EFFECTS OF HIGH-FAT DIET CONSUMPTION ON LOCOMOTION, EXPLORATION, AND NOVEL OBJECT RECOGNITION IN RATS

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SUMMARY

Objectives: *To evaluate the effects of high-fat diet (HFD) consumption on locomotion, exploration, and object recognition in rats.* ***Subjects and methods:*** *62 white male rats (8 - 9 weeks old) were caged individually and randomly divided into two groups by the diets of high-fat and standard food (control group) ones for 7 consecutive weeks. Their behaviors were then assessed with a battery of tests for locomotion and exploration in an open-field apparatus, and a test for novel object recognition.* ***Results:*** *HFD fed rats reduced the length and duration of exploration within the open-field in comparison to those in the control group (trajectory length: Control group: 1800.24 ± 438.92 cm; HFD group: 1317.29 ± 566.10 cm; p < 0.001; duration: Control group: 215.25 ± 32.38s; HFD group: 174.35 ± 67.34s; p = 0.004) and lowered the exploratory activity to the open-field's central zone (trajectory length: Control group: 91.09 ± 47.76 cm; HFD group: 64.51± 56.58 cm; p = 0.05; duration: Control group: 8.94 ± 6.67s; HFD group: 7.03 ± 5.74s; p = 0.23). Rats bred with HFD showed a deficit of short-term memory via novel object recognition test (object exploration spent time ratios, expressed as familiar and novel objects consequently: Control group: 41.29 ± 17.65% and 58.71 ± 17.65%, p < 0.001; HFD group: 50.32 ± 16.89% and 49.68 ± 16.89%; p = 0.88).* ***Conclusions:*** *Rats fed with HFD showed less locomotor and exploratory activities, more anxiety-like behavior in the open-field, and a defected short-term memory compared to their peer consumed standard food.*

** Keywords: High-fat diet; Locomotion; Exploration; Object recognition; Rats.*

INTRODUCTION

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health from children to adults [12, 14]. The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended, which is often seen as a combination of an increase in daily food intake rich in sugars and fat and a reduction in daily physical activities [4, 5, 12, 14]. Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2) [14].

Overweight and obesity, or raised BMI may lead to a higher risk for many cardiovascular and respiratory diseases, metabolic disorders, such as triglyceridemia,

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cholesterolemia, type 2 diabetes, and central nervous disfunctions, etc. The incidence of overweight and obesity is rising with an alarming rate in global worldwide, in both developed and developing countries [1, 4, 5, 12, 14].

Many reports from other countries on obesity in human and in animal models showed deficits in locomotor activities [5, 8, 9], learning, memory, and cognitive functions [4, 13, 15]. However, there are still rare studies in Vietnam on behavior changes of locomotor, memory and learning functions in experimental obesity models. In a contribution to obesity research, this study has been performed: *To evaluate effects of high-fat diet consumption on locomotion, exploration, and novel object recognition in rats*.

SUBJECTS AND METHODS

1. Subjects

62 male rats of 8 - 9 weeks old obtained from the Animal Center of Vietnam Military Medical University (VMMU) had been recruited. They were randomly divided into groups of control and obesity model as described previously [1] by their diets, the standard and the HFD ones (supplied by Nutricare Company, Hadong, Hanoi). The control group was fed by the standard diet having fat composition accounting for 15.1% of the total food energy, while the HFD group was given a high-fat diet with fat and cholesterol taking up 38.9% of the total food energy. Behavior assessments from those groups of rats had been conducted after 7 weeks of feeding by two dietary regimes, when they reached the age of 15 - 16 weeks old. Rats were caged individually in comfortable conditions with food and water ad libitum, and a 12/12 light/dark cycle. All experimental processes were in accordance with the current animal care and guidelines of VMMU.

2. Methods and experimental designs

The experimental framework (1,5 × 1,5 × 1,5 m) was isolated by black curtain and equipped with a ceiling-camera that connected to a computer with ANY-Maze 4.99 software installed (Stoelting, USA) for recording and analyzing animal behaviors. A cylindrical open-field (made of composite with diameter 80 cm and height 25 cm, black-painted inside) placed at the center of the framework and used for behavioral assessments of locomotion, exploration (the open-field test), and cognitive function (novel object recognition test).

