BROWN FAT AND NON-SHIVERING THERMOGENESIS IN THE GRAY MOUSE LEMUR (*MICROCEBUS MURINUS*)

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RH Non-shivering thermogenesis in the gray mouse lemur

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The gray mouse lemur *Microcebus murinus* is a rare example of a primate exhibiting daily torpor. In captive animals, we examined the metabolic rate during arousal from torpor and showed that this process involved non-shivering thermogenesis (NST). Under thermoneutrality (28°C), warming up from daily torpor (body temperature < 33°C) involved a rapid (< 5 min) increase of O₂ consumption that was proportional to the depth of torpor \((n = 8)\). The injection of a β-adrenergic agonist (isoproterenol) known to elicit NST induced a dose-dependent increase in metabolic rate \((n = 8)\). Moreover, maximum thermogenesis was increased by cold exposure. For the first time in this species, anatomical and histological examination using an antibody against uncoupling protein UCP, specifically demonstrated the presence of brown fat. Using Western blotting with the same antibody, we showed a likely increase in UCP expression after cold exposure, suggesting that NST is also used to survive low ambient temperatures in this tropical species.

*Key-words*: thermoregulation brown adipose tissue arousal from daily torpor isoproterenol uncoupling protein
Thermoregulation involves various strategies. High body mass, and increase of insulation with fat, down and fur enable animals to save heat. Some species tend to fit their daily rhythm of activity with the cycle of ambient temperature. During the resting phase, some species use micro-habitats, build nests and burrows, or huddle together. Endotherms are able to withstand extreme ambient temperatures by maintaining a constant high body temperature (3, 51).

Under thermoneutrality, all heat production is provided by the basal metabolic rate. In contrast, under cold exposure, extra heat production is required during inactivity (20, 25). In small mammals, two strategies are used for this extra heat production: shivering thermogenesis (SH) and non-shivering thermogenesis (NST) (19, 23, 27). Shivering thermogenesis involves skeletal muscles whereas non-shivering thermogenesis involves a unique thermogenic effector organ, brown adipose tissue (BAT) (35, 49). Brown fat is present in various species including hibernators and non-hibernators (44). Thermogenesis in BAT results from an increase in the rate of substrate oxidation in mitochondria caused by a proton conductance pathway though a 32 kDa “uncoupling protein” (UCP1) of the inner membrane (6, 35, 52). The tissue specificity of UCP1 in BAT has been widely used as a sensitive marker to identify whether BAT is present or not (21). Non-shivering thermogenesis has been shown to be induced by the sympathetic adrenal system, and injection of norepinephrine or β-adrenergic agonist (isoproterenol) has been used to induce non-shivering thermogenesis experimentally (17, 23, 24, 26, 53).

The cost of homeothermic thermoregulation is very high in cold and dry environments (50). Heterothermic endotherms use torpor to survive food and water shortage (3, 50). This energy saving state is expressed by lowering body temperature and metabolism that can last less than 24 h (daily torpor) or more (hibernation) (14, 50).
Torpor bouts are induced by both cold exposure and food or water restriction (8, 31). In addition, daily torpor frequency and/or depth often show seasonal changes with a reduction in summer (12, 29). The metabolic cost of arousal from torpor is high and can reach 80% of the total energy expenditure of daily torpor (11). Both shivering and non-shivering thermogenesis are used in the arousal process (13, 27). SH seems to precede NST during the arousal process (7, 27). A third mechanism using passive warming up has been found in marsupials (12, 13, 28).

The gray mouse lemur, *Microcebus murinus* (cheirogaleidae) is a small nocturnal primate exhibiting various adaptive traits to cope with the cool, dry winter occurring in its natural habitat of Western and Southern Madagascar, including sexual rest, autumn fattening, daily torpor and huddling behaviour (4, 5, 16, 36, 37, 40, 41, 46-48). Daily torpor is well documented in *Microcebus murinus* and studies on the reduction of metabolic rate (MR) and body temperature have been performed in the laboratory (4, 5, 10) as well as in the field (47). Torpor frequency and depth increase in the winter corresponding to short-photoperiod exposure in captivity (4, 36). Furthermore, the depth of shallow daily torpor has been shown to increase under cold exposure (5). Wild animals maintained in captivity and exposed to ambient temperatures show no increase in metabolic rate during arousal from spontaneous daily torpor and seem to use passive warming up (46). However, little is known about the involvement of NST during arousal from torpor and during cold exposure.

