High Body Mass Has No Effect on Cellular and Innate Immunity in Siberian Hamsters (*Phodopus sungorus*)

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ABSTRACT

Body mass is considered to be related with immune function in animals. Our aim was to test the hypothesis that cellular and innate immunity would be suppressed in high body mass of Siberian hamsters (*Phodopus sungorus*). Six heavier (high body mass, HBM) and six lighter (low body mass, LBM) hamsters were selected from 28 male hamsters. Body mass, body fat mass, wet spleen mass and blood glucose levels were significantly higher in the HBM group than in the LBM group. However, phytohaemagglutinin response, serum bacteria killing capacity and white blood cells did not differ between the two groups, suggesting cellular and innate immunity was not impaired in high body mass of hamsters. There was no correlation between cellular, innate immunity and body mass, body fat mass and glucose levels, suggesting cellular and innate immunity was not suppressed by higher body mass, body fat mass and glucose levels. In summary, cellular and innate immunity was not impaired in the HBM hamsters compared with the LBM hamsters.

1. INTRODUCTION

Obesity becomes more and more prevalent and it is associated with several chronic diseases [1-5]. It is also considered to be related with increased susceptibility to infections [6, 7]. The mechanism may be due to the changes of immune responses in obesity [8-10].

Effect of obesity on immune function has been carried out in some obese animals [11]. Cellular immunity is suppressed in *ob/ob* mice whose leptin gene was genetically mutated [12-14]. It is also impaired in *db/db* mice and *fa/fa* rats whose mutation in leptin receptor gene [15, 16]. Suppression of immune responses are also observed in diet-induced obese mice [17-19] or rats [20, 21]. Both cellular and humoral immunity are dampened in overweight Mongolian gerbils (*Meriones unguiculatus*) [22]. Likewise, obese human also show decrease in the capacity of lymphocyte proliferation [23] and humoral immunity [24, 25]. However, immune responses were not changed in diet-induced obese cats [26].
Serum bacterial killing capacity is usually examined to assess innate immunity, which is one arm of immune system [27-29]. This assay was performed in a sterile laminar flow cabinet to assess the functional response by the animal’s innate immune system against a relevant pathogen (i.e., Escherichia coli) [27-29]. Cellular immunity belongs to adaptive immune system which is responsible for controlling intracellular pathogens [9]. It is often evaluated by phytohaemagglutinin (PHA) response, which involves a subcutaneous injection of PHA that induces local T-cell stimulation and proliferation resulting in swelling [30, 31]. Immune organs including thymus and spleen are indirect immunological parameters [32, 33]. In addition, white blood cells (WBC) are also used to assess the overall health [34], and the increase of WBC is related with inflammation in obesity [3, 35].

Adipose tissues are not only energy reserves, but also are considered as important endocrine and immune organs [36-39]. Glucose plays a role in normal survival and function of lymphocytes, and glucose uptake and glycolysis increase in T cells during an immune response [40, 41], whereas hyperglycemia reduces immune function [42, 43].

The major problem in understanding the influence of overweight or obesity on immunity in human is that the obese population is too heterogeneous in various aspects, including dietary patterns, individual microbial and social environments [10]. Furthermore, the percentage of obesity caused by genetic deficiency is small [11]. Therefore, wild overweight or obese rodents perhaps are potential model to examine the impact of obesity on immunity independently of the confounding heterogeneity in human [10]. To date, however, little information is available about how obesity or overweight affects immune function in wild rodents.

Siberian hamsters (Phodopus sungorus) are small seasonal breeding and granivorous rodents mainly living in the desert and semi-arid regions of Mongolia in China [44, 45]. Previous work has demonstrated this species undergoes changes in immunity [46, 47] and decreases in body mass and fat mass in response to “winter-like” conditions [48, 49]. Reduction in fat mass harms humoral immunity in striped hamsters [50], implying that immunity may be related with body mass. In the present study, we are to test the hypothesis that innate and cellular immunity will be impaired in high body mass of Siberian hamsters.

2. MATERIALS AND METHODS

2.1. Animals and Experimental Design

All animal procedures were licensed under the Animal Care and Use Committee of Qu Normal University. Adult male Siberian hamsters used in this study were the offspring of hamsters in our laboratory colony. The hamsters were housed individually after weaning in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding under a constant photoperiod of 12 L:12 D (12 h:12 h light-dark cycle) and temperature of 23˚C ± 1˚C. Commercial standard rat pellets (Beijing KeAo Feed Co.) and water was provided at ad libitum throughout the experiment. The macronutrients of the diet were 6.2% crude fat, 18% crude protein, 23.1% neutral fiber, 5% crude fiber, 12.5% acid detergent fiber, and 10.0% ash, and the caloric value was 17.5 kJ/g. After body mass stabilized, we selected 6 heavier (21.4%) (high body mass, HBM) and 6 lighter (21.4%) (low body mass, LBM) hamsters from 28 males (age: 10 to 12 months).

