



**HAL**  
open science

**Daily rhythms of core temperature and locomotor activity indicate different adaptive strategies to cold exposure in adult and aged mouse lemurs acclimated to a summer-like photoperiod.**

Jeremy Terrien, Philippe Zizzari, Jacques Epelbaum, Martine Perret,  
Fabienne Aujard

► **To cite this version:**

Jeremy Terrien, Philippe Zizzari, Jacques Epelbaum, Martine Perret, Fabienne Aujard. Daily rhythms of core temperature and locomotor activity indicate different adaptive strategies to cold exposure in adult and aged mouse lemurs acclimated to a summer-like photoperiod.. *Chronobiology International*, 2009, 26 (5), pp.838-53. 10.1080/07420520903044281 . hal-00426201

**HAL Id: hal-00426201**

**<https://hal.science/hal-00426201>**

Submitted on 23 Oct 2009

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Submitted : 14/24/08 Returned for revision : 01/28/09 Accepted : 04/01/09**

**Daily rhythms of core temperature and locomotor activity indicate different adaptive strategies to cold exposure in adult and aged mouse lemurs acclimated to a summer-like photoperiod.**

**Terrien J.<sup>1</sup>, Zizzari P.<sup>2</sup>, Epelbaum J.<sup>2</sup>, Perret M.<sup>1</sup>, Aujard F<sup>1</sup>.**

<sup>1</sup> *Mécanismes Adaptatifs et Évolution, UMR CNRS/MNHN 7179, 4 avenue du Petit Château, 91800 Brunoy, France*

<sup>2</sup> *Neuroendocrinologie de la croissance et du vieillissement, UMR 894 INSERM Paris 5, 4ter rue d'Alésia, 75014 Paris, France*

Address correspondance to:

Jérémy TERRIEN

Mécanismes Adaptatifs et Évolution, UMR CNRS/MNHN 7179, 1 avenue du Petit Château, 91800 Brunoy, France.

Tel. +33 1 60 47 92 00 – Fax. +33 1 60 46 81 18

*E-Mail:* terrien@mnhn.fr

**ABSTRACT**

Daily variations in core temperature ( $T_c$ ) within the normothermic range imply thermoregulatory processes that are essential for optimal function and survival. Higher susceptibility towards cold exposure in older animals suggests these processes are disturbed with age. In the mouse lemur, a long-day breeder, we tested whether aging affected circadian rhythmicity of  $T_c$  and locomotor activity (LA) and energy balance under long-day conditions when exposed to cold. Adult ( $N = 7$ ) and aged ( $N = 5$ ) mouse lemurs acclimated to LD14/10 were exposed to 10-day periods at 25 and 12°C.  $T_c$  and LA rhythms were recorded by telemetry, and caloric intake (CI), body mass changes, and plasma IGF-1 were measured. During exposure to 25°C, both adult and aged mouse lemurs exhibited strong daily variations in  $T_c$ . Aged animals exhibited lower levels of nocturnal LA and nocturnal and diurnal  $T_c$  levels in comparison to adults. Body mass and IGF-1 levels remained unchanged with aging. Under cold exposure, torpor bout occurrence was never observed whatever the age category. Adult and aged mouse lemurs maintained their  $T_c$  in a normothermic range and a positive energy balance. All animals exhibited an increase in CI and a decrease in IGF-1 in response to cold. The decrease in IGF-1 was delayed in aged mouse lemurs compared to adults. Moreover, both adult and aged animals responded to cold exposure by increasing their diurnal LA compared to those under  $T_a = 25^\circ\text{C}$ . However, aged animals exhibited a strong decrease in nocturnal LA and  $T_c$ , whereas cold effects were only slight in adults. The temporal organization and amplitude of daily phase of low  $T_c$  were particularly well preserved under cold exposure in both age groups. Sexually active mouse lemurs exposed to cold seemed thus to prevent torpor exhibition and temporal disorganization of daily rhythms of  $T_c$ , even during aging. However, although energy balance was not impaired with age in mouse lemurs after cold exposure, aging was associated with lower LA and  $T_c$  during the night and delayed decrease in IGF-1. This might reflect that adaptive strategies to cold exposure differ with age

in mouse lemurs acclimated to a summer-like photoperiod. (Author correspondence: terrien@mnhn.fr).

**Key words:** Aging – LD14/10 – thermoregulation – IGF-1 – circadian rhythms – cold exposure – *Microcebus murinus*.

## INTRODUCTION

Daily variations in core temperature within the normothermic range imply thermoregulatory processes that are essential for optimal function and survival. The circadian rhythmicity in core temperature ( $T_c$ ) is the result of both intrinsic circadian variations in heat production and heat loss, and interactions of heat generating behaviors, such as locomotor activity (LA) or adapted postures (for review, see Van Someren et al., 2002). Its expression is under the control of the central circadian pacemaker which generates the endogenous rhythmicity and allows effective synchronization to the light dark cycle (Dardente & Cermakian, 2007). Thermoregulatory processes are seasonally dependent (Lovegrove, 2005), suggesting that the seasonal breeding state could interfere with  $T_c$  levels. In long-day breeders, acclimatization to a long photoperiod induces entrance into reproductive state with an increase in gonadal steroid hormones, which are well known for their thermogenic action (Hampl et al., 2006). This may partially explain why many mammals, such as European (Wollnik & Schmidt, 1995) and golden hamsters (Jefimow et al., 2004) exhibit higher  $T_c$  during the summer than winter. The influence of seasonal acclimatization on thermoregulatory mechanisms has also been demonstrated in voles (Zubidat et al., 2007). Thus, the sexually active state could allow animals to cope with low ambient temperatures ( $T_a$ ) and to efficiently prevent hypothermia. In fact, cold exposure interferes with circadian rhythmicity in  $T_c$  by inducing modulations in thermoregulatory processes. This implies hormonal changes (Larrouy et al., 1995). More specifically, Insulin-like Growth Factor type 1 (IGF-1) has been proposed to act in cold-induced thermogenesis processes in the rat (Duchamp et al., 1997; Yamashita et al., 1994).

With age, there are evidences of a decrease in the robustness of circadian rhythmicity in  $T_c$  (for review, see Van Someren et al., 2002; Weinert & Waterhouse, 2007). More, aging is associated with an increased prevalence of death caused by hypothermia (Stocks et al., 2004;

Ward & Cowley, 1999). However, data on daily Tc and LA rhythms during continuous exposure to cold are scarce (Palkova et al., 1999; Rensing & Ruoff, 2002; Tokura & Aschoff, 1983) and do not address the question of age-related disturbances. Thus, the reasons why some aged individuals become more sensitive to cold exposure are not entirely clear. Considering that aging induces changes in many hormonal factors (Ferrari et al., 2008; Vermeulen, 1995), seasonal variations in hormones might also be disturbed with aging (Vom Saal et al., 1994), with lower capacities for photoperiod-induced adjustments. IGF-1 secretion is influenced by photoperiod in golden hamsters (Laartz et al., 1994) and primates (Ganzhorn et al., 2003) and is also modified with aging (Sherlock & Toogood, 2007). Such endocrine processes could directly or indirectly contribute to age-related impaired thermoregulatory responses towards cold exposure. Based on these published findings and to the lack of study on the role of reproductive state on cold resistance, we explored whether age-associated Tc decrease in response to cold are preserved in a sexually active non-human primate, i.e., the mouse lemur (*Microcebus murinus*).

