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HIGH-FAT DIET FEEDING INDUCES HYPERTROPHIC RESPONSES IN SOME METABOLIC AND IMMUNE TISSUES IN SWISS MICE

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Abstract. Having profound awareness about obesity characteristics will be a strategy to treat obesity and its related diseases. In the current study, we fed mice with high-fat diet for 10 weeks and saw a significant increase in body weight of the high-fat diet fed mice compared to the regular diet fed mice. Consequently, the high fat-diet fed mice showed significant higher in the weight of the mesenteric adipose tissue and the white adipose tissue around brown adipose tissue than did the regular diet fed mice. Interestingly, there was a markedly increase in size and weight of spleen tissue of the high fat-diet fed mice compared with the regular fed mice. These results indicated that the high-fat diet feeding induced hypertrophic response not only in weight and size of several white adipose tissues but also in weight and size of immune tissue such as spleen. Therefore, strategies in controlling the changes in both white adipose tissue and immune tissue could be benefits in the battle against obesity and its related metabolic dysfunctions.

Keywords: High-fat diet, white adipose tissue, immune tissue.

1. Introduction

Prevalence of obesity is rising rapidly in the world not only in developed countries but also in developing countries. And the money paying in medical to beat obesity and its related metabolic disorders (e.g., type 2 diabetes, fatty liver diseases, and cardiovascular diseases) is becoming cost burden for many countries [1]. Obesity is associated with morphological changes in metabolic tissues such as adipose tissues, liver and skeletal muscle tissues [2, 3]. Especially, changes in white adipose tissue size and weight are important signs of obesity. Studies have reported that increased adipogenesis in obesity is related with increased pro-inflammatory cytokines (e.g., tumor necrosis factor alpha/TNF-α, interleukin 6/IL-6, monocyte chemoattractant protein 1/MCP-1) which disrupt insulin signaling, thus, contributing to insulin

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resistance and type 2 diabetes. Additionally, obesity-related adipose-hypertrophy leads to increased plasma free fatty acid level that presumably contributes to dyslipidemia and its related dysfunctions such as arthrosclerosis and nonalcoholic fatty liver diseases [4].

It is also worthy to note that changes in structure and function of immune tissues (such as spleen and mesenteric lymph nodes) have recently been recognized as playing a pivotal role in obesity and its related metabolic disorders. These are usually accompanied by changes in the local cytokines and populations of immune cells that at least partly lead to systemic chronic inflammatory responses [5, 6]. The latest responses are well known as important factors inducing obesity-related diseases [7].Thus, changes both in white adipose tissues and immune tissues are critical characteristics of obesity. Rodent obese models have been begun studying in Vietnam recently [8, 9] and, thus, establishing a stable obese model would be a strategy for further studies in treatment of obesity. In the present study, we fed mice with high-fat diet for 10 weeks and saw significant increases in body weight and in the weight and size of the mesenteric adipose tissue and the white adipose tissue around brown adipose tissue. These were accompanied with an increase in weight and size of spleen tissue in the mice fed with high-fat diet. These data suggested that changes in both white adipose tissue and immune tissue could be a marked sign of obesity.

2. Content

2.1. Materials and methods

**** Animals and diets***

Four-week old-male Swiss mice were purchased from the National Institute of Hygiene and Epidemiology (NIHE). Mice were housed in an animal facility, where 12-12 h light-dark cycle was maintained. The mice were fed for 10 weeks with a regular diet (RD) (5% energy from lipid) or with a high-fat diet (60% energy from lard) on the RD base. The RD was also purchased from the NIHE. The mice were fed restrictedly with food and arbitrarily with water and were weighted weekly to observer their body weight. After 10weeks, the animals were killed by decapitation. Several white adipose tissues and spleen tissues were dissected and measured.

**** Statistical Analysis***

The results were shown as means ± standard error of the mean (SEM). Comparisons of variables were carried out by using Student's *t* test. Differences were considered to be significant when $P < 0.05$.

