# Effects of age on thermoregulatory responses during cold exposure in a non-human primate, *Microcebus murinus*.

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Running head: Aging and cold resistance in a non-human primate

## ABSTRACT

Cold resistance appears altered with aging. Among existing hypothesis, the impaired capacity in response to cold could be related to an altered regulation of plasma insulin-like growth factor-1 (IGF-1) concentration. The combined effects of age and cold exposure were studied in a short-living primate, the gray mouse lemur (*Microcebus murinus)* which adjusts its energy balance using a daily torpor phase, to avoid high energy cost of normothermia maintenance. Changes in body mass, core temperature, locomotor activity and caloric intake

were monitored under 9-day exposures to 25 °C and 12 °C in captive animals in winter conditions. Short-term (after 2 days) and long-term (after 9 days) cold-induced changes in IGF-1 levels were also evaluated. In thermoneutral conditions (25 °C), general characteristics of the daily rhythm of core temperature were preserved with age. At 12 °C, age-related changes were mainly characterized by a deeper hypothermia and an increased frequency of torpor phases, associated with a loss of body mass. A short-term cold-induced decrease in plasma IGF-1 levels was observed. IGF-1 levels returned to basal values after 9 days of cold exposure. No significant effect of age could be evidenced on IGF-1 response. However, IGF-1 levels of cold-exposed aged animals were negatively correlated with the frequency of daily torpor. Responses exhibited by aged mouse lemurs exposed to cold revealed difficulties in the maintenance of normothermia and energy balance, and might involve modulations of IGF-1 levels.

**Key words**: autonomic thermoregulation – cold exposure – IGF-1 – aging – *Microcebus murinus*.

## INTRODUCTION

Although some studies concluded to a conservation of thermoregulatory capacities during aging in normothermic conditions [25, 27], appropriate coping to cold environment appears altered with age [16]. Experimental data are available on core temperature (Tc) response to cold exposure in aged animals but the mechanisms involved in this impaired cold resistance remain to be fully elucidated. In response to cold, homeothermic organisms, including mammals, enhance body heat production and conservation to maintain daily variations in Tc in a normothermic range. In rodents [47], The first responses to cold exposure are mostly behavioural. Autonomic thermoregulatory responses are secondarily involved and require the intervention of two processes: shivering and humoral thermogenesis. Shivering thermogenesis is the main autonomic response to cold in big mammals. Humoral thermogenesis can be divided into non-shivering thermogenesis (NST, [24]), and hormonal thermogenesis. NST corresponds to mitochondrial heat production by enhancement of cellular metabolism in brown adipose tissue (BAT, [43]). This process plays a major function in small mammals [24, 43]. A role of Insulin-like Growth Factor type 1 (IGF-1), a growth factor enhancing cellular proliferation and maturation, has been proposed to act in cold-induced BAT increase in the rat [12, 51]. Indeed, in these studies, IGF-I levels decreased in the plasma but increased markedly in BAT after several days of cold exposure. It was thus, suggested that the cold-induced plasma IGF-1 decrease could be induced by the massive recruitment of growth factors in BAT for a more efficient response to cold. Moreover, small mammals, which have low capacities of energy storage, exhibit a daily hypothermic phase, particularly strong when exposed to low ambient temperature (Ta). Heterotherms use daily hypothermia as an alternative strategy against high costs of normothermia maintenance by minimizing energy costs of heat production [18, 22, 28]. Daily hypothermia is associated with a low basal metabolism, a rapid

drop in Tc and a slow re-establishment to normothermic values which takes several hours. In the case of drastic environmental conditions (food rarefaction or cold Tas), the daily hypothermic phase becomes deeper and the Tc can reach values under 33 °C. This deep hypothermic state is defined as daily torpor [20]. Arousal from hypothermia or torpor mainly involves the activation of NST. Therefore, we postulated that IGF-1 might be important for small mammals which have to increase their Tc after this hypothermic episode.

