Dietary palmitate and linoleate oxidations, oxidative stress, and DNA damage differ according to season in mouse lemurs exposed to a chronic food deprivation

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Giroud S, Perret M, Gilbert C, Zahariev A, Goudable J, Le Maho Y, Oudart H, Momken I, Aujard F, Blanc S. Dietary palmitate and linoleate oxidations, oxidative stress, and DNA damage differ according to season in mouse lemurs exposed to a chronic food deprivation. Am J Physiol Regul Integr Comp Physiol 297: R950–R959, 2009. First published July 22, 2009; doi:10.1152/ajpregu.00214.2009.—This study investigated the extent to which the increase in torpor expression in the grey mouse lemur, due to graded food restriction, is modulated by a trade-off between a whole body sparing of polyunsaturated dietary fatty acids and the related oxidative stress generated during daily torpor. We measured changes in torpor frequency, total energy expenditure (TEE), linoleate (polyunsaturated fatty acid) and palmitate (saturated fatty acid) oxidation, hexanoyl-lysine (HEL; the product of linoleate peroxidation), and 8-hydroxydeoxyguanosine (8OHdG; a marker of DNA damage). Animals under summer-acclimated long days (LD) or winter-acclimated short days (SD) were exposed to a 40% (LD40 and SD40) and 80% (LD80 and SD80) 35-day calorie restriction (CR). During CR, all groups reduced their body mass, but LD80 animals reached survival-threatened levels at day 22 and were then excluded from the CR trial. Only SD mouse lemurs increased their torpor frequency with CR and displayed a decrease in their TEE adjusted for fat-free mass. After CR, SD40 mouse lemurs shifted the dietary fatty acid oxidation toward palmitate and spared linoleate. Such a shift was not observed in LD animals and during severe CR, during which oxidation of both dietary fatty acids was increased. Concomitantly, HEL increased in both LD40 and SD80 groups, whereas DNA damage was only seen in SD80 food-restricted animals. HEL correlated positively with linoleate oxidation confirming in vivo the substrate/product relationship demonstrated in vitro, and negatively with TEE adjusted for fat-free mass, suggesting higher oxidative stress associated with increased torpor expression. This suggests a seasonal-dependant, cost-benefit trade-off between maximizing torpor propensity and minimizing oxidative stress that is associated with a shift toward sparing of dietary polyunsaturated fatty acids that is dependent upon the expression of a winter phenotype.

polyunsaturated fatty acid; energy savings; Microcebus murinus

TO COPE WITH AN UNFAVORABLE environment, heterothermic mammals increase energy and water savings by extending their ability to display bouts of reduced body temperature (Tb) and metabolic rate (25), i.e., torpor or hibernation. This state of lowered Tb requires biochemical adjustments to ensure that physiological functions can be maintained at low temperatures (2). In ectotherms that are also able to tolerate low Tb, an important adaptation for low thermal tolerance appears to be a high proportion of unsaturated fatty acids in cell membranes because of their low melting point. In heterotherms, the role of unsaturated fatty acids for thermal tolerance is less clear cut than in ectotherms (29, 37), but they appear to play an important role in the maintenance of cell membrane function and white adipose tissue fluidity at varying tissue temperatures.

The importance of polyunsaturated fatty acids (PUFA) for mammalian heterotherms is highlighted by their positive effects on hibernation and daily torpor. Increased PUFA content in the diet enhances the propensity of animals to enter torpor and, at least in several studies, lowers minimal Tb tolerated and increases torpor bout duration and energy savings, as reported in hibernating marmots, ground squirrels, and chipmunks (18, 20, 28, 29, 59). This PUFA-induced increase in torpor propensity was associated with a rise of PUFA content in lipid membranes (17, 26). In a study by Geiser (26), deer mice consuming a saturated or unsaturated fat diet showed significant differences in the total unsaturated fatty acid content of depot fat (55.7 vs. 81.1%, respectively) and leg muscle (56.4 vs. 72.1%, respectively). In the echinida, fatty acid composition of fat pads during the prehibernation season was almost identical to that of the most abundant prey species, which included 60% of a monounsaturated lipid, oleic acid (17). Interestingly, such a differential distribution of lipids was also observed independently of dietary selection (30, 50). Indeed, modifications of torpor patterns (frequency and depth) triggered by a shift in photoperiodic regimen occur in concomitance with changes in total lipid composition of muscle tissue (30). This suggests that if dietary selection plays a role in the unsaturation processes of the cell membranes and adipose tissue, a season-dependent differential partitioning of dietary fatty acids between oxidative and synthetic pathways is likely to occur in seasonal heterothermic mammals. Echidnas, ground squirrels, and yellow-bellied marmots were indeed reported to rely on monounsaturated fatty acids and saturated fatty acids as fuel for hibernation to spare PUFA (17, 19, 23). Therefore, it is likely that tissue unsaturation, when the animal is on a fixed diet, is associated with differential changes in PUFA and