This study attempted to assess whether NST was involved during arousal from torpor and during cold exposure in *Microcebus murinus*. We examined thermogenic responses of gray mouse lemurs to various treatments known to elicit NST attributed to BAT in other small mammals (cold exposure and isoproterenol injection). In line with
the functional studies *in vivo*, the presence of BAT was evaluated by both histological examination and the use of an antibody to UCP1.

**MATERIAL AND METHODS**

*Animals.* Seventeen mouse lemurs were used in this study (13 males and 4 females). The animals were born in a laboratory breeding colony at Brunoy (Muséum National d’Histoire Naturelle, European Institutions Agreement N° 962773, France) from stock originally caught in southern Madagascar. This work follows the guiding principles for research involving animals defined by the American Physiological Society (1). All animals were adult 2-6 years old. General conditions of captivity have already been described and were maintained constant with respect to ambient temperature (25 ± 2°C) and relative humidity (55 %) (4). To avoid possible social influences, animals were housed individually in cages (0.5 x 0.3 x 0.3 m) provided with a nest-box and branches, and separated from each other. Animals had been exposed to short photoperiod (LD:10-14) for 3 months. All were reproductively inactive and showed the high body mass (BM) corresponding to the winter-like short photoperiod (BM> 90 g). Food and water were supplied *ad libitum*. Daily torpor bouts were induced by food restriction when animals were fed on 8-10 kcal / day (15). Animals were considered in daily torpor (DT) when minimum body temperature was below 33°C.

*Body temperature.* To observe the warming up corresponding to the spontaneous arousal from daily torpor, body temperature was monitored during 8 days of *ad libitum* feeding and 8 days of food restriction in one male using telemetry, as already described (41). A small telemetric transmitter (TA10TA-F40, 3.2 g DATA SCIENCE, Minn., USA) was implanted into the visceral cavity, under KETAMINE anesthesia (IMALGENE 500 mg, 10 mg / 100 g). Recovery occurred in 14 days and animals were
used at least one month after surgery. The receiver board (RLA1020, DATA SCIENCE, Minn., USA) was positioned in front of the nest-box. Body temperature was recorded every 5 minutes. Signals were transferred to a computer program (DATAQUEST LABPRO). The provoked arousal from torpor was induced when Tb reached stable minimum values, by quick handling of the animal.

Rectal temperature was measured using a digital thermometer (EIRELEC) with a flexible thermo-couple inserted 15 mm into the rectum (± 0.1°C, time constant 0.1 s). Rectal temperature was assumed to be equal to body temperature (4).

Oxygen consumption. Warming up at 28°C was induced by handling resting animals which was done rapidly (< 3 min) : animals were removed from their nest box, weighed (± 1 g), and rectal temperature was measured. The energy expenditure during provoked arousal from torpor was measured continuously for 70 minutes in 8 animals (4 males and 4 females) by the determination of O₂ consumption in a closed-circuit respirometer as already described (2). The respirometry chamber was placed in a water bath at the neutral temperature of 28.0°C. Soda lime and silica gel were used to remove carbon dioxide and water. The rise of a water column caused by the depression in the respirometer was detected by a photo cell that activated the injection of the same volume of ambient air (3.6 to 4.5 mlO₂). Oxygen consumption was measured by noting the time at each injection. Animals were transferred into the respirometer in their nest box : 1- during ad libitum feeding at least 6 h of daylight, while the animals were under normothermy ; 2- within the first 6 h of daylight, after 2 days of food restriction while the animals entered daily torpor bouts (Tb < 33°C). Possible movements of the animals could be checked through the respirometer which was transparent. Rectal temperature was measured again after each measurement of O₂ consumption. Since body mass might
change during increased metabolic rate, oxygen consumption was expressed, for each measurement, in mlO₂ / BM * h, where BM was initial body mass in grams.