The open-field maze is one of the most commonly used platforms to measure spontaneous exploration and anxiety-like behaviors in animals as they are exposed to a novel environment for the first time [8, 10]. For running this test (*figure 1A*), an ANY-Maze program is set to delineate the maze's surface into central zone (round shape, 25 cm diameter) and peripheral zone (donut shape, surrounding the central zone with 25 cm inner- and 80 cm outer-diameters). Rats were allowed to explore the maze freely for 5 minutes and its behaviors were recorded and analyzed by ANY-Maze program for parameters of locomotion length (the total trajectory in cm that rodent moved in the test) and locomotion duration (the total time in seconds that rodent moved in the test). Locomotion duration was further

calculated for the total time duration and the time of immobility defined as the rat standed still for at least 2 seconds.

The novel object recognition (NOR) test aimed to evaluate the declarative memory, which is based on the natural tendency of the animals to explore more a new object than a familiar object, in a familiar context [15]. The NOR test was conducted sequentially in three phases in two consecutive days, each phase lasted 5 minutes.

- Habituation phase: Conducted on the first day in the open-field. The animals were allowed to freely explore the environment. This phase was also the open-field test as described above (*figure 1A*).

- The sample phase: Tested on the next day, in which two identical objects in shape, material, and color were added bilaterally inside the open-field and the animals could explore and remember the environment (*figure 1B*).

- The test phase: 6 hours after the sample phase, the test phase was conducted with similar protocol except for a novel object (different shape but the same material, size and color) which had been replaced for one of the two objects in the sample phase. Rats were free to explore and remember the environment, including novel and familiar objects (*figure 1C*).

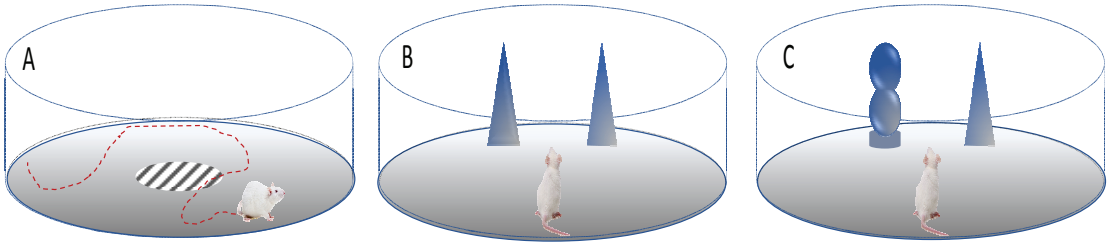

Figure 1: Phases of object recognition test in the open-field for rats.

A. The habituation phase (the open-field exploration test); stripped zone - central zone; dotted line - animal's trajectory.

B. The sample phase with two identical objects (cone - shaped).

C. The test phase with a novel object (snow-man - shaped on the left).

For both sample and test phase, two cone and snowman-shaped objects taken turns were made of white ABS plastic and had the same size (15.5 cm in height; 7.5 cm in base diameter). Rats were identified to have object exploration behavior whenever their head lied in a 5 cm-sized rim around the base of objects. The ability to remember and recognize familiar and novel objects was demonstrated by the ratio of time exploration, defined as the percentage (%) of time exploring the novel object to total time of exploring objects.

3. Data analysis

Research data has been analyzed by statistical algorithms on IBM SPSS Statistics 20 (IBM Inc., USA). Differences

within or between groups were compared by Student's T test followed analyses of data distribution. The significant value was set at $p \leq 0.05$.

RESULTS

** Locomotion and exploration in the open-field:*

Rats were exposed to the environment for the first time and let explore for 5 minutes. Their locomotor and exploratory activities were evaluated via the total and partial traveled length and velocity, as demonstrated in figure 2. The control rats with standard diet had clearly longer total length (*figure 2A-C*) and total locomotive duration (*figure 2D-F*) in the open-field than those of HFD rats (figure 2A for total trajectory length: Control group: 1800.24 ± 438.92 cm; HFD group: 1317.29 ± 566.10 cm; $p < 0.001$; figure 2D for total locomotive duration: Control group: 215.25 ± 32.38s; HFD group: 174.35 ± 67.34s; $p = 0.004$).