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saturated fatty acids oxidations. A study conducted by Paulsrud and Dryer (46) on bat brown adipose tissue demonstrated that the in vitro oxidation rate of palmitic acid is higher than that of oleic acid, when homogenates were incubated at temperatures below 20°C. Nevertheless, there are, so far, no direct in vivo measures of dietary fatty acid oxidation, varying according to their biochemical structures, in seasonal heterothermic species.

An undisputed adverse effect of high PUFA content is, however, the high susceptibility to peroxidation by radical oxygen species, which are massively generated during the mitochondrial respiration burst of the arousal phase from torpor (11, 60). Lipid peroxidation leads to deleterious products, such as reactive aldehydes that cause damages to membranes as well as enzymes and alters DNA and protein functions (for an overview, see Ref. 38). Frank and Storey (23) demonstrated that when the amounts of dietary linoleic acid (a PUFA highly susceptible to peroxidation), are either below or above natural levels, hibernation ability of golden-mantled ground squirrels is greatly reduced. Additionally, the activities of antioxidant enzymes were increased in brown adipose tissue, whereas trends for increases were observed in other tissues during the high-linoleic diet, suggesting a higher oxidative stress response. Therefore, it has been argued that optimal levels of PUFA intake and fat depots in hibernators result from a trade-off between their beneficial effects on membrane function and white adipose tissue fluidity at low Tb during torpor bouts, and the oxidative stress-related cellular damage (21, 23).

The relationship between the seasonal use of different dietary fatty acids and the oxidative stress in relation to torpor optimization has, however, not been thoroughly studied.

The grey mouse lemur (Microcebus murinus) is a good model to investigate such trade-offs because the factors regulating its heterothermia are well characterized and reproducible in captivity (1, 31, 48, 49, 57). This small primate uses daily torpor and shows marked seasonal rhythms (1, 32, 47). During short days (SD < 12 h of daylight), mouse lemurs enter a resting state, fasten, and increase their torpor depth and duration. Conversely, long days (LD > 12 h of daylight) trigger an increase in behavioral activities, a reduction of body mass, and a low expression of daily torpor. This season-dependent plasticity in torpor use was further demonstrated through food-restriction paradigms (31, 34).

Therefore, we hypothesize that the aforementioned cost-benefit trade-off between torpor optimization, differential lipid oxidative metabolism, and oxidative stress is dependent on season. As such, seasonal optimization is likely to occur only during moderate food deprivation in mouse lemurs with the winter phenotype. To test this hypothesis, we determined the extent to which 1) the oxidative metabolism of dietary palmitate (saturated) and linoleate (polyunsaturated) fatty acids varies seasonally when the torpor expression is extended by graded calorie restriction (CR); and 2) how it relates to oxidative stress, through the measurement hexanoyl-lysine (HEL), the product of linoleate peroxidation, and 8-hydroxydeoxyguanosine (8OHdG), a marker of DNA damage (16, 36).

MATERIAL AND METHODS

Animals

The 34 adult male grey mouse lemurs (Microcebus murinus, Cheirogaleidae, Primates) used in this study were adults (2 to 5 years old) and were born in the laboratory breeding colony of Brunoy (UMR7179 CNRS/MNHN, France; European Institutions agreement no. 962773) from a stock originally caught along the southwestern coast of Madagascar, 40 years ago. Seasonal Malagasy rhythms were reproduced by alternating 6-mo periods of long-days (14:10-h light-dark) and short-days (10:14-h light-dark). Mouse lemurs were transferred to our laboratory at Strasbourg (UMR7178 CNRS/ULP, France) and housed individually in cages (70 × 68 × 52 cm), visually separated from each other, to minimize social influences. The relative humidity in animal rooms was maintained constant (55%) and LD and SD mouse lemurs were kept at ambient room temperatures of 25°C under long-day and short-day exposures.

