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Mitochondrial dysfunction and oxidative damage in the brain of diet-induced obese rats but not in diet-resistant rats

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ABSTRACT

Aims: It has been suggested that obesity triggered by consuming a high-fat diet (HF) can account for oxidative damage and mitochondrial dysfunction. Thus, we aim to explore the oxidative stress and mitochondrial dysfunction detected in the brain of diet-induced obese (DIO) rats.
Main methods: Sprague–Dawley (SD) rats were fed either a HF diet or a normal-fat (NF) diet for 10 weeks to obtain the control (CON), DIO and diet-resistant (DR) rats. D-Galactose was injected subcutaneously for 10 weeks to establish oxidative stress model (MOD) rats. Then, the levels of total antioxidant capacity (T-AOC), lipid peroxidation (LPO), malondialdehyde (MDA), both in plasma and brain tissue, and catalase (CAT) in plasma were measured using enzymic assay kits and the levels of ghrelin, neuropeptide Y (NPY) and leptin in both plasma and brain tissue were measured by using enzyme-linked immunosorbent assay (ELISA) kits. Mitochondrial reactive oxygen species (ROS) formation in brain tissues was detected with 2, 7-dichlorofluorescein diacetate (DCFH2-DA) dyeing. The mitochondrial membrane potential (MMP) was measured with tetrachloro-tetraethyl benzimidazol carbocyanine iodide (JC-1) by a flow cytometer.
Key findings: HF diet leads to an obese or DR state characterized by increased or decreased adiposity. The HF diet increased brain LPO, which was accompanied by lower ghrelin levels in DIO rats compared with DR rats. In addition, the increased mitochondrial ROS and lower MMP were detected in DIO rat comparing with DR rats.
Significance: The current results demonstrated that mitochondrial dysfunction and oxidative damage in the brains of DIO rats, induced by HF diets, might be measurable.

Introduction

Obesity has emerged as a public health problem associated with a number of diseases. Increased intake of high energy-density foods, such as those seen in high-fat (HF) diets, is the main reason for obesity. The availability of useful animal models, such as diet-induced obese (DIO) and diet-resistant (DR) rats, is crucial in the search for novel compounds for the treatment of obesity. Levin (Levin et al., 1983) first found that when Sprague–Dawley rats were fed a HF diet, some rats developed obesity while others remained lean. This phenomenon has also been confirmed in our laboratory (Li et al., 2011; Zhao et al., 2008).

In recent years, increasing attention has been paid on the relationship between obesity and brain diseases (Shefer et al., 2013), such as Alzheimer's disease (AD). AD is characterized by increased beta-amyloid deposition and neuronal dysfunction (Ding et al., 2013). HF diets were reported to increase brain expression of beta-amyloid associated with memory impairment (Thirumangalakudi et al., 2008; Knight et al., 2014; Pistell et al., 2010). Epidemiological studies also showed that people with higher body mass index (BMI) are at greater risk for developing AD than the subjects with normal BMI (Fitzpatrick et al., 2009; Whitmer et al., 2007). The linkage between obesity and cognitive impairment is also strongly supported by epidemiological cross-sectional and prospective studies (Yaffe et al., 2004). However, the neurobiological damage caused by obesity is poorly understood.

It has been reported that obesity is associated with lower brain volumes in normal subjects (Ho pril et al., 2010), suggesting that the brain is involved in the pathogenesis of obesity. Obesity has been associated with structural abnormalities in the brain (Smucny et al., 2012). Animal studies also showed that high-energy diet can damage hippocampal structure and function (Davidson et al., 2009, 2012; Kanoski et al., 2010).

Recent studies indicate that various mechanisms linking obesity to cognitive dysfunction have been postulated, including oxidative stress. A HF diet is correlated with increased oxidative stress. Brain function is sensitive to oxidative pathways, the expression of which is enhanced in the obese state (Rege et al., 2013). Obesity linked to mitochondrial

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dysfunction in neurons can cause impairment in central glucose sensing (Parton et al., 2007), which indicates that the mitochondria may play an important role in obesity.

DIO mice differed from DR mice in a number of neurotransmitter systems, including decreased mRNA expression and increased NPY mRNA expression in the hypothalamic nucleus (Huang et al., 2003). However, the differences between the brain of DIO and DR rats have not been studied.

Therefore, the aim of the study is to explore the oxidative stress and mitochondrial dysfunction in the brain of DIO and DR rats. The study will provide a base to uncover the relationship between obesity and brain oxidative damage by comparing the difference between DIO and DR rats.

Materials and methods

Animals and diets

All experimental procedures were approved by the Animal Ethics Committee of Capital Medical University and conducted in compliance with the animal-use guidelines.

