Am J Physiol Regul Integr Comp Physiol 284: R811–R818, 2003.
First published November 21, 2002; 10.1152/ajpregu.00525.2002.

Brown fat and nonshivering thermogenesis in the gray mouse lemur (*Microcebus murinus*)

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Submitted 29 August 2002; accepted in final form 18 November 2002

Génin, F., M. Nibbelink, M. Galand, M. Perret, and L. Ambid. Brown fat and nonshivering thermogenesis in the gray mouse lemur (*Microcebus murinus*). *Am J Physiol Regul Integr Comp Physiol* 284: R811–R818, 2003. First published November 21, 2002; 10.1152/ajpregu.00525.2002.—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 nonshivering thermogenesis (NST). Under thermoneutrality (28°C), warming-up from daily torpor (body temperature <33°C) involved a rapid (<5 min) increase of O_2 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, anatomic and histological examination using an antibody against uncoupling protein (UCP) specifically demonstrated the presence of brown fat. With the use of 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.

thermoregulation; brown adipose tissue; arousal from daily torpor; isoproterenol; uncoupling protein

THERMOREGULATION INVOLVES various strategies. High body mass (BM) 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 microhabitats, build nests and burrows, or huddle together. Endotherms are able to withstand extreme ambient temperatures by maintaining a constant high body temperature (Tb) (3, 51).

Under thermoneutrality, all heat production is provided by the basal metabolic rate (MR). 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 nonshivering thermogenesis (NST) (19, 23, 27). SH involves skeletal muscles, whereas NST involves a unique thermogenic effector organ, brown adipose tissue (BAT) (35, 49). Brown fat is present in various species including hibernators and nonhibernators (44). Thermogenesis in BAT results from an increase in the rate of substrate oxidation in mitochondria caused by a proton conductance pathway through 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). NST has been shown to be induced by the sympathoadrenal system, and injection of norepinephrine or β-adrenergic agonist (isoproterenol) has been used to induce NST 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 Tb and metabolism that can last less than 24 h [daily torpor (DT)] or more (hibernation) (14, 50). Torpor bouts are induced by both cold exposure and food or water restriction (8, 31). In addition, DT 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 DT (11). Both SH and NST 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, DT, and huddling behavior (4, 5, 16, 36, 37, 40, 41, 46–48). DT is well documented in *Microcebus murinus,* and studies on the reduction of MR and Tb have been

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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 DT has been shown to increase under cold exposure (5). Wild animals maintained in captivity and exposed to ambient temperatures show no increase in MR during arousal from spontaneous DT 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.

MATERIALS 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 and Human Beings" by the American Physiological Society (2). All animals were 2- to 6-yr-old adults. 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 × 0.3 × 0.3 m) provided with a nest-box and branches and were separated from each other. Animals had been exposed to short photoperiod (10:14-h light-dark cycle) for 3 mo. All were reproductively inactive and showed the high BM corresponding to the winter-like short photoperiod (BM >90 g). Food and water were supplied ad libitum. DT bouts were induced by food restriction when animals were fed 8–10 kcal/day (15). Animals were considered in DT when minimum Tb was below 33°C.

Tb. To observe the warming-up corresponding to the spontaneous arousal from DT, Tb 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) was implanted into the visceral cavity, while the lemurs were under ketamine anesthesia (Imalgene 500 mg, 10 mg/100 g). Recovery occurred in 14 days and animals were used at least 1 mo after surgery. The receiver board (RLA1020, Data Science) was positioned in front of the nest-box. Tb was recorded every 5 min. 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 Tb (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 min in eight animals (4 males and 4 females) by the determination of O_2 consumption in a closed-circuit respirometer as already described (1). 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 ml O_2). 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 and *2*) within the first 6 h of daylight, after 2 days of food restriction while the animals entered DT bouts (Tb <33°C). Possible movements of the animals could be checked through the respirometer that was transparent. Rectal temperature was measured again after each measurement of O_2 consumption. Because BM might change during increased MR, oxygen consumption was expressed, for each measurement, in milliliters of O_2 per BM per hour, where BM was initial BM in grams.