2.2. Organs

Visceral organs were dissected as described previously [31]. In brief, after interscapular BAT (IBAT) was removed, the visceral organs, including heart, thymus, lungs, liver, spleen, kidneys, testes, epididymis, seminal vesicals and the digestive organs with contents (i.e., stomach, small intestine, caecum and colon) were dissected and weighed (±1 mg). The stomach, small intestine, caecum and colon were rinsed with saline to eliminate all the gut contents, before being weighed.

2.3. Body Composition

Perigonadal fat, mesenteric fat, retroperitoneal fat and subcutaneous fat were also dissected carefully and weighted. The mass of the four fat pads is considered as total body fat, and the fat content was calculated.
as perigonadal fat, mesenteric fat, retroperitoneal fat, subcutaneous fat and total fat mass divided by wet carcass mass [51].

2.4. Cellular Immunity Assays

PHA response (i.e., cellular immunity) was measured as described previously [30, 31]. Briefly, we measured the footpad thickness of their left hind feet with a micrometer (Tesa Shopcal, Swiss) to ±0.01 mm. Immediately thereafter, hamsters were injected subcutaneously 0.1 mg of PHA dissolved in 0.03 ml sterile saline in the middle of the footpad. After 6 h, 12 h, 24 h, 48 h injection, we measured footpad thickness. The PHA response was calculated as the difference between pre- and post-injection measurements divided by initial footpad thickness (PHA response = (post PHA − pre PHA)/pre PHA). Each measurement of PHA response was replicated six times on the same hamster [30, 31]. Only PHA responses after 6 h of PHA injection were included in the results because they were representative of the maximal response.

2.5. White Blood Cells Assays

At the end of the experiment, after collecting trunk blood, 20 µl whole blood was diluted immediately in 0.38 ml solution containing 1.5% glacial acetic acid, 1% crystal violet (Sigma) and the leukocytes were counted in an improved Neubauer chamber using microscope. The total number of WBC was determined by counting all leucocytes in the four corner large-squares of the Neubauer chamber, and multiplying the raw data by 5 × 10⁷ to obtain the final values (10⁹ cells/l) [52].

2.6. Innate Immunity

Serum bacterial killing capacity indicative of innate immunity was performed in a sterile laminar flow cabinet to assess the functional response by the animal’s innate immune system against a relevant pathogen, *Escherichia coli* [27-29]. Briefly, serum samples were diluted 1:20 in CO₂-independent medium (Gibco no. 18,045, Carlsbad, GA, USA). A standard number of colony-forming units (CFUs) of *E. coli* (ATCC no. 8739, Microbial Culture Collection Center of Guangdong Institute of Microbiology, China) was added to each sample in a ratio of 1:10, and the mixture (i.e., a number of *E. coli* dissolved in CO₂-independent medium, serum samples and CO₂-independent medium) was allowed to incubate at 37°C for 30 min to induce bacterial killing. After incubation, 50 µl of each sample was added to tryptic soy agar plates in duplicate. All plates were covered and left to incubate upside down at 37°C for 24 h, and then total CFUs were counted and bactericidal capacity was calculated as 100% minus the mean number of CFUs for each sample divided by the mean number of CFUs for the positive controls (containing only medium and standard bacterial solution), i.e. the percentage of bacteria killed relative to the positive control.

2.7. Blood Glucose Assays

Blood glucose levels were measured with FreeStyle Mini Blood Meter (Abbott Diabetes Care Inc. Alameda, USA) according to the manufacture’s instructions. The range tested of blood glucose was 1.1 - 27.8 mmol/l. The within-lot and -vial variabilities were <5.6% and <4.1%, respectively [31].

2.8. Statistical Analysis

Data were analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov-Smirnov and Levene tests, respectively. The ratio values including PHA response were subjected to arcsine transformation. The differences of body mass between the control and experimental groups at any time point were analyzed by independent-samples t-test. Group differences in wet organ mass with body mass as the covariate were analyzed by General Linear Model multivariate analysis followed by Bonferroni post hoc tests. Group differences in other parameters (body compositions, PHA response, WBC) were analyzed by
independent-samples t-test. Results were expressed as mean ± SE, and \( P < 0.05 \) was considered to be statistically significant.

3. RESULTS

3.1. Body Composition

The HBM hamsters had significantly higher body mass, wet carcass mass, perigonadal, mesenteric and total body fat mass than those of the LBM hamsters, while retroperitoneal and subcutaneous fat mass did not differ between the two groups (Table 1).

