



Inhibition of glucocorticoid synthesis alleviates cognitive impairment in high-fat diet-induced obese mice

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ABSTRACT

In addition to cardiovascular diseases, metabolic syndrome and type 2 diabetes mellitus, obesity is associated with cognitive deficits. In rodents, it has been shown that long-term high-fat diet (HFD) consumption leads to the alteration of hypothalamic-pituitary-adrenal (HPA) axis resulting in increased corticosterone release. However, mechanisms underpinning cognitive impairments induced by long-term HFD intake are unclear. Herein we evaluated the effects of systemic administration of glucocorticoid synthesis inhibitor metyrapone on cognitive performance assessed by novel object recognition test and plasma corticosterone levels evaluated by enzyme-linked immunosorbent assay in HFD-induced obese mice. We found that metyrapone treatment alleviated recognition memory impairments in HFD-induced obese mice. Furthermore, glucocorticoid synthesis inhibitor also lowered plasma corticosterone levels in HFD-induced obese mice. Our findings indicate that hyperactivation of HPA axis resulting in elevated circulating glucocorticoid levels leads to memory impairments in HFD-induced obese mice. We identify glucocorticoid system as a potential therapeutic target for treating cognitive deficits associated with obesity condition.

1. Introduction

Obesity has doubled worldwide in the last thirty years, becoming pandemic. The major causes of obesity are an imbalanced caloric intake with consumption of energy-dense food such as a high-fat diet (HFD), and a reduced physical activity. In addition to cardiovascular diseases, metabolic syndrome and type 2 diabetes mellitus, obesity is associated with cognitive deficits. Clinical and experimental studies indicate that learning and memory functions depending on the integrity of the hippocampus are particularly compromised in obese subjects [1]. However, mechanisms underlying cognitive impairments induced by obesity remain unclear.

Previous studies indicate that obesity is associated with the alteration of hypothalamic-pituitary-adrenal (HPA) axis [2] resulting in an increase of cortisol release in response to stress compared to non-obese subject. In rodents, db/db mice, a genetic mouse model of obesity, as well as HFD-induced obese mice, exhibited an elevation in plasma corticosterone at basal levels [3]. Moreover, it has been demonstrated that metyrapone improves memory deficits in a genetic mouse model of obesity and diabetes [3,4] and glucocorticoid receptor antagonist

rescues memory deficits in HFD-fed rats [5,6]. However, the impact of inhibition of glucocorticoid synthesis on cognitive deficits in obese mice induced by long-term HFD intake has not been investigated.

In the present study, we aimed to determine the effects of systemic administration of glucocorticoid synthesis inhibitor metyrapone on cognitive function evaluated by novel object recognition test in HFD-induced obese mice. We also investigated the effects of systemic administration of metyrapone on plasma corticosterone levels in obese mice induced by long-term HFD intake.

2. Material and method

2.1. Animal and diet

All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Burapha University (Permit Number: 42/2561). Thirty male C57BL/6 N mice obtained from Nomura Siam International, Thailand, aged three weeks old at arrival were housed in group of five per cage in a climatized room (24 ± 1 °C) and were maintained under a 12 h light/dark cycle. After one week of

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acclimatization, all animals were fed either a control diet (CD; $n = 10$) offering a total calorie of 3.04 kcal/g (containing 4.5% crude fat, 24% crude protein) (the National Animal Center, Salaya Campus, Mahidol University, Bangkok, Thailand) or a high-fat diet (HFD; $n = 20$) supplemented with 25% sucrose (containing 14.4% crude fat, 24.3% crude protein) (Quick fat; CLEA, Japan) offering a total calorie of 4.11 kcal/g for twenty-six weeks. After twenty-four weeks of feeding, half of mice receiving HFD were treated with metyrapone (HFD + Met) for one week (100 mg/kg, i.p., Sigma-Aldrich) every day between 9 and 11 a.m. This dose has been demonstrated to lower corticosterone levels, as described by previous studies [4]. Another half of HFD-fed mice and all CD-fed mice were injected with vehicle (saline with 20% polyethylene glycol). The drug and vehicle were injected in a volume of 10 ml/kg. During the drug treatment, all animals were continued on their diet. Body weight was monitored weekly across the experiment.