The thermogenic response to intra-muscular injection of 2 doses of a β-adrenergic agonist (isoproterenol) was measured in the same way for 60 minutes (53). The injection of 20 and 200 µg / 100 g of isoproterenol in 200 µl of saline vehicle, and a control injection of vehicle were performed in the same 4 normothermic animals (2 males and 2 females), on a different day, during the diurnal rest, at least 6 h of daylight. To assess maximum thermogenesis, maximum O₂ consumption occurring within the 10 minutes following the injection of isoproterenol (0, 2, 4, 20 and 200 µg / 100 g) was measured at the same time of day in 8 normothermic males in a closed-circuit respirometer, as already described (16). The animals were placed in a closed respiratory chamber of 1655 ml volume containing 10 ml of a 10 % KOH solution used to remove carbon dioxide and water. The chamber was placed in a water bath at 28°C. After 10 minutes, O₂ consumption was measured using a SERVOMEX 570 A paramagnetic gas analyser (CROWBOROUGH, UK) (42). The animals were injected each dose on a different day. A second injection of 200 µg of isoproterenol was performed after a 24 h-cold exposure (5°C).

Immunochemistry. For immunochemical analyses, as already described (33, 34), one male was exposed to cold (7°C) twice for 12 h. A control male was maintained at 23-25°C. Both were killed by decapitation after over-anesthesia by CO₂. Fat depots were sampled for histological analysis and western blotting of UCP1.

After overnight fixation, tissues were dehydrated and paraffin embedded. Sections (5 to 7 µm) were incubated for 1 hour at room temperature with 0.5 µg / ml anti UCP1 antibody (purified rabbit IgG against mouse UCP1 –UCP 11-A–, ALPHA
Second antibody (JACKSON IMMUNORESEARCH, West Grove, PA) coupled to alkaline phosphatase (1 : 200) was visualized using BCIP/NBT (K 598, DAKO CORPORATION, Carpinteria, CA). Endogenous alkaline phosphatase activity was inhibited by levamisole (X 3021, DAKO CORPORATION, Carpinteria, CA). Slides were counterstained with nuclear red. Control experiments were performed using purified rabbit IgG and yielded no staining.

**Western blot analysis.** Mitochondrial fractions were prepared by differential centrifugation of tissue homogenates as already described (9). 0.1 or 0.2 µg of tissue protein from total homogenate or mitochondrial fraction were electrophoresed in 10 % polyacrylamide S.D.S. gels, transferred (1 h) onto nitrocellulose (Hybond™-P, Transfer membranes, RPN 2020 F, AMERSHAM) and incubated overnight with the same rabbit IgG against mouse UCP1 (0.25 mg / ml). The peroxidase activity of the second antibody (donkey anti-rabbit IgG, peroxidase-linked species-specific whole antibody, NA 934, AMERSHAM), diluted 1 : 8000, was revealed using the kit ECL (AMERSHAM RPN 2106) and Hyper film ECL-TM (RPN 310-3H, AMERSHAM). The blots were exposed for 18 minutes. A positive control was performed by 0.1 µg of rat-BAT mitochondria protein. To avoid any contamination, the non-fluorescent molecular weight marker was placed between BAT deposit and positive control.

**Data analyses.** All values are means ± S.E. Normality of distributions was evaluated by calculating the skewness and the kurtosis. Log-transformation was used in cases of non-normal distributions. To compare the different measurements obtained in the same animals (normothermy *versus* daily torpor, responses to different doses of isoproterenol, effect of cold exposure), we used Student’s paired t-test , analysis of variance (ANOVA) or General Linear Models of analysis of variance (GLM) for repeated values. The latter method provides statistical comparisons in values (factor)
and in time (factor * time). To compare O2 consumption during the arousal from torpor and after the injection of isoproterenol, we used analysis of variance (ANOVA). Multiple pair-wise comparisons were made using Tukey's post-hoc test. Correlations between parameters were evaluated by linear regression analysis using the Pearson correlation coefficient.