3.2. Organs

Masses of lung, spleen, kidneys, stomach, colon with contents, colon, total digestive tract and the length of small intestine, colon, total digestive tract in the HBM group were higher than those of the LBM group (Table 2). Other organ masses including heart, thymus, liver, small intestine, caecum, testes, epididymis and seminal vesical were not different between the two groups (Table 2).

3.3. White Blood Cells

There was no significant difference of WBC between the HBM and LBM groups (\( t = -1.105, \, df = 10, \, P = 0.295 \)) (Figure 1).

3.4. Cellular Immune Response

No significant difference of PHA response between the HBM and LBM groups was observed (\( t = \))

Table 1. Body composition in the high and low body mass of Siberian hamsters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Statistical summary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBM</td>
<td>LBM</td>
</tr>
<tr>
<td>Initial body mass (g)</td>
<td>50.8 ± 0.8(^a)</td>
<td>29.2 ± 0.8(^b)</td>
</tr>
<tr>
<td>Final body mass (g)</td>
<td>51.2 ± 3.7(^a)</td>
<td>31.1 ± 1.2(^b)</td>
</tr>
<tr>
<td>Wet carcass mass (g)</td>
<td>36.7 ± 7.1(^a)</td>
<td>22.6 ± 2.6(^b)</td>
</tr>
<tr>
<td>Perigonadal fat (g)</td>
<td>1.273 ± 0.356(^a)</td>
<td>0.343 ± 0.141(^b)</td>
</tr>
<tr>
<td>Mesenteric fat (g)</td>
<td>0.546 ± 0.169(^a)</td>
<td>0.284 ± 0.067(^b)</td>
</tr>
<tr>
<td>Retroperitoneal fat (g)</td>
<td>0.231 ± 0.167</td>
<td>0.080 ± 0.053</td>
</tr>
<tr>
<td>Subcutaneous fat (g)</td>
<td>1.538 ± 1.556(^a)</td>
<td>0.823 ± 0.217(^b)</td>
</tr>
<tr>
<td>Total body fat (g)</td>
<td>3.588 ± 2.034(^a)</td>
<td>1.530 ± 0.450(^b)</td>
</tr>
<tr>
<td>Perigonadal fat content (%)</td>
<td>0.031 ± 0.006</td>
<td>0.014 ± 0.005</td>
</tr>
<tr>
<td>Mesenteric fat content (%)</td>
<td>0.013 ± 0.002</td>
<td>0.012 ± 0.003</td>
</tr>
<tr>
<td>Retroperitoneal fat content (%)</td>
<td>0.005 ± 0.003</td>
<td>0.003 ± 0.002</td>
</tr>
<tr>
<td>Subcutaneous fat content (%)</td>
<td>0.034 ± 0.033(^a)</td>
<td>0.033 ± 0.008(^b)</td>
</tr>
<tr>
<td>Total body fat content (%)</td>
<td>0.084 ± 0.039(^a)</td>
<td>0.062 ± 0.017(^b)</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6). Values for a specific parameter that shares different superscripts are significantly different at \( P < 0.05 \), determined by independent t-test analysis. HBM: high body mass; LBM: low body mass.
Table 2. Wet organ mass in the high and low body mass of Siberian hamsters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Statistical summary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBM</td>
<td>LBM</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>0.294 ± 0.494</td>
<td>0.204 ± 0.027</td>
</tr>
<tr>
<td>Lungs (g)</td>
<td>0.338 ± 0.020a</td>
<td>0.282 ± 0.057b</td>
</tr>
<tr>
<td>Thymus (g)</td>
<td>0.014 ± 0.008b</td>
<td>0.014 ± 0.006a</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>2.282 ± 0.624</td>
<td>1.319 ± 0.191</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>0.219 ± 0.041a</td>
<td>0.139 ± 0.047b</td>
</tr>
<tr>
<td>Kidneys (g)</td>
<td>0.807 ± 0.157a</td>
<td>0.386 ± 0.068b</td>
</tr>
<tr>
<td>Stomach with contents (g)</td>
<td>1.493 ± 0.331</td>
<td>0.991 ± 0.186</td>
</tr>
<tr>
<td>Stomach (g)</td>
<td>0.377 ± 0.060a</td>
<td>0.234 ± 0.084b</td>
</tr>
<tr>
<td>Small intestine with contents (g)</td>
<td>2.030 ± 0.187a</td>
<td>1.288 ± 0.142b</td>
</tr>
<tr>
<td>Small intestine (g)</td>
<td>0.539 ± 0.144</td>
<td>0.481 ± 0.105</td>
</tr>
<tr>
<td>Small intestine length (cm)</td>
<td>30.9 ± 2.5a</td>
<td>27.2 ± 1.5b</td>
</tr>
<tr>
<td>Caecum with contents (g)</td>
<td>1.092 ± 0.328</td>
<td>0.854 ± 0.131</td>
</tr>
<tr>
<td>Caecum (g)</td>
<td>0.191 ± 0.068</td>
<td>0.198 ± 0.063</td>
</tr>
<tr>
<td>Caecum length (cm)</td>
<td>4.9 ± 0.9</td>
<td>5.1 ± 1.9</td>
</tr>
<tr>
<td>Colon with contents (g)</td>
<td>1.011 ± 0.290a</td>
<td>0.603 ± 0.169b</td>
</tr>
<tr>
<td>Colon (g)</td>
<td>0.372 ± 0.134a</td>
<td>0.209 ± 0.054b</td>
</tr>
<tr>
<td>Colon length (cm)</td>
<td>16.9 ± 1.6a</td>
<td>12.5 ± 1.4b</td>
</tr>
<tr>
<td>Total digestive tract (g)</td>
<td>1.102 ± 0.222a</td>
<td>0.887 ± 0.157b</td>
</tr>
<tr>
<td>Total digestive tract length (cm)</td>
<td>52.7 ± 1.9a</td>
<td>44.7 ± 3.1b</td>
</tr>
<tr>
<td>Testes (g)</td>
<td>0.654 ± 0.138</td>
<td>0.164 ± 0.118</td>
</tr>
<tr>
<td>Epididymis (g)</td>
<td>0.088 ± 0.032</td>
<td>0.035 ± 0.019</td>
</tr>
<tr>
<td>Seminal vesical (g)</td>
<td>0.654 ± 0.232</td>
<td>0.058 ± 0.061</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6). Values for a specific parameter that shares different superscripts are significantly different at P < 0.05, determined by a multivariate analysis with body mass as the covariate. HBM: high body mass; LBM: low body mass.

