Chronic food shortage and seasonal modulations of daily torpor and locomotor activity in the grey mouse lemur (*Microcebus murinus*)

Sylvain Giroud,1,2 Stéphane Blanc,2 Fabienne Aujard,1 Frédéric Bertrand,3 Caroline Gilbert,2 and Martine Perret1

1Mécanismes Adaptatifs et Evolution, UMR 7179 Centre National de la Recherche Scientifique (CNRS), Muséum National d’Histoire Naturelle, Bruxy, France; 2Institut pluridisciplinaire Hubert Curien-Département d’Ecologie, Physiologie, Ethologie UMR 7178 CNRS, Université Louis Pasteur, Strasbourg, France; and 3Institut de Recherche en Mathématique Avancée, Université Louis Pasteur, Strasbourg, France

Submitted 31 October 2007; accepted in final form 16 April 2008

Giroud S, Blanc S, Aujard F, Bertrand F, Gilbert C, Perret M. Chronic food shortage and seasonal modulations of daily torpor and locomotor activity in the grey mouse lemur (*Microcebus murinus*). Am J Physiol Regul Integr Comp Physiol 294: R1958–R1967, 2008. First published April 23, 2008; doi:10.1152/ajpregu.00794.2007.—The extent to which seasonal plasticity in torpor displayed by one of the smallest Malagasy primates (*Microcebus murinus*) will help survival in the context of ongoing global change-induced chronic food shortage, is unknown. Body temperature (Tb) and locomotor activity were measured by telemetry in short- (SD, winter-acclimated) and long-days (LD, summer-acclimated) males (n = 24) during an experimental 35-day calorie restriction of 40 or 80%. Under SD exposure, regardless of calorie restriction intensity, mouse lemur immediately increased torpor depth and duration by 4.6-fold, and showed greater phase-advanced entry into torpor (2.4-fold). Tb adjustments were efficient under 40% calorie restriction to maintain body mass, whereas they did not prevent a 0.71 ± 0.11 g/day mass loss during 80% calorie restriction. The 40% food-deprived LD animals combined an early shallow deepening of torpor (1°C) and a late 18% decrease in locomotor activity, resulting in a moderate 6% mass loss. After 15 days of 80% calorie restriction, LD animals exhibited a SD phenotype by increasing their torpor duration and phase-advancing the entry of torpor (16 min/day). Those adjustments had no impact on mass loss (0.93 ± 0.07 g/day) as locomotor activity increased four-fold. Daily torpor allows *M. murinus* to face moderate food shortage whatever the photoperiod but poorly mitigates energy imbalance during severe food deprivation, especially under SD exposure. Although the behavioral thermoregulation role warrants further investigation in energy savings, *M. murinus* survival would be impaired during long-term food shortage in summer.

body temperature; daily rhythm; energy balance; photoperiod; climate change

GLOBAL CHANGE TRIGGERS ENVIRONMENTAL modifications that seriously threaten biodiversity and species survival, particularly those restricted in distribution to natural “habitat islands” (29). In this context, Madagascar has emerged as a critical hotspot to season, and the vulnerability of this primate species would differ according to species (1, 9, 37, 42, 44, 46). Indeed, the grey mouse lemur (*Microcebus murinus*), one of the smallest primates, shows marked biological rhythms characterized by the succession of an active state during the summer breeding season and, after an autumnal fattening, an optimization of their daily torpor during the winter resting period. Previous works of our group aimed to study the short-term physiological adjustments of these small primates confronted with food shortage both in the short-days (winter-) and in long-days (summer-) acclimated state. A 3-day food starvation (51) and 80% energy restriction over 8 days (14) revealed that short-term food restriction led to a significant increase in locomotor activity and in the frequency of daily torpor in both seasons. A greater plasticity of the body temperature (Tb) adjustments was, however, observed in animals under SD phenotype with torpor depth and duration being 1.5- and 2.4-fold higher, respectively, than in animals under LD exposure (14). These results are consolidated by the observation that the grey mouse lemur in the field enters torpor spontaneously during the dry season but not during the rainy period (49). This seasonal difference in ability to display torpor suggests that adaptive mechanisms developed by *M. murinus* would differ according to season, and the vulnerability of this primate species would mainly depend on seasonal modifications triggered by the ongoing global changes.