2.2. Results and discussion

2.2.1. Body weight change in high-fat diet fed mice

We first tested whether high-fat diet (HFD) feeding altered body weight gain. We fed 4 week old-male mice with a HFD or a regular diet (RD) for 10 weeks and we found that body weight gain of the HFD-fed mice was significantly higher than that of the RD-fed mice from week 2 to week 10 (Figure 1B). This tendency was also observed

in previous studies with arbitrarily HFD feeding for 12 weeks or restricted HFD for 30 days [8, 9].Consistent with the body weight gain, the size of mice fed with HFD showed much larger than did the size of mice fed with RD (Figure 1A).

Since all mice were fed with almost the same amount of food, the increase in body weight, thus, could be due to the additional fat of the HFD that caused abundant calorie intake of the HFD-fed mice.

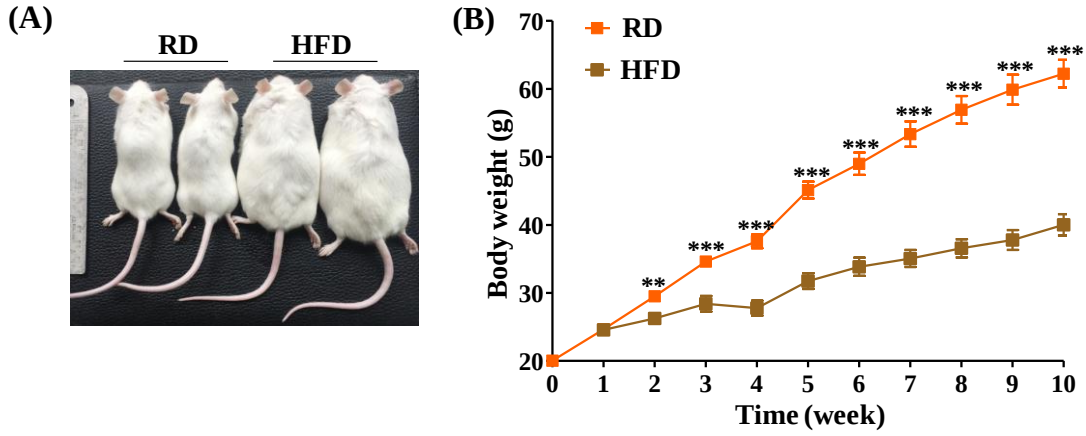


Figure 1. Changes in morphology and weight of HFD-fed mice

Four week-old male Swiss mice were fed a regular diet (RD) or a high-fat diet (HFD) for 10 weeks, (A) gross morphology of mice (B) daily body weight changes

Data are presented as means ± SEM; n = 6 in each group

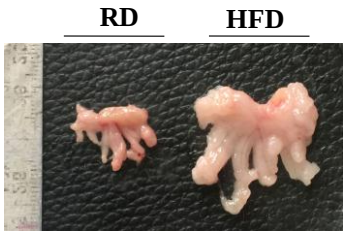
***P < 0.01, *** P < 0.001 compared with RD-fed mice*

2.2.2. Character of abdominal fat in high-fat diet fed mice

It has been reported that abdominal fat plays pivotal role in metabolic diseases such as fatty liver disease, type 2 diabetes [10, 11]. Here, we tested effects of the HFD-feeding on mesenteric adipose tissue that is a typical type of abdominal fats. The result shows that the size of mesenteric adipose tissue of the HFD-fed mice markedly bigger than that of the RD-fed mice did (Figure 2A). Similarly, the mesenteric adipose tissue weight of the HFD-fed mice was significantly higher than that of the RD-fed mice (Figure 2B and 2C).

Previous studies have shown that high-calorie diet feeding leads to increased plasma free fatty acid which ultimately deposit in not only under-skin adipose tissue but also abdominal adipose tissues [2, 12]. Therefore, the HFD-induced mesenteric adipose tissue in the current study may be at least partly caused by increased plasma free fatty acid levels. Since, increased mesenteric adipose tissue weight is associated with the HFD-induced obesity, thus, suppression of this adipose tissue hypertrophy could be efficiency in reduction of obesity and its related metabolic syndromes.