Energy Intake and CR

After a month of acclimatization to their new housing, individual calorie intake was measured during a 10-day period to calculate subsequent food-restricted energy allotments. Animals were fed, in ad libitum conditions, on fresh bananas and a standardized homemade mixture containing baby cereals, spice bread, egg, concentrated milk, white cheese, vitamins, and dietary minerals (Vitapaulia/MR, Intervet; France and Toison d’orR, Clément Thékan, France). Since grey mouse lemurs, and particularly those with the winter phenotype, tend to overfeed when isolated and thus gain mass during the ad libitum period, energy intake was clamped to the level required to stabilize their body masses. This protocol was required to avoid a significant underestimation of the CR intensities. Each individual was initially fed ad libitum with banana and the homemade mixture, and progressively, daily energy intake was narrowed according to the body mass time course (34).

During CR, LD and SD mouse lemurs were then provided either with 60% (40% CR) or 20% (80% CR) of their individually derived energy requirements. Food-restricted allotments were available every day at the very onset of the dark phase, and water was always provided ad libitum. Daily food intake was calculated from the difference between provided and remaining food masses and was corrected for dehydration. Energy equivalents of 3.7 kJ/g for the banana and 4.6 kJ/g for the mixture allowed us to convert grams of food intake to kilojoules. Throughout the food restriction period, mouse lemurs under 40% CR received an energy allotment of 47.5 ± 1.3 kJ/day (for LD animals, named LD40) and 45.8 ± 3.3 kJ/day (for SD animals, designated SD40). The 80% food-restricted animals were provided with an energy allotment of 16.5 ± 2.5 kJ/day (for LD animals, named LD80) and 15.5 ± 0.6 kJ/day (for SD animals, designated SD80). All LD80 mouse lemurs reached, on the day 22, a body mass of 52 ± 1 g (vs. 78 ± 2 g under ad libitum diet), which we defined as survival-threatening body mass level, based on the lowest value (50 g) reported in the colony of Brunoy for this photoperiod (49). Therefore, these animals were excluded from the study before the end of the food-deprivation trial and returned to the colony on an ad libitum diet. Neither urine samples nor energetic measurements were therefore performed in the CR period for this group and no data can be reported.

Protocol Overview

Each animal was studied during the ad libitum period and after 35 days of CR. The tests were identical in both conditions and consisted of the measurement of total energy expenditure (TEE) and fat-free mass (FFM) by the doubly labeled water (DLW) method, 24-h palmitate and linoleate oxidation by using stable and radioisotope labeling, and HEL and 8OHdG concentrations in 24-h pooled urine. Torpor frequency was measured by telemetry using implanted Tb recorders (characteristics detailed below) in a different group of animals submitted to the same protocol. The Research was conducted under the authorization 67–223 from the Direction Départementale des Services Vétérinaires du Bas Rhin and the Internal Review Board.
of the UMR 7179 CNRS, Mécanismes Adaptatifs et Evolution, Muséum National d’Histoire Naturelle, Brunoy.

TEE and FFM

TEE was determined during a 2-day period by the multipoint DLW methodology (56). A urine sample was collected by providing gentle pressure on the bladder. A premixed two grams per kilogram (estimated total body water, TBW) dose of DLW was then intraperitoneally injected into the animals. The dose was composed of 0.55 g/kg estimated TBW 97% 2H2O (Rotem Industries, Israel) and 0.15 g/kg estimated body 18O2 (Cambridge Isotope Laboratories, Andover, MA) and was diluted with 3% NaCl to physiological osmolality. We assumed a percentage of hydration of 0.60 and 0.55 for LD40 and LD50, respectively, to calculate an in vivo enrichment of about 250 and 1,200‰ (13) by the plateau methodology (56). A urine sample was collected at 1 h postdose from quick sampling in the saphenous vein. Immediately after collection, blood-containing capillaries were rapidly flame sealed. Mouse lemurs were then released inside their own cages, and urine samples were taken 24 and 48 h after the equilibration time in cryogenically stable tubes. Blood and urine samples were stored at 5°C and −20°C, respectively, until analyses by isotope ratio mass spectrometry.

Water from serum and urine samples was extracted by cryodistillation, as previously described (66). Then, 0.1 μl of water was reduced to hydrogen and carbon monoxide by reduction on a glassy carbon reactor held at 1,400°C in an elemental analyzer (model Flash 1112, ThermoQuest). Isotopic equilibration in body water was determined through a blood sample collected at 1 h postdose from quick sampling in the saphenous vein. Immediately after collection, blood-containing capillaries were rapidly flame sealed. Mouse lemurs were then released inside their own cages, and urine samples were taken 24 and 48 h after the equilibration time in cryogenically stable tubes. Blood and urine samples were stored at 5°C and −20°C, respectively, until analyses by isotope ratio mass spectrometry.

