concentrations of glucose and insulin

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting blood glucose (mg/dL)</th>
<th>Insulin (ng/mL)</th>
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<tbody>
<tr>
<td>M + Sham</td>
<td>144.27 ± 14.27</td>
<td>1.35 ± 0.13</td>
</tr>
<tr>
<td>F + Sham</td>
<td>122.27 ± 12.27</td>
<td>1.08 ± 0.04</td>
</tr>
<tr>
<td>F + OVX</td>
<td>123.71 ± 12.71</td>
<td>1.12 ± 0.06</td>
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Table 2. CLS & Macrophage infiltration

<table>
<thead>
<tr>
<th>Group</th>
<th>CLS number/cm²</th>
<th>Macrophage infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>M + Sham</td>
<td>139 ± 37a</td>
<td>1.6 ± 0.24a</td>
</tr>
<tr>
<td>F + Sham</td>
<td>3 ± 3b</td>
<td>0.4 ± 0.40b</td>
</tr>
<tr>
<td>F + OVX</td>
<td>56 ± 11b</td>
<td>1.4 ± 0.24b</td>
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Figure 3. Adipocyte size and H&E staining of gonadal adipose tissue

- The adipocyte size was significantly increased in M + Sham and F + OVX groups. M + Sham group showed the highest number of CLS (the black arrows in figure 3) and the estrogen depletion in female animals increased the CLS number. The score of macrophage infiltration in adipose tissue was also significantly higher in the M + Sham and F + OVX groups than that of the F + Sham group.

Figure 4. Protein expression of energy metabolism-related genes in adipose tissue and liver tissue

- M + Sham and F + OVX groups had higher expression of lipogenesis-associated proteins (p-ACC, FAS, LPL) in adipose tissue than F + Sham group, while lipolysis-associated proteins (p-HSL) showed opposite results.

- Interestingly, liver tissue expression of p-P38K and p-Akt confirmed that the male’s insulin sensitivity were significantly lower compared to that of females in both groups. The absence of significance between female groups indicates that expression of these proteins are not affected by estrogen depletion.

Figure 5. Alpha-diversity and beta-diversity of gut microbiota

- The alpha-diversity in M + Sham group is significantly lower than the diversity in female groups, and the beta-diversity indicated that female ovarioectomy changes the distribution of microbiota similar to male.

- In addition, there were a number of taxa showing sex-dependent difference in their proportion which were either estrogen-dependent or estrogen-independent (data not shown).

Conclusions

- Non-estrogenic effects may partly contribute to sex differences in energy metabolism and gut microbiota composition and there is tissue specificities.

- The results of this study can be used as a scientific basis to establish gender-specific strategies to prevent and treat obesity contributing human well-being. (relevant SDGs: 1, 4, 5, 6, 11, 17)

Acknowledgement

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