RESULTS

Warming up from daily torpor. Body temperature (Tb) showed strong daily variations both under ad libitum feeding and under food restriction. Tb was high during nocturnal activity and dropped at the end of the night during daily torpor (Fig. 1). Whatever the food intake, minimum Tb was reached during the first 3 hours of the day. However, duration and depth of daily torpor was increased by food restriction. Whereas Tb remained above 33°C during ad libitum feeding, food restriction induced deeper and longer hypothermia after 2 days (daily torpor bouts). Fig. 1 shows an example of a daily torpor that occurred after 4 days of food restriction. Spontaneous warming up was rapid and linear, Tb rising from 27.7°C to 34.7°C within 3 h ($r = 0.959$, $n = 4$). Afterward, Tb reached a plateau above 35°C corresponding to basal metabolic rate. The same pattern of arousal was observed after handling of the animal (provoked arousal).

Increase of O2 consumption during provoked arousal from torpor. The initial body mass of the animals averaged 112 ± 2 g ($n = 8$). Although they stayed almost motionless, animals previously normothermic significantly increased their body temperature within the 80 minutes following handling, Tb rising from 35.0 ± 0.4°C to 37.1 ± 0.3°C ($t = 3.5$, df = 7, $P = 0.01$). After 2 days of food restriction, all animals were torpid prior to their transfer into the respirometer. A warming up corresponding to
arousal from daily torpor was observed within the 70 minutes of O₂ consumption measurements, Tb rising from 30.4 ± 0.6°C to 36.4 ± 0.4°C (t = 9.2, df = 7, P < 0.0001). During warming up, maximum O₂ consumption occurred within the first 10 minutes following handling, and was significantly higher during arousal from daily torpor than during normothermy, and reached 2.49 ± 0.22 mlO₂ / g * h versus 1.54 ± 0.16 mlO₂ / g * h, respectively (t = 3.5, df = 7, P = 0.01, Fig. 2). During both warming up from daily torpor and from normothermy, O₂ consumption decreased within the following 10 minutes (F = 34.3, df = 1/7, P = 0.001). From the 20th to 30th minute following handling, O₂ consumption did not change significantly (F = 1.6, df = 1/7, P = 0.25). From the 30th to the 80th minute following handling, O₂ consumption remained at a low level, minimum O₂ consumption reaching 0.71 ± 0.06 mlO₂ / g * h, corresponding to resting metabolic rate (table 1). Resting metabolic rate was significantly correlated with body mass (r = 0.715, n = 8). The increase of body temperature was correlated with total O₂ consumption, but only during arousal from daily torpor (r = 0.759, n = 8). The maximum rate of metabolism was observed in the warming up from the deepest torpor bout, Tb rising from 27°C to 36°C, maximum O₂ consumption reaching 2.86 mlO₂ / g * h, and total O₂ consumption reaching 1.77 mlO₂ / g * h. No significant sex-specific difference was observed either in the rate of metabolism or in warming up (O₂ consumption: F = 0.05, df = 1/14, P = 0.81; Tb: F = 0.1, df = 1/14, P = 0.72). The peak of O₂ consumption was extremely transient and generally occurred as early as the first air injection, suggesting that warming up had started before the transfer into the respirometer. In 4 cases, maximum O₂ consumption was observed at the second air injection and a kinetic curve was
obtained: Fig. 3 showed that warming up actually occurred within the 5 minutes following handling of the animals.