*Microcebus murinus* is a small nocturnal primate (body weight: 60 – 90 g) originating from Madagascar. The life span of this species is about 8 yrs in captivity (Perret, 1997). In thermoneutral conditions, mouse lemurs exhibit robust daily rhythms in Tc, particularly characterized by a phase of low Tc (assimilated as a drop to a minimal Tc followed by an increase in Tc) during the first half of the light period (Perret & Aujard, 2001). Daily exposure to light for longer than 12 h promotes sustained behavioral and reproductive activity (Perret, 1992). Entrance into the reproductive state appears to protect normothermia maintenance in the mouse lemur. For example, when testing behavioral thermoregulatory responses, adult animals under LD14/10 did not need to select warm environments to efficiently maintain normothermia. Furthermore, the effects of a moderate food deprivation, known to induce a decrease in Tc, were minimal in adult mouse lemurs exposed to LD14/10

(Giroud et al., 2008). In contrast, aged animals always chose the warmest ambient temperatures during the behavioral test. Such differences in adaptive strategies between young adults and aged mouse lemurs highlight subtle changes in thermoregulatory capacities that were undetected when studying daily rhythms of Tc in reference Ta (25°C). Indeed, a marked rhythm of Tc is maintained in aged animals with, however, a delayed time of occurrence of minimal Tc with age (Aujard et al., 2007). Finally, aging is also associated in the mouse lemur with a decrease in amplitude of the seasonal variations in body mass, gonadal hormones (Aujard & Perret, 1998), melatonin (Aujard et al., 2001), and DHEA-S (Perret & Aujard, 2005, 2006). Based on these findings, age-related effects on thermoregulatory responses towards cold were investigated by monitoring daily Tc and LA rhythms, body mass, caloric intake, and plasma IGF-1 in sexually active mouse lemurs.

## **METHODS**

### Animals and housing conditions:

All the gray mouse lemurs studied were males, born in the laboratory breeding colony of Brunoy (MNHN, France, license approval N° A91.114.1) and were pathogen free. General conditions of captivity were maintained constant: Ta (24 - 26°C) and relative humidity (55%). Food (including fresh fruits and a milky mixture) and water were available *ad libitum*. In captivity, seasonal variations of physiological functions can be entrained by alternating 6-month periods of summer-like long photoperiod (14 h of light/day) and winter-like short photoperiod (10 h of light/day) under artificial light (fluorescent tubes during the day and dim red light during the night). In the present study, male mouse lemurs were studied during the long-day season (LD14/10), at least two months after the onset of the summer-like photoperiod. This ensured stabilization of the physiological status of the animals. Long days correspond to the mating season (high testosterone levels and large testis size), associated with high levels of activity and significant body mass decrease. General conditions of

captivity were applied and animals were maintained in social groups before and after experimenting. In the breeding colony of the Brunoy laboratory, analysis of survival from 254 male mouse lemurs determined the mean life span (mean  $\pm$  SEM) to be  $6.0 \pm 0.2$  yrs, the mean life span of the 10% of longest lived animals to be  $10.0 \pm 0.2$  yrs, and the observed maximal survival duration to be 12.0 yrs. In the present study, randomly chosen adults ( $N = 7$ ; mean age  $\pm$  SEM:  $1.6 \pm 0.3$  yrs, range: 1.0 – 2.4 yrs) and aged mouse lemurs ( $N = 5$ ; mean age  $\pm$  SEM:  $7.4 \pm 0.2$  yrs, range: 6.4 – 8.3 yrs) were used throughout all experiments. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and the ethical standards of the journal (Portaluppi et al., 2008). All efforts were made to minimize nociception.

#### Core temperature and locomotor activity recording:

Animals were maintained in climate chambers (Sanyo incubator MIR-253), in which air was filtered and light was provided by cool fluorescent lamps. Mouse lemurs were acclimated to the experimental device for 10 days at  $T_a = 25^\circ\text{C}$ . They were then studied for 10 days at the reference  $T_a$  of  $25^\circ\text{C}$  and then exposed to a cold environment (10 days at  $12^\circ\text{C}$ ). Core temperature ( $T_c$ ) was measured using a telemetric device: a 2.5 g transmitter (TA10TA-F20, Data Science Co. Ltd, Minnesota, USA) was implanted under general anesthesia (Valium, 2mg/100g i.m.; Ketamine Imalgem, 10mg/100g i.p.) in the visceral cavity of the animals. Calibrations for each transmitter were provided by the manufacturer. Experiments were performed after at least 2 weeks of recovery. Mouse lemurs were isolated in individual cages provided with branches and a wooden nest. A receiving plate (RPC-1, Data Science Co Ltd, Minnesota, USA) localized in the cage permitted the recording of data sent by the transmitter.  $T_c$  (in  $^\circ\text{C}$ ) was recorded every 10 min and locomotor activity (LA in arbitrary units a.u.) was continuously recorded and summed within this interval by two antennas located in the



receptor plate and detecting vertical and horizontal movements (X-Y coordinate system, Dataquest Lab Pro v. 3.0, Data Science Co. Ltd, Minnesota, USA).

The following parameters were analyzed: mean Tc during the active nocturnal phase ( $T_{c_{night}}$ ), mean Tc during the resting diurnal phase ( $T_{c_{day}}$ ), minimal Tc value ( $T_{c_{min}}$ ), time of occurrence of  $T_{c_{min}}$  ( $H_{min}$ ), and time of onset of Tc drop ( $H_{decr}$ ). The last two parameters were expressed in minutes relative to lights on and determined day after day on graphic determination.  $H_{decr}$  was determined each day as the first time point after which at least 3 successive bins of Tc decrease occurred. Similarly,  $H_{min}$  was determined each day as the time point occurring at least after 30 min of decrease and preceding at least 30 min of Tc increase. Consequently,  $T_{c_{min}}$  corresponded to the Tc value pointed at the  $H_{min}$  time point.  $T_{c_{min}}$ ,  $H_{decr}$ , and  $H_{min}$  were representative parameters of the daily phase of low Tc. Finally, LA values were averaged during the nocturnal active phase ( $LA_{night}$ ) and the diurnal resting phase ( $LA_{day}$ ). Actograms were also generated using Clocklab software (Actimetrics Inc., Evanston, IL). LA onset and offset (in min) were defined as the time of occurrence of the first or last (respectively) 3 successive bins when activity was greater or lower (respectively) than  $LA_{day}$ . LA onset and offset were calculated in reference to the time of lights-off and lights-on, respectively. The duration of the LA active phase (alpha, in min) corresponded to the time duration between LA onset and offset values. For all temporal parameters, phase advances were expressed by positive values and phase delays by negative values in reference to their respective reference points. All telemetric parameters were averaged for each thermal exposure.