Similarly, there was a longer trajectory length for exploring the central zone between two groups of rats (Control group: 91.09 ± 47.76 cm; HFD group: 64.51± 56.58 cm; $p = 0.05$; *figure 2B*), but no remarkable difference in time spent in the central zone between the control and HDF rats (Control group: 8.94 ± 6.67s; HFD group: 7.03 ± 5.74s; $p = 0.23$; *figure 2E*).

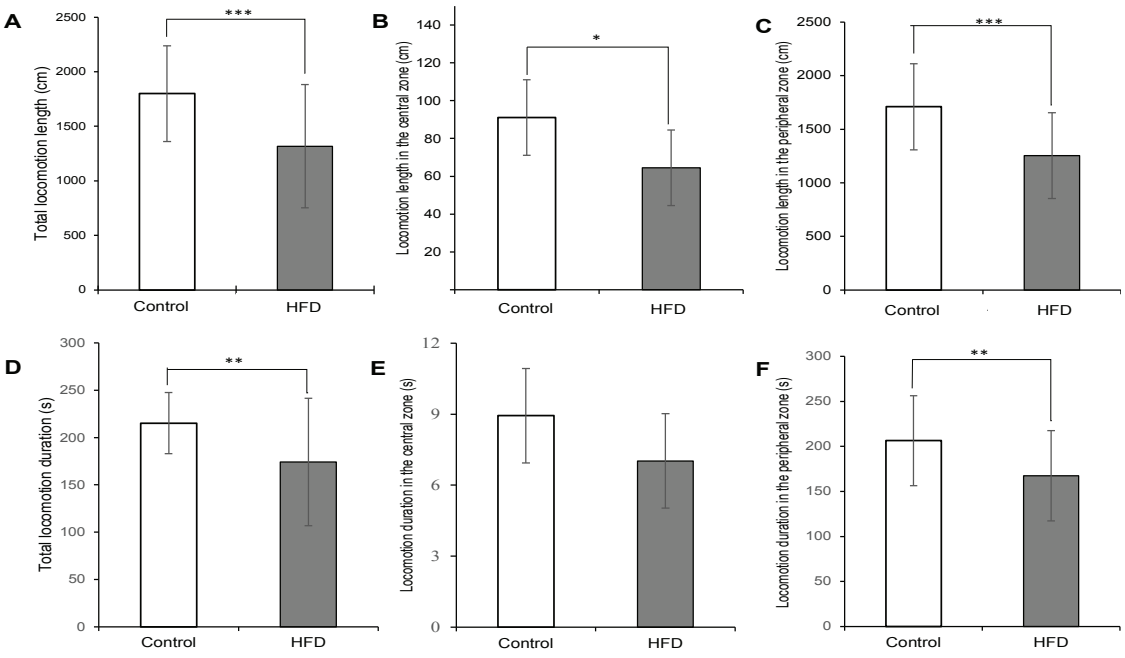

Figure 2: Locomotion and exploration of rats fed with the two diets in the open-field.

A - C: Locomotion length.

D - F: Locomotion duration in total, central and peripheral zones of the open-field.

(, **, *** as p ≤ 0.05; 0.01, and 0.001)*

Locomotion and exploration behaviors in the peripheral zone showed strong differences between the two groups of animals in both trajectory length (Control group: 1709.14 ± 421.32 cm; HFD group: 1252.77 ± 533.06 cm; p < 0.001; *figure 2C*) and trajectory duration (Control group: 206.30 ± 32.09s; HFD group: 167.32 ± 63.80s; p = 0.004; *figure 2F*).

** Object recognition:*

Figure 3 demonstrated the ratios of time that animals explored objects in the open-field through two phases of experiment, sample and test. In the sample phase (*figure 3A*), animals of both diet groups explored two identical objects with similar proportions, no side bias tendency (compared with random exploration rate value of 50%, the discrimination ratios of two objects in control group: 48.16 ± 16.17% and 51.84 ± 16.17%, p = 0.41; in HFD group: 53.75 ± 18.61% and 46.25 ± 18.61%; p = 0.09).