An impaired ability to efficiently thermoregulate during cold exposure has been established in aged humans and laboratory animals (for review see [16]) but experimental data on this matter are not always consistent. For example, in aged rats [26], mean body temperature levels do not differ significantly between young and aged rats, but age-related differences can be observed in regards to changes in amplitude of the daily body temperature rhythm. In fact, mean body temperature levels show significant decrease in senescent rats only. More recently, studies have demonstrated that aging induces subtle but robust changes in thermoregulation [10, 48]. To date, the reasons why some aged individuals become more fragile towards cold exposure are not entirely clear. In aged rats, exposure to cold also induces a blunted NST response [15]. A relationship between IGF-1 and the observed age-related alteration of NST response to cold may occur, since the IGF-1 axis is deficient during aging (for review see [45]). In order to understand the mechanisms and adaptive strategies implied in age-related impairments in thermal resistance, we investigated, in a small nocturnal Malagasy primate, the mouse lemur (*Microcebus murinus*, Primates), whether Tc rhythm and blood IGF-1 modulations in response to cold exposure were similar between adult and aged individuals.

The mouse lemur is exposed, in its natural habitat, to strong seasonal variations of Ta and food availability, particularly in winter when Ta values are the lowest and thermal amplitude between night and day is very high [40]. To resist to cold Tas, the mouse lemur developed different behavioral (gregarism, nesting in buffered holes) and autonomic (daily torpor)

responses. These advanced thermoregulatory processes are mainly characterized by the modulation of hypothermia, which is daily exhibited in normothermic conditions. In captive animals, modulation of the hypothermic phase varies according to photoperiod and Ta [4, 6, 35, 42], food availability [42] and social factors [41]. In this primate, the expression of a very robust daily rhythm of Tc involves the use of NST by activation of BAT [19]. In this species, age-related physiological changes have been well studied. With aging, declines in sex hormones [3], melatonin [2] and DHEA-S levels [31, 33] are observed. Moreover, age-related deficits in behavioral thermoregulation were recently demonstrated in this species [5]. In the present study, we tested the hypothesis that normothermia maintenance could be deteriorated with aging in the mouse lemur. Age-related effects on autonomic cold-resistance capacities were investigated by the follow-up of daily Tc rhythm, body mass, caloric intake and plasma IGF-1 levels in adult and aged mouse lemurs exposed to cold.

## 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), 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 short-day season, when animals have to cope with strong ambient conditions. During the

short-day season, mouse lemurs spontaneously enter in sexual rest and exhibit a significant gain in body mass. General conditions of captivity were applied and animals were maintained in social groups before and after testing. In the breeding colony of the Brunoy laboratory, analysis of survival from 254 male mouse lemurs allowed to determine the mean life span (mean ± SEM: 6.0 ± 0.2 years), the mean life span of the 10% of the most long lived animals (10.0 ± 0.2 years) and the observed maximal survival duration (12,0 years). Among age-associated pathologies, the incidence of cataracts was studied in the mouse lemur colony. Cataracts were very rare in adults but were diagnosed in more than 50% of animals over 7 years of age [7]. In the present study, adults (N = 6; mean age ± SEM: 2.3 ± 0.3 years, range: 1.2 – 3.0) and aged mouse lemurs (N = 6; mean age ± SEM: 7.1 ± 0.2 years, range: 6.5 – 7.8) were used. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC). All efforts were made to minimize nociception. Individual life span of aged animals used in this study was monitored in order to evaluate if those animals were or not in a senescent stage of their life at the time of the experiments. Aged animals lived on average 1.3 ± 0.2 years after the end of the study.