The thermogenic response to intramuscular injection of two doses of a β-adrenergic agonist (isoproterenol) was measured in the same way for 60 min (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 four 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 O_2 consumption occurring within the 10 min following the injection of isoproterenol (0, 2, 4, 20, and 200 μg/100 g) was measured at the same time of day in eight normothermic males in a closed-circuit respirometer, as already described (16). The animals were placed in a closed respiratory chamber of 1,655-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 min, O_2 consumption was measured using a Servomex 570 A paramagnetic gas analyzer (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 an overdose of CO_2. 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 h at room temperature with 0.5 μg/ml anti-UCP1 antibody (purified rabbit IgG against mouse UCP1-UCP 11-A, Alpha Diagnostic International, San Antonio, TX). Second antibody (Jackson Immunoresearch, West Grove, PA) coupled to alkaline phosphatase (1:200) was visualized using BCIP/NBT (K 598, Dako, Carpinteria, CA). Endogenous alkaline phosphatase activity was inhibited by levamisole (X 3021, Dako). 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). Zero point one or zero point two micrograms of tissue protein from total homogenate or mitochondrial fraction were electrophoresed in 10% polyacrylamide SDS. 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 1:8,000, was revealed using the kit ECL (Amersham RPN 2106) and Hyper film ECL-TM (RPN 310–3H, Amersham). The blots were exposed for 18 min. A positive control was performed by 0.1 μg of rat-BAT mitochondria protein. To avoid any contamination, the nonfluorescent molecular weight marker was placed between BAT deposit and positive control.

Data analyses. All values are means ± SE. 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 vs. DT, responses to different doses of isoproterenol, effect of cold exposure), we used Student's paired *t*-test, 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 O_2 consumption during the arousal from torpor and after the injection of isoproterenol, we used ANOVA. Multiple pairwise 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 DT. 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 DT (Fig. 1). Whatever the food intake, minimum Tb was reached during the first 3 h of the day. However, duration and depth of DT were increased by food restriction. Although Tb remained above 33°C during ad libitum feeding, food restriction induced deeper and longer hypothermia after 2 days (DT bouts). Figure 1 shows an example of a DT that occurred after 4 days of food restriction. Spontaneous warming-up was rapid and linear, Tb rising from 27.7 to 34.7°C within 3 h (r = 0.959, n = 4). Afterward, Tb reached a plateau above 35°C corresponding to basal MR. The same pattern of arousal was observed after handling of the animal (provoked arousal).

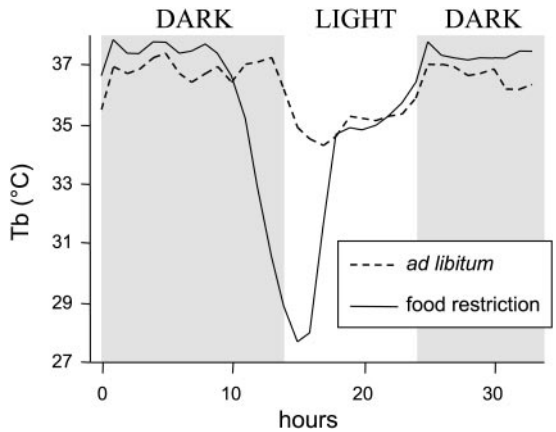

Fig. 1. Daily variations of body temperature (Tb) of the same male gray mouse lemur during ad libitum feeding and after 4 days of food restriction (~30% of ad libitum feeding) leading to daily torpor.

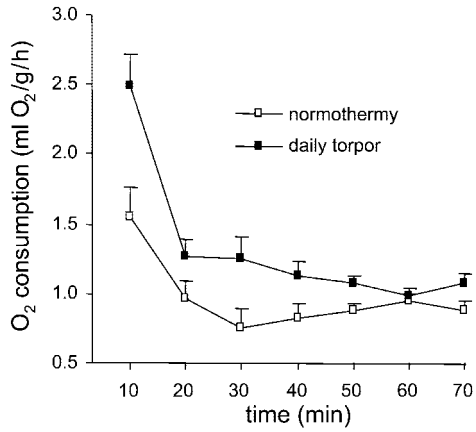

Fig. 2. Oxygen consumption vs. time (minutes) after handling in 8 gray mouse lemurs during provoked arousal from daily torpor and after the handling of the same animals under normothermy, which led to lower warming-up. Hypothermia was induced by a 2-day food restriction (~30% of ad libitum feeding).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