#### Body mass, caloric intake:

Before the experiment, body mass (BM) of adult (mean  $\pm$  SEM:  $80.4 \pm 4.9$  g) and aged mouse lemurs (mean  $\pm$  SEM:  $97.1 \pm 8.7$  g) did not differ significantly (one-way ANOVA,  $F_1$ ,

$t_{13} = 3.0$ ,  $p = 0.11$ ). BM was measured every 2 days throughout the experiment and the body mass gain (BMG) was calculated as a mean ratio (in g/day) during the whole exposures. Animals were routinely fed *ad libitum* on a diet including fresh banana (393 kJ/100g) and a homemade milky mixture containing baby cereals, eggs, and milk (435 kJ/100g). Daily caloric intake (CI) was calculated by subtracting the remaining food from the total food mass given. CI was expressed in kJ according to the Diem table (Diem, 1963) and normalized to the BM of the animal (kJ/day\*100g BM). The evaporation-related loss was taken into account in the calculation of CI (Seguy & Perret, 2005) to ensure reliable comparisons between 25 and 12°C, since evaporative loss varied between the two Tas.

#### Plasma IGF-1 levels:

To assess the IGF-1 response to thermal stress, blood was taken from animals 3 h before the beginning of the nocturnal phase at the reference  $T_a$  of 25°C and then 2 days (short-term response) and 9 days (long-term response) after the beginning of cold exposure. About 100  $\mu$ l of blood was drawn from the saphenous vein into heparinized tubes without anesthesia. After centrifugation, plasma was immediately collected and preserved at -20°C until the radioimmunoassay, which was performed according to the manufacturer instructions (Immunotech IGF-I IRMA; Beckman Coulter, Paris, France). Intra- and between-series variation rates were <7% and the minimal detectable value was 2 ng/ml. To avoid any potential influence of animal handling on  $T_c$  values, the telemetric data corresponding to the 4 h after the blood samples were removed. Plasma IGF-1 is expressed in ng/ml. IGF-1 is known to co-vary with BM since it influences body composition, particularly body fat (Engstrom et al., 2006; Onder et al., 2006); therefore, BM was included in all statistical models analyzing IGF-1.

### Statistical analysis

All dependent variables were analyzed with Linear Mixed Effect models (LME), built with the “nlme” function (Pinheiro et al., 2005). All dependent variables were checked for normality with the residuals of the models. Non-normal variables were transformed to reach normality, i.e., log-transformation for  $LA_{\text{night}}$ ,  $H_{\text{min}}$ , and  $Tc_{\text{night}}$  and square-root transformation for LA onset and alpha. To take into account inter-individual variability, the effect of individual identity was declared as a random effect. In addition, since the same individuals were used at 12 and 25°C, we allowed inter-individual variation to depend on temperature by declaring the slope of the effect of Ta as a random factor. Statistical models that included the additive effects of age (two levels, adult *versus* old) and Ta (two levels, 12 and 25°C) and their interaction were constructed. Significance of effects were assessed by F-tests (Bolker et al., 2009) with software R Version 2.6.0 (R Development Core, 2004).

## **RESULTS**

### **Effects of age and Ta on energy balance and IGF-1 levels:**

Energy balance was studied by quantifying CI, BMG, and plasma IGF-1 levels. CI was affected by cold exposure, independently of age (Figure 1A). Adult animals ingested on average  $138 \pm 12$  kJ/day\*100g BM at 25°C and  $183 \pm 7$  kJ/day\*100g BM at 12°C (33% increase from 25°C to 12°C). Aged animals exhibited an increase of 14% in CI after cold exposure, from  $131 \pm 22$  kJ/day\*100g BM at 25°C to  $149 \pm 30$  kJ/day\*100g BM at 12°C. BM remained stable throughout the experiment without significant effects of age or Ta exposure (Figure 1B), although the decrease in BMG between 25 and 12°C was close to significance.

When taking into account variations in BM, IGF-1 was affected by an interaction between age and Ta effects (Figure 2). IGF-1 levels were similar at both ages at 25°C, but they were differently modified after cold exposure. The IGF-1 level decreased from  $878 \pm 70$  ng/ml to  $685 \pm 68$  ng/ml after 2 days of cold exposure in adult animals, whereas such a

decrease was only observed after 9 days in aged animals (from  $944 \pm 131$  to  $808 \pm 109$  ng/ml). Therefore, the IGF-1 level was higher in aged than in adult animals at the beginning of cold exposure, but it was similar at the end of the 10-day cold exposure (Figure 2).

### **Effects of age and cold exposure on Tc and LA rhythms parameters:**

LA daily rhythms, for both age groups during both exposures to 25 and 12°C, are represented in Figure 3. The  $LA_{\text{night}}$  level significantly differed by interaction between age and Ta effects (Figure 3 & Table 1). At 25°C, aged animals exhibited a lower LA than adults during both the nocturnal and diurnal phases (Table 1). After cold exposure, adult animals slightly decreased their nocturnal activity compared to 25°C, whereas a 39% decrease occurred in aged animals between 25 and 12°C (Figure 3). Therefore, the  $LA_{\text{night}}$  level remained lower in aged compared to the young adult animals at 12°C as observed at 25°C. Cold exposure induced a 2.1-fold increase in  $LA_{\text{day}}$  in adult animals, whereas a 1.5-fold increase was observed between 25 and 12°C in aged animals. In addition, a 10% lengthening of LA alpha was observed in both young adult and aged animals between 25 and 12°C (Table 1). This effect of cold exposure was due to a strong phase advance in LA onset in both adult and aged mouse lemurs at 12°C, whereas LA offset was not affected by age or cold exposure. The nocturnal organization of LA was also modulated in both age groups in response to cold. The amount of LA decreased in the middle of the night and increased around the periods of lights on and lights off into distinct early and late peaks of activity (Figure 3).

Tc daily rhythms of both age groups during the exposures to 25 and 12°C, are represented in Figure 4. At each Ta (25 or 12°C), adult and aged animals exhibited robust daily rhythms of Tc, with high values during the nocturnal active phase and lower values during the diurnal resting phase. Daily rhythms of Tc throughout the 10 days of exposure to 25 and 12°C are represented in Figure 4. Tc levels at 25°C were lower in aged lemurs

compared to young adults ones, except for the minimal levels of  $T_c$  that were not affected by age at 25°C (Table 2). Nocturnal  $T_c$  responses to cold exposure strongly differed according to the age category. Indeed, only a slight decrease in  $T_{c_{night}}$  was observed in young adult animals after exposure to 12 compared to 25°C (Table 2). In contrast, in aged animals,  $T_{c_{night}}$  strongly decreased by 0.8°C between mean values at 25 and 12°C (Figure 4). Mean values of  $T_{c_{min}}$  were not significantly lowered, and neither adult nor aged animals exhibited torpor phases (i.e.,  $T_c$  dropping <33°C).  $T_{c_{min}}$  values ranged from 33.8 to 35.4°C in young adult mouse lemurs and from 33.2 and 35.8°C in aged animals at 12°C. However, during the day, levels of  $T_c$  remained lower in aged than in adult animals. The difference between the two ages was greatly accentuated by cold exposure. Moreover, the amplitude between the nocturnal and diurnal levels of  $T_c$  was lowered after cold exposure ( $F_{(1,11)} = 8.96$ ,  $p = 0.01$ ), independently of age ( $F_{(1,11)} = 0.10$ ,  $p = 0.76$ ). In fact, the difference between night and day decreased from  $1.2 \pm 0.1$  to  $1.0 \pm 0.1$ °C in adult mouse lemurs, and from  $1.2 \pm 0.1$  to  $0.8 \pm 0.1$ °C in aged animals.