Dietary Palmitate and Linoleate Oxidations

The dietary fat oxidation tests were all performed 5 days after the DLW test to avoid deuterium isotopic interferences in the TEE and FFM calculations by DLW. After the collection of basal urine samples and right before the dark phase, the homemade diet mixture, including 40 mg/kg body wt [d31]palmitate (Cambridge Isotope Laboratories, Andover, MA) and 1.40 μCi/Kg body wt [9,10,12,13-3H]linoleate (American Radiolabeled Chemicals, St. Louis, MO), was orally administered. The dose of deuterated palmitate was twice the dose given in humans to ensure an in vivo enrichment well above the enriched baseline due to the prior DLW dose. In the ad libitum animal, the enrichment 24-h postdose was on average 80‰ above an average enrichment of 250‰ for 18-oxygen calculated from Coward et al. (13) by the plateau methodology (56). A urine sample was collected at 1 h postdose from quick sampling in the saphenous vein. Immediately after collection, blood-containing capillaries were rapidly flame sealed. Mouse lemurs were then released inside their own cages, and urine samples were taken 24 and 48 h after the equilibration time in cryogenically stable tubes. Blood and urine samples were stored at 5°C and −20°C, respectively, until analyses by isotope ratio mass spectrometry.

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CO2 production was calculated according to the single pool equation of Speakman (58): rateCO2 = [(N/2.078)(Ks − K0) − 0.0062·K0·N], where N represents the average isotope dilution space of 18-oxygen calculated from Coward et al. (13) by the plateau method using the 1-h postdose sample. Ks and K0 represent the isotope constant elimination rates calculated by linear regression of the natural logarithm of isotope enrichment as a function of elapsed time from day 1 samples. TEE was calculated by the Weir equation (64) using a food quotient of 0.823 estimated from the animal’s diet. TEE was measured from the dilution space of 18-oxygen after correction for exchange by the factor 1,007 (51). FFM was calculated from TBW by assuming a hydration coefficient of 73.2% that is not affected by chronic CR (4).

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and resting metabolic rate. This group of animals was under the same laboratory conditions. In our study, a torpor bout was defined when Tb dropped below 33°C, and torpor frequency, which corresponds to the number of torpor bouts per week, for each animal group was then calculated during the 5 wk of food restriction. Frequency of torpor was used as the only parameter to appreciate the torpor expression of food-deprived mouse lemurs, since it represented the changes across the 5 wk of food restriction in other parameters (duration and depth) of torpor patterns, as previously described (34).

Data Analysis and Statistics

Throughout the analysis, the sample size of the analyzed data varied to a small extent due to limitations imposed by the 24-h urine volume collected or to the difficulty encountered in collecting spot urine or blood samples, especially after CR. In LD40, LD80, SD40, and SD80 animal groups, the sample sizes for each variable are indicated in the legends of each figure. All data were normally distributed, and parametric tests were used, with the exception of torpor frequency, which showed a poisson distribution. During the ad libitum period, differences between LD and SD groups were assessed by using Student’s t-test. In each animal group, Student’s paired t-test compared the ad libitum and food-restricted levels for each parameter studied. To determine differences between food-restricted animal groups an analysis of variance was used, and Fisher’s protected least significant difference tests were performed. A generalized linear model with a poisson error distribution and a log-link function was used to analyze the effects of photoperiod (LD vs. SD) and CR (40% vs. 80%) on the time courses of torpor frequencies during the 5 wk of food restriction. Bonferroni tests were used to compare weeks of food restriction with ad libitum condition. A Mann-Whitney U-test compared difference in torpor frequency between LD and SD mouse lemurs during the control period. An overall difference in palmitate oxidation, after food restriction, was reported between animal groups (F = 4.2, nLD40 = 8, nSD40 = 7, nLD80 = 8, P < 0.05) and post hoc analysis revealed that palmitate oxidation rates of SD40 and SD80 mouse lemurs differed from each other (20.8 ± 3.6 vs. 33.2 ± 3.2% of recovery, P < 0.05).