Increase of O_2 consumption during provoked arousal from torpor. The initial BM of the animals averaged 112 ± 2 g (n = 8). Although they stayed almost motionless, animals previously normothermic significantly increased their Tb within the 80 min following handling, Tb rising from 35.0 ± 0.4 to 37.1 ± 0.3°C (t = 3.5, df = 7, P = 0.01). After 2 days of food restriction, all animals were torpid before their transfer into the respirometer. A warming-up corresponding to arousal from DT was observed within the 70 min of O_2 consumption measurements, Tb rising from 30.4 ± 0.6 to 36.4 ± 0.4°C (t = 9.2, df = 7, $P < 0.0001$). During warming-up, maximum O_2 consumption occurred within the first 10 min following handling and was significantly higher during arousal from DT than during normothermy and reached 2.49 ± 0.22 vs. 1.54 ± 0.16 ml $O_2 \cdot g^{-1} \cdot h^{-1}$, respectively ($t$ = 3.5, df = 7, P = 0.01; Fig. 2). During both warming-up from DT and from normothermy, O_2 consumption decreased within the following 10 min (F = 34.3, df = 1/7, P = 0.001). From the 20th to 30th minute following handling, O_2 consumption did not change significantly (F = 1.6, df = 1/7, P = 0.25). From the 30th to the 80th minute following handling, O_2 consumption remained at a low level, minimum O_2 consumption reaching 0.71 ± 0.06 ml $O_2 \cdot g^{-1} \cdot h^{-1}$, corresponding to resting MR (Table 1). Resting MR was significantly correlated with BM (r = 0.715, n = 8). The increase of Tb was correlated with total O_2 consumption but only during arousal from DT (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 to 36°C, maximum O_2 consumption reaching 2.86 ml $O_2 \cdot g^{-1} \cdot h^{-1}$, and total O_2 consumption reaching 1.77 ml $O_2 \cdot g^{-1} \cdot h^{-1}$. No significant sex-specific difference was observed either in the rate of metabolism or in warming-up (O_2 consump-

Table 1. *O_2 consumption during daily torpor, diurnal rest, and increased thermogenesis*

	Maximum Thermogenesis	Arousal From Torpor		
		This study $n = 8$		Schmid, 2000* $n = 10$
	This study $n = 8$	N	DT	DT
Body mass, g	114 ± 7	112 ± 2	108 ± 3	62.2 ± 13.4
TMR, ml O_2/h				28.4 ± 13.2
RMR, ml O_2/h	164.9 ± 9.2	94.5 ± 4.2	88.5 ± 8.2	132.6 ± 50.5
MMR, ml O_2/h	250.1 ± 10.3 (25°C) 267.3 ± 9.3 (5°C)		267.8 ± 20.9	169.5 ± 75.2

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 semicaptive conditions (46).

tion: $F = 0.05$, df = 1/14, $P = 0.81$; Tb: $F = 0.1$, df = 1/14, $P = 0.72$). The peak of O_2 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 four cases, maximum O_2 consumption was observed at the second air injection and a kinetic curve was obtained. Figure 3 showed that warming-up actually occurred within the 5 min following handling of the animals.

