EVALUATING THE MORPHOLOGICAL CHARACTERISTICS AND VISCERAL FAT OF MALE MICE GENERATED OBESITY MODEL BY A HIGH-FAT DIET

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ABSTRACT

Background: *Obesity is a global health issue caused by consuming too much energy compared to the body's needs, which negatively impacts almost every organ of the body. As a result, generating an experimental obesity model is critical for researching new drugs to improve obesity and its consequences.* ***Objectives****: Generating obesity model of male mice over a 6-week period of feeding by a high-fat diet.* ***Materials and methods****: In this experimental research, 24 male mice (Swiss albino) of 3,5 weeks old were divided into 2 groups, each containing 12 mice: control group fed by a normal-fat diet (NFD) and obesity group fed by a high-fat diet (HFD) in a period of 6 weeks. Evaluation was conducted at the beginning of research, every 7 days and at the end of the study.* ***Results****: The mean of body weight, weight gain, body length, body mass index (BMI), chest and waist circumference of HFD group had a statistically significant difference from the end of week 2. The morphological difference was more remarkable at week 3 (p<0.001). After 6 weeks of study, the weight gain rate of HFD group was 131.32% higher than the NFD group. It was also noticeable that the weight of omental fat and renal fat (both sides) in the HFD group was significantly higher than that of the NFD group (p<0,001).* ***Conclusions****: Research has successfully generated an obesity model in male mice after a period of 6 weeks by a HFD (640Kcal/100g, 52-53% lipid).*

Keywords: *Swiss albino, male mice, obesity, high-fat diet.*

I. INTRODUCTION

Obesity is a global health issue that is rapidly becoming a pandemic, with 1.6 billion adults classified as overweight and 400 million adults classified as obese. There are evidences that an animal model of parental obesity increases the risk of obesity and diabetes in offspring, suggesting a possible mechanism in the amplification of these chronic diseases [3], [7]. Obesity is the result of consuming too much energy compared to the body's needs.

Changes in BMI, serum lipid profile including total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein are factors of obesity. Obesity may adversely affect almost all systems of the body and causes some conditions such as coronary heart disease, hypertension, type 2 diabetes và different types of cancer. If current trends continue, it is estimated that 38% and 20% of the world's population will be overweight and obese by 2030, respectively [3].

Today, scientists are concentrating their efforts on developing new drugs that can improve obesity and its consequences. To test the pharmacological effects of new drugs, it is necessary to build an experimental obesity model. Many domestic and international studies have successfully generated experimental obesity models, including the Metabolic Syndrome model in high reproductive performance mice by a high-fat diet (30% lipid) in 19 weeks [1], obesity model in 4-week-old mice (Swiss albino*)* by a high-fat diet (46.36Kcal/mouse/day) after fed in 6 weeks [2], obesity model in 6 – 8-week-old male rats (Wistar) by a high-fat diet (46% lipid) in 16 weeks [3], type 2 diabetes model generated by obesity model in mice (Swiss albino) in 4 weeks [4], obesity Swiss albino mice model in 24 weeks by a high-fat diet (60% lipid) [5], obesity model in male rats (Wistar) by a high-fat diet (38.9% lipid) [6], obesity *Mus musculus* var. *albino* model by a high-fat diet and a sedentary existence on 30 days [8], type 2 diabetes model generated by obesity Swiss albino mice model by a high-fat diet in 4 weeks [9]. Nowadays, studies on building obesity models on mice in the Mekong Delta in particular and in Vietnam in general still have some limitations and incomplete published results. Therefore, our research team conducted this study with the goal of generating obesity model of male mice over a 6-week period of feeding by a high-fat diet.

II. MATERIALS AND METHODS

2.1. Study animals

3.5-week-old male mice, which were the purebred Swiss albino line, were provided by the Institute of Vaccines and Biological Medical (IVAC). Their lot number was CT0511. Mice were raised in a stable environment for three days after being transported to acclimate to their new surroundings.

2.1.1. Standards for the selection

Individuals chosen for the study must be from the same lot, at the same age, free of deformities, have never been mated and weighed from 18 to 24 grams.

2.1.2. Standards for elimination

We eliminated individuals which suffered from respiratory, digestive and infectious diseases during the research; died or showed behavioral disturbances during the study; failed in the process of modeling or taking samples for research.