Concerning the temporal organization of the daily phase of low  $T_c$ ,  $H_{min}$  was not significantly affected by age or cold exposure (Figure 5A).  $T_{c_{min}}$  occurred in adult mouse lemurs  $-156 \pm 36$  min and  $-209 \pm 30$  min after lights-on at 25 and 12°C, respectively. Aged animals exhibited  $H_{min}$  values of  $-198 \pm 22$  min and  $-280 \pm 18$  min at 25 and 12°C, respectively. In contrast,  $H_{decr}$  was significantly lowered after cold exposure, whatever the age, and the  $T_c$  drop was thus delayed at 12°C compared to 25°C (Figure 5B). At 25 °C,  $H_{decr}$  occurred  $51 \pm 14$  min before lights-off in young adult animals and  $68 \pm 22$  min before lights-off in aged mouse lemurs. After cold exposure,  $H_{decr}$  was delayed in aged ( $44 \pm 18$  min) and in adult ( $47 \pm 14$  min) animals. No age effect could be detected at either 25 or 12°C.

## DISCUSSION

Both young adult and aged mouse lemurs exhibited strong daily variations in  $T_c$  at 25°C as previously described (Aujard et al., 2006, 2007). In adult mouse lemurs under LD14/10, behavioral thermoregulation appeared to be less crucial for maintenance of normothermia than what was observed under LD 10/14 (Aujard et al., 2006). Thus, autonomic mechanisms and hormonal status of breeding season seemed sufficient to maintain normothermia under long-day exposure. This is corroborated by the fact that, in the present study, no torpor bout was observed at 25°C, whatever the age category, and that age effects on  $T_c$  and LA rhythms were only slight. Aged animals exhibited a decreased nocturnal LA as seen previously in mouse lemurs (Aujard et al., 2007; Cayetanot et al., 2005) and in humans (Huang et al., 2002). For the first time, the recording of daily rhythms was associated with a quantification of caloric intake (CI) and body mass variation in adult and aged animals. Interestingly, body mass stabilization was achieved by aged animals in a similar manner as in adult animals. Moreover, there is some evidence of age-related decline in sexual endocrine function in the mouse lemur during the long-day season (Aujard et al., 2001; Aujard & Perret, 1998; Perret & Aujard, 2005, 2006). However, no significant effect of age on endocrine function was detected at 25°C in the present study, since IGF-1 level, which can be considered as a good biomarker for aging (Kappeler & Epelbaum, 2005), remained similar between adult and aged mouse lemurs. It would be interesting to test the effect of age on others hormones involved in energy balance to further define the ability of aged mouse lemurs to maintain energy balance and body temperature within a normothermic range at a  $T_a$  of 25°C under LD 14/10.

Exposure to 12°C only induced slight variations in  $T_c$  in adult mouse lemurs. Diurnal and minimal  $T_c$  levels remained unchanged and only nocturnal  $T_c$  was significantly lowered between 25 and 12°C. Moreover, torpor bouts were not observed during the whole exposure at 12°C, in contrast to animals acclimated under LD10/14 (Terrien et al., 2008). Gonadal hormonal status, particularly enhanced during the reproductive season, would thus allow

protective normothermia and torpor avoidance. In the present study, mouse lemurs increased their caloric intake after cold exposure, as previously described in pigs (Schenck et al., 1992). In contrast with this species, body mass did not vary significantly in mouse lemurs, and this could be related to their stable energy balance in summer-like photoperiodic conditions. The concomitant increase in CI and diurnal LA suggest that adult animals strongly focus energy expenditure into the enhancement of LA to produce body heat and prevent Tc decrease. IGF-1 level decreased after cold exposure, and this may implicate IGF-1 in cold resistance through non-shivering thermogenesis (NST) process, as already described in mouse lemurs acclimated to LD10/14 (Terrien et al., 2008). The daily organization of the Tc and LA rhythms appeared slightly disturbed in young adult mouse lemurs after cold exposure. In contrast with results observed in LD10/14, daily adjustments were manifested during the diurnal active phase. Adult animals strongly anticipated lights-off, therefore extending their locomotor activity periods. In fact, adult animals delayed the beginning of the Tc drop at the end of the activity period, and minimized their activity during the early phase of diurnal Tc decrease. But from the second part of the day to the beginning of night, their activity progressively increased. The time of occurrence of the minimal Tc value was unaffected by exposure to 12°C. Daily rhythmicity remained highly present in adult mouse lemurs under LD14/10, as also observed in squirrel monkeys (Robinson & Fuller, 1999). The sexually active state of mouse lemurs under summer-like photoperiod might enable a strong protection of Tc rhythm stability.

Age-related differences in Tc were also slight after cold exposure in mouse lemurs and appeared clearly minor compared to results observed under LD10/14 (Terrien et al., 2008). In the present study, nocturnal Tc levels dropped between 25 and 12°C in association with a concomitant decrease in LA, probably reflecting the masking effect of LA on Tc. In aged animals, the increase in diurnal LA, combined with the decrease in nocturnal LA, led to a decreased amplitude of the daily rhythm in LA. In this manner, aged mouse lemurs could

compensate diurnal costs of LA, allowing normothermia maintenance and torpor avoidance, by lowering the costs of LA during the night. Moreover, aged animals prevented energy imbalance and compensated energy expended by increasing their CI. These results revealed that aged mouse lemurs enhance LA-induced production of body heat with a lower propensity than young adults. This could explain the lower nocturnal and diurnal levels of  $T_c$  observed in aged animals compared to young adults at 12°C. Otherwise, it is commonly accepted that aging is associated with a decreased stabilization and synchronization in  $T_c$  rhythms (Van Someren et al., 2002; Weinert & Waterhouse, 2007). In the present work, daily temporal organization in  $T_c$  rhythms were slightly modulated in aged mouse lemurs after cold exposure, as observed in adult animals.  $H_{\text{decr}}$  occurred later in aged animals at 12 than at 25°C, which can be related to the delayed peak of LA observed at the end of the dark phase at such an ambient temperature. The time occurrence of minimal  $T_c$  was unaffected by cold exposure. Moreover, aged animals seemed to adapt their LA like the young adults did, by extending the LA duration to cope with  $T_a$  lowering. Finally, the decrease in plasma IGF-1 level observed after cold exposure occurred later in aged than in young adult mouse lemurs. It was not associated with a differential time-course of day-after-day variations in  $T_c$  levels between both ages, but it could lead to impaired responses towards extreme cold during aging. Furthermore, this could be related to the impairment of hormonal status observed during aging (Ferrari et al., 2008; Vermeulen, 1995) and could play a role in the circadian instability, however not really evidenced in the present study.