Metabolic response to injection of isoproterenol. Oxygen consumption was measured in four previously normothermic animals. The initial BM of the animals averaged 109 ± 1 g ($n = 4$). The control injection did not lead to any change in O_2 consumption that averaged 0.79 ± 0.10 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ ($F = 0.92$, df = 5/15, $P = 0.5$; Fig. 4). By contrast, after injection of 20 μg/100 g of isoproterenol, O_2 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 min and reached 2.32 ± 0.05 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ ($F = 0.0$, df = 1/3, $P = 0.9$). A significant decrease was noted afterward, O_2 consumption reaching 1.22 ± 0.11 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ the 60th minute after the injection ($F = 61.9$, df = 1/3, $P = 0.004$). This value was not significantly different from O_2 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 BM led to a high increase of O_2 consumption that was significantly different from increase following 20 μg injection ($F = 4.6$, df = 20/15, $P = 0.002$). Within the 10 min following the injection of 200 μg isoproterenol/100 g, O_2 consumption increased significantly and reached its maximum value of 3.04 ± 0.14 ml $O_2 \cdot g^{-1} \cdot h^{-1}$, significantly higher than after the 20-μg injection ($F = 15.2$, df = 4/3, $P < 0.03$). Oxygen consumption decreased significantly within the following 10 min 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 60th minutes following the injection, O_2 consumption was still higher than after the 20-μg injection ($F = 9.9$, df = 4/3, $P < 0.05$).

Because the thermogenic response to isoproterenol was maximum within the 10 min following the injection, maximum thermogenesis was assessed by 10-min measurements of O_2 consumption. Initial BM of the animals averaged 118 ± 8 g ($n = 8$). All the animals tested were previously normothermic with no signifi-

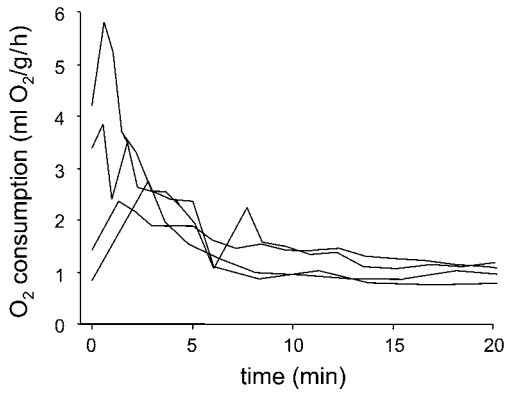

Fig. 3. Oxygen consumption vs. time (minutes) in 4 gray mouse lemurs during provoked arousal from daily torpor (Tb from 29 to 32°C). Oxygen consumption was measured by noting the time at each injection of the same volume of ambient air into the respirometry chamber. Hypothermia was induced by a 2-day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

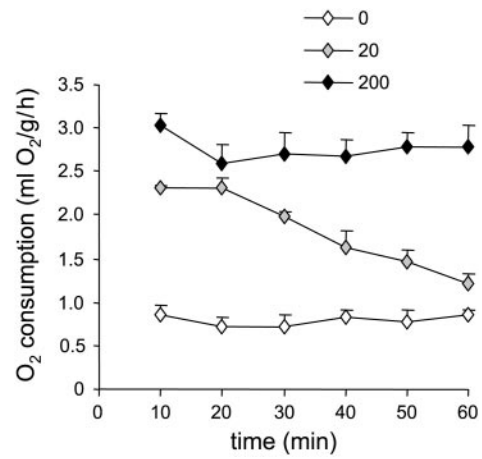

Fig. 4. Oxygen consumption vs. time (minutes) in 4 normothermic gray mouse lemurs after the injection of 2 doses of β-adrenergic agonist isoproterenol, 20 μg/100 g body mass (20) and 200 μg/100 g body mass (200), and after a control injection of saline vehicle (0). Values are means ± SE.

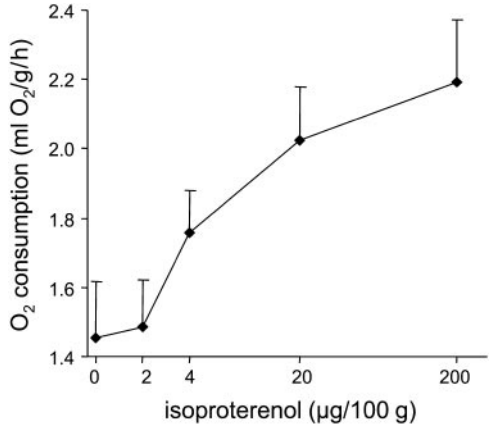

Fig. 5. Dose-response curve obtained with the injection of 4 different doses of isoproterenol (2, 4, 20, and 200 μg/100 g) and a control injection of saline vehicle in 8 male gray mouse lemurs. Oxygen consumption was measured during the first 10 min following the injection. Values are means ± SE.