Metabolic response to injection of isoproterenol. Oxygen consumption was measured in 4 previously normothermic animals. The initial body mass of the animals averaged 109 ± 1 g (n = 4). The control injection did not lead to any change in O₂ consumption which averaged 0.79 ± 0.10 mlO₂ / g * h (F = 0.92, df = 5/15, P = 0.5, Fig. 4). By contrast, after injection of 20 µg / 100 g of isoproterenol, O₂ consumption increased significantly (F = 19.7, df = 5/15, P < 0.0001), reaching values significantly higher than after the control injection (F = 23.6, df = 4/3, P < 0.02). Maximum was observed during the first 20 minutes and reached 2.32 ± 0.05 mlO₂ / g * h (F = 0.0, df = 1/3, P = 0.9). A significant decrease was noted afterward, O₂ consumption reaching 1.22 ± 0.11 mlO₂ / g * h the 60th minute after the injection (F = 61.9, df = 1/3, P = 0.004). This value was not significantly different from O₂ consumption observed the 60th minute after the control injection (F = 6.0, df = 4/3, P = 0.09). The injection of 200 µg isoproterenol / 100 g body mass led to a high increase of O₂ consumption that was significantly different from increase following 20 µg injection (F = 4.6, df = 20/15, P = 0.002). Within the 10 minutes following the injection of 200 µg isoproterenol / 100 g, O₂ consumption increased significantly and reached its maximum value of 3.04 ± 0.14 mlO₂ / g * h, significantly higher than after 20 µg injection (F = 15.2, df = 4/3, P < 0.03). Oxygen consumption decreased significantly within the following 10 minutes and remained at a high level until the 60th minute following the injection (respectively, F = 11.1, df = 1/3, P < 0.05; F = 1.8, df = 1/3, P = 0.3). Between the 50th and the 60th minutes following the injection, O₂ consumption was still higher than after 20 µg injection (F = 9.9, df = 4/3, P < 0.05).
Since the thermogenic response to isoproterenol was maximum within the 10 minutes following the injection, maximum thermogenesis was assessed by 10 minute-measurements of oxygen consumption. Initial body mass of the animals averaged $118 \pm 8$ g ($n = 8$). All the animals tested were previously normothermic with no significant difference between the doses injected, and Tb averaged $35.8 \pm 0.1^\circ C$ ($F = 0.7$, $df = 4/28$, $P = 0.57$). Injection of isoproterenol led to an increase of $O_2$ consumption within the first 10 minutes and this response was dose-dependent ($F = 26.4$, $df = 4/28$, $P < 0.0001$, Fig. 5). The maximum metabolic response, obtained with the highest dose of isoproterenol (200 µg / 100 g), averaged $2.19 \pm 0.18$ mlO$_2$/g * h ($n = 8$), which was not significantly different from maximum values obtained during warming up from daily torpor ($F = 1.1$, $df = 1/14$, $P = 0.30$, table 1). This dose led to a significant increase of body temperature, from $36.1 \pm 0.4^\circ C$ to $37.4 \pm 0.3^\circ C$ after 10 minutes ($F = 23.1$, $df = 1/7$, $P = 0.002$). After 24 h of cold exposure ($5^\circ C$), the maximum metabolic rate obtained with the injection of 200 µg of isoproterenol increased significantly, reaching $2.40 \pm 0.17$ mlO$_2$/g * h ($t = 5.8$, $df = 1/7$, $P = 0.001$, table 1). The increase of Tb was not significantly different before and after cold exposure ($F = 0.7$, $df = 8/7$, $P = 0.71$). However, both initial and final body temperatures were lower after cold exposure, although measurements were performed at $28^\circ C$ (initial, $F = 10.0$, $df = 1/7$, $P < 0.02$; final, $F = 15.6$, $df = 1/7$, $P < 0.01$). After cold exposure, the warming up led to significant weight loss ($114 \pm 7$ to $112 \pm 7$g, $F = 19.4$, $df = 1/7$, $P = 0.003$). Furthermore, panting, sweating and intense salivation were noted 1 hour after the injection.

**Macroscopic analysis of brown fat.** Brown fat anatomical locations in mouse lemurs were found to be the axillary, cervical and interscapular regions, around the
heart and the aorta and in the abdominal cavity (along the aorta and around the kidneys). At each of these sites the fat tissue collected clearly looked like typical brown fat from rodents. Epididymal, inguinal, bladder and tail depots were white adipose tissue.