One of the main reasons for Malagasy species’ disappearance is the fragmentation of specific habitat due to anthropization (16). In addition to the human impact, global climate change models predict that Madagascar’s climate will experience an increased intensity and frequency of abnormal drought and/or precipitation, as well as cyclone and storm occurrence throughout the year (3, 20). Currently, the wetter conditions in Madagascar, and thus resource availabilities were globally shown to coincide with El Niño phenomenon. More precisely, there is a strong negative correlation between vegetation density indexes and El Niño Southern Oscillation (ENSO). During severe episodes of El Niño, certain regions of the island experience drought conditions lasting up to several months, even in the summer, as observed in 1982–1983 and 1994–1995

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: Institut Pluridisciplinaire Hubert Curien-Département d’Ecologie, Physiologie, Ethologie UMR CNRS Université Louis Pasteur 7178, 23 rue Becquerel 67087 Strasbourg, France. (e-mail: stephane.blanc@c-strasbourg.fr).

The extent to which seasonal changes impact on the body temperature (Tb) of this small primate was measured in short- and long-days (LD, summer-acclimated) males (n = 24) during an experimental 35-day calorie restriction of 40 or 80%. Under SD exposure, regardless of calorie restriction intensity, mouse lemur immediately increased torpor depth and duration by 4.6-fold, and showed greater phase-advanced entry into torpor. Tb adjustments were efficient under 40% calorie restriction to maintain body mass, whereas they did not prevent a 0.71 ± 0.11 g/day mass loss during 80% calorie restriction. The food-deprived LD animals combined an early shallow deepening of torpor and a late 18% decrease in locomotor activity, resulting in a moderate 6% mass loss. After 15 days of 80% calorie restriction, LD animals exhibited a SD phenotype by increasing their torpor duration and phase-advancing the entry of torpor (16 min/day). Those adjustments had no impact on mass loss (0.93 ± 0.07 g/day) as locomotor activity increased four-fold. Daily torpor allows *M. murinus* to face moderate food shortage whatever the photoperiod but poorly mitigates energy imbalance during severe food deprivation, especially under SD exposure. Although the behavioral thermoregulation role warrants further investigation in energy savings, *M. murinus* survival would be impaired during long-term food shortage in summer.

body temperature; daily rhythm; energy balance; photoperiod; climate change

GLOBAL CHANGE TRIGGERS ENVIRONMENTAL modifications that seriously threaten biodiversity and species survival, particularly those restricted in distribution to natural “habitat islands” (29). In this context, Madagascar has emerged as a critical hotspot for biodiversity conservation due to its high level of endemism (17), particularly in primate species.

The Madagascar climate juxtaposes a warm and wet summer with large food availability to a cold and dry winter with drastic food shortage, which endemic species have adapted through efficient physiological and behavioral strategies of energy and water economy. This is particularly the case for the primate members of the family Cheirogaleidae, which show strong seasonal variations in body mass and periods of hibernation or torpor, as reported in the *Cheirogaleus medius* (10, 42) and *Microcebus* species (1, 9, 37, 42, 44, 46). Indeed, the grey mouse lemur (*Microcebus murinus*), one of the smallest primates, shows marked biological rhythms characterized by the succession of an active state during the summer breeding season and, after an autumnal fattening, an optimization of their daily torpor during the winter resting period. Previous works of our group aimed to study the short-term physiological adjustments of these small primates confronted with food shortage both in the short-days (winter-) and in long-days (summer-) acclimated state. A 3-day food starvation (51) and 80% energy restriction over 8 days (14) revealed that short-term food restriction led to a significant increase in locomotor activity and in the frequency of daily torpor in both seasons. A greater plasticity of the body temperature (Tb) adjustments was, however, observed in animals under SD phenotype with torpor depth and duration being 1.5- and 2.4-fold higher, respectively, than in animals under LD exposure (14). These results are consolidated by the observation that the grey mouse lemur in the field enters torpor spontaneously during the dry season but not during the rainy period (49). This seasonal difference in ability to display torpor suggests that adaptive mechanisms developed by *M. murinus* would differ according to season, and the vulnerability of this primate species would mainly depend on seasonal modifications triggered by the ongoing global changes.

One of the main reasons for Malagasy species’ disappearance is the fragmentation of specific habitat due to anthropization (16). In addition to the human impact, global climate change models predict that Madagascar’s climate will experience an increased intensity and frequency of abnormal drought and/or precipitation, as well as cyclone and storm occurrence throughout the year (3, 20). Currently, the wetter conditions in Madagascar, and thus resource availabilities were globally shown to coincide with El Niño phenomenon. More precisely, there is a strong negative correlation between vegetation density indexes and El Niño Southern Oscillation (ENSO). During severe episodes of El Niño, certain regions of the island experience drought conditions lasting up to several months, even in the summer, as observed in 1982–1983 and 1994–1995
El Niño episodes (19), which results in unpredictable food shortage during the food-abundant season. Consequently, given its wide distribution on the west coast of the island, it is likely that the grey mouse lemur uses efficient strategies to cope with both predictable and unpredictable food shortages. The direct corollary is that the summer heterothermy used by these primates may be more plastic than previously thought from short-term food deprivation studies or that it is used in combination with other efficient physiological or behavioural strategies. Such strategies may involve modulation of locomotor activity pattern as demonstrated from rodent studies (18) and suggested from the short-term food deprivation studies on *M. murinus* (14).