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 Ta = 25 °C. They were then studied for 9 days at the reference Ta of 25 °C and then exposed to a cold environment (9 days at 12 °C). Core temperature (Tc) 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 to register the data given by the transmitter. The Tc (in °C) was recorded every 5 minutes and the locomotor activity (LA in arbitrary units a.u.) was continuously recorded by two antennas located in the receptor plate and detecting vertical and horizontal movements (X-Y coordinate system). Data were computed by a software (Dataquest Lab Pro v. 3.0, Data Science Co. Ltd, Minnesota, USA). Daily profiles were smoothed by a 10-min moving average. The following parameters were analyzed on these smoothed profiles: mean Tc during the active nocturnal phase ($Tc_{night}$), mean Tc during the resting diurnal phase ($Tc_{day}$), the minimal Tc value ($Tc_{min}$), time of occurrence of $Tc_{min}$ ($H_{min}$) and time of occurrence of the beginning of Tc decrease ($H_{decr}$). The last two parameters were expressed in minutes relative to lights on. Times of occurrence preceding lights on (phase advances) were expressed by positive values. Inversely, phase delays were expressed by negative values. $Tc_{min}$, $H_{decr}$, and $H_{min}$ were representative parameters of the daily hypothermic phase. The frequency of torpor ($F_{torp}$) over the 9 days of exposure to a given Ta was determined for each animal at each Ta, according to the fact that hypothermia was considered as torpor when Tc dropped below 33 °C [20]. Finally, LA values were summed during the nocturnal active phase ($LA_{night}$) and the diurnal resting phase ($LA_{day}$). All telemetric parameters were first averaged for each thermal exposure, and then analyzed as a day-by-day time course over each thermal exposure.

Body mass, caloric intake:

Before the experiment, body mass (BM) of adult (mean ± SEM: 103.5 ± 7.3 g) and aged mouse lemurs (mean ± SEM: 97.2 ± 7.7 g) did not differ significantly (One-way ANOVA, $F_{1, 10} = 0.36$, NS). BM was measured every 2 days throughout the experiment. 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 to the food mass given. CI was expressed in kJ [49] according to the Diem table [11] 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 at each Ta. The Body Mass Gain (BMG) was also followed and calculated as a mean ratio (in g/day) during the whole exposure.

Plasma IGF-1 levels:

To assess IGF-1 levels in response to thermal stress, blood was taken from all animals at the reference Ta of 25 °C and then 2 days (short-term response) and 9 days (long-term response) after the beginning of cold exposure. About 100 µl of blood was drawn from the saphenous vein into heparinized capillaries without anesthesia. After centrifugation, plasma was immediately collected and preserved at –20 °C until the radioimmunoassay, which was performed in reference to the manufacturer instructions (Immunotech IGF-I IRMA; Bechman Coulter, Paris, France). Intra- and between-series variation rates were lower than 7% and minimal detectable values were 2 ng/ml. One data corresponding to an aged mouse lemur missed for the second day of cold exposure because of a technical failure during the sealing of capillaries. As Tc rhythms could be disturbed by animal handling, the telemetric data corresponding to the 4 hours after the blood samples were removed. IGF-1 plasma levels were related to the body mass of the animal studied in accordance to the fact that IGF-1, by its property of growth factor, can interact with body composition, and particularly with body fat [13, 29].

Statistical analysis

Results are given in mean ± SEM. All data, except $F_{torp}$, were normally distributed, according to the Shapiro-Wilk normality test. Linear Mixed Effect models (LME) were performed by using the "nlme" function [36] in software R Version 2.6.0 [37]. Tests of effects resulted from comparisons of more complex models with the most parsimonious model (i.e. the model including significant effects only). Then, mean energy values (BM, BMG and CI) and IGF-1 levels were analysed with models including the additive effects of age (two levels, young *versus* old) and Ta (two levels, 12 °C and 25 °C), and their interaction. Second, temporal variations of the telemetric parameters ($Tb_{night}$, $Tb_{day}$, $Tb_{min}$, $H_{decr}$, $H_{min}$, $LA_{night}$ and $LA_{day}$) during exposures to temperature treatments were analysed. The unconstrained effect of time (nine days), plus two-term and three-term interactions, were added to the effects of age and Ta in the LME models. To account for repeated measures on the same randomly chosen animals, individual identities (twelve levels) were declared as random effect in LME models. In addition, since the same individuals were used to test for the effect of the two temperature treatments, the additive effect of temperature was also included in the random term. As $F_{torp}$, that was not normally distributed, was a probability and followed a binomial distribution, we logit-transformed and analysed it with linear mixed model (nlme function under "R"), testing coupling effects of age and Ta exposures.

Finally, Spearman correlations were also performed to test the presence of correlations between IGF-1 blood levels and frequency of torpor occurrence.