(A)



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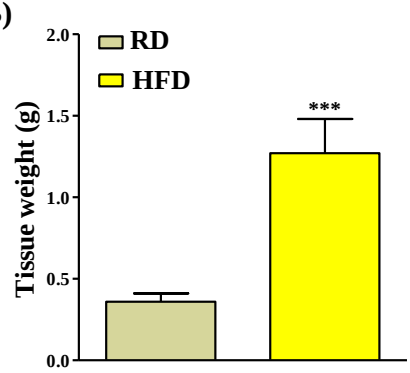


(C)

n = 6	RD	HFD
1	0.175	1.620
2	0.424	0.992
3	0.264	1.190
4	0.498	1.880
5	0.467	1.680
6	0.330	0.613
X	**0.360**	**1.271**
SEM	**0.051**	**0.210**
P	***< 0.001***	

Figure 2. Changes in mesenteric adipose tissue of the HFD-fed mice

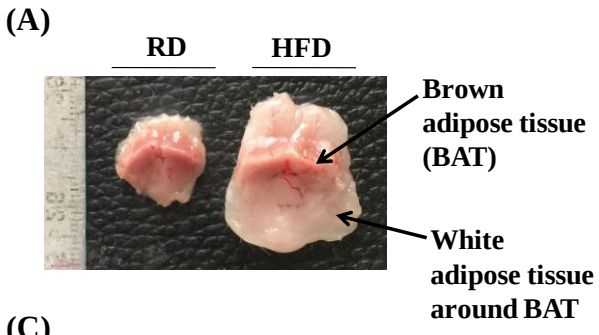
Four week-old male Swiss mice were fed a regular diet (RD) or a high-fat diet (HFD) for 10 weeks, (A) gross morphology of mesenteric adipose tissues, (B) mesenteric adipose tissue weight, (C) data analysis of mesenteric adipose tissue weights, Data are presented as means ± SEM; n = 6 in each group

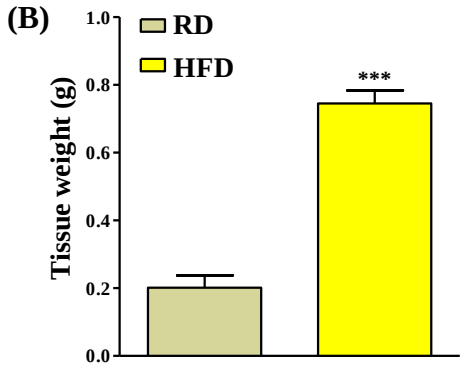
**** P < 0.001 compared with RD-fed mice*

2.2.3. Character of white adipose tissue around brown adipose tissue in high-fat diet fed mice

Change in brown adipose tissue (BAT) morphology and function has been considered as an important factor that is associated with metabolic dysfunctions [13]. Ectopic white adipose tissue deposition in and/or around BAT may contribute to alteration of brown fat morphology and function at least partly by increased pro-inflammatory cytokine (e.g., TNF-α) production [14]. The next was, thus, to examine whether HFD-feeding had effects on white adipose tissue around BAT. The result shows that the HFD-fed mice contained much larger white adipose tissue around BAT than did the RD-fed mice (Figure 3A). Consistently, the weight of white adipose tissue around BAT of the HFD-fed mice was significantly higher than that of the RD-fed control mice (Figure 3B and 3C).

It has been noted that the mice fed with HFD show decreased PGC-1α (Peroxisome proliferator activated receptor gamma coactivator 1 alpha) signaling pathway response that leads to decreased BAT activity [15]. Since, BAT recruits lipids for its activities in thermogenic process to produce heat, thus, increased white fat accumulation around BAT in the HFD-fed mice may be due to decreased thermogenesis.





(C)

n = 6	RD	HFD
1	0.060	0.585
2	0.257	0.630
3	0.213	0.790
4	0.280	0.650
5	0.131	0.790
6	0.267	0.867
X	**0.201**	**0.745**
SEM	**0.036**	**0.038**
P	***< 0.001***	

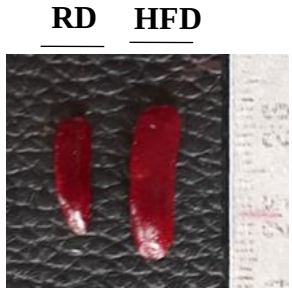
Figure 3. Changes in white adipose tissue around BAT of the HFD-fed mice.