Therefore, in the context of global climate change, the objective of the study was to determine the nature and the limits of the seasonal adaptive strategies used by *M. murinus* to face a chronic food shortage. Strategies were assessed through the thermomodulation and locomotor activity responses to moderate and severe experimental food scarcities of 35 days.

**MATERIAL AND METHODS**

**Animals**

The 24 male grey mouse lemurs (*Microcebus murinus*, Cheirogaleidae, Primates) in this study were adults (2 to 5 years old) and were born in the laboratory-breeding colony of the National Museum of Natural History (Brunoy, UMR7179 CNRS/MNHN, France; European Institutions Agreement # 962773) from a stock originally caught in southern Madagascar 35 years ago. Behavioral and physiological seasonal changes of mouse lemurs are dependent on photoperiod and are easily reproduced in captivity (2, 15, 40, 41). None of the animals was studied twice in the present experiment. In the breeding colony, animals were exposed to an artificial photoperiodic regime consisting of alternating 6-mo periods of Malagasy winter-like short-day lengths (L:D 10:14) and of Malagasy summer-like long-day lengths (L:D 14:10). To minimize social influences, animals were housed individually in cages (50 × 30 cm) visually separated from each other. Relative humidity was maintained at a constant 55%, and individuals under LD and SD exposure were kept at ambient room temperature of 30 and 25°C, respectively. Indeed, monthly mean temperatures in Madagascar are 30–31°C at the midsummer (January) and 24–26°C at the mid-winter (July) (21).

**Energy Intake During the Control Period and Calculation of Calorie Restriction**

Before calorie restriction, individual energy requirement was measured during a 10-day control period to determine food-restricted allotments. In ad libitum conditions, animals were fed on fresh banana and a standardized homemade mixture containing baby cereals, spice bread, egg, concentrated milk, white cheese, water, and vitamins and dietary minerals (VitaPalulia/M, Intervet, France and Toison d’or, Clément Thékan, France) in proportions of 0.10 and 0.03% of the total mass of the mixture, respectively. All these ingredients were blended together, so that animals were not able to specifically eat only some of them. The macronutrient composition of the mixture was 50% carbohydrates, 20% proteins, and 30% lipids. Because isolated animals, and particularly those under SD exposure, tend to overfeed and gain mass during the control period, energy intake was clamped to the level required to stabilize their body masses. This procedure was required to avoid a significant underestimation of the calorie restriction intensities. Each individual was initially fed ad libitum and progressively; daily energy intake was narrowed according to the body mass time course. Patterns of Tb and locomotor activity were not modified during the control period, and none of the animals lost weight.

Half of the animals in each photoperiod were then provided 60% (=40% calorie restriction) or 20% (=80% calorie restriction) of individually derived energy requirements. Food-restricted individuals were fed daily with the reference mixture at the onset of the dark phase to avoid disturbances of biological rhythms. Animals ate their food ration in one go at the beginning of the night. Water was provided ad libitum. During the control and food-restricted periods, daily food intake was calculated from the difference between provided and remaining food masses and was corrected for water evaporation, when necessary, that is, when the animal did not eat all of its food ration. Grams of food intake were converted to kilojoules using equivalents of 3.7 kJ/g for the banana and 4.6 kJ/g for the mixture. During the 35-day food restriction, the 40% calorie restriction received an energy allotment of 57 ± 2 kJ/day and 52 ± 6 kJ/day in the long (LD) and short days (SD), respectively (named LD40 and SD40). Comparatively, the 80% calorie restriction corresponded to an energy allotment of 17 ± 0 kJ/day and 19 ± 2 kJ/day in LD80 and SD80 groups, respectively.

**Body Mass**

During the calorie restriction period, the body mass of each animal was measured every two days. For ethical reasons, special attention was paid to the body mass time course of the LD80 group because of their leanness at inclusion. Animals were excluded from the study when body masses reached the lowest value (50 g) reported in the colony for this photoperiod (41). Practically, this affected only two animals: one was excluded on day 28 and a second on day 33 of calorie restriction.