## RESULTS

**Coupling effects of cold exposure, age and duration of treatments on Tc and LA rhythms parameters:**

Mean values of Tc and LA rhythms parameters measured for both Ta and both age groups are represented in Table 1. At each thermal exposure (25 °C or 12 °C), adult and aged animals exhibited very robust mean daily rhythms of Tc (Figure 1) with high values during the nocturnal active phase and lower values during the diurnal resting phase. All Tc parameters ($Tc_{night}$, $Tc_{day}$ and $Tc_{min}$) were significantly affected by the triple interaction age, Ta and duration of Ta exposure (Table 2). Tc levels at 25 °C were similar between the two experimented ages but strongly differed after exposure to 12 °C. Aged animals exhibited lower values of nocturnal and diurnal Tc than adult ones at 12 °C (Table 1), revealing differences in the strength of effect of Ta between adult and aged animals. Indeed, effects of Ta on Tc levels were major in aged animals whereas they were minor in adult animals, as attested in Figure 1. Aged animals were also characterized by a higher inter-individual variability. For example, at Ta = 12 °C, individual average $Tc_{min}$ values ranged from 24.3 °C to 33.9 °C in aged mouse lemurs, whereas they ranged only from 34.0 °C to 35.5 °C in adult ones. At Ta = 25 °C, Tc values did not vary during the whole duration of experiment, whatever the age group. Considering the fact that $Tc_{min}$ variations were the most representative parameter of hypothermia modulation, they were represented day to day on Figure 2. At Ta = 12 °C, $Tc_{min}$ of adult animals did not vary significantly during the whole cold exposure and the levels were similar to those exhibited at 25 °C. In aged animals, $Tc_{min}$ immediately decreased to an average value of 28.8 ± 2.1 °C on the first day of cold exposure. Thereafter, $Tc_{min}$ values slowly decreased from day 2 ($Tc_{min}$ = 32.1 ± 1.1 °C) to day 6 ($Tc_{min}$ = 24.5 ± 3.1 °C) and increased from day 6 to 7, to reach an average value of 33.2 ± 1.1 °C. Finally, aged animals restored reference levels of $Tc_{min}$ from day 7 to the end of the cold

exposure. The same observations could be made for $Tc_{day}$ and $Tc_{night}$, but with a smaller amplitude of variation.

Concerning the temporal organization of daily hypothermia, no significant effect of age or duration of Ta exposure could be evidenced on $H_{decr}$ (Table 2). Nevertheless, a slight effect of Ta was observed with a decrease between 25 °C and 12 °C in adult animals (62 ± 6 min vs 48 ± 5 min) and in aged animals (86 ± 4 min vs 55 ± 6 min). By contrast, a strong effect of Ta was observed on $H_{min}$ (Figure 3 – Table 2). On average, $Tc_{min}$ occurred later at 12 °C than at 25 °C. No effect of duration was directly evidenced on the two temporal parameters. However, as shown on Figure 3, inter-individual variability increased under cold exposure in comparison to 25 °C, in both age categories.

Mean amounts of $LA_{night}$ did not differ significantly neither between adult and aged animals, nor between 25 °C and 12 °C (Table 2). Nevertheless, a significant effect of duration of Ta exposure was observed for $LA_{night}$, characterized by a progressive decrease of 33% between day 1 and day 7, followed by a slow increase to initial values from the $7^{th}$ day under cold exposure. Finally, $LA_{day}$ was strongly affected by the change in temperature. Indeed, aged animals exhibited higher levels of $LA_{day}$ at each thermal exposure. Independently of age, mouse lemurs were less active after exposure to 12 °C than at 25 °C. Moreover, the levels of $LA_{day}$ remained relatively constant in adult animals during both thermal exposures whereas aged animals exhibited a strong 73% increase on the second day at 12 °C, levels restored to reference levels at day 3.

**Effects of age and Ta on mean parameters of torpor frequency, energy balance and IGF-1 levels:**

Logit-transformed torpor frequencies differed significantly according to coupling effects of age and Ta ($\chi^2_{(1)} = 7.58$, $p = 0.006$). Aged animals exhibited a largely higher frequency of

torpor than adult animals at 12 °C (67 ± 13 % vs 11 ± 4 %) whereas this difference was slight at 25 °C (11 ± 7 % vs 3 ± 3 %). This result confirmed the higher susceptibility of aged animals towards cold exposure. In fact, a small increase in $F_{torp}$ was observed in adult animals after cold exposure, while a 9-time increase was observed in aged animals (Table 1).