cant difference between the doses injected, and Tb averaged 35.8 ± 0.1°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 min 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 ± 0.18 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ ($n = 8$), which was not significantly different from maximum values obtained during warming-up from DT ($F = 1.1$, df = 1/14, $P = 0.30$; Table 1). This dose led to a significant increase of Tb, from 36.1 ± 0.4 to 37.4 ± 0.3°C after 10 min ($F = 23.1$, df = 1/7, $P = 0.002$). After 24 h of cold exposure (5°C), the maximum MR obtained with the injection of 200 μg of isoproterenol increased significantly, reaching 2.40 ± 0.17 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ ($t = 5.8$, df 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 Tbs were lower after cold exposure, although measurements were performed at 28°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 ± 7 to 112 ± 7 g, $F = 19.4$, df = 1/7, $P = 0.003$). Furthermore, panting, sweating, and intense salivation were noted 1 h after the injection.

Macroscopic analysis of brown fat. Brown fat anatomic locations in mouse lemurs were found to be the axillary, cervical, and interscapular regions, around the heart and 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. 6, *A–D*) and white adipose tissue (Fig. 6, *E* and *F*). All cells from BAT were multilocular. Figure 6, *A* and *B*, shows sections of axillary deposit from a mouse lemur exposed to 25°C (W). Figure 6*A* was a control showing no staining, whereas Fig. 6*B* showed blue staining revealing the presence of UCP1. This feature of staining appeared in perirenal adipose tissue from mouse lemur W (Fig. 6*C*) and mouse lemur C (Fig. 6*D*). The specific labeling of UCP1 was increased in perirenal BAT of mouse lemur C, which 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. 6, *E* and *F*).

Western blotting of UCP1. With the use of anti-UCP1 antibodies, we screened Western blots of mitochondrial protein from BAT pads of two mouse lemurs. The

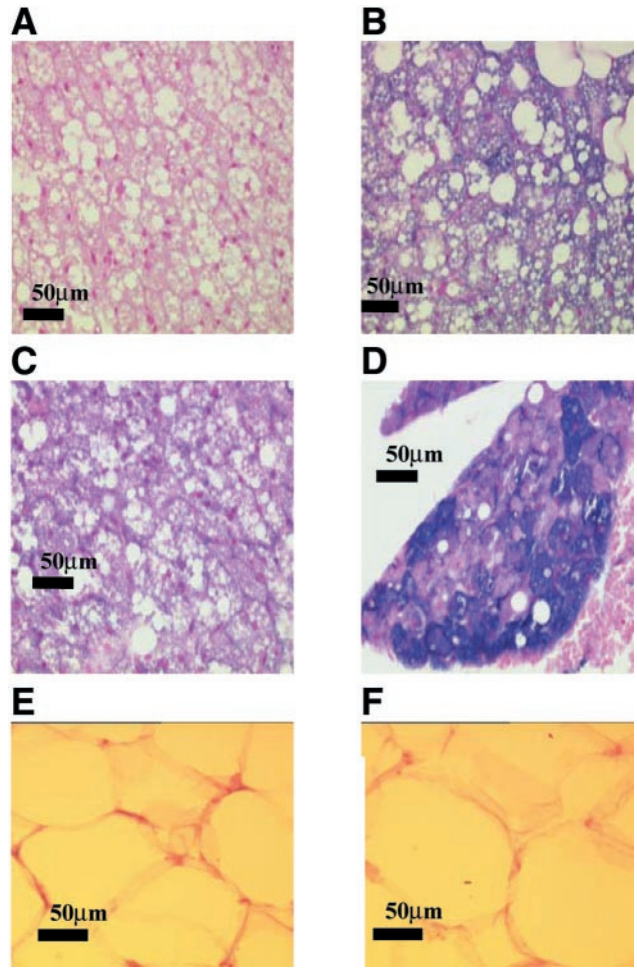

Fig. 6. In histochemical experiments, dark blue precipitates indicate 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT) deposit following alkaline phosphatase reaction coupled to second antibody. Counterstain was nuclear red. Histological sections of brown adipose tissue (BAT) from 2 male mouse lemurs: an antibody against rat uncoupling protein (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.