2.2. Novel object recognition test

The experimental apparatus used for the object recognition test was an open-field box (40 cm \times 40 cm) with 25 cm high walls, placed in the center of experimental room (light intensity of 15 Lux). The objects to be discriminated were Lego® and flask. The test consisted of three phases: habituation phase, training phase and test phase, previously described with some modifications [7]. During the habituation phase, mice were exposed to the open field in the absence of objects for 5 min. Mice were then returned to their home cage. On the following day (training phase), mice were placed in the same open field with two identical objects and were allowed to freely explore the environment and objects for 10 min 24 h after (testing phase), mice were placed back in the open field and at this time, mice were allowed to explore a novel object and a familiar object for 10 min. A recognition index was calculated as time spent exploring the novel object divided by total time spent exploring both objects \times 100.

2.3. Measurement of plasma corticosterone

Blood was collected into microtube containing 10% EDTA by cardiac puncture in the morning (between 9 and 12 a.m.) at baseline under isoflurane as anesthetic agent. Samples were centrifuged at 5000 g, 4 °C for 10 min, and the supernatant (plasma) was stored at -20 °C. Quantification of plasma corticosterone enzyme-link immunosorbent assay

(ELISA) was carried out by using a commercially available kit (ABclonal Technology, Wuhan, China) according to the manufacturer's instructions. Plasma samples were diluted in the sample diluent provided with a kit at 1:1.

2.4. Data analysis

All data are expressed as means \pm SEM and were analyzed using one-way ANOVA with post hoc Tukey's multiple comparison test or Two-way repeated measures ANOVA when appropriate. Analyses were performed in GraphPad Prism version 5.0 and $p < 0.05$ was considered statistically significant.

3. Results

We first evaluated the effect of chronic HFD consumption on body weight in these mice. An analysis of diet exposure for 24 weeks found significant effect of diet ($F_{(1,28)} = 26.11, p < 0.001$), time ($F_{(23,644)} = 396.00, p < 0.001$) and an interaction between diet and time ($F_{(23,644)} = 24.27, p < 0.001$). Post hoc analyses indicated that HFD-fed mice were significantly heavier than CD-fed mice since the 9th week under the diet ($p < 0.05$) (Fig. 1A). After one week of metyrapone treatment, one-way ANOVA followed by post hoc analysis indicated that both saline-treated and metyrapone-treated HFD-fed mice were significantly heavier than saline-treated CD-fed mice ($F_{(2,27)} = 16.90, p < 0.0001$) (Fig. 1B). However, the body weight of metyrapone-treated HFD-fed mice did not differ from that of saline-treated HFD-fed mice ($p > 0.05$) (Fig. 1B) suggesting that one week of metyrapone treatment did not alter the body weight of HFD-fed mice.

We next evaluated the effect of chronic HFD consumption and glucocorticoid synthesis inhibitor metyrapone treatment on cognition. One-way ANOVA for the percentage of recognition index revealed a significance of group effect ($F_{(2,25)} = 5.58, p < 0.01$). Post hoc analysis indicated that saline-treated HFD-fed mice showed a decrease in recognition index compared to that of saline-treated CD-fed mice ($p < 0.05$) indicating the alteration of recognition memory in HFD-fed mice. HFD-fed mice treated with glucocorticoid synthesis inhibitor exhibited greater recognition index when compared to that of saline-treated HFD-fed mice ($p < 0.05$) (Fig. 1C). However, the percentage of recognition index during training did not differ between groups ($F_{(2,25)} = 0.47, p > 0.05$) (Supplementary Fig. 1). The total time of objects exploration