During the 9 days of exposure to 25 °C and 12 °C, CI was affected neither by age ($\chi^2_{(1)}$ = 1.11, p = 0.29), nor by Ta ($\chi^2_{(1)}$ = 0.98, p = 0.32) and animals ate an average amount of 132 ± 18 kJ/day*100g BM (Figure 4). However, even if animals did not change their feeding behavior under conditions of age or Ta, BMG varied according to age ($\chi^2_{(1)}$ = 7.57, p = 0.01) and Ta ($\chi^2_{(1)}$ = 7.16, p = 0.01). BMG was always lower in aged animals compared to adults, and a decrease between 25 °C and 12 °C was observed in both age groups, leading to a negative energy balance in aged animals exposed to cold (-0.7 ± 0.4 g/day).

IGF-1 values differed between the 3 sampled time points with a short-term decrease 2 days after cold exposure when compared to basal values at 25 °C (Table 3). Then, basal IGF-1 levels were restored after 9 days of exposure to cold. Even if no effect of age was observed, an increase of the intra-individual variability was evidenced in aged animals at 12 °C. Indeed, SEM was higher in aged than in adult animals exposed to cold and could reach until 3 times the values measured in adult animals. IGF-1 levels and $F_{torp}$ were not correlated in both age groups at Ta = 25 °C (adults: $r_s$ = 0.655, NS; aged: $r_s$ = -0.370, NS) and at the beginning of cold exposure (adults: $r_s$ = 0.794, NS; aged: $r_s$ = -0.410, NS). By contrast, after 9 days at Ta = 12 °C, although no correlation was evidenced in adult animals ($r_s$ = 0.412, NS), IGF-1 blood levels were negatively correlated with the frequency of torpors in aged animals ($r_s$ = -0.870, $p<0.05$). High frequency of torpor was associated with low plasma levels of IGF-1.

## DISCUSSION

In thermoneutral conditions, adult mouse lemurs exhibited robust daily rhythms of Tc, particularly characterized by hypothermia during the first half of the light period [34]. Mouse lemurs, active during the night, nested in anticipation of lights on, for the whole duration of the day. Tc decreased more than 2 hours before the onset of light and $Tc_{min}$ was reached within the next 2 hours following lights on. Tc progressively increased during the second part of the day. The decrease in Tc during hypothermia cannot be considered as a passive failure of normothermia maintenance but more as an active and regulated response, as already described in rodents [38, 46]. Thus, Tc was maintained in adult animals upon a specific minimal value, determined by the hypothalamus, through an homeostatic process [14]. Adult mouse lemurs exhibited a significant increase in body mass, corresponding to the classical gain observed in animals during their short day period [20].

Under cold exposure, adult animals maintained normothermia and the use of torpor, described as a strong hypothermic phase during which Tc drops below 33 °C [20], was rare. The increase in caloric intake observed in pigs after cold exposure [39], in compensation for the increase in energy expenditure necessary for maintaining normothermia, was not observed in adult mouse lemurs. Cold exposure induced a decrease in body mass gain. Tc began to decrease later at 12 °C in adult mouse lemurs in comparison to 25 °C. Even if the amount of nocturnal locomotor activity was not higher in adult animals at 12 °C in comparison to 25 °C, we suspect that the total energy expenditure increased during cold exposure. Indeed, a previous study described an increase of basal metabolic rate after a cold exposure in adult animals maintained in an experimental device adapted for oxygen consumption [4]. Compensatory strategies, such as an effective non-shivering thermogenesis or endocrine efficient modulations, may be developed in adult animals to avoid hypothermia. In rats, modulations of blood IGF-1 levels by cold exposure are reported in some [12] but not all

