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Non-shivering thermogenesis activation and maintenance in the aging gray mouse lemur (*Microcebus murinus*)

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ABSTRACT

The cold-induced enhancement of non-shivering thermogenesis (NST), involving brown-adipose tissue (BAT) metabolism, could participate to impair energy balance in the aged gray mouse lemur (*Microcebus murinus*). We first investigated the age-related modulations of cold-stimulated BAT cell morphology and contents. Then, NST was pharmacologically stimulated to assess whether aging impaired NST activation in the mouse lemur.

In reference conditions, the ability to activate NST was preserved during aging in the mouse lemur as BAT morphology and UCP-1 presence did not differ between adult and aged mouse lemurs. Also, the pharmacological activation of NST revealed similar increased levels of O_2 consumption in adult and aged animals, confirming that no age effect could be evidenced on NST activation at 25 °C. However, preliminary histological data revealed a lack of lipid resources in one aged individual during cold exposure. Surprisingly, the pharmacological activation of NST revealed an impaired evacuation of the excess body heat in aged animals, associated with increased energy expenditure.

Thus, aging seems to be related to decreased capacities in the maintenance of NST rather than in its activation. Energy mobilization could be impaired in the aging mouse lemur but remains to be demonstrated.

1. Introduction

Non-shivering thermogenesis (NST), which mainly depends on the thermogenic metabolism of brown-adipose tissue (BAT), is widely used in heterothermic mammals (Himms-Hagen, 1984). BAT and NST are also assumed to be functional in humans, but only at fetal and neonatal stages (Symonds and Lomax, 1992). Until recently, there was no evidence for a functional activity of such thermogenesis in adult humans, while other thermogenic pathways (shivering, behaviors...) seemed to be preferred (Leppaluoto et al., 2005). However, a growing interest raised a few years ago on BAT metabolism, since the potential metabolic role of this thermogenic tissue has been recently described in adult humans (Christensen et al., 2006; Crisan et al., 2008; Cypess et al., 2009; Nedergaard et al., 2007; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009).

NST consists in a huge production of body heat originated from activation of mitochondrial activity in BAT (Cannon and Nedergaard, 2004; Ricquier, 2006; Sell et al., 2004). Indeed, brown adipocytes are constituted of multilocular lipid storing cells containing a massive amount of mitochondria and densely innervated by the sympathetic nervous system. Beta-adrenergic receptors are activated by noradrenaline release induced by the sympathetic influx. A cascade of metabolic events occurs, leading to the activation of uncoupling protein 1 (UCP-1). Free fatty acids are released from lipolysis of triglyceride droplets and are then used by UCP-1. This aerobic process is very demanding in oxygen implying that the respiratory metabolism is also highly increased. Body heat production through BAT metabolism is particularly efficient and allows a rapid rewarming, and prompt arousal from torpid state in small mammals (Klingenspor, 2003). In addition, when environmental conditions become harsher, for example when ambient temperatures (T_{as}) drop to low values, NST is used to avoid hypothermia. Thus, cold exposure has been shown to enhance NST activation at different levels (for review, see Klingenspor, 2003; Watanabe et al., 2008). Typically, brown adipocytes are massively recruited after cold exposure. These cells become hypertrophic and hyperplasic, while UCP-1 synthesis increases in association with thermogenic mitochondrial biogenesis.

Aging has been described to potentially impair the NST response in rodents, by reducing the amount of functional BAT and its thermogenic capacity by 50–60% (Horan et al., 1988; McDonald and Horwitz, 1999). However, the age-related decrease in NST capacity does not seem to be related to a decrease in sympathetic signalling (Gabaldon et al., 2003; McDonald et al., 1991, 1993), but

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rather to a decrease in glucose utilization efficiency (McDonald et al., 1994) or in UCP-1 presence (Florez-Duquet and McDonald, 1998). Alternatively, an age-related impairment in fuel mobilization could explain the decrease in NST capacity during aging, at least in rodents (Florez-Duquet and McDonald, 1998; Kontani et al., 2005; Ueno et al., 1998), but this remains to be demonstrated in other species. In the case of non-human primate species, available data on the effects of aging on BAT function are scarce, whereas such data could bring valuable information on the potential role of BAT in humans.

In this context, the gray mouse lemur (*Microcebus murinus*, Primates) appears to be a biological model particularly interesting. The life span of this species is about 8 years in captivity (Perret, 1997), what is rare for such a small-sized species. This nocturnal primate originating from Madagascar exhibits daily heterothermia, which is characterized by the occurrence of a daily phase of low core temperature (T_c) during the first hours of daytime (Perret and Aujard, 2001). This physiological mechanism is particularly well adapted to compensate for small energy stores (Aujard and Vasseur, 2001) and leads to energy savings during the phase of T_c decrease. However, daily arousals imply the enhancement of NST and BAT metabolism in this species (Genin et al., 2003) and might induce high energy costs for rewarming. In captive gray mouse lemurs, daily modulations of T_c vary according to photoperiod and ambient temperature (Aujard et al., 1998; Seguy and Perret, 2005). More especially, it has been recently demonstrated that the daily phase of low T_c was strongly deepened during winter in aged mouse lemurs exposed to cold (Terrien et al., 2008). This was associated with impaired energy balance during aging, and we suggested that the energy costs of NST could be related to such impairment. For instance, no data is available on the effects of aging on NST function in the mouse lemur. Based on these findings, age-related effects on NST capacity were investigated in the mouse lemur by first investigating the effects of aging on cold-stimulated BAT to assess whether aging was associated with a modulation in BAT cell morphology or contents. Then, NST was pharmacologically stimulated to assess whether aging impaired NST activation in the mouse lemur.

