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Food restriction attenuates oxidative stress in brown adipose tissue of striped hamsters acclimated to a warm temperature

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ABSTRACT

It has been suggested that the up-regulation of uncoupling proteins (UCPs) decreases reactive oxygen species (ROS) production, in which case there should be a negative relationship between UCPs expression and ROS levels. In this study, the effects of temperature and food restriction on ROS levels and metabolic rate, UCP1 mRNA expression and antioxidant levels were examined in the brown adipose tissue (BAT) of the striped hamsters (*Cricetulus barabensis*). The metabolic rate and food intake of hamsters which had been restricted to 80% of ad libitum food intake, and acclimated to a warm temperature (30 °C), decreased significantly compared to a control group. Hydrogen peroxide (H_2O_2) levels were 42.9% lower in food restricted hamsters than in the control. Malonadialdehyde (MDA) levels of hamsters acclimated to 30 °C that were fed ad libitum were significantly higher than those of the control group, but 60.1% lower than hamsters that had been acclimated to the same temperature but subject to food restriction. There were significantly positive correlations between H_2O_2 and, MDA levels, catalase activity, and total antioxidant capacity. Cytochrome c oxidase activity and UCP1 mRNA expression significantly decreased in food restricted hamsters compared to the control. These results suggest that warmer temperatures increase oxidative stress in BAT by causing the down-regulation of UCP_1 expression and decreased antioxidant activity, but food restriction may attenuate the effects.

1. Introduction

Reactive oxygen species (ROS) are produced continuously as a by-product of aerobic metabolism (Miwa and Brand, 2003). Oxidative stress occurs when the production of ROS exceeds the capacity of antioxidant protection and repair mechanisms to neutralize them, resulting in oxidative damage to molecules such as lipids, proteins and DNA (Selman et al., 2002; Speakman and Garratt, 2014). For example, superoxide produced as a result of the one-electron reduction system within mitochondria can be converted into H_2O_2 and then into highly reactive hydroxyl radicals, resulting in lipid peroxidation (Miwa and Brand, 2003).

The "rate of living – free-radical" hypothesis proposes that risk of oxidative stress increases during periods of elevated metabolic rate, and therefore predicts a positive relationship between metabolic rate and ROS production (Harman, 1956; Selman et al., 2002; Speakman et al., 2002). In contrast, the "uncoupling to survive hypothesis" postulates that ROS levels are strongly exacerbated by increased protonmotive force in the inner mitochondria membrane (Toimea and Brand, 2010), and that the futile cycle of proton pumping and proton leakage is used by mitochondria to attenuate ROS production, in which case there should be a negative relationship between metabolic rate and ROS production (Korshunov et al., 1997; Brand, 2000; Toimea and Brand, 2010; Liu et al., 2009). So far, the results from relevant studies on endotherms have been contradictory (Pearl, 1928; Beckman and Ames, 1998; Brand, 2000; Selman et al., 2000, 2002, 2008, 2012; Speakman et al., 2002, 2004; Venditti et al., 2004; Brzęk et al., 2014; Chen et al., 2014; Duarte and Speakman, 2014).

Brown adipose tissue (BAT) is the main site of metabolic thermogenesis in small mammals, where 60% of total oxygen consumption occurs during exposure to cold temperatures (Foster and Frydman, 1979; Selman et al., 2002; Klingenspor, 2003). Uncoupling protein 1 (UCP1) is expressed exclusively in BAT and comprise up to 8% of the total mitochondrial protein of this tissue. UCP1 generates a proton leak in the mitochondrial inner membrane, dissipating proton motive force as heat (Nicholls and Locke, 1984; Brand, 2000; Oelkrug et al., 2010; Rousset et al., 2004). Metabolic activity in BAT, as indicated by UCP1 mRNA and protein levels is inversely correlated with temperature, decreasing as the temperature rises (Nedergaard et al., 2001; Zhang and Wang, 2006). It has been observed that oxidative stress in BAT, indicated

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by higher protein carbonyl levels and lower proteasome activity than in other tissues, increases in cold-exposed animals (Barja de Quiroga et al., 1991; Barja de Quiroga, 1992; Selman et al., 2002), a result that is contrary to that expected under the "uncoupling to survive hypothesis" (Selman et al., 2000, 2002, 2008; Venditti et al., 2004; Yang et al., 2013; Chen et al., 2014; Xu et al., 2014).

Organisms possess a variety of defense mechanisms to protect them against oxidative stress and maintain a correct oxidant-antioxidant balance (Yu, 1994; Halliwell and Gutteridge, 1999; Garratt et al., 2011). Any increases in ROS production, or weakening in these defense can ultimately lead to oxidative stress and cellular damage (Selman et al., 2000). A significant increase in BAT catalase (CAT), an important antioxidant, has been observed in cold-exposed rats (*Rattus norvegicus*), suggesting that this enzyme plays a role in protecting against oxidative stress (Davidovi,ć et al., 1999; Siems et al., 1999; Selman et al., 2000). In the short-tailed field vole (*Microtus agrestis*) exposure to cold caused significant increases in both metabolic rate and antioxidant activity in BAT, which could protect this tissue from accumulated oxidative damage (Selman et al., 2002). This protection may be also a very important factor in sustaining uncoupled respiration (Selman et al., 2002). This suggests that interactions between the antioxidant defense system and the up-regulation of UCPs expression could be an important to protecting BAT against oxidative stress.

