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Shallow hypothermia depends on the level of fatty acid unsaturation in adipose and liver tissues in a tropical heterothermic primate

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ABSTRACT

Optimal levels of unsaturated fatty acids have positive impacts on the use of prolonged bouts of hypothermia in mammalian hibernators, which generally have to face low winter ambient temperatures. Unsaturated fatty acids can maintain the fluidity of fat and membrane phospholipids at low body temperatures. However, less attention has been paid to their role in the regulation of shallow hypothermia, and in tropical species, which may be challenged more by seasonal energetic and/or water shortages than by low temperatures. The present study assessed the relationship between the fatty acids content of white adipose and liver tissues and the expression of shallow hypothermia in a tropical heterothermic primate, the gray mouse lemur (*Microcebus murinus*). The adipose tissue is the main tissue for fat storage and the liver is involved in lipid metabolism, so both tissues were expected to influence hypothermia dependence on fatty acids. As mouse lemurs largely avoid deep hypothermia (i.e. torpor) use under standard captive conditions, the expression of hypothermia was triggered by food-restricting experimental animals. Hypothermia depth increased with time, with a stronger increase for individuals that exhibited higher contents of unsaturated fatty acids suggesting that they were more flexible in their use of hypothermia. However these same animals delayed the use of long hypothermia bouts relative to individuals with a higher level of saturated fatty acids. This study evidences for the first time that body fatty acids unsaturation levels influence the regulation of body temperature not only in cold-exposed hibernators but also in tropical, facultative heterotherms.

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1. Introduction

In contrast to saturated (SFA) and monounsaturated (MUFA) fatty acids, polyunsaturated fatty acids (PUFA) are essential nutrients that mammals are unable to synthesize *de novo* Florant, 1998), and can only acquire from their food resources. They exert multiple key physiological functions by modulating the architecture and function of cellular membranes, cell signaling pathways and gene expression (Spector, 1999). In animals, specifically in ectotherm and heterotherm vertebrates, an important function of unsaturated fatty acids is the maintenance of cell membrane functioning at varying tissue temperatures (Moyes and Ballantyne, 2011). Thus, unsaturated fatty acids do play a role in the modulation of physiological functions in heterotherms. Two main theories are proposed: the membrane

fluidity hypothesis and the membrane protein–lipid interaction hypothesis (reviewed in Munro and Thomas, 2004). In the first theory, the degree of fatty acid unsaturation influences the melting point of the fat stored in adipose tissue (Murray et al., 2010) and, as a consequence, the bioavailability of fat stores to sustain metabolic needs at low temperatures via lipolysis pathway (Geiser et al., 1994). Because PUFA have a high number of double bounds (unsaturation), they have low melting points, which can help maintaining the fluidity of fats and membrane phospholipids at low body temperatures (Munro and Thomas, 2004; Ruf and Arnold, 2008). The second theory is the membrane protein–lipid interaction in which PUFA are thought to modulate the functioning of transmembrane proteins and thus to play a key role in biochemical adaptations to low body temperatures in organisms that regularly enter into hypothermia.

Heterothermic mammals are known to increase the proportion of unsaturated fatty acids in their body fat before entering into torpor during the lean season, and cold-exposed species exhibit higher proportion of unsaturated fatty acids in their body fat than those from warmer climates (Munro and Thomas, 2004). This is directly related to the fact that unsaturated fatty acids would facilitate the decrease of

body temperature (hypothermia depth), the maintenance of low body temperature over an extended period of time (hypothermia bout duration), as well as the reduction of metabolic rate during hibernation. PUFA and especially n-6 PUFA contribute to the maintenance of cardiac activity at low body temperature (Giroud et al., 2013), for instance. Thus, diets with optimal content of unsaturated fatty acids favor torpor expression (Frank and Storey, 1995; Munro et al., 2005), and in turn energy savings (reviewed in Dark, 2005; Munro and Thomas, 2004; Ruf and Arnold, 2008). At the contrary, SFA have a negative effect on mammalian torpor (Geiser et al., 1994). The role of fatty acid composition of essential tissues involved in storage and metabolism of lipids has mainly been investigated for hibernation, and in a lesser extent, for daily torpor. However, the role of fatty acid composition for shallow hypothermia remains largely unknown. The gray mouse lemur (*Microcebus murinus*) is a small nocturnal primate originating from Madagascar. It uses to forage on fruits, insects and gums (Dammhahn and Kappeler, 2008), a diet that is reproduced in captivity by providing a homemade mixture (content is detailed hereafter). This primate exhibits biological seasonal rhythms that depend on photoperiod and is characterized by an important body mass gain before the dry season and a decreased resting metabolic rate during this season (Perret and Aujard, 2001; Séguy and Perret, 2005). In captivity, this primate has a life expectancy of around 8 years (Languille et al., 2012), and body mass ranges from 80 g in summer-like photoperiod to 120 g in winter-like photoperiod, on average. This species is a facultative heterotherm since it can spontaneously enter into torpor in response to a reduction of ambient temperature and/or food availability, in the wild as in the laboratory (Canale et al., 2011; Schmid, 2000, 2001; Schmid and Speakman, 2000; Vuarin et al., 2013), although it avoids the use of torpor in absence of energetic constraints. The ability of animals to enter into hypothermia depends on the presence of active brown adipose tissue which is used during the rewarming process (Terrien et al., 2010). Thus, because mouse lemurs are characterized by a strong seasonal fat mass gain and because fat reserves play a key role in torpor expression, they represent an appropriate model to study the mechanisms of lipid mobilization in the regulation of daily facultative use of hypothermia (Dal-Pan et al., 2010; Giroud et al., 2009).