2. Methods

2.1. Animals and housing conditions

All the gray mouse lemurs studied were males, born in the laboratory breeding colony of UMR 7179 (CNRS/MNHN, France, license approval No. A91.114.1) and were pathogen free. Before the experiment, general conditions of captivity were maintained constant: T_a (24–26 °C), relative humidity (55%). Food and water were available *ad libitum*. In captivity, seasonal variations of physiological functions can be entrained by alternating 6-month periods of summer-like long photoperiod (14 h of light/day) and winter-like short photoperiod (10 h of light/day) under artificial light (fluorescent tubes during the day and dim red light during the night). In the present study, male mouse lemurs were studied under short photoperiod during which animals are supposed to intensively use NST (Terrien et al., 2008). Animals were routinely fed *ad libitum* on a diet including fresh banana (393 kJ/100 g) and a homemade milky mixture containing baby cereals, eggs and milk (435 kJ/100 g). General conditions of captivity were applied and animals were maintained in social groups before and after experiments. Survival data from 254 male mouse lemurs from our breeding colony were used to determine the mean lifespan (mean ± SEM: 6.0 ± 0.2 years), the mean lifespan of the 10% of the most long lived animals (10.0 ± 0.2 years) and the maximal observed survival (12.0 years). After reaching 5 years of age, mouse lemurs exhibit typical morphological and physiological modifications related to aging: graying of the fur and decreased amplitude in seasonal variations of body mass (Perret, 1997; Perret and Aujard, 2005). Moreover, in the male mouse lemur, both behavioral and physiological parameters of reproductive function show clear decrease with aging after the age of 5 years. Indeed, reductions in aggressive, marking and sexual behaviors are commonly observed in aged mouse lemurs and are concurrent with a decline in sexual hormone levels (Aujard and Perret, 1998; Perret and Aujard, 2005). For these reasons, animals younger than 5 years were considered adult in the present study, whereas those older than 5 years were considered aged. Hence, adults (First experiment: $N = 6$, mean age ± SEM = 2.5 ± 0.2 years; Second experiment: $N = 6$, mean age ± SEM = 2.7 ± 0.2 years old) and aged mouse lemurs (First experiment: $N = 6$, mean age ± SEM = 7.1 ± 0.2 years; Second experiment: $N = 6$, mean age ± SEM = 5.8 ± 0.2 years old) were used during this work. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC). All efforts were made to minimize nociception.

2.1.1. Cold-induced activation of NST

2.1.1.1. Core temperature recording. Adults and aged mouse lemurs were maintained in climate chambers (Sanyo incubator MIR-253), in which air was filtered and light was provided by cool fluorescent lamps. Prior to experiment, body mass averaged 98.3 ± 4.9 g and 79.1 ± 7.0 g in adult and aged animals, respectively. Mouse lemurs were habituated to the experimental device for 10 days at $T_a = 25$ °C and then exposed to a cold environment (2 days at 12 °C). Core temperature (T_c) was measured using Thermochron iButtons (DS1921H-F50; Dallas Semiconductor MAXIM, Dallas, Texas, USA; http://www.ibutton.com) according to the technic previously described (Davidson et al., 2003). The data loggers were implanted under general anesthesia (Valium, 2 mg/100 g i.m.; Ketamine Imalgen, 10 mg/100 g i.p.) in the visceral cavity of the animals. Calibrations for each transmitter were provided by the manufacturer. Experiments were performed after at least 2 weeks of recovery after checking for complete healing and stabilization of T_c rhythm. Mouse lemurs were isolated in individual cages provided with branches and a wooden nest. T_c (in °C) was recorded every 10 min. Data were computed by a software (iButton Viewer v. 3.21, Dallas Semiconductor MAXIM, Dallas, Texas, USA). The following parameters were analyzed: mean T_c during the active nocturnal phase ($T_{c_{night}}$), mean T_c during the resting diurnal phase ($T_{c_{day}}$) and minimal T_c value ($T_{c_{min}}$).

2.1.1.2. Immuno-histological analysis. Adult ($N = 2$ at each T_a) and aged ($N = 2$ at each T_a) mouse lemurs were removed from the experiment after the habituation period at 25 °C and after the 2-day exposure to 12 °C, and then deeply anesthetized. BAT fragments were sampled from the different locations already described (Genin et al., 2003) and stored for histological analysis and Western blotting of UCP-1. Brown-adipose pads were removed, fixed on 95% ethanol and embedded on paraffin until sectioned. Five-micrometer sections were deparaffinized, hydrated and endogenous peroxidase activity was removed by incubating with H_2O_2, 3% in TBS for 10 min. UCP-1 protein was detected using a 5 µg/ml dilution of rabbit polyclonal antibody (alpha diagnostic UCP-1-A). ImmPress (Vector MP-7401) was used as secondary antibody. Finally, sections were stained using 3-Amino-9-ethylcarbazole and counterstained by haematoxylin. A control experiment was performed using purified rabbit IgG and yielded no staining. Slides were counterstained with nuclear red.

2.1.1.3. Western blot analysis. Mitochondrial fractions were prepared by differential centrifugation of tissue homogenates as already described (Casteilla et al., 1987). 0.1 to 0.5 µg of tissue