**Histological analysis of brown fat.** To identify cells expressing UCP1, we performed immunocytochemical experiments on previously dissected brown (Fig. 6A to 6D) and white adipose tissue (Fig. 6E, F). All cells from brown adipose tissue (BAT) were multilocular. Fig. 6A and 6B show sections of axillary deposit from a mouse lemur exposed to 25°C (W). Fig. 6A was a control showing no staining whereas Fig. 6B showed blue staining revealing the presence of UCP1. This feature of staining appeared in perirenal adipose tissue from mouse lemur W (Fig. 6C) and mouse lemur C (Fig. 5D). The specific labeling of UCP1 was increased in perirenal BAT of mouse lemur C, that had been exposed to cold (7°C).

Tail and bladder deposits both showed characteristics specific to typical white adipose tissue with a large central droplet surrounded by red-stained cytoplasm. No sections ever exhibited UCP staining (Fig. 6E-F).

**Western blotting of UCP1.** Using anti-UCP1 antibodies, we screened western blots of mitochondrial protein from BAT pads of two mouse lemurs. The antibody raised against rabbit antimouse UCP1 cross-reacted with a mouse lemur fat mitochondrial protein (Fig. 7). This protein had a molecular mass corresponding to the apparent molecular mass of UCP (#32kDa). Perirenal and axillary adipose tissues displayed highly positive UCP signals in the two animals tested. The exposure to cold (7°C) increased positive signals in axillary BAT (Fig. 7).

**DISCUSSION**
This study provides the first evidence of the presence of brown fat and the use of non-shivering thermogenesis in the gray mouse lemur *Microcebus murinus*. The brown fat, that looked like typical BAT from rodents, was anatomically located in axillary, cervical, and interscapular regions, and around the heart, the aorta and the kidneys. The uncoupling protein UCP1, was specifically identified using antibodies against mouse UCP1. Histological identification of BAT has been documented in primates, mainly in new born animals (22, 44, 45). In gray mouse lemurs, non-shivering thermogenesis was stimulated by the β-adrenergic agonist isoproterenol. The dose-response curve appeared very similar to those obtained in rodents (17, 53). Maximum oxygen consumption obtained with the highest dose of isoproterenol used to give maximum thermogenesis, was about 3 times higher than the resting metabolic rate. Among primates, non-shivering thermogenesis has only been evidenced in diet-induced thermogenesis of the common marmoset (44).

Non-shivering thermogenesis is used in the arousal from daily torpor in the gray mouse lemur. In constant ambient temperatures of 23-25°C, spontaneous arousal from food restriction-induced torpor occurred rapidly. Thermogenic activity was evaluated by the comparison of O₂ consumption of the same animals from normothermy and from daily torpor. Handling of torpid animals induced an increase of O₂ consumption that resulted in rapid warming up of over 10 minutes. The same animals from normothermy showed a lower increase of oxygen consumption that also induced an increase of body temperature. Kinetic curves showed that the peak of oxygen consumption occurred within the 5 minutes following handling. Likewise, the high metabolic rates obtained with the control injection, compared to the resting metabolic rate, can be attributed to stress and secretion of adrenaline (38, 39). Non-shivering thermogenesis may thus be
interpreted as an antipredator adaptive feature. Indeed, several predators of mouse lemurs have been reported to visit resting sites (18, 30).

The arousal from daily torpor has been described in wild animals as a two-step process with an initial passive climb of body temperature following the increase of ambient temperature, and active heat production initiated when a body temperature of about 25°C is reached (46). Instantaneous measurements were used in this study, and the extremely transient increase of O₂ consumption may have been missed in some cases, whereas a peak of O₂ consumption of about 200 mlO₂ / h was observed in at least one case (46). In fact, passive warming up should occur only in high ambient temperatures. Table 1 gives a comparison between our study and measurements performed on wild animals maintained in semi-captivity. In fact, due to their low body mass, maximum specific metabolic rate of wild animals was not lower than in captive animals and reached 2.7 ± 0.8 mlO₂ / g * h. Note that resting metabolic rate is much higher in wild animals than in captive animals (46). Moreover, cold-exposed animals shivered suggesting that shivering may be used under low ambient temperature. Shivering thermogenesis may be used during the first step of arousal from daily torpor, as observed in cold-exposed torpid animals (Perret, pers. com.) and as already shown in rodents (7, 27).

