Comparative Biochemistry and Physiology, Part A 189 (2015) 84–90

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Effect of temperature on oxidative stress, antioxidant levels and uncoupling protein expression in striped hamsters

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ARTICLE INFO

Article history:
Received 12 January 2015
Received in revised form 29 July 2015
Accepted 29 July 2015
Available online 2 August 2015

Keywords:
Antioxidants
Brown adipose tissue (BAT)
Oxidative stress
Striped hamster
Temperature
Uncoupling proteins

ABSTRACT

According to the rate of living-free radical hypothesis, higher metabolic rates should increase reactive oxygen species (ROS) production. However, the "uncoupling to survive" hypothesis postulates that uncoupling proteins (UCPs) can decrease ROS production by lowering the potential of the inner mitochondrial membrane, in which case the correlation between metabolic rate and ROS levels would be a negative rather than positive. In this study, we examined energy intake, oxidative stress levels, antioxidant activity and the expression of UCPs in brown adipose tissue (BAT), and in the liver, heart, skeletal muscle and brain, of striped hamsters (*Cricetulus barabensis*) acclimated to either 5 °C or 32.5 °C. The energy intake of hamsters acclimated to 5 °C increased by 70.7%, whereas the energy intake of hamsters acclimated to 32.5 °C decreased by 31.3%, relative to hamsters kept at room temperature (21 °C) ($P < 0.05$). Malonadialdehyde (MDA) levels, total antioxidant capacity (T-AOC) and glutathione peroxidase (GSH-PX) activity in BAT significantly decreased in 5 °C group, but increased in 32.5 °C group, relative to the 21 °C group. Neither ROS levels (i.e. H_2O_2 levels), nor antioxidants in skeletal muscle, liver, heart or brain tissue, were affected by temperature. UCP_1 expression in BAT was significantly up-regulated in 5 °C group, but down-regulated in 32.5 °C group, relative to the 21 °C group. UCP_3 expression of skeletal muscle was also up-regulated significantly in hamsters acclimated to 5 °C. These results suggest that the relationship between ROS levels and metabolic rate was negative, rather than positive. UCP_1 expression in BAT may have played a role in lowering ROS levels.

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1. Introduction

Several authors have suggested a relationship between metabolic rate and lifespan (Pearl, 1928; Sohal, 2002; Speakman et al., 2002). The rate of living-free radical hypothesis proposes that higher metabolic rates are correlated with shorter lifespans at both inter-specific and intra-specific levels (Pearl, 1928; Harman, 1956; Sohal, 2002; Speakman et al., 2004; Mookerjee et al., 2010). The mechanism thought to be responsible for this apparent relationship is that the increase in mitochondrial oxidative phosphorylation required to achieve higher metabolic rates leads to increased production of free radicals, more specifically, reactive oxygen species (ROS) (Speakman et al., 2004; Selman et al., 2013). High ROS levels are thought to increase mortality rates by increasing oxidative damage to macromolecules such as proteins, lipids and DNA (Beckman and Ames, 1998; Selman et al., 2002). Support for this hypothesis comes from comparative research on mammals that indicates that those with longer life-spans usually have lower metabolic rates after corrected for their body mass (Pearl, 1928; Harman, 1956; Speakman et al., 2004; Selman et al., 2012).

However, research on birds indicates that species with relatively high metabolic rates can also have relatively long lifespans (Nagy et al., 1999; Holmes et al., 2001; Speakman et al., 2004). Furthermore, rats exposed to relatively cold temperatures were found to have significantly higher metabolic rates, and longer lifespans, than counterparts maintained at room temperature (Holloszy and Smith, 1986). Increased metabolic rate in response to cold temperatures is not, however, always associated with elevated ROS levels in skeletal muscle, and in certain organs, including the liver, heart, lungs, spleen, kidneys and digestive tract (Selman et al., 2002; Chen et al., 2014). One potential explanation for the apparent negative correlation between increased metabolic rate ROS production found in some studies is the "uncoupling to survive" hypothesis, which proposes that reducing the proton motive force protects against ROS production, and that this can be achieved by activating UCPs (Brand, 2000; Echtay et al., 2002; Ježek, 2002; Speakman et al., 2002, 2004).