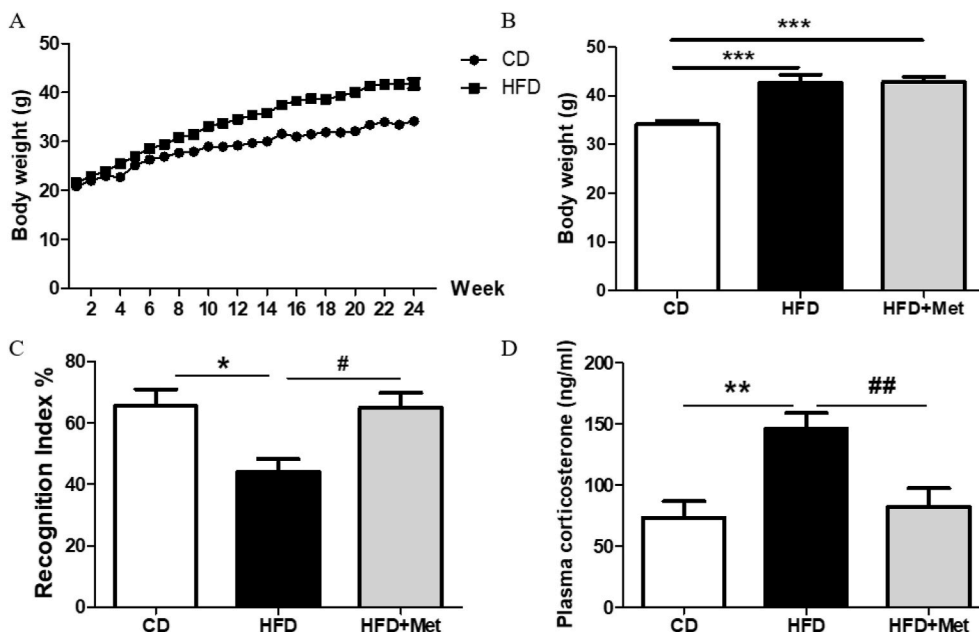


Fig. 1. Effects of systemic glucocorticoid synthesis inhibitor administration on recognition memory and plasma corticosterone levels in HFD-induced obese mice. (A) the body weight of CD-fed mice (CD) and HFD-fed mice (HFD). (B) Effect of systemic glucocorticoid synthesis inhibitor on the body weight in HFD-fed mice. (C) Systemic glucocorticoid synthesis inhibitor alleviated recognition memory in HFD-fed mice. (D) Systemic glucocorticoid synthesis inhibitor lowered plasma corticosterone levels in HFD-fed mice. Saline-treated CD-fed mice (CD), saline-treated HFD-fed mice (HFD), metyrapone-treated HFD-fed mice (HFD + Met), ($n = 8-10$, per group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to CD-fed mice, # $p < 0.05$, ## $p < 0.01$ compared to saline-treated HFD-fed mice.

during training ($F_{(2,25)} = 0.06, p > 0.05$) (Supplementary Table 1) and testing ($F_{(2,25)} = 1.05, p > 0.05$) (Supplementary Table 2) did not differ between groups. This result suggests that one week of glucocorticoid synthesis inhibitor metyrapone treatment rescues HFD-induced cognitive impairments.

We further investigated the effects of long-term HFD intake and metyrapone treatment on plasma corticosterone levels. One-way ANOVA for plasma corticosterone levels revealed a significance of group effect ($F_{(2,27)} = 8.19, p < 0.01$). *Post hoc* analysis revealed that saline-treated HFD-fed mice had a higher level of plasma corticosterone compared to that of saline-treated CD-fed mice ($p < 0.01$). Interestingly, systemic treatment of glucocorticoid synthesis inhibitor metyrapone significantly decreased plasma corticosterone levels in HFD-fed mice ($P < 0.01$) (Fig. 1D).