1.272, df = 10, P = 0.232) (Figure 2). PHA response was not correlated with body mass (r = 0.140, P = 0.665) and total body fat mass (r = 0.069, P = 0.831).

3.5. Innate Immunity

Bacteria killing capacity indicative of innate immunity did not differ between the HBM and LBM groups (t = −1.167, df = 10, P = 0.271) (Figure 3). No correlation was observed between innate immunity and body mass (r = 0.396, P = 0.202), total body fat mass (r = 0.118, P = 0.716).

3.6. Blood Glucose Levels

The HBM hamsters had higher blood glucose levels than those of the LBM hamsters (t = 3.672, df = 10, P < 0.05) (Figure 4). Blood glucose levels were not correlated with cellular (r = 0.337, P = 0.283) and
innate immunity, but positively correlated with body mass and total body fat mass ($r = 0.750, P = 0.005$) ($r = 0.855, P < 0.001$).

4. DISCUSSION

Our main results showed that PHA response, serum bacteria killing capacity and white blood cells did
not differ between the high and low body mass groups, indicating that both cellular and innate immunity were not suppressed in the high body mass hamsters. This result did not agree with our previous study in which cellular and humoral immunity were impaired in overweight Mongolian gerbils [22]. Higher body mass, body fat mass, and blood glucose levels might not explain these results. Spleen mass was higher in HBM hamsters than LBM hamsters, while thymus mass was not different between the two groups. This finding was inconsistent with other research in which both thymus and spleen mass were reduced in ob/ob mice [53].

Cellular or humoral immunity may be suppressed due to excessive body mass or fat loss during starvation [31, 54, 55] or experimental reductions in body fat [50]. Similarly, immune function can be impaired owing to excessive body mass or fat gain such as in obesity [6, 10, 56]. In the present experiment, we found that high body mass did not influence cellular and innate immunity in hamsters. Additionally, PHA response and innate immunity were not correlated with body mass and body fat mass in hamsters, indicating cellular and innate immunity were not influenced by higher body mass and body fat mass in hamsters. The small sample size in our study might be also responsible for these results.

Glucose provides energy for immune responses [40, 41]. However, hyperglycemia may harm immunity through non-enzymatic glycosylation of circulating immunoglobulins [42, 43]. In our study, no significant correlation was observed between glucose levels and cellular, innate immunity, suggesting higher levels of glucose in the HBM hamsters had no effect on immune function in contrast with the LBM hamsters. Blood glucose levels were positively correlated with body mass and body fat mass, indicating that higher body mass and body fat mass contributed to hyperglycemia in the HBM hamsters.

In summary, body mass, body fat mass and blood glucose levels were higher in the HBM hamsters than in the LBM hamsters. Both cellular and innate immunities were not affected by body mass in hamsters, indicating healthy immune function in high body mass hamsters.

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