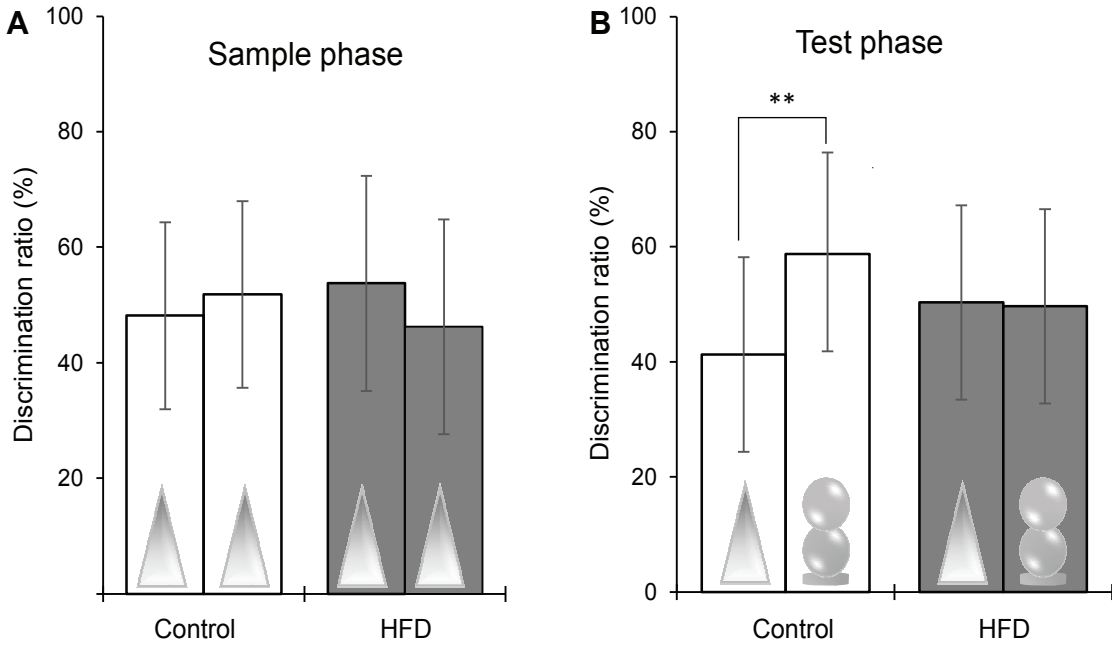

Figure 3: The discrimination ratios of rats fed with the two diets in the open-field in the sample phase (A) and the test phase (B) (***: p = 0.01*).

In the test phase (*figure 3B*), the control-diet fed rats were able to remember and recognize the novel object, as shown by a significantly longer time spent for exploring the novel object than that for the familiarized object. While HFD fed rats explored either novel or familiar objects almost randomly - 50% (discrimination ratios in control diet group: familiar object 41.29 ± 17.65%, and novel object 58.71 ± 17.65%, p < 0.001; in HFD group: familiar object 50.32 ± 16.89%, and novel object 49.68 ± 16.89%; p = 0.88).

DISCUSSIONS

** Locomotion and exploration in the open-field:*

The open field maze is commonly used to measure behaviors in animal models. It is a fast and relatively easy test that provides a variety of behavioral information ranging from general ambulatory ability to data regarding the emotionality of the subject animal [10].

The locomotion and exploring test in the open-field was initially developed to measure emotionality in rodents [10]. The parameters for evaluating locomotion and exploring related to emotions and anxiety-like behaviors in an open-field often include length and duration of trajectory in total or proportions of the open-field.

The results had showed that rats that were overweight/obese by HFD feeding in 7 consecutive weeks decreased locomotion and exploration activity in the open-field (shorter trajectory - Figure 2A, B, C; less time mobile - Figure 2D, F) as compared with standard food fed rats, but there was no difference in anxiety-like behavior when rats exposed to a novel environment, as shown by the similarity in the exploration time in the central zone between the two groups (*figure 2E*).