Mouse lemurs may use non-shivering thermogenesis under cold exposure. Maximum thermogenesis, obtained with the injection of 200 µg of isoproterenol was significantly increased by 24 h of cold exposure (5°C). In a single individual compared to a control animal, expression of UCP uncoupling protein seemed to be increased by cold exposure (7°C). Animals treated with the highest dose of isoproterenol kept an extremely high metabolic rate for 70 min and showed a cooling response involving
intense sweating, panting and salivation as already found in a strepsirhine primate submitted to high ambient temperatures (32).

The animals used in the present study were born in a captive colony from stock originally caught in Madagascar over 30 years ago. In constant conditions of ambient temperature, the animals have kept their thermogenic capacity, as well as their annual rhythm of reproductive function and body mass changes. In the present study, the stimulation of non-shivering thermogenesis by isoproterenol led to a decrease of body mass probably corresponding to water loss. As already hypothesized in rodents, non-shivering thermogenesis may contribute to the control of body mass (43). Indeed, the transfer from short photoperiod to long photoperiod leads to a decrease of body mass associated with a slow increase of food intake (16). Conversely, the transfer from long photoperiod to short photoperiod leads to a rapid increase in body mass due to an increase of food intake and possibly to a decrease of energy expenditure (16). Thus, non-shivering thermogenesis is likely to play a central role in the regulation of energy balance in the gray mouse lemur.

Perspectives

The present work focuses on animals exposed to short photoperiod. The gray mouse lemur has been shown to be highly seasonal and further studies dealing with seasonal changes in BAT and UCP production may be of interest. Further studies should also investigate molecular regulation of non-shivering thermogenesis, well known in rodents, and control of lipolysis and flux of fat from white adipose tissue to brown adipose tissue. Thermoregulation may have implications for feeding behavior and use of dietary fatty acids. Moreover, passive warming up may be obtained experimentally with increasing ambient temperature. We suggest interspecific comparisons be made between mouse lemur species and the sibling fat-tailed dwarf
lemur *Cheirogaleus medius*, which is a true hibernator. Finally, UCP1 has recently been found in mouse longitudinal smooth muscle cells of sexual and gastrointestinal tracts (34), suggesting that UCP1 may be involved in the relaxation of smooth muscle layers. Gray mouse lemurs appear as a convenient model of primate for studies on BAT and extra-BAT uncoupling proteins.
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FIGURE LEGENDS

Fig. 1 Daily variations of body temperature (Tb) of the same male gray mouse lemur during *ad libitum* feeding and after 4 days of food restriction (~30 % of *ad libitum* feeding) leading to daily torpor.

Fig. 2 Oxygen consumption *versus* time (min) after handling in 8 gray mouse lemurs during provoked arousal from daily torpor (daily torpor) and after the handling of the same animals under normothermy (normothermy), that lead to lower warming up. Hypothermia was induced by a 2-day food restriction (~30 % of *ad libitum* feeding). Body temperature was above 33°C under normothermy and below 33°C (from 27 to 32°C) in daily torpor. Values are means ± SE.

Fig. 3 Oxygen consumption *versus* time (min) 4 gray mouse lemurs during provoked arousal from daily torpor (body temperature from 29 to 32°C). Oxygen consumption was measured by noting the time at each injection of the same volume of ambient air into the respirometry chamber. Hypothermia was induced by a 2-day food restriction (~30 % of *ad libitum* feeding). Warming up was provoked by handling of the animals which started about 3 minutes before transfer into the respirometer.