**Tb and Locomotor Activity Recording**

A telemetric transmitter (TA10TAF20, 3.2 g; Data Science, St. Paul, MN) was implanted into the abdominal cavity, under general anesthesia (preanesthesia: valium 10 mg, 2 mg/100 g im; anesthesia: Ketamine Imalgene 500 mg, 10 mg/100 g im) as routinely done in the laboratory (51). Animals started the experimental protocol 1 mo after surgery. Before being implanted in the animals, transmitters were calibrated individually by the manufacturer in two points of temperature, 35 and 39°C. Moreover, the linearity of the logger response was further calibrated in our laboratory, between 21°C and 42°C, using a thermostated water bath. The receiver board (RPC-1; Data Science, St. Paul, MN) was positioned in front of the nest-box to collect the radio frequency signals. Tb was recorded for 10 s every 5 min. Locomotor activity was recorded continuously, and the sum of activity counts, from the entire previous 5 min, was reported in arbitrary units (au). Activity counts are recorded when the animal moved in three dimensions, the number of counts generated depending on both distance and speed of movement. Data were analyzed using the Dataquest software (LabPro Data Science). After the study, the transmitters were removed via surgery, and the animals were returned to their breeding groups.

**Data and Statistical Analyses**

Because of unexpected transmitter failures, three individuals (one in SD40 and two in LD80) were excluded from the data analysis.

**Parameters studied.** For each individual, six parameters were calculated from the locomotor activity and Tb records (Fig. 1) to characterize the strategies used by mouse lemurs to face calorie restriction. The parameters were divided into two types: those characterizing the active period and those relating to the resting period. The active period was defined as the period that lasted from the start of the dark phase to the time of entry in torpor, from which started the resting period that finished at the onset of the following dark phase. The locomotor activity level during the active period was calculated. The time of entry in torpor, indicated by negative numbers (in minutes), represents the onset of a continuous Tb drop, which goes below the average Tb of the active period, until the minimal Tb. Therefore, the more negative the time of entry in torpor was, the more...
phase-advanced was the entry into torpor. The other parameters of the resting period included the duration of the Tb drop from the entry into torpor until the appearance of minimal Tb, the minimal Tb, and the duration of the torpor bout characterized by a Tb below 33°C. Therefore, the part of the Tb drop above 33°C (shallow torpor) was not accounted in the calculation of the torpor duration. We also calculated the locomotor activity level during the light phase. Locomotor activity levels during the active period and light phase were expressed in arbitrary units per hour of the active period or light phase, respectively, and were also represented every 10 min on a double-plotted actogram, using the Clocklab software (Actimetrics, Evanston, IL) to highlight changes in patterns.

Main effects of the trend analysis. The variables were analyzed with the generalized linear model (GLZ), with a gamma error distribution and log-link function, as described by Geister and collaborators (13). The GLZ was used to analyze effects of photoperiod and calorie restriction intensity on the nonlinear time courses of calorie restriction response for each telemetry parameter. Thus, photoperiod (LD vs. SD), calorie restriction intensities (40% vs. 80%), and time were entered as main factors in the GLZ, and therefore, the time effect is taken into account from a nonparametric point of view. The type of distribution followed by each variable was analyzed by the Analysis of Processes module of Statistica (V7.1.515.0, Statsoft France). The GLZ was used in other studies (5, 36). A breakpoint corresponded to the intercept of the two linear segments that characterized the nonlinear time course of one parameter. When the slopes did not differ from zero, average Tb or locomotor activity values were used. When appropriate, between and within-group differences were compared by Mann-Whitney U-test and Wilcoxon test, respectively, both corrected by a Bonferroni P-value adjustment procedure.

Multiple regression analysis. The LD and SD grey mouse lemurs showed an early and a late response, respectively, to food deprivation. Thus, to investigate the determinants of the loss of body mass and of minimal Tb, multiple regression analyses were performed on both the 7th and 25th day of calorie restriction to highlight the early and the late responses, respectively. In the model for body mass loss, the 24-h locomotor activity level, minimal Tb, torpor duration, and the ingested energy were selected as explanatory variables, as presumably they were the most important contributors to explain the model. In the model for minimal Tb, the duration of the Tb drop, the Tb during the active phase, and the ingested energy were selected as the explanatory variables, as they seemed to be the main contributors to explain the model.

All reported values are means ± SE, and P < 0.05 was considered significant. All the statistical computations were performed by Statistica (V7.1.515.0, Statsoft France).