The present study aimed to assess the relationship between white adipose and liver tissues fatty acids contents and the spontaneous use of daily, shallow hypothermia in gray mouse lemurs. The adipose tissue is the main tissue for fat storage, and the liver is largely involved in lipid metabolism. Even though they are both involved in the regulation of body lipids, the adipose tissue and liver are characterized by different functions and different fatty acid compositions. Therefore, they are both susceptible to drive heterothermy dependence on unsaturated fatty acids. Daily hypothermia expression of gray mouse lemurs was related to their adipose tissue and liver fatty acids contents (see Pifferi et al., 2012 for details on the methodology and complete characterization of lipid contents). To increase the probability that animals undergo hypothermic bouts during resting time, they were exposed to a moderate calorie restriction. Mouse lemurs were expected to gradually increase their use of hypothermia through time, as a consequence of the cumulative effect of calorie restriction, and individuals with higher contents of unsaturated fatty acids were expected to express deeper and/or longer hypothermia bouts than those with low unsaturation levels.

2. Material and methods

2.1. Animals

Six adult female gray mouse lemurs (Cheirogaleidae, Primates) aged of 23 ± 4 months were maintained in constant conditions relative to temperature (25 ± 1 °C) and humidity ($55 \pm 1\%$).

Throughout the two weeks of the experiment, they were exposed to a moderate food restriction (animals received 60% of the ad libitum portion) and a winter-like photoperiod (10:14 h light–darkness), conditions that trigger the expression of torpor, by mimicking the ecological conditions of the dry season (Ortmann et al., 1997; Schmid, 2001). Females were preferred because they are more prone to enter into hypothermia than males (Perret and Aujard, 2001a, 2001b). The animals that were used for this study were originally used for the purpose of another experiment (Pifferi et al., 2012). The meal was composed of fresh fruits and a laboratory-made mixture of cereals, fresh cheese, milk and egg, and contained about 1 g of total fatty acids per 100 g of meal. 18:1 n-9 represented 225.2 mg/100 g of diet. PUFA from both n-3 and n-6 series were present, mainly in the form of 18 carbons precursors, linoleic acid (18:2 n-6, LA: 103.8 mg/100 g) and α-linolenic acid (18:3 n-3, ALA: 9.9 mg/100 g). The long-chain PUFA were also present, mainly arachidonic acid (20:4 n-6, AA: 3.2 mg/100 g) and docosahexaenoic acid (DHA, 22:6 n-3: 1.4 mg/100 g) (Table 1). Mean body mass (BM) of the animals was 106.2 ± 12.9 g (ranging from 92 to 127 g).

2.2. Body temperature measurements and tissues sampling

Body temperature (T_b, °C) was recorded using thermo-sensitive telemetric transmitters (TA10TA-F20, 3.2 g, Datascience) that had been implanted in the visceral cavity under general anesthesia (following the surgery procedure used by Pifferi et al., 2011 and following the recommendations of Dausmann, 2005). Initial accuracy: 0.1 °C; resolution: 0.05 °C; temperature drift first two weeks = 0.1 °C per 6 months after = 0.1 °C; temperature operating range: 34–41 °C. Transmitters may work over wider ranges with reduced accuracy according to manufacturer. Calibration to lower temperatures (25 °C) was performed in house. Emitted frequencies were collected for 5 s every 10 min thanks to RPC-1 receiver boards placed in the cages, and then converted to T_b values by interpolation of the calibration curves for each transmitter. After a week of recovery, T_b was monitored for two weeks, at the end of which the 6 females were sacrificed when reaching the deepest part of their daily hypothermia bout (in the morning). Euthanasia was justified for the purpose of another experiment (Pifferi et al., 2012). For each individual, a sample of adipose tissue was excised from the tail (white adipose tissue being mainly located in the tail during winter mass gain in the gray mouse lemur) and a sample of liver was taken. Both tissue samples were frozen in liquid nitrogen and then stored at -80 °C. Since analyzing heterothermy use with a fixed threshold of body temperature has methodological drawbacks (Boyles et al., 2011; Brigham et al., 2011; Canale et al., 2012), daily shallow hypothermia depth and bout duration were determined on individual T_b profiles. Hypothermia depth corresponded to daily minimal body temperature ($T_{b\ min}$, °C). Hypothermia bout duration (D_{hyp}, h) was calculated using the following parameters: the beginning of daily hypothermia was defined as the first 3 successive bins of 10 min in which T_b was lower than the mean night T_b, and the end of hypothermia was defined as the first 3 successive bins of 10 min in which T_b was greater than the mean night T_b.

2.3. Analysis of fatty acid composition of white adipose and liver tissues

The total lipids were extracted from the adipose tissue samples with 4 mL of chloroform–methanol (2:1, vol:vol). The liver ethanolamine phosphoglycerolipids were isolated by solid-phase extraction on an aminopropylbonded silica cartridge (see Pifferi et al., 2012 for details on the methodology). Ethanolamine phosphoglycerolipids have been selected because they correspond to