2.1.3. Study address and time

The study was conducted in the laboratory of the Department of Physiology, Can Tho University of Medicine and Pharmacy from November 2021 to January 2022.

2.2. Methods

2.2.1. Study design

This was a controlled experimental, descriptive cross-sectional, and longitudinal study follow-up over 6 weeks of nourishment.

2.2.2. Study size

The study included 2 groups, each containing 12 mice: the control group (NFD group) and the obesity group (HFD group). Mice from the same group were kept in the same cage (6 mice/cage, size 43x28x15cm), and all groups were raised and cared under the same conditions of temperature, humidity, living environment, and natural light with a 12:12h light-dark cycle.

2.2.3. Sampling method

Mice were numbered to distinguish between individuals by marking the front legs (right, left), hind legs (right, left), cheeks (right, left), head, back, and tail. We drew randomly the ordinal numbers of mice and divided them into 2 study groups.

2.2.4. Study contents

Morphological characteristics

We evaluated the morphological characteristics at the beginning of the study and every 7 days for 6 weeks, including bodyweight: the total body weight of fasting mice (g), weight gain: the difference of the current and the initial week's weight (g), length: the longest size measured from nose to anus (mm), BMI: the result of body weight divided by the square of length (kg/m^2), chest circumference: the largest circumference across the mice's chest area (mm), waist circumference: the largest circumference through the mice's abdomen area (mm).

The weight gain rate and weight of visceral fat after a 6-week period

At the end of the study, the mean of weight gain rate (%, in comparison with the initial week) was evaluated. The mice were then anesthetized and separated to weigh the mean of omental and both sides renal fat separately (g).

2.2.5. Study materials

Table 1. Nutritional composition of AniFood's product.

Ingredient	Wheat flour, soybean meal, cod liver oil, fish meal, rice flour, cornstarch, rice bran, trace minerals, multivitamins... which contains all kinds of amino acids and vitamins needed for the growth of experimental animals.					
Nutrient	**Protid**	**Lipid**	**Fiber**	**Minerals**	**Humidity**	**Energy**
Ratio	≥21%	5-7%	5-6%	6-8%	<10%	384Kcal/100g

Suoi Dau breeding facility under IVAC provided AniFood's product (**Table 1**). The NFD group (NFD) only consumed AniFood's product at a daily dose of 15g/100g bodyweight. HFD group (HFD) had a mixture of lipid-rich foods (52-53%) including 50% AniFood and 50% pork fat (896Kcal/100g edible portion), then the total energy value was 640Kcal/100g.

Distilled water was prepared in a mice-special bottle. Mice's body weight was determined by an electronic scale (model WH-B series electronic kitchen, Guangzhou WeiH-eng Electronics Co., Ltd; range/graduation: 1kg/0.1g). Mice's body length, chest circumference and waist circumference were determined by a 150cm tape measure (accuracy to millimeters). Mice were anesthetized by diethyl ether (Merck Brand, Germany), and impregnated with cotton wool in sufficient quantity before dissecting. The weight of mice's organs was measured by an analytical balance (Sartorius Brand, Germany; max: 220g, d=0.1mg). The organs were gently peeled, cut and placed immediately on filter papers, which soaked in physiological saline in Petri dishes to prevent drying. Then we removed the surrounding tissue, blotted with paper before putting it on the weighing plate and recorded the results.

2.2.6. Statistical analysis

According to the medical statistics method, all of the data obtained from the research were processed by SPSS 20.0 software. To check the normal distribution of data, the Kolmogorov-Smirnov test was used. Quantitative variables were presented as mean values ± standard deviation if normally distributed. Student T-Test with statistical significance determined at $p \leq 0.05$ was used to compare the mean difference between 2 groups.

2.2.7. Ethics approval

All of the laboratory animals participating in the study were given reasonable care in accordance with the research objectives. In a laboratory, captive conditions were always guaranteed to be safe, clean, and minimize stress factors. Prior to proceeding, all phases of the research were subjected to a thorough animal ethical review. We reduced the number of manipulations that might cause significant pain in the study animals. The use of animals and the collection of specimens are only for scientific research purposes.