### ***Conclusions and perspectives***

Modulations of rhythm parameters were relative minor after cold exposure in LD14/10, even in aged animals. This might suggest the active reproductive state of animals protects circadian rhythmicity and prevents animals from  $T_c$  decrease when coping with low ambient temperatures and that this property is well preserved with age. However, additional



experiments such as effects of castration or injection of testosterone during LD10/14 are necessary to determine if this process is only due to gonadal function. Finally, challenges in energy savings appeared strongly dependent on seasons, since the present results strongly differed from those observed under LD10/14 (Aujard et al., 2006; Terrien et al., 2008). In fact, the increase in diurnal LA, compensated with an increase in CI, appeared efficient to avoid Tc decrease below the normothermic level. However, even though energy balance was not impaired with age in mouse lemurs after cold exposure, aging was associated with lower LA and Tc levels during the night and delayed decrease in plasma IGF-1. This might reflect different adaptive strategies to cold exposure that vary by age in mouse lemurs acclimated to a summer-like photoperiod.

#### **ACKNOWLEDGEMENTS**

We would like to thank Pierre-Yves Henry for his help for statistical analysis. This work was carried out with the financial support of the "Institut de la Longévité et du Vieillissement », the « Fondation pour la Recherche Médicale (FRM) » and the « ANR- Agence Nationale de la Recherche - The French National Research Agency » under the « Programme National de Recherche en Alimentation et nutrition humaine », project « ANR-06-PNRA-010-01».

**REFERENCES**

- Aujard F, Cayetanot F, Terrien J, Van Someren EJ. (2007). Attenuated effect of increased daylength on activity rhythm in the old mouse lemur, a non-human primate. *Exp. Gerontol.* 42: 1079-1087.
- Aujard F, Dkhissi-Benyahya O, Fournier I, Claustrat B, Schilling A, Cooper HM, Perret M. (2001). Artificially accelerated aging by shortened photoperiod alters early gene expression (Fos) in the suprachiasmatic nucleus and sulfatoxymelatonin excretion in a small primate, *Microcebus murinus*. *Neuroscience* 105: 403-412.
- Aujard F, Perret M. (1998). Age-related effects on reproductive function and sexual competition in the male prosimian primate, *Microcebus murinus*. *Physiol. Behav.* 64: 513-519.
- Aujard F, Seguy M, Terrien J, Botalla R, Blanc S, Perret M. (2006). Behavioral thermoregulation in a non human primate: effects of age and photoperiod on temperature selection. *Exp. Gerontol.* 41: 784-792.
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MH, White JS. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24: 127-135.
- Cayetanot F, Van Someren EJ, Perret M, Aujard F. (2005). Shortened seasonal photoperiodic cycles accelerate aging of the diurnal and circadian locomotor activity rhythms in a primate. *J. Biol. Rhythms* 20: 461-469.
- Dardente H, Cermakian N. (2007). Molecular circadian rhythms in central and peripheral clocks in mammals. *Chronobiology international* 24: 195-213.
- Diem K. (1963). Les vitamines liposolubles. In *Tables scientifiques*, Bâle: K. Geigy (Eds), pp 459-463.
- Duchamp C, Burton KA, Geloan A, Dauncey MJ. (1997). Transient upregulation of IGF-I gene expression in brown adipose tissue of cold-exposed rats. *Am. J. Physiol.* 272: E453-460.
- Engstrom BE, Burman P, Holdstock C, Ohrvall M, Sundbom M, Karlsson FA. (2006). Effects of gastric bypass on the GH/IGF-I axis in severe obesity - and a comparison with GH deficiency. *Eur. J. Endocrinol.* 154: 53-59.
- Ferrari E, Cravello L, Falvo F, Barili L, Solerte SB, Fioravanti M, Magri F. (2008). Neuroendocrine features in extreme longevity. *Exp. Gerontol.* 43: 88-94.
- Ganzhorn JU, Klaus S, Ortmann S, Schmid J. (2003). Adaptations to seasonality: Some Primate and Nonprimate Examples. In PM Kappeler, ME Pereira (Eds), *Primate Life Histories and Socioecology*, Chicago: University of Chicago Press, pp 132-144.
- Giroud S, Blanc S, Aujard F, Bertrand F, Gilbert C, Perret M. (2008). 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: R1958-1967.

- Hampl R, Starka L, Jansky L. (2006). Steroids and thermogenesis. *Physiol. Res.* 55: 123-131.
- Huang YL, Liu RY, Wang QS, Van Someren EJW, Xu H, Zhou JN. (2002). Age-associated difference in circadian sleep-wake and rest-activity rhythms. *Physiol. Behav.* 76: 597-603.
- Jefimow M, Wojciechowski M, Tegowska E. (2004). Seasonal changes in the thermoregulation of laboratory golden hamsters during acclimation to seminatural outdoor conditions. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 139: 379-388.
- Kappeler L, Epelbaum J. (2005). [Biological aspects of longevity and ageing]. *Rev. Epidemiol. Sante Publique* 53: 235-241.
- Laartz B, Losee-Olson S, Ge YR, Turek FW. (1994). Diurnal, photoperiodic, and age-related changes in plasma growth hormone levels in the golden hamster. *J. Biol. Rhythms* 9: 111-123.
- Larrouy D, Ambid L, Richard D. (1995). *La Thermorégulation*, Paris, 128 pp.
- Lovegrove BG. (2005). Seasonal thermoregulatory responses in mammals. *J. Comp. Physiol. [B]* 175: 231-247.
- Onder G, Liperoti R, Russo A, Soldato M, Capoluongo E, Volpato S, Cesari M, Ameglio F, Bernabei R, Landi F. (2006). Body mass index, free insulin-like growth factor I, and physical function among older adults: results from the iSIRENTE study. *Am. J. Physiol. Endocrinol. Metab.* 291: E829-E834.
- Palkova M, Sigmund L, Erkert HG. (1999). Effect of ambient temperature on the circadian activity rhythm in common marmosets, *Callithrix j. jacchus* (primates). *Chronobiol. Int.* 16: 149-161.
- Perret M. (1992). Environmental and social determinants of sexual function in the male lesser mouse lemur (*Microcebus murinus*). *Folia Primatol. (Basel)* 59: 1-25.
- Perret M. (1997). Change in photoperiodic cycle affects life span in a prosimian primate (*Microcebus murinus*). *J. Biol. Rhythms* 12: 136-145.
- Perret M, Aujard F. (2001). Daily hypothermia and torpor in a tropical primate: synchronization by 24-h light-dark cycle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 281: R1925-1933.
- Perret M, Aujard F. (2005). Aging and season affect plasma dehydroepiandrosterone sulfate (DHEA-S) levels in a primate. *Exp. Gerontol.* 40: 582-587.
- Perret M, Aujard F. (2006). [Aging and biological rhythms in primates]. *Med. Sci.* 22: 279-283.
- Pinheiro J, Bates D, DebRoy S, Sarkar D. (2005). nlme: linear and nonlinear mixed effects models. *R package version 3.1-85*.