RESULTS

Baseline Data During the Control Period

Baseline parameters are shown in Table 1. As expected from previous work (15), LD grey mouse lemurs had a 22% lower body mass than SD ones. No difference was reported on food intake during the control period. During their active period, LD animals showed a 30% greater locomotor activity level than SD animals. During their light phase, LD animals displayed higher minimal Tb than SD animals, while their locomotor activity levels did not differ between groups. In contrast to LD mouse lemurs that did not show Tb below 33°C, SD animals displayed torpor bouts (Tb < 33°C) with a 3.8-fold earlier entrance in torpor state than the LD ones.
Table 1. **Body masses, energy intake, and parameters of body temperature, and locomotor activity of long days (summer) and short days (winter) animals during the control period**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Long Days (n = 10)</th>
<th>Short Days (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>84±1</td>
<td>108±4</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Energy intake, kJ/day</td>
<td>90±3</td>
<td>89±7</td>
<td>NS</td>
</tr>
<tr>
<td>Active period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotor activity level, au</td>
<td>2098±150</td>
<td>1461±137</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Light phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locomotor activity level, au</td>
<td>302±62</td>
<td>225±38</td>
<td>NS</td>
</tr>
<tr>
<td>Resting period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal Tb, °C</td>
<td>35.6±0.2</td>
<td>34.1±0.5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Time of entry in torpor, min</td>
<td>−26±4</td>
<td>−98±11</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Duration of torpor, min</td>
<td>0±0</td>
<td>83±33</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. au, arbitrary unit. Between-group differences were compared by a Mann-Whitney U-test and P < 0.05 was considered significant.

**Body Mass**

Both the LD80 and the SD80 animals lost weight at a similar rate of 0.8 ± 0.1 g/day during calorie restriction (U = 7, nLD80 = 4, nSD80 = 6, P = 0.14; Fig. 2). In contrast, the 40% calorie restriction induced a different pattern of mass loss in LD and SD animals. Indeed, during the first 11 ± 1 days, both LD40 and SD40 animals had a similar rate of mass loss of 0.3 ± 0.1 g/day (U = 11, nLD40 = 5, nSD40 = 6, P = 0.54). Afterward, whereas body mass stabilized in the SD40 group at 101 ± 7 g, the LD40 animal’s mass further dropped at a rate of 0.20 ± 0.04 g/day. Overall, the 40% calorie restriction resulted in an accumulated mass loss of 7% in LD animals and no body mass change in SD mouse lemurs, whereas the 80% calorie restriction induced a 20% and 31% reduction in SD and LD animals, respectively. This difference was essentially explained by the initial body masses of each group and not by the rate of body mass loss.

**Levels and Patterns of Locomotor Activity**

**Locomotor activity during the active phase.** Under SD, calorie restriction had no effect on locomotor activity levels during the active phase, independent of calorie restriction intensity (Fig. 3, **top**). The LD40 group showed an 18% decrease in locomotor activity level by the 20th (±3) day. Although the locomotor activity level in the LD80 group showed a trend to increase, no significant modifications were reported due to the high interindividual variability. Nevertheless, the differences in locomotor activity level were maintained between photoperiods.

**Locomotor activity during the light phase.** Locomotor activity levels gradually decreased by 48% and 50% in SD40 and SD80 groups, respectively (Fig. 3, **bottom**). In contrast, the LD40 animals displayed no change in their activity level, whereas the LD80 animals showed a four-fold increase from the 14th (±4) day before reaching a plateau on the 24th day.

**Locomotor activity patterns.** The LD40 animals did not redistribute their locomotor activity level over a nycthemere (Fig. 4). Conversely, from day 15, the LD80 animals decreased their locomotor activity level by 31% during the last 4 h of the night and increased it five-fold in the 4 h before dusk. Considering the SD40 and SD80 animals, we observed a gradual concentration of the locomotor activity level in the first 6 h of the active phase until the 15th day of calorie restriction, corresponding to an increase of 7% and 34%, respectively. These redistributions of locomotor activity in the LD80, SD40, and SD80 groups matched the increased phase-advance of the entry into torpor, as described later in the text.

**Effects of Calorie Restriction on Torpor Parameters**

**Time of entry in torpor.** Both the SD40 and SD80 animals displayed, from the very start of the calorie restriction, a significant 10 ± 3 min/day phase advance of the entry into torpor during 14 ± 1 days (Fig. 5, **top**). Then, both groups stabilized the time of entry in torpor at a value of −238 ± 35 min. In contrast, although no modifications of the time of entry in torpor occurred in the LD40 group during the calorie restriction period, the LD80 animals showed a significant phase-advance of the entry into torpor from the 16th (±2) day, at a rate of −16 ± 6 min/day.

**Torpor bout duration.** Although LD40 mouse lemurs did not show any torpor bouts over the 5-wk food shortage, those facing an 80% calorie restriction significantly increased their torpor duration at a rate of 9 ± 5 min/day during the first 25 ± 0 days of calorie restriction, stabilizing it at a value of 253 ± 61 min (Fig. 5, **bottom**). Conversely, both SD40 and SD80 animals increased their torpor duration at a rate of 30 ± 6 min/day during the first 14 ± 1 days of calorie restriction. Then, SD animals stabilized their torpor duration at similar
values of 409 ± 129 min for SD40 and 357 ± 128 min for SD80 (U = 14.0, nSD40 = 5, nSD80 = 6, P = 0.86).