The striped hamster (*Cricetulus barabensis*) is a common rodent in northern China, Russia, Mongolia, and Korea. This species can be found in farmlands and grasslands but prefers sandy areas. The climate within its range is characterized by warm and dry summers (maximum, 42 °C) and cold winters (< -20 °C) (Zhao et al., 2010a, 2010b). The striped hamster is granivorous, nocturnal and feeds mainly on stems and leaves of plants during summer and seeds in winter (Zhang and Wang, 1998; Zhang and Zhao, 2015). It does not store food during summer, and therefore must cope with both reduced food abundance and hot temperature in this season.

The striped hamster is characterized by a relatively high metabolic rate after correcting for body size (145% of that predicted by Hayssen and Lacy (1985) and 170% of that predicted by McNab (1988)) (Zhang and Wang, 1998; Song and Wang, 2003). It displays physiological plasticity in both metabolic rate and UCP1 expression in response to seasonal changes in climate, particularly with respect to temperature and food availability (Zhao et al., 2010a, 2010b; Liu et al., 2003). We previously found that the metabolic rates of striped hamster decreased after acclimation to a warm temperature and restricting its diet to 80% of its ad libitum food intake. In contrast to previous studies on cold-exposed animals, in this paper we assess the "rate of living – free-radical" and "uncoupling to survive" hypotheses by examining the effects of both food restriction and temperature on the metabolic rate, UCP1 gene expression, antioxidant activity, and oxidative stress, in striped hamster BAT.

2. Materials and methods

2.1. Animals

Striped hamsters were obtained from our laboratory-breeding colony, which is comprised of the descendants of animals initially trapped in farmland in the center of Hebei province (115°13′E, 38°12′S), on the North China Plain. All animals were initially maintained at 21 ± 1 °C under a 12 L:12D (light:dark, lights on at 0800 h) photoperiod. Food (standard rodent chow; Beijing KeAo Feed Company, Beijing, China) and water were provided ad libitum. All experimental procedures were in compliance with the Animal Care and Use Committee of the Institute of zoology, Chinese Academy of Sciences.

2.2. Experiment protocol

Sixty male hamsters, 3.5–4.5 months of age, were randomly assigned to either a control (room temperature), or warm temperature, treatment group, which were kept at 21 ± 1 °C and 30 ± 1 °C, respectively, for 8 weeks (d 1–56). The hamsters in these groups were then randomly assigned into two subgroups, an ad libitum (AL) group, which was fed ad libitum throughout the experiment (d 1–56), and a food restricted (FR) group, which was fed ad libitum from day 1 to 28 but was then put on a restricted diet from day 29 to 56. The restricted diet was 80% of the averaged ad libitum food intake between day 22 and 28. As described previously (Zhao, 2012), most animals completed this period of food restriction with significant decreases in body mass. There were thus four experimental groups in this study: 21 °C-AL, 21 °C-FR, 30 °C-AL and 30 °C-FR, each comprised of 15 animals. Food intake was calculated as the weight of food missing from the hopper every day, minus any food found among the bedding material.

2.3. Resting metabolic rate (RMR)

RMR, the rate of oxygen consumption, was quantified on day 56 using an open-flow respirometry system (Sable system, USA). Briefly, air was pumped through a cylindrical sealed Perspex chamber at a rate of 650–750 ml/min and transferred to an incubator (± 0.5 °C). Gases leaving the chamber were dried with silica gel and passed through an oxygen analyzer at a flow rate of 150–175 ml/min. RMR data were collected and averaged every 10 s by a computer connected by an analogue-to-digital converter (STD-UI2, Sable system), and analyzed using standard (Sable system) software. RMR was measured for at least 2.5 h at 30 ± 0.5 °C within the striped hamster's thermal neutral zone (Song and Wang, 2003; Zhao et al., 2010a). RMR was calculated from the lowest rate of oxygen consumption over 5 min, corrected to standard temperature and air pressure conditions and expressed as ml O_2/h (Zhao et al., 2010a, 2010b). All measurements were made between 1000 and 1700 h.

2.4. Hydrogen peroxide (H_2O_2) and malonadialdehyde (MDA) levels in brown adipose tissue (BAT)

Animals were euthanized by decapitation at the end of the experiment. Interscapular BAT was quickly removed, weighed (to 0.1 mg), and stored in liquid nitrogen. As described previously, BAT was homogenized and its H_2O_2 levels analyzed using commercial kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. H_2O_2 levels (405 nm) were expressed as mmol/g protein (Chen et al., 2014). Lipid peroxidation levels were inferred from MDA levels, which are a marker of oxidative damage in lipid (Garratt et al., 2013). MDA is the end product of lipid peroxidation and reacts with thibarbituric acid (TBA) as a thiobarbituric reactive species (TBARS) to produce a pink colored complex that has peak absorbance at 532 nm (Manivannan et al., 2013). MDA levels of BAT were expressed as nmol/mg protein (Zheng et al., 2015). The kit had been previously demonstrated to be effective for striped hamsters (Chen et al., 2014).

2.5. BAT catalase (CAT) and total antioxidant capacity (T-AOC) activity

CAT and T-AOC activity were also determined using commercial kits (produced by Nanjing Jiancheng Bioengineering Institute), according to the manufacturer's instructions. One unit of CAT activity was defined as the decomposition of 1 μmol H_2O_2 per min. One unit of T-AOC activity was defined as the increment in absorbance (A520) of 0.01/min/mg protein at 37 °C. T-AOC was