Dietary Lipid Oxidation

Palmitate. During the control period, palmitate oxidation rates of 18.2 ± 3.4 and 14.7 ± 2.3% of recovery in LD and SD groups, respectively, did not differ (t = 0.9, nLD = 11, nSD = 18, P = 0.39) (Fig. 1A). After a 35-day food restriction, SD mouse lemurs significantly increased their palmitate oxidation. LD40 animals also increased palmitate oxidation by 52%, but this increase was associated with only a statistical trend (P = 0.09). An overall difference in palmitate oxidation, after food restriction, was compared the ad libitum and food-restricted levels for each parameter (duration and depth) of torpor patterns, as previously described (34).

Linoleate. Under ad libitum conditions, LD and SD mouse lemurs showed similar values of linoleate oxidation rates of 38.9 ± 3.0 and 39.6 ± 2.7% of recovery, respectively (t = −0.16, nLD = 10, nSD = 18, P = 0.87) (Fig. 1B). After food restriction, only SD40 animals did not change the rate of linoleate oxidation compared with the baseline value (P = 0.86). Conversely, both LD40 and SD80 mouse lemurs increased their linoleate oxidation rate by 47% and 92%, respectively, after food deprivation, compared with control values.

Oxidative Stress

HEL. Mouse lemurs under summer and winter phenotypes displayed similar levels of HEL during the control period (4.4 ± 0.3 vs. 4.6 ± 0.4 nmol/mmol·creatinine, t = −0.2, nLD = 11, nSD = 17, P = 0.82) (Fig. 2A). After 35 days of food restriction, HEL levels increased in both LD40 and SD80 mouse lemurs by 48% and 160%, respectively, compared with baseline values. Conversely, the HEL level of food-restricted SD40 animals only showed a trend to increase that did not reach significance, compared with control values (P = 0.44).

8OHdG. During the control period, the 8OHdG level was similar in LD and SD mouse lemurs (2.9 ± 0.4 vs. 2.9 ± 0.4 nmol/mmol·creatinine, t = 0.1, nLD = 11, nSD = 17, P = 0.93) (Fig. 2B). After food restriction, only SD80 animals showed a threefold increase in 8OHdG level, compared with control values, whereas both LD40 and SD40 groups did not show significant increases in 8OHdG level.

**RESULTS**

**Body Mass**

During the control period, SD mouse lemurs showed a 31% higher average body mass value compared with LD animals (79 ± 2 vs. 118 ± 3 g, t = −10.0, nLD = 10, nSD = 17, P < 0.001) (Table 1). All animal groups significantly reduced their body mass at the end of the food-deprived trial, compared with baseline values. This corresponds to a body mass loss of 15%, 33%, 8%, and 23% for LD40, LD80, SD40, and SD80 mouse lemurs, respectively, at the end of the 35-day food restriction.

| Table 1. Body mass, TEE, and TEEFM of long (LD)- and short (SD)-day mouse lemurs exposed to either a 40% moderate calorie restriction (LD40 and SD40, respectively) or 80% severe energy deprivation (LD80 and SD80, respectively) |
|---|---|---|---|---|
| | Summer-Acclimated Animals | Winter-Acclimated Animals | |
| | LD40 | LD80 | SD40 | SD80 |
| n | 7 | 3 | 8 | 8 |
| Body mass, g | 79±2 | 69±4† | 52±1† | 118±3‡ | 105±5* | 94±5† |
| TEE, kJ/day | 82.1±6.3 | 61.2±3.8* | 77.8±3.7 | 63.9±2.3* | 43.0±3.8† | 45.8±3.9† |
| TEEFM, kJ/day | 80.5±6.1 | 68.3±5.4 | 73.5±3.4 | 62.8±3.8* | 43.0±3.8† | 45.8±3.9† |

