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# Bitter melon seed oil may reduce the adiposity through the hypothalamus mTOR signaling in mice fed a high fat diet

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ABSTRACT

Bitter melon seed oil (BMSO) was found to have an advantageous effect on anti-obesity. Up to date, the mechanisms underlying this process have been extensively investigated. However, there are very few reports focusing on the roles of central nervous system (CNS) involved. In this study, Golgi-Cox staining and western blotting assays were used to examine the hypothalamic spine density and the expression levels of NMDA-2B receptor and P-S6 protein, respectively. A significant reduction concerning hypothalamic spine density was observed in HFD mice, a phenomenon that could be partially rescued by the BMSO administration. Furthermore, it was found that BMSO could also reverse the changes upon the phosphorylation levels of S6, a typical protein involved in mTOR signaling pathway, indicating that mTOR signaling potentially participated in this metabolism regulation. Besides, NMDA-2B levels were up-regulated in HFD mice, which could not be considerably influenced by BMSO. In summary, this study first proposed aberrant hypothalamic plasticity as CNS's roles in BMSO's fat-reducing effects, favoring the better recognition and treatment of the intractable hypothalamic obesity.



## 1. Introduction

Obesity, a complex metabolic disorder, has become one of the main health and public issues in both developed and developing countries [1–3]. In the US, over one-third adults and 16.7% children were obese in 2009–2010, which costs up to 200 billion US dollars annually [4]. And in China, there were about 200 million people who were affected by obesity in 2007 [5]. Therefore, it's urgent to find some alternative natural substances to address this serious issue.

Recently, we have revealed that the supplementation of bitter melon seed oil (BMSO) could efficiently reduce the body weight of the tested mice (unpublished data), which is consistent with the results from other studies [6–8]. Given that, a series of studies were carried out to elaborate the mechanisms underlying this beneficial effect. Currently, several mechanisms have been proposed: increased fatty acid transport, mitochondrial uncoupling, prevention of adipocyte hypertrophy, adipose tissues' apoptosis, etc [8,9]. However, these propositions were primarily associated with the changes of periphery tissues, with CNS's roles during the process largely ignored.

Hypothalamus is a portion of brain mainly responsible for the regulation of energy homeostasis [10]. Since obesity was tightly correlated with the aberrant energy metabolism, hypothalamus should be regarded as a competent candidate for investigating the obesity-led Central Nervous System (CNS) damages. Actually, in contrast with pure obesity, the hypothalamic obesity was a type of more intractable disease, due to the dysfunction of this regulation center [11]. A couple of recent studies showed that obesity could accompany the entry of immune cells into CNS, leading to the inflammation and activation of glia cells [10,12]. Nonetheless, whether hypothalamic neurons, as well as their physiological behaviors, were involved in the BMSO's advantageous effect, remains unknown.

The purpose of this study is to explore neurobiological mechanisms of the anti-obesity effect of BMSO. This is the first paper, to our knowledge, to present the bitter melon seed oil on the obesity associated with neuronal regulation, shedding new light on the interpretation and treatment of hypothalamic obesity.

## 2. Methods

### 2.1. Preparation of BMSO

Bitter melon seeds (purchased from Chengxin Herb Corporation,

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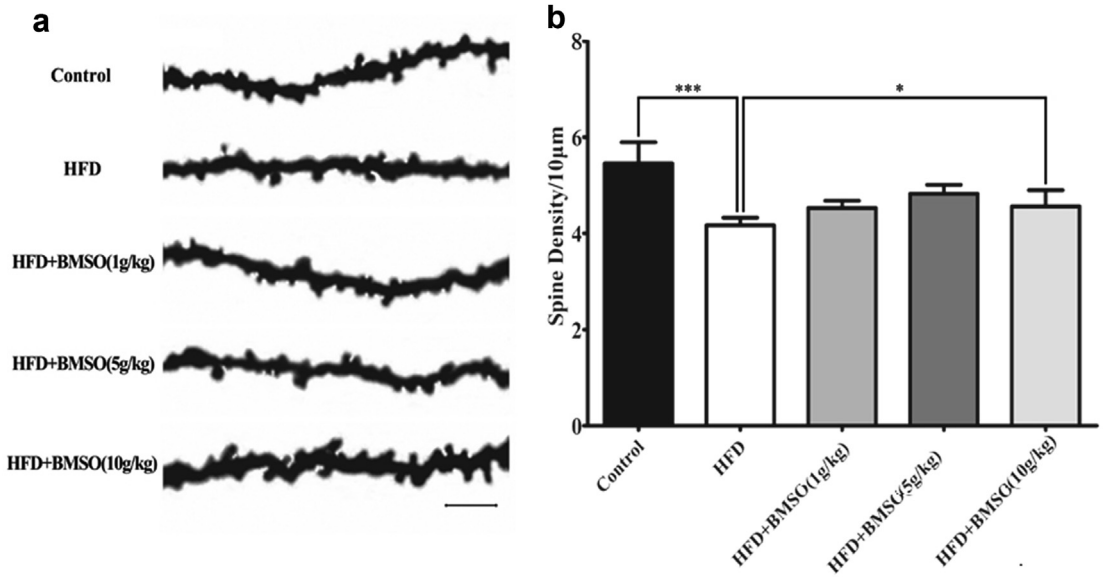