Minimal Tb. Both SD40 and SD80 animals displayed a drop in minimal Tb of 6.2°C until the 12th (±2) day (Fig. 6). Thereafter, minimal Tb at the 12th and 13th day did not differ from their final respective one (SD40: 27.6 ± 1.7°C vs. 27.6 ± 0.6°C, W = 0.4, n = 5, P = 0.59; SD80: 27.6 ± 0.7°C vs. 28.0 ± 1.0°C, W = 0.5, n = 6, P = 0.60). The LD40 group displayed a transient but significant drop in their minimal Tb of 1°C during the first 8 ± 2 days of calorie restriction. Then, the minimal Tb at the 8th day reached a stable value of 34.8 ± 0.3°C until the end of the calorie restriction. The LD80 animals showed a decrease in minimal Tb over the first 24 ± 1 days and then stabilized at 32.9 ± 0.6°C. During the early adaptation

Fig. 3. Locomotor activity during the active and light phases. The Wald statistics, mentioned on the right side of each graph, show overall general effects of P and CRI on the evolution of locomotor activity levels over a 30-day calorie restriction (time) in LD and SD animals under 40% or 80% calorie restriction (LD40, n = 6; LD80, n = 4; SD40, n = 5; SD80, n = 6). When mentioned, symbol # or * on a curve indicates the time of rupture in the time course of locomotor activity of the 40% or 80% food-deprived group, respectively. Values are expressed as means ± SE.
phase first (7th day of calorie restriction), the model obtained from multiple regression analysis demonstrated no significant determinants for the minimal Tb. Conversely, during the late response (25th day of calorie restriction), the only variable determining the minimal Tb in the LD mouse lemurs appeared to be the energy intake (Table 2). In SD primates, whereas the duration of the Tb drop (from the onset of the torpor until the occurrence time of the minimal Tb) appeared to be the main explanatory variable of the minimal Tb at the 7th day of calorie restriction, no determinants for the minimal Tb reach significance during the late response (25th day of calorie restriction, Table 2).

Determinants of Changes in Body Mass

Multiple regression analysis showed that energy intake was the only variable explaining the loss of mass in the LD grey mouse lemurs for the entire calorie restriction period;

Fig. 5. Onset and duration of torpor bout. The Wald statistics, mentioned on the right side of each graph, show overall general effects of P and CRi on changes of the initiation time and duration of torpor bout over a 30-day calorie restriction (time) in LD and SD animals under 40% or 80% calorie restriction (LD40, n = 6; LD80, n = 4; SD40, n = 5; SD80, n = 6). #Time of rupture in the time course of onset or duration of torpor bout of the 40% food-deprived group. *Time of rupture in the time course of onset or duration of torpor bout of the 80% food-deprived group. Values are expressed as means ± SE.

Fig. 6. Minimal body temperature. The Wald statistics, mentioned on the right side of each graph, show overall general effects of P and CRi on evolutions of the minimal body temperature over a 30-day calorie restriction (time) in LD and SD animals under 40% or 80% calorie restriction (LD40, n = 6; LD80, n = 4; SD40, n = 5; SD80, n = 6). #Time of rupture in the time course of minimal body temperature of the 40% food-deprived group or *Time of rupture in the time course of minimal body temperature of the 80% food-deprived group. Values are expressed as means ± SE.
DISCUSSION

From previous studies on *M. murinus* (14, 51), the onset of a short-term food restriction induces a progressive increase in torpor depth. In addition, an 80% food deprivation during 8 days results in greater phase-advance of the entry into torpor and increased torpor bout duration (14). In our study, the grey mouse lemurs that were acclimated to short days responded immediately to food deprivation, by exhibiting a deeper (22% increased) and 2.4-fold more phase-advanced entry into torpor under both moderate and severe food deprivations. The early adjustment of daily Tb allows for immediate energy saving for the grey mouse lemur that enters into a behavioral and sexual rest, optimizing a torpor bout after an autumnal fattening to face seasonal food shortage (39). Moreover, seasonal Tb adjustments have been reported in the wild mouse lemur, housed in outdoor enclosures for the measurements (37, 38, 44, 45), and all are more significant when the ambient temperature (Ta) reaches extreme minimal values during the torpor phase: around 15°C and down to 4°C during the night on Madagascar. This feature allows mouse lemurs to display deeper torpor bouts, increasing energy savings in the wild compared with our laboratory study. However, although the frequency of such extreme Ta (4°C) on the island is too low to be of significance in terms of energy economy, an extreme average minimal Ta of 15°C would have a much more substantial effect on energy metabolism, almost twice than that observed under a Ta of 25°C. Like mouse lemurs, several small mammals show seasonal heterothermy to conserve energy when faced with environmental stresses (24, 26, 57), as reported in food-deprived free-ranging elephant shrew (*Elephantulus myurus*) (25, 32).