III. RESULTS

3.1. Morphological characteristics

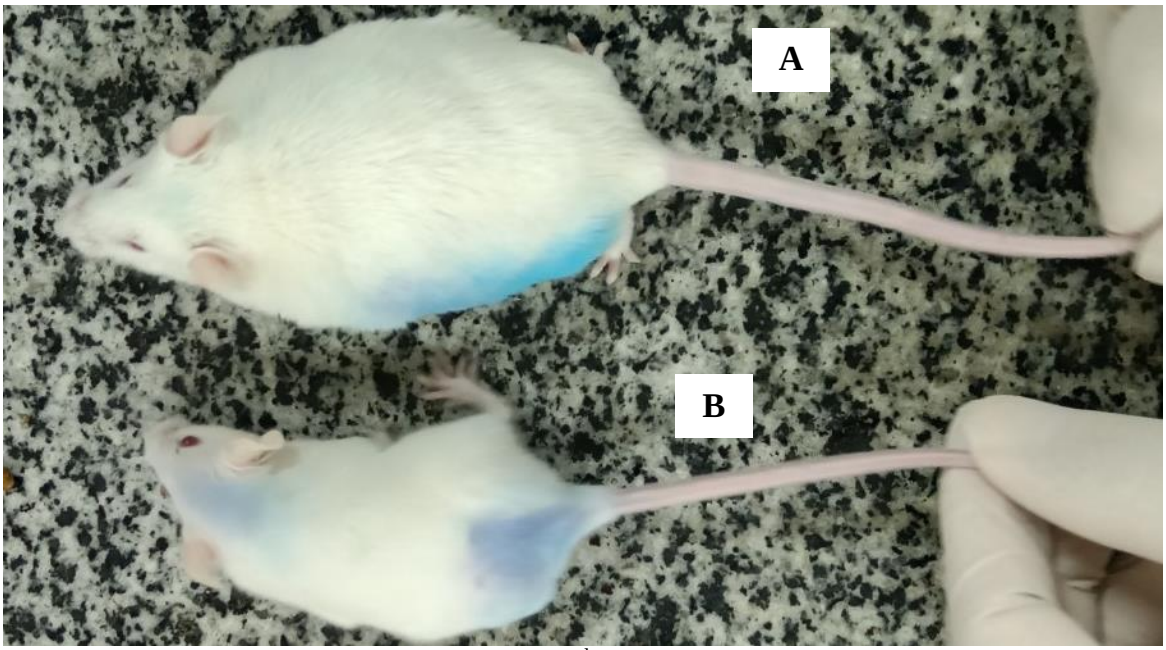

Figure 1. Morphology of the mice on the 6th weekend (A: HFD group, B: NFD group).

It is remarkable that the significant difference in morphology of the HFD group compared to NFD group at the end of the study.

Table 2. The morphological characteristics of mice in 2 groups every week.

Para-meter	Group	Week						
		Initial	1	2	3	4	5	6
Body weight (g)	NFD (n=12)	22.92 ±0.90	23.83 ±0.94	25.08 ±0.10	27.75 ±2.01	28.92 ±1.88	31.42 ±1.88	33.25 ±1.71
	HFD (n=12)	22.17 ±1.64	25.58 ±2.50	33.83 ±2.66	46.67 ±1.83	53.25 ±1.48	57.92 ±1.68	61.00 ±1.60

Parameter	Group	Week						
		Initial	1	2	3	4	5	6
p-value		0.183	**0.04**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Weight gain (g)	NFD (n=12)	-	0.92 ±0.51	2.17 ±1.11	4.83 ±1.85	6.00 ±1.76	8.50 ±1.98	10.33 ±1.78
	HFD (n=12)	-	3.42 ±1.78	11.67 ±2.10	24.50 ±1.93	31.08 ±1.68	35.75 ±1.66	38.83 ±2.21
p-value		-	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Body length (mm)	NFD (n=12)	79.50 ±2.88	80.33 ±2.90	80.67 ±3.20	80.75 ±3.11	83.58 ±4.01	85.08 ±3.63	87.08 ±3.32
	HFD (n=12)	76.50 ±6.34	81.42 ±5.84	87.08 ±3.34	89.42 ±2.23	97.50 ±4.52	102.67 ±2.50	103.92 ±2.31
p-value		0.156	0.573	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
BMI (kg/m^2)	NFD (n=12)	3.63 ±0.24	3.71 ±0.30	3.87 ±0.28	4.26 ±0.31	4.16 ±0.44	4.36 ±0.47	4.40 ±0.37
	HFD (n=12)	3.83 ±0.45	3.88 ±0.36	4.46 ±0.28	5.85 ±0.34	5.63 ±0.51	5.50 ±0.30	5.66 ±0.30
p-value		0.207	0.221	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Chest circum-ference (mm)	NFD (n=12)	65.42 ±3.20	68.83 ±3.69	71.92 ±3.26	73.08 ±3.85	73.75 ±4.49	75.50 ±5.35	77.83 ±4.55
	HFD (n=12)	65.00 ±2.13	70.75 ±4.18	78.17 ±4.28	83.92 ±4.21	86.50 ±5.37	91.00 ±3.33	92.08 ±3.34
p-value		0.712	0.246	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Waist circum-ference (mm)	NFD (n=12)	75.08 ±3.15	77.92 ±4.58	82.58 ±4.10	86.25 ±4.86	87.75 ±4.45	88.58 ±4.17	90.58 ±4.19
	HFD (n=12)	74.58 ±2.75	81.92 ±3.06	88.83 ±3.43	98.00 ±2.86	100.67 ±4.25	104.42± 5.26	108.33± 4.92
p-value		0.682	**0.02**	**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