Lalanza et al [8] compared locomotion activity between rats fed with normal food (control) and those fed with cafeteria food (CAF); and evaluated changes in metabolism and behaviors of locomotor activity, exploration, anxiety-like behavior, and social interaction via appropriate tests. The results showed that CAF induced disorders of fat and carbohydrate metabolism, reduced locomotor activity, reduced anxiety-like behavior, and increased exploration and social interaction [8]. The concordance of motor activity reduction in rodents with high-fat diet was reported by others, as in studies by Arika et al [2] and Schroeder et al [9].

Impaired motor function in animal models of obesity by chronic exposure to the obesogens (HFD, cafeteria food, etc.) [2, 6, 8] has been linked to impairments in dopamine synthesis, release, and receptor function, particularly in the striatum [7]. HFDs potentiate an oxidative attack on brain resident cells resulting in activation of cholinergic motor inhibitory system, alteration of acetylcholinesterase (AchE) activity, and damage to the peripheral muscle. Necrosis of skeletal muscle fibers enhances the reduction of locomotor activity in animal models; or reduction of expression levels of brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor (TrkB) in hypothalamic nuclei affects the strength of synaptic connections or dendritic spine density leading to altered satiety signals and locomotor activity [2, 7].

The cholinergic and dopaminergic pathways are signaling in many higher brain functions as learning ability and emotional, therefore, the anxiety-like exploration in the open-field might also affected. The anxiety-like emotion when an animal is being exposed to a novel environment is indicated by trajectory length and locomotive duration in the central zone, which is often longer than those in the periphery. Moreover, in the open-field arena, this behavioral domain (anxiety) may be mediated by two key factors, namely, agoraphobia and individual

testing [2] as in social - lifestyle species like rodents.

Our rats raised with HFD reduced trajectory length (p = 0.05) in the central zone and the locomotive duration also tended to be shorter (p = 0.23) compared to rats received standard diet. These results are similar to many reports on rodent models evaluating the effects of diet on obesity, such as Arika et al's (2019) [2] and Keleher et al's findings (2018) [6], but differ from Lalanza et al's experiment on CAF fed rats showed female animals reduced anxiety-like behaviour by increasing activity in the open arms of elevated maze [8]. Thus, further studies on emotional exploration behavior are needed.

** Object recognition behavior:*

Animals have a natural tendency to explore and remember novel elements in a familiar environment/context. The object recognition test is designed to evaluate the animal's declarative memory, which is performed through two basic phases: the sample phase (remember phase with two identical objects - the cone shape in *figure 1B* and *figure 3*) and the test phase (recall phase with one familiarized object in the previous phase and a novel object - the snowman shape in *figure 1C*). The ability to recognize and remember familiar-novel object reflects the function of encoding and storing information of the hippocampal formation or cerebral cortices, especially the anterior frontal cortex and periolfactory cortex, which are more related to the object recognition [3]. Our present study showed that both groups of rats had similar discrimination ratios of identical objects in the sample phase (*figure 3A*), but the ability to remember and recall to recognize the novel object in control rats was better than that of HFD rats (*figure 3B*). This finding proved that HFD impaired learning-memory and cognitive ability in rodents.

Zanini et al (2017) applied the object recognition test to assess effects of obesity-inducing diets on exploration and cognition ability using the total time spent exploring and discrimination ratios. Their results indicated that rats raised with HFD had a decrease in object recognition compared with that of control animals. This cognitive impairment could be associated with leptin and insulin resistances [15].

Sims-Robinson et al (2016) investigated the long-term impact of a HFD on hippocampal insulin signaling and memory using three different diet groups of mice: a standard diet (control), a HFD, or a HFD for 16 weeks and then the standard diet for 8 weeks (HFD16). The results indicated that HFD-induced impairments in glucose tolerance and hippocampal insulin signaling occurred concurrently with deficits in both short- and long-term memory. Furthermore, these conditions were improved with dietary intervention. However, in the group of HFD16 insulin receptor expression in the hippocampus improved dietary intervention [11].

CONCLUSIONS

Rats fed with HFD showed less locomotor and exploratory activity, more anxiety-like behavior in the open-field, and a defected short-term memory in comparison to those of their peers fed with standard food.

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