**Fig. 1.** Dendritic spine alteration of hypothalamus neurons after BMSO treatment on fat diet-induced obesity mice. (A) Visualization of spines on dendrites in mice of five groups. The scale bar represents 10 μm. (B) Quantification by image analysis of spine density. Data was expressed as mean ± SEM. $P < 0.05$ was considered as statistical difference ($n = 8$ in each group *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).

Hebei province, China) were collected from ripe bitter melons only, with similar size, color, and no physical damage. The seeds were oven-dried on a stainless steel tray at 40 °C in a dehydrator for 3 h, got the shell off and ground using a sample grinder. Then the seeds were filtered to obtain a uniform particle size. Seed oil was extracted by supercritical $CO_2$. The extraction were carried out under the pressure of 25 MPa, with the temperature of 50 °C for 100 min. The extracted BMSO was stored separately in −20 °C.

### 2.2. Animals and diets

The four-week-old C57BL/6J male mice were obtained from the Laboratory Animal Center, Anhui Medical University, P.R. China, and raised according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by institutional animal care and use committee of Hefei University of Technology, China. Mice were randomly divided into two groups: one group raised with High-Fat Diet (HFD; lard oil 10%, egg yolk power 10%, butter 10%, peanut butter 10%; $n = 50$) and the other with chow diet (control; $n = 7$). Food intake and body weight were measured weekly. When the average weight of HFD group was 20% higher than that of control group, it was then regarded as the successful obesity model [13]. Animals were treated by BMSO for three weeks, and they were deeply anesthetized with $CO_2$ followed by cervical dislocation.

HFD group were divided into four sub-groups randomly, which were HFD group ($n = 8$), HFD+BMSO (1 g/kg; $n = 9$), HFD+BMSO (5 g/kg; $n = 9$), and HFD+BMSO (10 g/kg; $n = 9$). kg refers to body weight of the mice. BMSO treatment was applied by intragastric administration while control and HFD groups were treated with

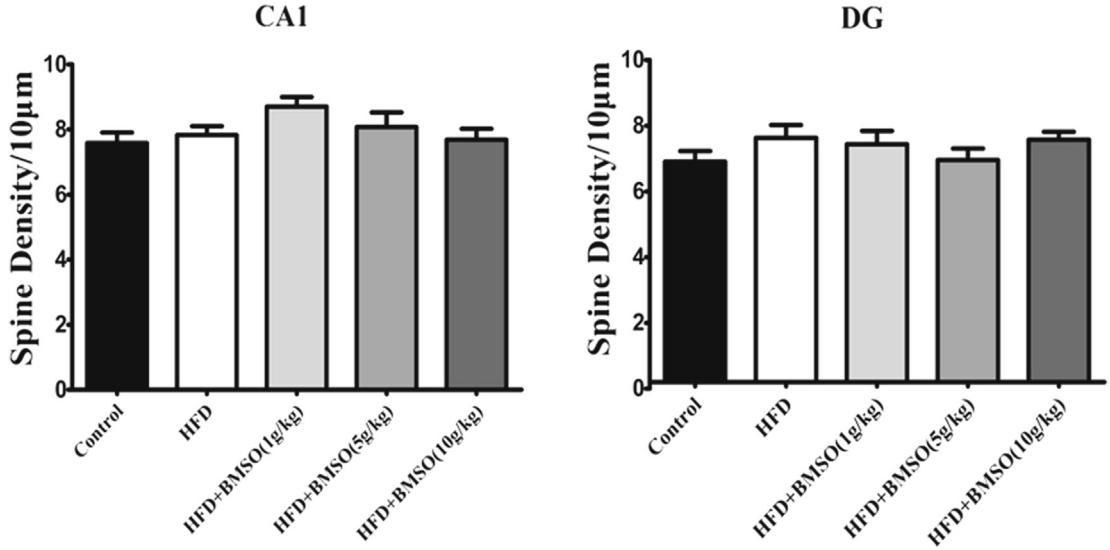


**Fig. 2.** Dendritic spine alteration of hippocampal neurons after BMSO treatment on fat diet-induced obesity mice. Quantification by image analysis of spine density of CA1 and DG area. Data was expressed as mean ± SEM. $P < 0.05$ was considered as statistical difference ($n = 8$ in each group *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$).