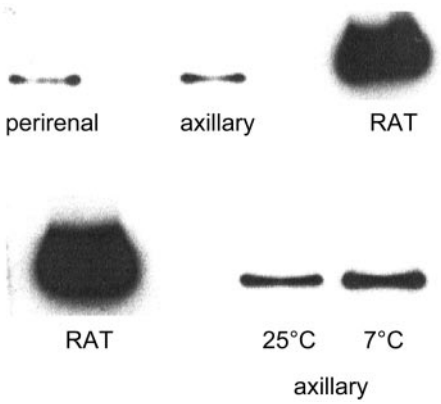

Fig. 7. Immunological identification of UCP1 in adipose tissue mitochondria by Western blotting: gray mouse lemur perirenal and axillary BAT and rat interscapular BAT. 25 And 7°C: axillary deposit of a mouse lemur exposed to ambient temperature (~25°C) and a mouse lemur exposed to cold (7°C). Detection was enhanced by chemiluminescence, with an 18-min exposure to film. Zero point one micrograms of mitochondrial protein were applied to each lane.

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 (32 kDa). 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 NST in the gray mouse lemur *Microcebus murinus*. The brown fat, which looked like typical BAT from rodents, was anatomically located in axillary, cervical, and interscapular regions and around the heart, aorta, and kidneys. UCP1 was specifically identified using antibodies against mouse UCP1. Histological identification of BAT has been documented in primates, mainly in newborn animals (22, 44, 45). In gray mouse lemurs, NST 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 three times higher than the resting MR. Among primates, NST has only been evidenced in diet-induced thermogenesis of the common marmoset (44).

NST is used in the arousal from DT 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 O_2 consumption of the same animals from normothermy and from DT. Handling of torpid animals induced an increase of O_2 consumption that resulted in rapid warming-up of over 10 min. The same animals from normothermy showed a lower increase of O_2 consumption that also induced an increase of Tb. Kinetic curves showed that the peak of O_2 consumption occurred within the 5 min following handling. Likewise, the high MRs obtained with the control injection, compared with the resting MR, can be attributed to stress and secretion of epinephrine (38, 39). NST may thus be interpreted as an anti-predator adaptive feature. Indeed, several predators of mouse lemurs have been reported to visit resting sites (18, 30).

The arousal from DT has been described in wild animals as a two-step process with an initial passive climb of Tb following the increase of ambient temperature and active heat production initiated when a Tb of ~25°C is reached (46). Instantaneous measurements were used in this study, and the extremely transient increase of O_2 consumption may have been missed in some cases, whereas a peak of O_2 consumption of ~200 ml O_2/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 semicaptivity. In fact, due to their low BM, maximum specific MR of wild animals was not lower than in captive animals and reached 2.7 ± 0.8 ml $O_2 \cdot g^{-1} \cdot h^{-1}$. Note that resting MR 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. SH may be used during the first step of arousal from DT, as observed in cold-exposed torpid animals (M. Perret, personal communication) and as already shown in rodents (7, 27).

Mouse lemurs may use NST 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 with a control animal, expression of UCP seemed to be increased by cold exposure (7°C). Animals treated with the highest dose of isoproterenol kept an extremely high MR 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 BM changes. In the present study, the stimulation of NST by isoproterenol led to a decrease of BM probably corresponding to water loss. As already hypothesized in rodents, NST may contribute to the control of BM (43). Indeed, the transfer from short photoperiod to long photoperiod leads to a decrease of BM associated with a slow increase of food intake (16). Conversely, the transfer from long photoperiod to short photoperiod leads to a rapid increase in BM due to an increase of food intake and possibly to a decrease of energy expenditure (16). Thus, NST 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 NST, well known in rodents, and control of lipolysis and flux of fat from white adipose tissue to BAT. 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 UCPs.

The authors thank P. Guillou for help in the histological study and C. Carpéné for critically reading the manuscript.

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