Male 6-week-old Sprague–Dawley rats (n = 50; body weight 140–160 g; SPF degree) were purchased from Academy of Military Medical Sciences (Beijing, China). All of the rats were housed in plastic boxes individually at 20–23 °C with available food and water. A 12:12 h light dark cycle with lights on at 8:00 am was maintained. Rats were fed with standard laboratory chow for the first week to adapt to the new environment. In the following experimental period, rats were given either a NF diet (345.3 kcal/100 g) or a HF diet (435.96 kcal/100 g) (Table 1). The HF and NF diet formulations (SPF degree), made from semi-synthetic materials, were purchased form Academy of Military Medical Sciences (Beijing, China).

Experimental protocol

The experimental schedule and study groups are shown in Fig. 1. After the acclimation period, 10 rats were randomly assigned to receive a NF diet according to their body weights, and D-galactose (120 mg/kg·day) was injected subcutaneously through the back of the neck for 10 weeks to establish model (MOD) rats. Another 40 rats were placed on a HF diet for 2 weeks. Then, the 10 intermediate weight gainers were switched back to a NF diet and were designated as controls (CON). Another 30 rats were fed a HF diet continually, and 8 weeks later the upper tertile (n = 10) in body weight gained was designated as DIO, and the lower tertile (n = 10) in body weight gained was designated as diet-resistant (DR). Those in the middle tertile of body weight gained were removed from the experiment. The rats were anesthetized and the blood was collected from the heart. The brain, heart, liver, spleen, perirenal fat, epididymal fat and omental fat were all removed and weighed.

Preparation of plasma samples

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg per body weight), and blood samples were collected from the heart and immediately transferred into chilled polypropylene tubes for plasma preparation. These tubes were gently rocked several times immediately after blood collection to obtain an even mixture and to prevent coagulation. Blood samples were centrifuged at 3500 *g* for 15 min at 4 °C. Plasma was collected and stored at −80°C until the assay.

Table 1
Composition of the normal fat and high fat diets.[a]

Diet component	Energy (NF diet, %)	Energy (HF diet, %)
Total crude protein	24	16
Crude fat	10.7	40.5
Crude carbohydrate	65.3	43.5

The HF diet is composed of 84 g NF diet and 16 g lard oil (added with 0.2 g cholesterol). NF, normal fat; HF, high fat.

[a] In the diets, all micronutrients, proteins and fiber were balanced by energy according to the Chinese standard.

Isolation of mitochondria of brain tissue

Mitochondrial/cytoplasmatic protein extraction kits (Calbiochem, America) were used to isolate the mitochondria. Brain tissues were washed with ice-cold PBS and added to a 1 ml cytosol extraction buffer mix and incubated on ice for 10 min. We homogenized the tissues using an ice-cold Dounce tissue homogenizer. The tissues were maintained on ice during the homogenization procedure. In general, 30–50 passes with the grinder were used, as recommended. The homogenate was transferred to a 1.5 ml microcentrifuge tube and centrifuged at 700 *g* for 10 min at 4 °C. The supernatant was then transferred to a 1.5 ml microcentrifuge tube and centrifuged at 10,000 *g* for 30 min at 4 °C. The supernatant was finally transferred to a clean tube, and what remains is the cytosolic fraction and the precipitates are the mitochondria of brain tissues.

Measurement of T-AOC, LPO, MDA and CAT in plasma

The T-AOC, LPO, MDA and CAT level in plasma were measured using assay kits purchased from Nanjing Jiancheng Biotechnology Company (China). The activity of T-AOC, the LPO concentration, the MDA concentration and the CAT activity were calculated.

Measurement of ghrelin, NPY and leptin in plasma and brain tissue

Since all the rats were fasting for 12 h, the ghrelin, NPY and leptin level in plasma and brain tissue were measured using assay kits purchased from Ray Biotechnology Company (America). Sigma Plot software, which can perform four-parameter logistic regression models, was used to calculate the concentration of ghrelin, NPY and leptin.

Measurement of mitochondrial ROS in brain tissues

Mitochondrial reactive oxygen species (ROS) formation was detected with 2, 7-dichlorofluorescein diacetate (DCFH2-DA), a fluorescent probe, according to the instruction of ROS assay kit (Beyotime Institute of Biotechnology, China) with a slight change. The isolated mitochondria were incubated with 10 μM DCFH2-DA dissolved in none-serum DMEM at 37 °C for 20 min. The fluorescence was then measured at 488 nm excitation and 525 nm emission by a Cary Eclipse fluorescence spectrometer (Agilent Technologies, USA).

Measurement of mitochondrial membrane potential

For assessment of changes in mitochondrial membrane potential (MMP), the brain mitochondria were incubated for 20 min with JC-1 (BD Bioscience, USA) at 37 °C. The fluorescence signals of cells were excited at 488 nm and emission was monitored at 525 nm wavelength in a flow cytometer, corresponding to the fluorescence peak of the monomer and that of the aggregate. The value of fluorescence intensity was analyzed by the FCS Express Version 3.0 software (De Novo Software, Canada).

Statistical analysis

Data are presented as means and their standard errors. All statistical analyses were performed using SPSS 13.0. All data were analyzed by a one-way ANOVA, followed by LSD test and Dunnett T3 test.