- Portaluppi F, Touitou Y, Smolensky MH. (2008). Ethical and methodological standards for laboratory and medical biological rhythm research. *Chronobiol. Int.* 25: 999-1016.
- R Development Core T (2004). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rensing L, Ruoff P. (2002). Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. *Chronobiol. Int.* 19: 807-864.
- Robinson EL, Fuller CA. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *Am. J. Physiol.* 276: R1397-1407.
- Schenck BC, Stahly TS, Cromwell GL. (1992). Interactive effects of thermal environment and dietary amino acid and fat levels on rate and efficiency of growth of pigs housed in a conventional nursery. *J. Anim. Sci.* 70: 3803-3811.
- Seguy M, Perret M. (2005). Changes in olfactory inputs modify the energy balance response to short days in male gray mouse lemurs. *Physiol. Behav.* 84: 23-31.
- Sherlock M, Toogood AA. (2007). Aging and the growth hormone/insulin like growth factor-I axis. *Pituitary* 10: 189-203.
- Stocks JM, Taylor NA, Tipton MJ, Greenleaf JE. (2004). Human physiological responses to cold exposure. *Aviat. Space Environ. Med.* 75: 444-457.
- Terrien J, Zizzari P, Bluet-Pajot MT, Henry PY, Perret M, Epelbaum J, Aujard F. (2008). Effects of age on thermoregulatory responses during cold exposure in a nonhuman primate, *Microcebus murinus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295: R696-703.
- Tokura H, Aschoff J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *Am. J. Physiol.* 245: R800-804.
- Van Someren EJ, Raymann RJ, Scherder EJ, Daanen HA, Swaab DF. (2002). Circadian and age-related modulation of thermoreception and temperature regulation: mechanisms and functional implications. *Ageing Res. Rev.* 1: 721-778.
- Vermeulen A. (1995). Dehydroepiandrosterone sulfate and aging. *Ann. N. Y. Acad. Sci.* 774: 121-127.
- Vom Saal FS, Finch CE, Nelson JF. (1994). Natural History and Mechanisms of Reproductive Aging in Humans, Laboratory Rodents, and Other Selected Vertebrates. In E Knobil, JD Neill (Eds), *The Physiology of Reproduction*, New-York: Raven Press, pp 1213-1314.
- Ward ME, Cowley AR. (1999). Hypothermia: a natural cause of death. *Am. J. Forensic Med. Pathol.* 20: 383-386.
- Weinert D, Waterhouse J. (2007). The circadian rhythm of core temperature: effects of physical activity and aging. *Physiol. Behav.* 90: 246-256.

Wollnik F, Schmidt B. (1995). Seasonal and daily rhythms of body temperature in the European hamster (*Cricetus cricetus*) under semi-natural conditions. *J. Comp. Physiol. [B]* 165: 171-182.

Yamashita H, Kizaki T, Ookawara T, Sato Y, Yamamoto M, Ohira Y, Ohno H. (1994). Is insulin-like growth factor I involved in brown adipose tissue enlargement? *Life Sci.* 55: 141-148.

Zubidat AE, Ben-Shlomo R, Haim A. (2007). Thermoregulatory and endocrine responses to light pulses in short-day acclimated social voles (*Microtus socialis*). *Chronobiol. Int.* 24: 269-288.

**FIGURE CAPTIONS**

Figure 1: **A.** Mean ( $\pm$  SEM) body mass (BM) corrected caloric intake (CI in kJ/day\*100g BM) and **B.** Body mass gain (BMG in g/day) observed in young adult (N = 7) and aged (N = 5) mouse lemurs acclimated to LD14/10 and exposed to 25 and 12 °C. Linear Mixed Effects models were performed to test effects of Ta and Age.

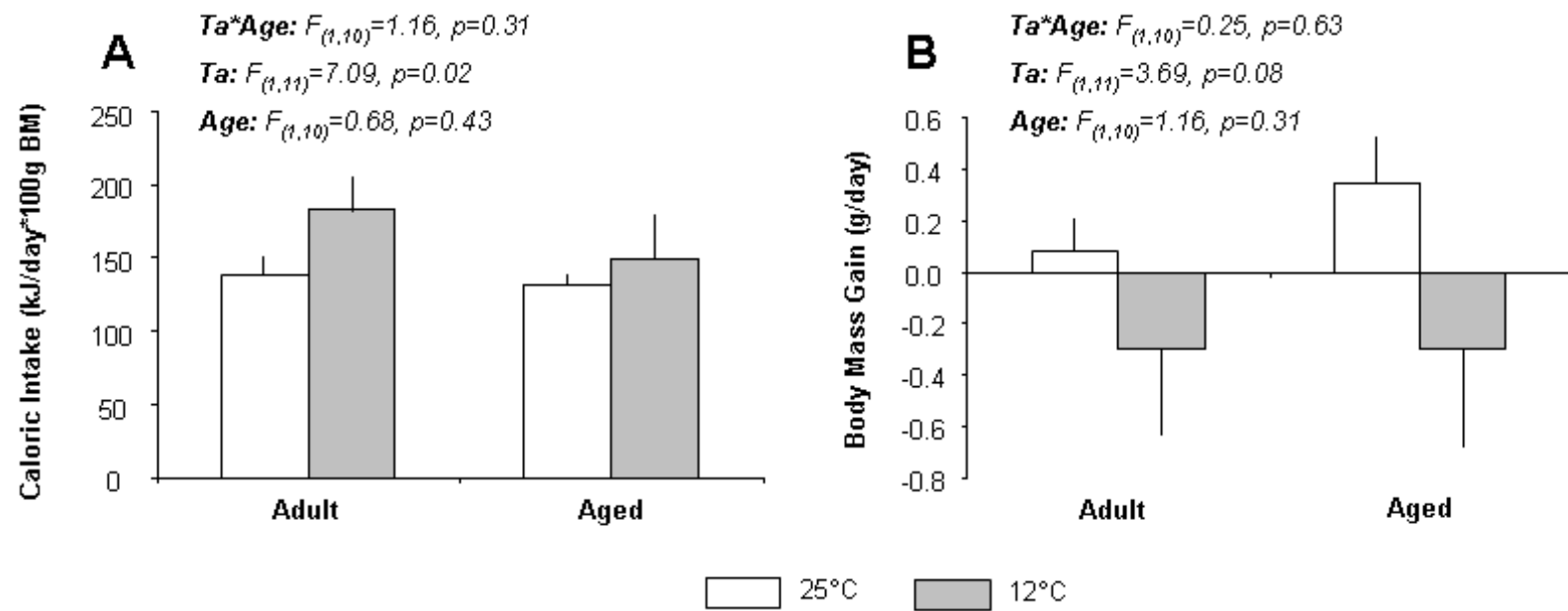
Figure 2: Mean ( $\pm$  SEM) levels of plasma IGF-1 (ng/ml) measured in young adult (N = 7) and aged (N = 5) mouse lemurs acclimated to LD14/10 and exposed to 25 and 12 °C. Linear Mixed Effects models were performed to test effects of Ta and Age.