LD-AL and SD-AL, long- and short-days animals under ad libitum conditions. The food-deprived trial lasted 35 days for LD40, SD40, and SD80 animals and 22 days for LD80 mouse lemurs. No data on total energy expenditure (TEE) and fat-free mass adjusted TEE (TEEFM) were available for the LD80 animals' group. *P < 0.05, †P < 0.01 vs. control; ‡P < 0.05 vs. LD-AL; †except for the body mass where n = 9 (SD40).
kJ/day, \( t = 0.1 \), \( n_{LD} = 7 \), \( n_{SD} = 17 \), \( P = 0.93 \); Table 1). In terms of food restriction, all animal groups reduced their TEE by 25%, 21%, and 47% in LD40, SD40, and SD80, respectively. However, these decreases in TEE were mainly explained by reductions of FFM, particularly in LD40 and SD80 mouse lemurs. Under ad libitum condition, SD mouse lemurs showed a lower, although nonsignificant, FFM-adjusted TEE of 75.8 ± 4.2 kJ/day, compared with LD animals, which displayed a value of 80.1 ± 6.2 kJ/day (\( t = 0.57, n_{LD} = 7, n_{SD} = 17, P = 0.58 \); Table 1). After food restriction, only SD40 and SD80 mouse lemurs reduced their FFM-adjusted TEE by 13% and 39%, respectively. An overall difference in FFM-adjusted TEE was reported between food-restricted groups (\( F = 9.5, n_{LD} = 7, n_{SD40} = 8, n_{SD80} = 8, P < 0.01 \)), and post hoc analysis revealed that SD80 mouse lemurs showed lower FFM-adjusted TEE values compared with LD40 and SD40 animals, after food deprivation (SD80 vs. LD40: 43.0 ± 3.8 vs. 61.2 ± 3.8 kJ/day, \( P < 0.01 \); SD80 vs. SD40: 43.0 ± 3.8 vs. 63.9 ± 2.3 kJ/day, \( P < 0.05 \)).

We observed a significant negative correlation between TEE and HEL level (\( R^2 = 0.26, P < 0.001 \); Fig. 3A). As changes in FFM, the active metabolic mass, account for TEE variations, it was interesting to note that the relation between HEL and FFM-adjusted TEE remains significant (\( R^2 = 0.14, P < 0.05 \); Fig. 3B). A significant positive correlation between linoleate oxidation and HEL level was also reported (\( R^2 = 0.30, P < 0.001 \); Fig. 4).

**DISCUSSION**

Our study investigated the extent to which the increase in torpor expression in the grey mouse lemur during graded food restriction is associated with a trade-off between a whole body sparing of polyunsaturated dietary fatty acids and the related oxidative stress generated during daily torpor. Our results support the existence of such a trade-off on a diet with a fixed macronutrient composition that is dependent on both the season and the degree of energy restriction.

**Torpor Frequencies**

LD and SD mouse lemurs did not display significantly different torpor frequencies during the control period (0.0 ± 0.0 vs. 1.3 ± 0.7, \( U = 24, n_{LD} = 6, n_{SD} = 11, P = 0.4 \) (Fig. 5). However, a differential effect of the 5 wk of food deprivation, according to photoperiod, can be observed (\( W = 2,508.4, df = 5, n_{LD40} = 6, n_{SD40} = 5, n_{SD80} = 6, P < 0.001 \)). Post hoc tests revealed that torpor frequency increased only in SD food-deprived mouse lemurs compared with control values and reached significance from the second week of food restriction (SD40: 5.8 ± 0.7 vs. 1.4 ± 0.8, \( P < 0.001 \); SD80: 6.5 ± 0.2 vs. 1.2 ± 1.1, \( P < 0.001 \)).

**Fig. 1.** A 35-day food restriction induced changes in palmitate (A) and linoleate (B) oxidations in summer-like long days (LD) and winter-like short days (SD) in mouse lemurs. Palmitate and linoleate levels of SD mouse lemurs under a moderate 40% (SD40) food restriction (a) significantly differed from that of SD animals facing a severe 80% (SD80) food restriction (b). Values are means ± SE. *\( P < 0.05 \); **\( P < 0.01 \) vs. control.

**Fig. 2.** Changes in hexanoyl-lysine (HEL; A) and 8-hydroxydeoxyguanosine (80HdG; B) levels after a 35-day food deprivation in mouse lemurs under LD and SD. HEL level of SD40 mouse lemurs (a) and that of SD80 animals (b) significantly differed from each other. Values are means ± SE. *\( P < 0.05 \); **\( P < 0.01 \) vs. control.
Differential Use of Lipid-Type Occurs Only in Mouse Lemurs in Winter Under Moderate Food Shortage

A shift in dietary fat oxidation emerges after moderate food restriction in mouse lemurs expressing the winter phenotype. An increased reliance upon dietary saturated palmitate for oxidation together with a full sparing of dietary polyunsaturated linoleate was observed in winter-acclimated animals subjected to moderate food shortage. This was in conjunction with a sevenfold increase in torpor frequency. Although the same magnitude of torpor expression was observed during severe CR, the shift in dietary fatty acid oxidation did not pertain; oxidation of both dietary fatty acids was increased and contributed equally to the fuel mix being oxidized. The same results were noted in the summer-acclimated animals, but torpor frequency was unaffected by the moderate CR.