At the start, the morphology of mice in the HFD group was similar to that of mice in the NFD group. The statistically significant difference was obvious in all research indicators from the second week and lasted until the sixth week of the study ($p<0.01$). Differences in body weight, weight gain, and waist circumference were also observed by the end of the first week of rearing ($p<0.05$).

3.2. The weight gain rate and weight of visceral fat after a 6-week period

Table 3. The figures for weight gain rate and weight of visceral fat of mice in 2 groups at the end of study (after a 6-week period).

Parameter		Weight gain rate (%)	Omental fat (g)	Renal fat (g)	
				Right	Left
Group	NFD (n=12)	45.25 ±8.54	0.6230 ±0.0595	0.0227 ±0.0019	0.0232 ±0.0022

Parameter		Weight gain rate (%)	Omental fat (g)	Renal fat (g)	
				Right	Left
	HFD (n=12)	176.57 ±21.68	3.5179 ±0.1684	0.1854 ±0.0192	0.1814 ±0.0192
p-value		**<0.001**	**<0.001**	**<0.001**	**<0.001**

The weight gain of the HFD group was 131.32% higher than the NFD group in the last week of this study. The weight of omental fat and renal fat (both sides) in the HFD group were significantly higher than that of the NFD group (p<0.001).

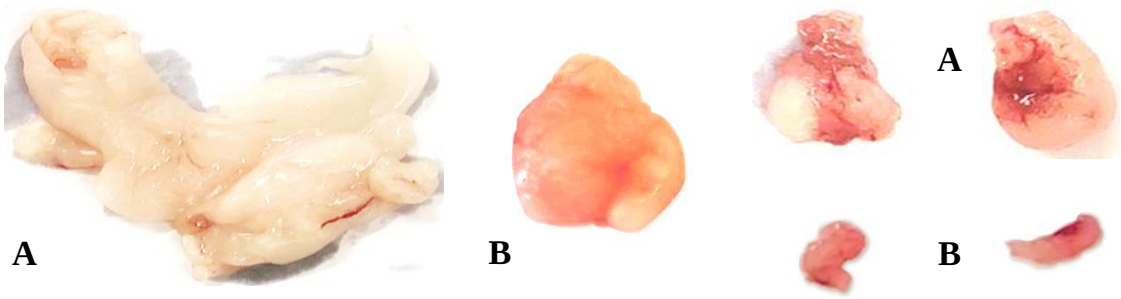

Figure 2. Visceral fat of the mice on the 6th weekend (A: HFD group, B: NFD group).