In our study, *M. murinus* acclimated to short days and facing a 40% food restriction stabilized energy balance, but when faced with a severe 80% lowered food availability, did not. Although it was expected that no compensation would occur at 80% food restriction, this small primate may use other strategies in their natural habitat to save energy during the winter period.

In contrast to our experimental design in which each animal was kept alone in a cage, wild mouse lemurs regroup themselves in tree holes during the night (27, 43, 48). This huddling process may represent an important strategy to limit energetic costs during the diurnal sleeping period due to social thermoregulation (22, 39, 43), notably allowing *M. murinus* to face a severe food shortage in winter more efficiently. Another energy-saving mechanism corresponds to the use of passive reheating during torpor arousal since considerable energy is required to arouse from a low Tb (34). During the cold and dry season in the Kirindy forest, *M. murinus* and *M. myoxinus* arouse from torpor through a two-step process, consisting of an initial passive climb in Tb in relation to Ta followed by an active rise of Tb to normothermic level (45, 47). This was also observed in small captive marsupials (*Sminthopsis macroura*) and free-ranging rock elephant shrew (*Elephantulus myurus*) (12).

In our experiment, the Ta of the summer-like long-days animal’s room was kept at 30°C. This feature theoretically allowed mouse lemurs to display minimal Tb ~32°C, since heterothermic mammals can generally show Tb 1–2°C above Ta during their torpor state (53, 54, 58). However, these animals did not display torpor bouts (Tb < 33°C) to increase energy conservation during the food-restricted period. Since mouse lemurs display an active breeding state during summer, this lack of torpor reported in food-restricted long-days animals can be due to their high level of reproductive hormones, which influences thermoregulation and torpor, as reported in pouch mice (33) and European hamsters (7). Furthermore, the low body mass loss of these long-days acclimated mouse lemurs under moderate food shortage of 40% might not be fully explained by torpor-induced energy saving. Thus, *M. murinus* under long-days exposure may combine other energy-saving

Table 3. Determinants of the changes in body mass

<table>
<thead>
<tr>
<th>Day of Calorie Restriction</th>
<th>R²</th>
<th>24 h LA Level, au</th>
<th>Minimal Tb, °C</th>
<th>Torpor Bout Duration, min</th>
<th>Ingested Energy, KJ/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long days, n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.95 P = 0.007</td>
<td>−0.02±0.13 P = 0.862</td>
<td>−0.10±0.18 P = 0.633</td>
<td>−0.06±0.17 P = 0.741</td>
<td>0.94±0.12 P = 0.001</td>
</tr>
<tr>
<td>25</td>
<td>0.95 P = 0.002</td>
<td>−0.22±0.12 P = 0.131</td>
<td>0.03±0.15 P = 0.860</td>
<td>0.01±0.12 P = 0.936</td>
<td>0.81±0.15 P = 0.006</td>
</tr>
<tr>
<td>Short days, n = 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.98 P &lt; 0.001</td>
<td>−0.33±0.08 P = 0.006</td>
<td>0.66±0.16 P = 0.007</td>
<td>0.71±0.16 P = 0.004</td>
<td>0.93±0.06 P &lt; 0.001</td>
</tr>
<tr>
<td>25</td>
<td>0.97 P &lt; 0.001</td>
<td>−0.24±0.08 P = 0.021</td>
<td>0.15±0.10 P = 0.183</td>
<td>0.24±0.10 P = 0.058</td>
<td>0.83±0.08 P &lt; 0.001</td>
</tr>
</tbody>
</table>

Values for β (regression coefficient) are means ± SE. Tb, body temperature; 24 h LA, total locomotor activity. β represents the relative contributions of the independent variables to the explanation of the dependent variable, the body mass loss. Bold characters represent significant results.
strategy in addition to their thermomodulation responses, to efficiently cope with a 40% food restriction. A possible mechanism may reside in the strategy used by the golden spiny mouse (Acomys russatus) facing 2 wk of 50% energy restriction. This mouse “switches down” its resting metabolism and is able to survive and maintain its body mass indefinitely on a 50% limited ration. The reduction in metabolism occurs without a decrease in Tb or in activity level (30) but may be limited to 50% of the total resting energy expenditure in man (55). In heterotherms, this physiological inhibitory mechanism, which occurs in addition to the temperature effect, must be involved in the reduction of the metabolic rate, as pointed out by Geiser (11) in his recent review. Therefore, the long-days acclimated grey mouse lemur may be able to decrease its metabolic rate to a step below the one predicted by its Tb drop during the torpor, saving additional energy. Alternatively, mouse lemur undergoes a large reduction of body mass in summer, an observation known as the Dehnel effect (31), and notably, mouse lemur under long-days exposure do not show a large amount of fat mass compared with short-days-acclimated animals. Therefore, the proportion of the fat-free mass loss would be higher in long-days animals compared with short-days ones. Fat-free mass being the main determinants of resting metabolic rate, it is likely that energy expenditure will also be decreased to a larger extent than in animals under short-days acclimation. Similarly, the cost of activity per gram of body mass will also be decreased. However, such season-related changes in body composition in response to calorie restriction require further studies.