The differential fatty acid oxidation may reflect changes in lipid stores that were previously reported when heterothermic animals are placed on a cafeteria diet (22). It has been recently reported in deer mice (30) that functional changes in torpor occurrence and length, induced by photoperiod or season and linked to changes in the composition of somatic fatty acids, can be observed independently of dietary selection. In particular, unsaturated fatty acids (including PUFA: melting point in the range of −1 to −15°C) increased in the short photoperiod group compared with the equinox and long photoperiod groups. Conversely, saturated fatty acids (melting point of +70°C) were two times more abundant during the long photoperiod than the short photoperiod. Therefore, these differences in lipid tissue composition reflected the differential lipid-type utilization between animals under long and short photoperiods that we observed in the present study. In addition, since ω3 and ω6 PUFA (such as linolenic and linoleic acid, respectively) are not naturally synthesized by mammals and are therefore required in the daily diet, storage, and catabolism of these fatty acids might be particularly important in heterotherms. In support of our results, a study on yellow-bellied marmots throughout the hibernation season, showed a tendency for a preferential metabolism of saturated fatty acids compared with essential fatty acids of the ω6 series, such as linoleate (18:2 ω6) (19).

Further experiments will be necessary to characterize the mechanisms by which such a shift in dietary fat use is achieved. Several enzymes are known to have different affinities for fatty acids, varying in chain length and saturation degrees, but their in vivo activity as well as their seasonal modulation is unknown. Among the potential protagonists, we can cite the steroyl-CoA desaturase 1, the carnitine palmitoyl transferase 1, the diacylglycerol acyltransferase, and the more recently characterized mitochondrial glycerol-3-phosphate acyltransferase, which is colocalized with carnitine palmitoyl transferase 1 in the outer mitochondrial membrane, and showed inversed affinity toward saturated and unsaturated activated fatty acids (24, 40).

Our present results and published data from the literature suggest that during moderate CR, mouse lemurs displaying a winter phenotype showed an increased torpor frequency that coincided with a selective reliance on saturated dietary fat for oxidation and a sparing of polyunsaturated fatty acids.

Fig. 3. Correlations between HEL and total energy expenditure (TEE; A) and fat-free mass (FFM)-adjusted TEE (B). Correlative data includes values LDAL and SDAL mouse lemurs under ad libitum (AL) and food-restricted condition.

Fig. 4. Correlation between HEL and linoleate oxidation.

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Increased Oxidative Stress in Mouse Lemurs That Do not Express a Shift in Dietary Fat Oxidation

Our results further suggest an association between the type of fatty acids oxidized and the degree of oxidative damages. The early stage of oxidative damage was measured through the urinary excretion of HEL. Oxidative stress produced hydrogen peroxide that interacts with free linoleate to produce linoleate hydroperoxide. This compound was shown to react covalently with lysine residues to form HEL, which was suggested to be hydrogen peroxide. This process was shown to react covalently with lysine residues to form HEL, which was suggested to be hydroperoxide. This compound was shown to react covalently with lysine residues to form HEL, which was suggested to be

Undoubtedly, such a mechanism would limit the generation of oxidative stress during the rewarming phase. Nevertheless, the use of passive arousal in *M. musculus* only concerns a part of the rewarming process (from minimal Tb to ~28°C) and the subsequent increase to normothermic values remains under active thermogenic control (54, 55).

Interestingly, HEL did not change in mouse lemurs expressing the shift in dietary fat oxidation, i.e., in animals fully expressing a winter phenotype and subjected to a moderate CR. Conversely, HEL urine excretion was increased in winter- and summer-acclimated animals under severe and moderate CR, respectively, i.e., in animals in which no linoleate sparing was observed. Thus, DNA damages that can be seen as ultimate oxidative damages increased only during long-term extreme CR. These results suggest synergistic mechanisms in winter-acclimated mouse lemurs between the linoleate oxidative metabolism, oxidative stress, and torpor optimization.