Fig. 4 Oxygen consumption *versus* time (min) in 4 normothermic gray mouse lemurs after the injection of 2 doses of a β-adrenergic agonist, isoproterenol,
20 µg / 100 g body mass (20) and 200 µg / 100 g (200), and after a control injection of saline vehicle (0). Values are means ± SE.

**Fig. 5** Dose-response curve obtained with the injection of 4 different doses of isoproterenol (2 µg/100g, 4 µg/100g, 20µg/100g, 200µg/100g) and a control injection of saline vehicle in 8 male gray mouse lemurs. Oxygen consumption was measured during the first 10 minutes following the injection. Values are means ± SE.

**Fig. 6** In histochemical experiments, *dark blue* precipitates indicate BCIP/NBT deposit following alkaline phosphatase reaction coupled to second antibody. Counterstain was *nuclear red*. Histological sections of brown adipose tissue (BAT) from two male mouse lemurs: an antibody against rat UCP1 was used for immunochemical revealing (B-D) and compared with a control without antibody (A): A and B, axillary BAT; C and D, perirenal BAT. One animal was exposed to cold (D) and the other maintained at ~25°C (A to C). Histological sections of white adipose tissue using the same antibody are added for comparison (E, bladder, F, tail) and did not show any blue labeling.

**Fig. 7** Immunological identification of UCP1 in adipose tissue mitochondria by western blotting: gray mouse lemur perirenal and axillary brown adipose tissue (BAT); rat interscapular BAT. 25°C, 7°C: axillary deposit of a mouse lemur exposed to ambient temperature (~25°C) and a mouse lemur exposed to cold
(7°C). Detection was enhanced by chemiluminescence, with 18 min exposure to film. 0.1 µg of mitochondrial protein was applied to each lane.
FOOTNOTES FOR TABLE

Table 1  Values are means ± SE. N, normothermy, DT: daily torpor; TMR: torpid metabolic rate; RMR: resting metabolic rate; MMR: maximum metabolic rate during arousal from torpor or after injection of 200 µg / g of β-adrenergic agonist (isoproterenol); 25°C: measurements after exposure to ambient temperature; 5°C: measurements after cold exposure (5°C). * Values obtained from wild animals in semi-captive conditions (46).
Fig. 1

The figure shows the body temperature (Tb) over time under different conditions: dark and light periods. The shaded areas represent the dark periods, and the curves represent the temperature changes with and without food restriction. The dashed line indicates the ad libitum condition, while the solid line shows the food restriction condition. The temperature fluctuates significantly during the light period.
Fig. 2

The graph illustrates the oxygen consumption (mlO₂/g*h) over time (min) in two conditions: normothermy and daily torpor. The oxygen consumption decreases sharply in normothermy, while in daily torpor, it remains relatively stable with a slight increase over time.
Fig. 3
Fig. 4

O₂ consumption (mL O₂/g·h)

- 0
- 20
- 200

0 0.5 1.0 1.5 2.0 2.5 3.0 3.5

0 10 20 30 40 50 60

time (min)
Fig. 5

O₂ consumption (mL O₂/g*hr) vs. isoproterenol (µg/100g)
Table 1. \(O_2\) consumption during daily torpor, diurnal rest, and increased thermogenesis

<table>
<thead>
<tr>
<th>maximum thermogenesis</th>
<th>arousal from torpor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>this study</td>
</tr>
<tr>
<td></td>
<td>N = 8</td>
</tr>
<tr>
<td></td>
<td>body mass (g)</td>
</tr>
<tr>
<td></td>
<td>114 ± 7</td>
</tr>
<tr>
<td></td>
<td>TMR (mlO_2/h)</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>RMR (mlO_2/h)</td>
</tr>
<tr>
<td></td>
<td>164.9 ± 9.2</td>
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<td></td>
<td>MMR (mlO_2/h)</td>
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<tr>
<td></td>
<td>250.1 ± 10.3</td>
</tr>
<tr>
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<td>(25°C)</td>
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