Figure 3: Average daily profiles of locomotor activity (LA in arbitrary units a.u.) in adult (N = 7) and aged (N = 5) mouse lemurs acclimated to LD14/10 and exposed for 10 days to 25 and 12 °C. Data presented as mean  $\pm$  SEM.

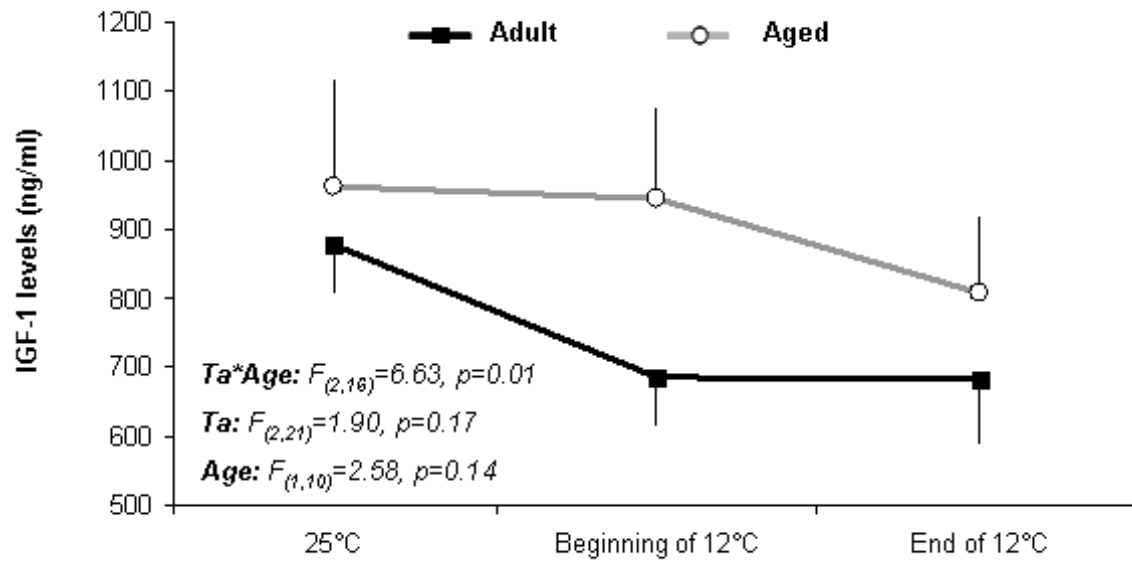
Figure 4: Time course of mean ( $\pm$  SEM) core temperature (Tc in °C) in adult (N = 7) and aged (N = 5) mouse lemurs acclimated to LD14/10 and exposed for 10 days at 25 and 12 °C.

Figure 5: **A.** Time of minimal core temperature occurrence ( $H_{\min}$  in min) and **B.** of core temperature decrease onset ( $H_{\text{decr}}$  in min) in young adult (N = 7) and aged (N = 5) mouse lemurs acclimated to LD14/10 and exposed to 25 and 12 °C. Data presented as mean  $\pm$  SEM. Linear Mixed Effects models were performed to test effects of Ta and Age.