A previous study of 80% food deprivation during 8 days on long-days acclimated M. murinus showed that mouse lemur displayed deeper and longer torpor bouts associated with an increase in physical activity level (14). Conversely, animals in our study showed Tb changes only from the 15th day of food restriction. In addition to these Tb modifications, these primates greatly phase-advanced the dark phase, increasing their locomotor activity level by 3.6-fold 4 h before dusk when food allotments became available. Therefore, it is likely that the energy saved by Tb adjustments was compensated by increased absolute levels of physical activity, resulting in an unmodified rate of body mass loss. Several studies reported that the observed increase in physical activity levels in response to food scarcity might represent an increase in foraging behavior (6, 8, 28, 52). The increased locomotor activity in the 80% food-deprived mouse lemur under long-days exposure likely corresponds to a programmed behavioral response for searching food that was exacerbated by our experimental design, that is, animals were spatially limited to their own cage, and food was provided at the beginning of the dark phase. Indeed, timed meal feeding associated with calorie restriction, that is, timed food restriction, is a powerful entraining agent as it phase advances nocturnal component of locomotor activity rhythm by 6 h, in Wistar rats (4). Therefore, timed availability of food allotments would act as a zeitgeber in the food anticipatory activity in long-days-acclimated mouse lemur under an 80% food restriction. In spite of this synchronization role of timed food restriction, in our study, the higher level of locomotor activity before food availability only resulted in higher energy expenditure. In male Wistar rats, body mass must fall to some relatively fixed critical level before activity substantially increases (23), and there is a correlation between predeprivation body mass and the occurrence time of the day of the activity peak (50). A trigger for the increased locomotor activity level may involve plasma leptin level. It was found that in rats, leptin suppresses semistarvation-induced hyperactivity (8). Therefore, it was suggested that hypoactivity, as a result of food restriction, may represent the initial trigger for the increased activity levels in food-restricted rats (8). This may be a possible explanation for the 3.6-fold increase in locomotor activity level of the long-days-acclimated primates under an 80% calorie restriction. In addition, other hormones sensitive to energy homeostasis, named “gut hormones”, such as ghrelin, pancreatic polypeptide, and peptide YY (PYY), are positively correlated with behavioral activity level in mice (35).

**Perspectives and Significance**

Apart from the fundamental approach in ecophysiology, calorie restriction received a great deal of attention in homeothermic species because undernutrition without malnutrition is, so far, the only paradigm that increases life span in all the species tested. On the basis of the strong similitude that exists between the effects of calorie restriction in homeothermic species and the processes of torpor/hibernation, Walford and Spindler (56) suggested in 1997 that the life-extending properties of restriction in energy are part of larger processes of energy saving developed to face food shortage that has been conserved across evolution and thus, may be seen solely as a laboratory artefact. It is evident that the responses to calorie restriction may vary across animal species, such as hyperlocomotor or hypolocomotor activity or shallow or deep hypothermia, those responses being probably selected according to environmental constraints. Similarly, differences exist between the effects of food shortage and the deep hibernators, but the convergence between the life-extending properties of calorie restriction and the mechanisms of torpor, as seen in estivation or in the primate of the present study, are clearly worthy of investigation. As such, comparative studies between the effects of moderate calorie restriction in heterotherms and homeotherms may provide original information on the mechanisms by which calorie restriction increases life span. This is currently being tested in our laboratory, where a colony of Microcebus murinus is being submitted to moderate calorie restriction since adulthood to natural death. We hope that this longitudinal study, named RESTRIKAL, in a primate heterotherm will open a new area of research on the biology of aging.

**ACKNOWLEDGMENTS**

We would like to thank Dr. Susanne Votruba for editing this article.

**GRANTS**

S. Giroud was financially supported by a fellowship of the French Ministry of Research. This study was supported by an Action Thématique Incitative sur Programme from the Centre National de la Recherche Scientifique (S. Blanc), the Bettencourt Schueller Fondation (S. Blanc), the Groupement d’Intérêt Scientifique-Longévité (S. Blanc) and the Agence Nationale pour la Recherche Alimentation & Nutrition Humaine (F. Aujard, M. Perret, and S. Blanc).

**REFERENCES**