*Four week-old male Swiss mice were fed a regular diet (RD) or a high-fat diet (HFD) for 10 weeks, (A) gross morphology of white adipose tissues around BATs, (B) weight of white adipose tissue around BAT, (C) data analysis of weights of white adipose tissue around BAT. Data are presented as means ± SEM; n = 6 in each group*** $P < 0.001$ compared with RD-fed mice*

2.2.4. Character of spleen tissue in high-fat diet fed mice

There are evidences supporting that obesity are not only characterized by changes in metabolic tissues (e.g., white adipose tissues, liver tissues) but also characterized by an increase in chronic inflammatory responses [7]. Obesity-related chronic inflammation is associated with increased pro-inflammatory cytokine levels (e.g., TNF-α, IL-6, MCP-1) in plasma and tissues such as white adipose tissues, liver, and skeletal muscle tissues [16]. However, it is interesting to note that those cytokines are also well-known productions of immune cells such as macrophages and lymphocytes [16]. Hence, we here examined the changes in spleen tissue, a typical immune tissue, in the HFD feeding. Surprisingly, spleen size of the HFD-fed mice was strongly bigger than that of the RD-fed mice (Figure 4A). In consistent with this, the spleen weight of the HFD-fed mice was significantly higher than that of the RD-fed mice (Figure 4B and 4C).

(A)



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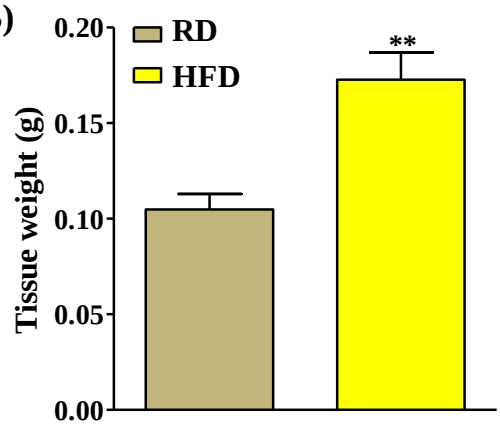

(C)

n = 6	RD	HFD
1	0.073	0.188
2	0.129	0.138
3	0.121	0.228
4	0.102	0.143
5	0.098	0.188
6	0.107	0.151
X	**0.105**	**0.173**
SEM	**0.008**	**0.014**
P	***0.002***	

Figure 4. Changes in spleen of the HFD-fed mice

Four week-old male Swiss mice were fed a regular diet (RD) or a high-fat diet (HFD) for 10 weeks, (A) gross morphology of spleens, (B) spleen weight, (C) data analysis of spleen weights, data are presented as means ± SEM; n = 6 in each group

*** P < 0.01 compared with RD-fed mice*

Spleen is a lymph node which contains mainly immune cells such as B and T lymphocytes and other white blood cells. Obese condition is accompanied by a chronic inflammatory state which is specified by increase in pro-inflammatory cytokine and free fatty acid levels in blood [16]. These molecules in turn stimulate immune cell proliferation and activation, thus, leading to hypertrophy of immune tissue such as spleen [17, 18]. Alternatively, increased size and weight of spleen is also a characteristic of the HFD-fed mice.

3. Conclusions

In conclusion, our current study demonstrated that 10 week feeding with the HFD markedly increased mouse body weight. This was associated with increases in size and weight of mesenteric white adipose tissue and white adipose tissue around brown adipose tissue as well as spleen tissue. The HFD feeding-induced those effects may be derived from increased adipogenesis in white adipose tissues and increased proliferation and activation of immune cells in spleen, respectively. As a consequence, enhanced white adipose tissue and spleen hypertrophy should be a pivotal sign of the HFD-

induced obesity. Therefore, controlling the changes in both white adipose tissues and immune tissues can be potential to reduce the risk of obesity and its related diseases.

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