studies [15]. In the present study, blood IGF-1 levels were significantly modulated in adult mouse lemurs during cold exposure. A slight short-term decrease of plasma IGF-1 levels was induced after 2 days of cold exposure and was followed by a re-establishment to basal values after 9 days of cold exposure, suggesting a physiological acclimation to cold. In rats, an increase in IGF-1 synthesis in BAT has been also described after the beginning of cold exposure [12]. Thus, the IGF-1 could act at two levels for cold resistance: the blood IGF-1 would be used in BAT for tissue enlargement before the increase of IGF-1 synthesis in BAT and other peripheral tissues. This two-step reaction could be sufficient to prevent hypothermia in mouse lemurs during the 9 days of cold exposure. However, the increase in IGF-1 synthesis in BAT remains to be demonstrated in the mouse lemur, by assessing BAT metabolism during cold exposure. The modulations of blood IGF-1 levels as a short-term response to cold exposure may confirm a role for this endocrine parameter in thermal resistance, particularly in the occurrence and the organization of the hypothermic phase, which was markedly affected by cold. Circulating IGF-1 levels could be efficient at the beginning of cold exposure to maintain normothermia and could be reinforced by another cellular pathway to maintain the resistance and permit acclimatization. For instance, $Tc_{min}$ was only briefly modified by cold exposure, suggesting a rapid efficiency of Tc modulation for prevention of hypothermia.

Concerning the effects of aging on thermoregulation, some studies [50] evidenced age-related alterations in Tc rhythms while others did not [27]. In fact, the difference between aging and senescence needs to be taken into account, since major changes in Tc rhythms observed in aged animals under thermo-neutrality (25 °C) seem to appear with the first signs of senescence, as demonstrated in rats [26]. Although age-related deficits in thermoregulation were not consistently demonstrated in both humans and non humans in the past, recent studies corroborate the fact that some subtle age-associated changes in thermoregulatory responses can be seen even under mild cooling condition [10] and can be influenced by external factors

such as the photoperiod [5]. Compared to the first published survival data of mouse lemurs from the Brunoy colony [30], the actual data base has permitted to obtain more precise survival data from a greater number of animals. These data corroborate the previously published data. Based on survival data, the experimented aged animals belong to an age category composed of more than 30% of living individuals. In rats, clear thermoregulatory alterations appeared only at the start of senescence [26], when animals began to lose weight. It is unlikely to be the case in the present study since no difference in body mass could be found between adult and aged mouse lemurs, and since aged animals survived more than one year after the end of the experiments. In thermoneutral conditions, no Tc rhythm disturbance was observed with age in mouse lemurs. Some previous studies in laboratory animals [23] described an age-associated decline in the amplitude of Tc rhythm. In the present study, the Tc rhythm amplitude did not seem to be disturbed, although the occurrence of minimal value of Tc was delayed in aged compared to adult animals. Moreover, locomotor activity was not affected by age. This is in agreement with the data of Cayetanot *et al.* [9] and Aujard *et al.* [1] showing that only animals exposed to long daylength exhibited age-related disturbances of the locomotor activity rhythm, whereas animals exposed to short photoperiod did not. This can be related to the high seasonality of physiological and behavioral functions of this species. Indeed, animals acclimated to winter are in a resting state, leading to low metabolic rate and body mass gain [21] and consequently to the decrease of activity levels. Animals acclimated to summer increase their metabolic rate, lose body mass [21] and enhance their locomotor activity to prepare the reproductive season. Even if no evidence of age-associated thermoregulatory deterioration could be seen in the present study, age-related thermal resistance deficiencies were demonstrated in a previous study using a paradigm of behavioral thermoregulation [5]. In that experiment, aged animals in short photoperiod spontaneously selected higher Tas than adult animals, in order to compensate eventual age-related increase

in heat loss, as also described in rodents [8, 16, 17]. Behavioral difference could be interpreted as a subtle change, as already related above.