**Figure 1.**

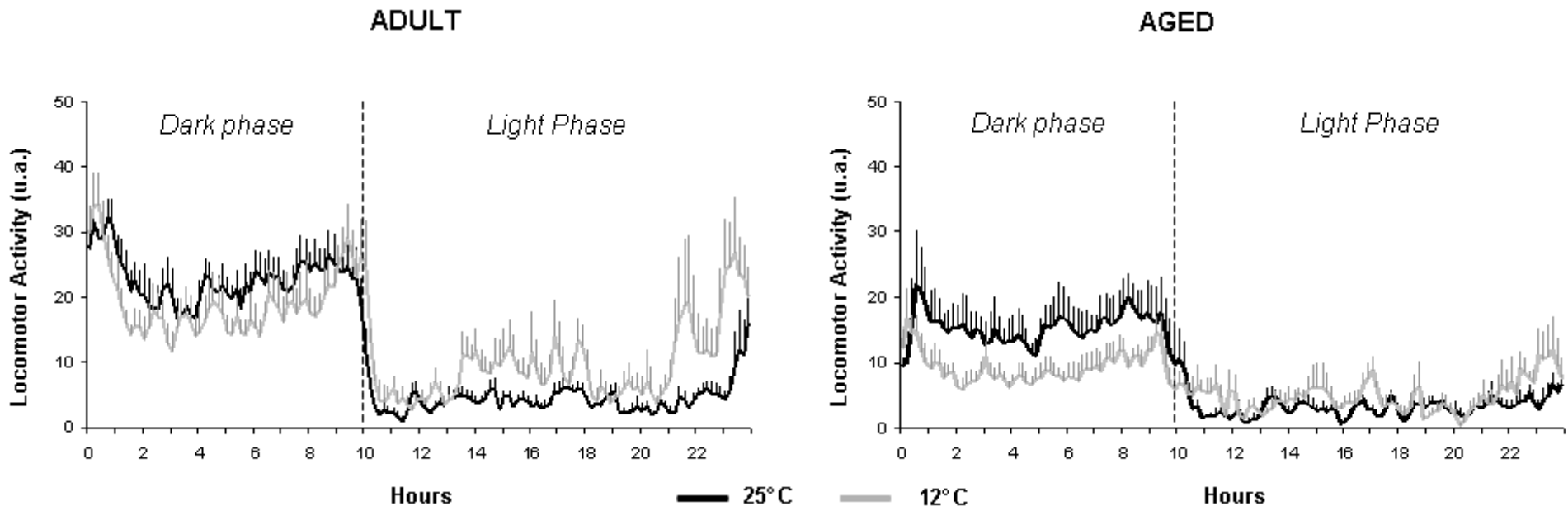


**Figure 2.**





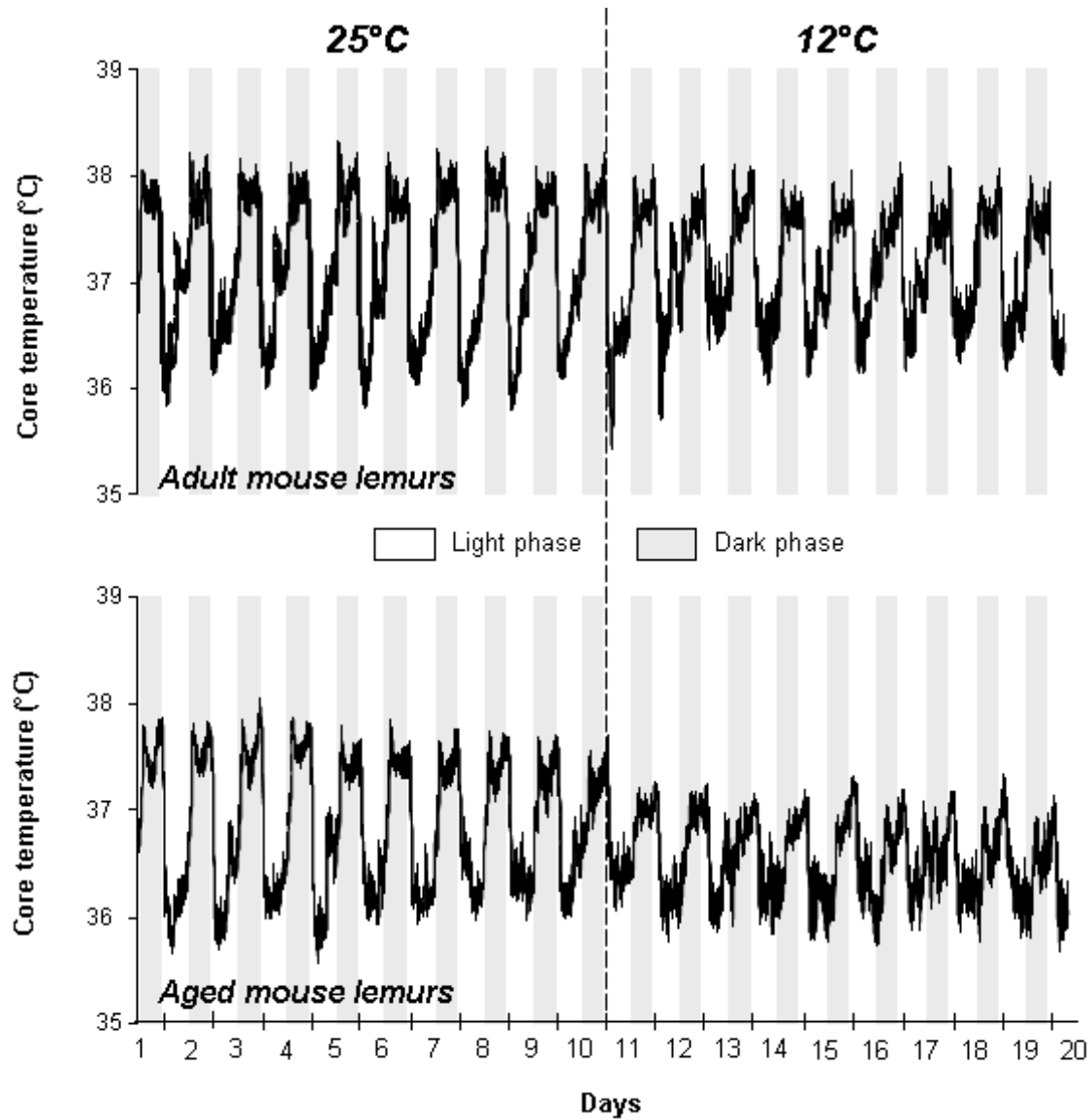
**Figure 3.**



**Table 1.** Rhythm parameters (means values  $\pm$  SEM) of locomotor activity (LA) registered in adult and aged mouse lemurs acclimated under LD14/10 during the 10-day exposures to 25 °C and 12 °C. LME were performed and F and p values are notified.

Parameters	Age	25° C	12° C	LME		
				Ta effect	Age effect	Ta*Age effect
<b>LA<sub>night</sub></b> <b>(u.a.)</b>	<b>Adult</b>	22.7 $\pm$ 2.5	19.2 $\pm$ 2.8	<b>F<sub>(1,10)</sub>=8.66,</b> <b>p=0.01</b>	<b>F<sub>(1,9)</sub>=22.86,</b> <b>p=0.00</b>	<b>F<sub>(1,9)</sub>=12.49,</b> <b>p=0.01</b>
	<b>Aged</b>	15.3 $\pm$ 4.6	9.3 $\pm$ 1.8			
<b>LA<sub>day</sub></b> <b>(u.a.)</b>	<b>Adult</b>	4.5 $\pm$ 0.4	9.6 $\pm$ 2.2	F <sub>(1,10)</sub> =0.37, p=0.56	F <sub>(1,9)</sub> =0.09, p=0.77	<b>F<sub>(1,9)</sub>=14.05,</b> <b>p=0.00</b>
	<b>Aged</b>	3.3 $\pm$ 0.7	4.9 $\pm$ 1.4			
<b>LA onset</b> <b>(min)</b>	<b>Adult</b>	51 $\pm$ 14	107 $\pm$ 25	<b>F<sub>(1,10)</sub>=6.97,</b> <b>p=0.02</b>	F <sub>(1,9)</sub> =0.39, p=0.55	F <sub>(1,9)</sub> =0.50, p=0.83
	<b>Aged</b>	76 $\pm$ 40	128 $\pm$ 39			
<b>LA offset</b> <b>(min)</b>	<b>Adult</b>	0 $\pm$ 8	-11 $\pm$ 8	F <sub>(1,10)</sub> =2.98, p=0.11	F <sub>(1,9)</sub> =0.61, p=0.45	F <sub>(1,9)</sub> =1.06, p=0.33
	<b>Aged</b>	-4 $\pm$ 16	-40 $\pm$ 32			
<b>LA alpha</b> <b>(min)</b>	<b>Adult</b>	651 $\pm$ 12	719 $\pm$ 26	<b>F<sub>(1,10)</sub>=6.21,</b> <b>p=0.03</b>	F <sub>(1,9)</sub> =2.53, p=0.15	F <sub>(1,9)</sub> =0.20, p=0.66
	<b>Aged</b>	680 $\pm$ 25	768 $\pm$ 48			

**Figure 4.**



**Table 2.** Rhythm parameters (means values  $\pm$  SEM) of core temperature (Tc) registered in adult and aged mouse lemurs acclimated under LD14/10 during the 10-day exposures to 25 °C and 12 °C. LME were performed and F and p values are notified.

Parameters	Age	25° C	12° C	LME		
				Ta effect	Age effect	Ta*Age effect
Tc <sub>night</sub> (°C)	Adult	37.8 $\pm$ 0.1	37.5 $\pm$ 0.1	F <sub>(1,11)</sub> = <b>16.00</b> , p= <b>0.00</b>	F <sub>(1,10)</sub> = <b>39.00</b> , p= <b>0.00</b>	F <sub>(1,10)</sub> = <b>11.00</b> , p= <b>0.01</b>
	Aged	37.5 $\pm$ 0.1	36.7 $\pm$ 0.1			
Tc <sub>day</sub> (°C)	Adult	36.6 $\pm$ 0.0	36.6 $\pm$ 0.1	F <sub>(1,11)</sub> =2.40, p=0.15	F <sub>(1,10)</sub> = <b>19.30</b> , p= <b>0.01</b>	F <sub>(1,10)</sub> =3.50, p=0.09
	Aged	36.3 $\pm$ 0.1	35.9 $\pm$ 0.1			
Tc <sub>min</sub> (°C)	Adult	35.8 $\pm$ 0.1	35.9 $\pm$ 0.2	F <sub>(1,11)</sub> =0.72, p=0.42	F <sub>(1,10)</sub> =0.83, p=0.38	F <sub>(1,10)</sub> =1.38, p=0.27
	Aged	35.8 $\pm$ 0.1	35.4 $\pm$ 0.1			

**Figure 5.**