Exceeded Seasonal Antioxidant Defenses in Mouse Lemurs in Winter Under Severe Food Shortage and in Animals in Summer

It has been well described in the literature that heterotherms developed endogenous defense mechanisms, which make them tolerant to oxidative stress during their torpor or hibernation bouts (for a review, see Ref. 10). In Arctic and 13-lined ground squirrels, plasma ascorbate may function as an antioxidant during the hibernation season since its plasma levels increase three- to fivefold in both species during torpor and return to euthermic levels upon arousal (15, 60). Conversely, tissue ascorbate levels increase significantly during arousal in the liver and spleen, which may reflect a redistribution of plasma ascorbate pools to counteract the increased radical oxygen species production generated by the rapid increase in mitochondrial activity during torpor arousal (60). The activities of several antioxidant enzymes are also increased in brown adipose tissue in hibernating European ground squirrels. Brown adipose tissue, which undergoes dramatic increases in mitochondrial activity and blood flow during arousal, displays higher activities of superoxide dismutase, ascorbate, and glutathione peroxidase during hibernation (8, 9). As oxidative...
stress results from a balance between antioxidant and oxidative molecules generated by the energy metabolism, other mechanisms do exist to reduce oxidative stress. Clearly, we cannot determine from the present study whether it is the mechanisms of defenses against radical oxygen species that are increased or whether it is their production that is decreased in summer-adapted animals. Our results nevertheless suggest that the seasonal modulations of dietary fat use are closely associated with oxidative stress.

Interestingly, summer-like long-day-exposed mouse lemurs increased oxidation rates of both dietary fatty acids and excretion of HEL. This suggests that summer phenotype mouse lemurs are more susceptible to lipid peroxidation than winter-acclimated animals. Seasonal differences in the antioxidant defense system have been reported in several studies (7, 9, 65). In the brain of European ground squirrels, the highest activity of antioxidant defense enzymes (superoxide dismutase and catalase) was found in the spring and was much lower in summer. Moreover, the highest levels of low-molecular-weight antioxidants (ascorbic acid and glutathione) were recorded in winter compared with spring and summer (7). Whether or not the incapacity to increase torpor episodes in summer-acclimated mouse lemurs is a consequence of low oxidative defense capacities cannot be answered. Interestingly, overconsumption of a diet rich in polyunsaturated fatty acids reduces hibernating expression as has been reported in captive golden-mantled ground squirrels (20, 21, 23) and very recently in free-ranging Arctic ground squirrels (22). Rather than a low antioxidant ability, an alternative explanation may be the high level of reproductive and thermogenic hormones during the mating season (summer) and their inhibiting effect on thermoregulation and torpor (14, 41, 45). Steroid hormones, particularly testosterone, have indeed been shown to inhibit hibernation in rodents (35). Furthermore, it has been suggested in rodents that the increase in testosterone, before the reproduction period in spring, may result in termination of the hibernation season (14).

Taken together, the results of the present study suggest a seasonal optimization of the strategies of energy economy in the mouse lemur, in which torpor optimization converges with a sparing of polyunsaturated fatty acid, likely to increase membrane and white adipose tissue fluidity at low Tb, but in the absence of oxidative stress damages. Whether or not the limited capacity of summer-adapted animals to increase torpor in response to CR is aimed at preventing the consequences of oxidative damages due to incapacity to shift the profile of dietary fat use cannot be answered, but the literature reported above suggests this hypothesis might partly explain the results. Overall, the results are consistent with the hypothesis of a cost-benefit trade-off between maximizing torpor propensity and minimizing oxidative stress, at least, in mouse lemurs facing a moderate food shortage in winter.

**Perspectives and Significance**

The seasonal fattening and torpor expression patterns expressed by the mouse lemurs represent a unique strategy of energy economy for a tropical primate to face the contrasted climate of Madagascar that juxtaposes 6 mo of dry winter with few resources with 6 mo of summer. In the context of global changes, different scenarios predict that the Madagascar biodiversity hotspot will face an increased occurrence of unprecedented episodes of food shortage throughout the year (12). From a conservation point of view, it is critical not only to study the strategies of energy economy used by endemic species but also the limits of plasticity of these strategies. This study demonstrates a relationship between torpor extension, dietary fat use, and oxidative damages that seems essentially limited to the winter phenotype and moderate food shortages. In a future study, it will be relevant to examine the nutritional contents, specifically in terms of types of fatty acids, of the mouse lemurs in the wild, especially in winter. Except for severe food restriction studies, on which no adaptation was clearly expected after 35 days of treatment, further studies are needed to determine the extent to which survival and fitness will be affected in this endangered species.

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Dietary Lipid Oxidation in Mouse Lemurs