When exposed to low Tas, alterations in cold resistance did occur in humans [44, 52]. In the present study, marked age-associated changes in thermoregulatory responses were also observed under cold exposure. The main differences between adult and aged mouse lemurs at 12 °C were revealed around the hypothermic phase. The depth of hypothermia and the frequency of torpors were significantly higher in aged animals compared to adult ones. This is likely to result in differences in energy management, and particularly during Tc increase, as also suggested by the increase of basal metabolism described in adult mouse lemurs during the Tc increase following torpor episodes [19]. Blood IGF-1 levels varied in aged mouse lemurs after cold exposure, in accordance with modulations observed in adult animals. This suggests an efficient mobilization of IGF-1 by thermogenic tissues in aged animals. However, a higher inter-individual variability was observed in the aged group in response to cold. Moreover, animals with the lowest IGF-1 levels after 9 days at 12 °C exhibited the highest frequencies of torpor. Taken together, these data suggest that some aged animals efficiently responded to cold while others did not, and that IGF-1 might be involved in this process. Such hypotheses could be verified in the future on a higher number of experimented mouse lemurs, and could eventually permit to evidence the existence of a healthy aged population and a frail one. In addition, because most biomarkers of aging validated in this species sometimes begin to decline at relatively early ages [32], future experiments on middle-aged animals may bring new information about the time course of apparition of such age-associated changes. This could also permit to observe correlations between Tc parameters disturbances and appearance of endocrine failures. Moreover, the time necessary for some aged animals to recruit all autonomic effectors in order to efficiently respond to cold could be longer than the one necessary for adult animals. In accordance with this hypothesis, Tc parameters in aged

animals decreased mostly during the first days of cold exposure and were apparently restored after 7 days of cold exposure.

Adult and aged animals seemed to develop different energy strategies to resist to cold exposure. Adult animals succeeded in maintaining normothermia without losing body mass. In contrast, aged animals developed torpor strategy to avoid the high costs of normothermia maintenance. The depth of hypothermic phases in aged animals might be a critical factor for energy management, particularly during Tc increase after the occurrence of the minimal Tc value. Indeed, in aged animals exposed to cold, the thermoregulatory “compromise”, leading to torpor, could not prevent body mass loss. This suggests that an energetic cost could not be avoided, although the energy economy induced by the torpor phases seems necessary for survival. Even if no direct effect of age could be evidenced on IGF-1 response, the increase of inter-individual variability in plasma IGF-levels with aging during the whole cold exposure likely attests the existence of an impaired endocrine response in deficient aged animals.

## Perspectives and significance

Responses exhibited by aged mouse lemurs exposed to cold revealed difficulties in the maintenance of normothermia and energy balance, which might be partially related to IGF-1 modulation failures. The understanding of age-related impairment in cold response is not totally elucidated in both humans and non human mammals, but the present data open interesting ways to explore this phenomenon in further experiments. The follow-up of non-shivering thermogenesis constitute the main challenge to test the existence of an age-related impairment in IGF-1 modulations within brown adipose tissue. The endocrine regulation of energy balance, such as gut hormones levels, could also be monitored to evidence (or not) an age-related impairment in energy mobilization during cold resistance, as suggested by the

negative energy balance observed in aged animals. Gender- or season-dependent responses may also be evidenced since sexual endocrine parameters might also be involved.

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**FIGURE CAPTIONS**

Figure 1: Daily rhythms of core temperature (Tc in °C) and locomotor activity (LA in arbitrary units a.u.) in adult and aged mouse lemurs exposed to 25 °C (A) and 12 °C (B) under short photoperiod. Data are given as mean ± SEM. N = 6 animals per group. Curves represent Tc rhythms and areas correspond to LA rhythms.

Figure 2: Time course of minimal core temperature ($Tc_{min}$ in °C) in adult and aged mouse lemurs exposed for 9 days to 25 °C and 12 °C. Data are given as mean ± SEM. N = 6 animals per group. D = day.

Figure 3: Time course of occurrence hour of minimal core temperature ($H_{min}$ in min) in adult and aged mouse lemurs exposed for 9 days to 25 °C and 12 °C. Data are given as mean ± SEM. N = 6 animals per group. D = day.

Figure 4 : Caloric intake (CI) and Body Mass Gain (BMG) in adult and aged mouse lemurs exposed to 25 °C and 12 °C under short photoperiod. Data are given as mean ± SEM. N = 6 animals per group. Caloric intake is given in kJ/day*100g Body Mass and Body Mass Gain in g/day. Linear Mixed Effects models were performed to test the effects of Ta on the modulation of CI and BMG.