IV. DISCUSSION

At the beginning of the study, the indicators of mice morphology did not differ between the two groups. There were differences in body weight, weight gain, and waist circumference during the first week of feeding (p<0.05). All study indexes of the HFD group were significantly higher than those of the NFD group in the second week of rearing (p<0.01); The differences continued to be noticed in the following weeks. Obesity mice had the highest body weight, height, BMI, chest circumference and waist circumference at the last week of the study. Meanwhile, the highest weight gain was at week 3, and the lowest one was at week 6 due to the reduction of food consumption and weight gain rate with age. Our results are compatible with many different studies conducted both domestically and internationally. In an 4-week-old obesity mice model research, mice were fed with a mixture of food pellets 29.36Kcal/mouse/day, cheese 8.5Kcal/mouse/day, and sausage 8.5Kcal/mouse/day for 6 weeks, the weight of mice in NFD group was 48.15±0.48g and HFD group was 57.71±1.24g (p<0.05) [2]. In another study about inducing obesity mice in 38 days; the NFD group reported a weight of 31.5±1.2g while the HFD group's record was 45.0±1.3g [4]. In the Swiss albino mice model of obesity due to a 60% lipid diet based on the experiment of Della Vedova et al (2016) on the C57BL/6J mouse strain, the weight of the HFD group was 31.15±2.69g which was significantly higher, compared to 27.23±2.95g of NFD group. After 24 weeks, the weight of mice in the HFD group was 46.41±8.24g, while the figure of NFD group was just 38.30±1.90 [5]. A similar study in rats (Wistar) showed that HFD mice gained more weight than the NFD group from the end of the 5th week of rearing (p<0.05), the length of HFD mice increased more than that of NFD over 7 weeks of nourishment and the result was clear from the end of week 2 (p<0.05) [6]. Another

research on the 8-week-old obesity mice model (*Mus musculus var. albino*) had an initial weight at 30±1g, after 30 days of rearing in sedentary conditions and eating a high-fat diet, the weight of HFD group was higher than the NFD group (42.18g in comparison with 38.58g) [8]. In a similar study in mice (male mice, Swiss strain), initial weight of 13-14g was induced in 4 weeks. The mean bodyweight of the HFD group was 46.38g compared to 29.26g of the NFD group [9]. The research results have discrepancies due to differences in research design (animal species, initial weight, rearing time, nutritional composition, lipid ratio, habitat). However, those studies have shown the feasibility and effectiveness of a model of obesity in mice and rats by a high-fat diet.

After evaluating the mice's weight, the ratio of fat to body mass is an indicator that need to be noticed. Fat is stored throughout the body in cells called adipocytes. Fat cells can increase or decrease in size depending on how much fat the body is storing. Consuming large amounts of fat from food causes fat accumulation and weight gain in rats [5]. After 6 weeks, the bodyweight of the HFD group increased by 38.83±2.21g (176.57±21.68%) compared with 10.33±1.78g (45.25±8.54%) in the NFD group ($p<0.001$). The figures for the weight of visceral fat of mice, which differ significantly between the two groups, are the basis for evaluating the success of this study. Another study of obesity in Swiss albino also showed that after 6 weeks body weight increased by 141.60% in the NFD group and 199.02% in the HFD group (57.42% higher in comparison with the NFD group) [2]. In the study of obesity mice model in 16 weeks, the morphological parameters (BMI, weight gain, renal fat, omental fat), leptin level and serum lipid profile were statistically significantly higher than the control group [3]. In another model of obesity in mice by lipid-rich foods, mice had a body mass greater than the NFD group of 13.5g, equivalent to an increase of 42.9% compared to the NFD group at the same time ($p=0.001$) [4]. Similar results on an obesity model in mice showed that there was no difference in the fat percentage of the HFD group and NFD group after 12 weeks. By week 16, the fat percentage of the HFD group was higher than that of the NFD group by 22.27±1.36% and 17.60±0.57%, respectively [5]. Mice were raised by a high-fat diet in a small area, so that the mice were sedentary for 30 days, the weight of mice increased by 40.5% compared to the beginning of the experiment [8]. A similar study in male mice demonstrated that the weight of the HFD group increased 36.91% more than that of the NFD group after 4 weeks of culture ($p<0.05$) [9]. Thus, successful experimental obesity models are based on the weight gain of HFD in comparison with the NFD group (from 36.91% to 57.42%). Meanwhile, the data of our study came up to 131.32% after 6 weeks of modeling and we evaluated the difference in visceral fat of mice in 2 groups at the end of the study (some previous studies did not proceed).

V. CONCLUSIONS

The research has successfully generated an obesity model in male mice (*Swiss albino*) by a high-fat diet (640Kcal/100g, 52-53% lipid) after 6 weeks of feeding. The mean of body weight, weight gain, body length, BMI, chest and waist circumference had a statistically significant difference from the end of week 2. At the end of the study, the figure for the weight gain of the HFD group was 131.32% higher than the NFD group and the weight of visceral fat was statistically higher than that of the NFD group.

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