4. Discussion

This study aimed to investigate the effects of glucocorticoid synthesis inhibitor metyrapone on cognitive function in obese mice induced by high-fat diet (HFD) consumption. Herein, we show that systemic administration of glucocorticoid synthesis inhibitor alleviates cognitive impairment associated with a reduction in plasma corticosterone levels in HFD-induced obese mice. We demonstrate that HFD-induced obese mice showed the alteration in object recognition memory which depends on levels of plasma glucocorticoid. Our findings were consistent with the previous studies reporting HFD-induced memory impairments associated with elevated levels of plasma corticosterone [8]. In the present study, we exposed animals to HFD after weaning for a long period of time (26 weeks of diet exposure) and it seems that 26 weeks of postweaning HFD exposure present a stronger effect on baseline corticosterone levels at the nadir than previous studies using shorter postweaning HFD exposure (1–12 weeks) [5,6,9]. In addition, we found that metyrapone improved memory deficits associated with a reduction in plasma corticosterone levels in a model of diet-induced obesity. Our results were similar to the studies demonstrated previously in a genetic mouse model of obesity and diabetes (db/db mice) [3,4] suggesting that elevated circulating glucocorticoid levels leads to memory impairments in HFD-induced obese mice.

Glucocorticoids exert numerous biological effects on the brain by modulating neurotransmission, metabolism, neuronal structure, maturation and survival of neurons. Although the acute increase in glucocorticoids is adaptive, chronically elevated levels of glucocorticoid are deleterious to the brain [10]. The hippocampus has a pivotal role in the long-term memories formation, highly expresses receptors for glucocorticoids in both rodents and humans [11], and is particularly sensitive to the chronic excess of glucocorticoids. Recently, it has been shown that twenty weeks of HFD exposure in mice lead to an increase in corticosterone levels in the hippocampus [12]. So, further study is needed to evaluate whether systemic metyrapone treatment could also normalize the hippocampal corticosterone levels in HFD-induced obese mice.

Glucocorticoid concentrations in the tissues including the brain are determined not only by plasma glucocorticoid levels, but also by intracellular 11- β hydroxysteroid dehydrogenases (11 β -HSDs), which interconvert active glucocorticoids and inert 11-keto forms within specific target cells. The type 2 isozyme (11 β -HSD2) is a potent NAD-dependent dehydrogenase that catalyzes the rapid inactivation of glucocorticoids whereas the type 1 isozyme (11 β -HSD1) acts in the reverse (reductase) direction in intact cells, thus regenerating active glucocorticoids. Previous studies indicated that hippocampal 11 β -HSD1 has been involved in spatial memory deficits in aged mice [13] and it also implicated in cognitive impairments in diabetic rats [14]. Thus, Evaluating the potential effects of 11 β -HSD1 inhibition in restoring cognitive deficits of HFD-induced obese mice is an interesting perspective.

In the present study, we used only males to evaluate the effects of metyrapone on cognitive function in HFD-induced obesity and this could be considered as a limitation when sex was taken into account as a

biological variable. Sex differences in the effects of HFD-induced obesity on cognitive deficits are still unclear. However, it has been shown that chronic HFD exposure after weaning impaired memory performance only in male mice indicating males were more vulnerable than females to the effect of juvenile HFD on cognitive function [15].

In conclusion, systemic treatment of glucocorticoid synthesis inhibitor metyrapone could prevent cognitive deficits in HFD-induced obese mice. The results obtained from this work will facilitate the development of new pharmacological interventions for treating cognitive impairments associated with obesity condition.

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Credit author statement

Yootana Janthakhin: Conceptualization, Investigation, Methodology, Project administration, Formal analysis, Writing original draft, Review & editing. **Sutin Kingtong:** Investigation, Review & editing. **Sirikran Juntapremjit:** Investigation, Project administration, Review & editing.

Declaration of competing interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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