So far, five mitochondrial UCPs have been found in small mammals; UCP_1, which is expressed exclusively in brown adipose tissue (BAT); UCP_2, which is expressed ubiquitously; UCP_3, expressed predominantly in muscle, and UCP_4 and UCP_5, which are expressed in the brain (Ježek, 2002). In BAT, proton leakage through UCP1 is a tightly regulated

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http://dx.doi.org/10.1016/j.cbpa.2015.07.017
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mechanism that utilizes energy from the oxidation of intermediate substrates to generate heat (Argyropoulos and Harper, 2002; Echtay et al., 2002; Dietrich and Horvath, 2010). There is evidence that exposure to cold causes significantly elevated UCP mRNA expression and protein levels, in particular UCP_1 and UCP_3 expression (Nedergaard et al., 2001; Simonyan et al., 2001; Jakus et al., 2002).

The "uncoupling to survive" hypothesis predicts that cold-exposed animals should have reduced ROS production in tissues where UCPs are highly expressed. However, several studies have documented tissue-specific increases in ROS levels (Davidović et al., 1999; Selman et al., 2002; Venditti et al., 2004). For example, hydroperoxide and carbonyl levels significantly increased in the liver, heart and skeletal muscle of cold-exposed rats (Venditti et al., 2004) and carbonyl levels increased in the muscle and liver tissue of short-tailed field voles (*Microtus agrestis*) after exposure to cold (Selman et al., 2002). Such results are inconsistent with the "uncoupling to survive" hypothesis.

In general, the energy metabolism and energy intake of mammals increase under cold conditions, but decrease under warm conditions, such as the temperature within their thermal neutral zone (TNZ). Numerous studies have examined the relationship between temperature, metabolic rate, oxidative stress and antioxidative protection in cold-exposed animals, whereas few studies focus on the relationship in animals acclimated to warm conditions (Davidović et al., 1999; Selman et al., 2002; Venditti et al., 2004). Animals that maintain a lower metabolic rate after acclimation to a temperature within their TNZ are ideal subjects to examine these relationships (Gordon, 1993, 2012; Nespolo et al., 2002; Liu et al., 2003; Song and Wang, 2003; Zhao et al., 2010a, 2010b).

We present the results of experiments designed to determine the effect of temperature on energy intake, ROS levels (i.e. via H_2O_2), antioxidative protection, and UCP mRNA expression, in the striped hamster (*Cricetulus barabensis*). We had previously found that the metabolic rate of striped hamsters was negatively correlated with temperature below the lower point of their TNZ. Another previous study found that exposure of hamsters to moderate cold and warm temperatures did not cause significant changes in either oxidative stress or antioxidant activity (Chen et al., 2014). The aim of the current study was to examine the relationship between temperature, energy intake, oxidative stress and antioxidant activity in striped hamsters. The antioxidant system has been demonstrated to be effective to defend the increased oxidative stress, whereas in this study we hypothesize the defense was tissue dependent. As the role of UCPs in regulations of ROS levels and antioxidant activity were examined in living organism rather than in isolated tissue mitochondria, the current study is novel. Based on the "uncoupling to survive" hypothesis, we predicted that ROS levels would be reduced in tissues where UCPs were highly expressed in hamsters acclimated to cold temperatures compared to those acclimated to warm temperatures. Alternately, if the rate of living hypothesis is true in this case, we predicted that cold-acclimated hamsters would have higher metabolic rates, and also higher ROS levels, than warm-acclimated counterparts.

2. Materials and methods

2.1. Animals

This research was conducted with the approval of the Animal Care and Use Committee, Institute of Zoology, Chinese Academy of Sciences. Striped hamsters were obtained from the colony at Wenzhou University, 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. Animals were singly housed in plastic cages (29 × 15 × 18 cm) with sawdust bedding. Food (standard rodent chow; Beijing KeAo Feed Co., Beijing, China) and water were provided *ad libitum*. All animals were maintained at 21 ± 1 °C (just below the lower critical ambient temperatures of TNZ) under a 12 L:12D (light:dark, lights on at 0800 h) photoperiod.

Twenty-five adult hamsters, 3.5–4.5 months of age, were randomly assigned to one of three experimental groups; a cold ($n = 9$) and a warm-temperature treatment group ($n = 8$) (hereafter referred to as the 5 °C and 32.5 °C groups), which were acclimated to 5 ± 1 °C and 32.5 ± 1 °C, respectively, for six weeks, and a 21 °C group ($n = 8$), which was kept at room temperature (21 ± 1 °C). The body mass and food intake of all animals were measured every two days. Food intake was calculated as the weight of food missing from the hopper each two day interval, minus any uneaten food found in the bedding material.

2.2. Gross energy intake (GEI), digestive energy intake (DEI) and digestibility

In order to quantify the metabolic rate of hamsters, we quantified digestive energy intake from over the last two days of the experiment. GEI and DEI were measured over the last two days of the experiment. Uneaten food and feces were manually separated from bedding material in each cage and dried to a constant mass at 60 °C. The gross energy content of the food and feces was then determined using a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA). GEI, DEI and digestibility were calculated using the following equations:

$$\mathrm{GEI\,(kJ/d)} = [\mathrm{food\ intake\,(g/d)} \times \mathrm{dry\ matter\ content\ of\ food\,(\%)}] \times \mathrm{gross\ energy\ content\ of\ food\,(kJ/g)};$$

$$\mathrm{DEI\,(kJ/d)} = \mathrm{GEI} - [\mathrm{dry\ feces\ mass\,(g/d)} \times \mathrm{gross\ energy\ content\ of\ feces\,(kJ/g)}];$$

$$\mathrm{Digestibility\,(\%)} = \mathrm{DEI} / \mathrm{GEI} \times 100\%$$

(Grodzinski and Wunder, 1975; Zhao et al., 2014a).

2.3. Oxidative stress markers and antioxidant enzymes

Animals were killed by decapitation at the end of the experiment. Interscapular BAT, leg skeletal muscle and the liver, heart, and brain of each animal, were removed quickly, and stored in liquid nitrogen. Tissues were homogenized in ice-cold 0.9% NaCl solution after which homogenates were centrifuged at 3000 *g* for 15 min and the resultant supernatant kept for subsequent assays. Protein concentration was determined using the method described by Lowry et al. (1951), using bovine serum albumin (BSA) as a standard.

ROS levels were measured in the tissues by measuring hydrogen peroxide (H_2O_2) levels. H_2O_2 levels were analyzed using a commercial kit (Nanjing Jiancheng Bioengineering Institute) that had proven effective for striped hamsters (Chen et al., 2014), in accordance with the manufacturer's instructions and guidelines. Levels of H_2O_2 (405 nm) were expressed as mmol/g protein.

Malonadialdehyde (MDA) was used as an indicator of lipid peroxidation. MDA is the end product of lipid peroxidation and reacts with thibabituric acid (TBA) to produce a pink colored complex with peak absorbance at 532 nm (Buege and Aust, 1978; Manivannan et al., 2013). Levels of tissue MDA were expressed as nmol/mg protein.

2.4. Total antioxidant capacity (T-AOC) and glutathione peroxidase (GSH-PX) activity

T-AOC and GSH-PX activity were also determined using commercial kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. The coefficients of variation of inter- and intra-assays for the T-AOC kit were 3.6% and 6.4%, respectively. One unit of T-AOC activity was defined by a 0.01 OD increment in absorbance per min.

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