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Of fruits and fats: high-sugar diets restore fatty acid profiles in the white adipose tissue of captive dwarf lemurs

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Fat-storing hibernators rely on fatty acids from white adipose tissue (WAT) as an energy source to sustain hibernation. Whereas arctic and temperate hibernators preferentially recruit dietary polyunsaturated fatty acids (PUFAs), tropical hibernators can rely on monounsaturated fatty acids that produce fewer lipid peroxides during oxidation. Nevertheless, compositional data on WAT from tropical hibernators are scant and questions remain regarding fat recruitment and metabolism under different environmental conditions. We analyse fatty acid profiles from the WAT of captive dwarf lemurs (*Cheirogaleus medius*) subjected to high-sugar or high-fat diets during fattening and cold or warm conditions during hibernation. Dwarf lemurs fed high-sugar (compared to high-fat) diets displayed WAT profiles more comparable to wild lemurs that fatten on fruits and better depleted their fat reserves during hibernation. One PUFA, linoleic acid, remained elevated before hibernation, potentially lingering from the diets provisioned prior to fattening. That dwarf lemurs preferentially recruit the PUFA linoleic acid from diets that are naturally low in availability could explain the discrepancy between captive and wild lemurs' WAT. While demonstrating that minor dietary changes can produce major changes in seasonal fat deposition and depletion, our results highlight the complex role for PUFA metabolism in the ecology of tropical hibernators.

1. Introduction

Hibernators can sustain prolonged periods of depressed metabolism by relying on pre-stored energy in the form of food caches or body fat depots. During hibernation, individuals undergo a series of multi-day torpor bouts, a state of decreased metabolic rate and low body temperature (T_b) [1]. With few exceptions [2], these torpor bouts are interrupted by short periods of euthermia (i.e. when individuals achieve body temperature characteristic of the active state) that require significant fat metabolism to sustain high energy demands [3,4].

In preparation for hibernation, arctic and temperate, fat-storing animals become hyperphagic and shift their dietary preferences to optimize the quantity and quality of fatty acids that are deposited as white adipose tissue (WAT) [5]. Fatty acids play two major roles during torpor: they are the fuel that sustains metabolism during hibernation and they maintain membrane function when T_b is lowered to critical levels [6,7]. Importantly, fatty acids, stored as triglycerides in adipose tissue, become bioavailable depending on their molecular size and degree of saturation, which partly explains the versatility in WAT composition from hibernators living under different ambient temperature (T_a) settings. For instance, polyunsaturated fatty acids (PUFAs) have lower melting points than do monounsaturated fatty acids (MUFAs). MUFAs, in turn, have lower melting points than do saturated fatty acids (SFA) of comparable molecular sizes [8]. Thus, while hibernating at low T_a, hibernators such as ground

squirrels (*Callospermophilus lateralis*) rely primarily on PUFAs to maintain the fluidity of tissues and membranes. Predictably, hibernators with higher PUFA content in WAT display lower minimal T_bs during torpor, have lower metabolic rates and undergo longer torpor bouts than do individuals with lower PUFA content, which translates into lower total energy expenditure and better body condition upon emergence from hibernation [7,9–15].

PUFAs, however, cannot be synthesized *de novo* by mammals and must be obtained from the diet [16]. During the hyperphagic, pre-hibernation fattening period, arctic or temperate hibernators select PUFA-rich items, most commonly containing linoleic (18 : 2n6) and α-linolenic acids (18 : 3n3) [5,6]. Although greater PUFA deposits are associated with deeper and longer torpor bouts in many hibernating species, there are limits above which those benefits are lost [10,15]. Among fatty acids, PUFAs are particularly predisposed to autoxidation, i.e. they produce lipid hydroperoxides that can increase cellular oxidative stress and inflammation [17,18]. Greater production of peroxides by PUFAs render them potentially more 'toxic' to the cellular machinery than are other fatty acids [10]. Accordingly, PUFA content in WAT appears determined by a trade-off between maintaining cellular integrity and fat fluidity at cold T_b while minimizing cellular peroxidation.

The fine-tuning of PUFA recruitment and metabolism in arctic or temperate hibernators is implied by the fact that PUFA content in WAT is consistent across individuals with different dietary repertoires. Hibernators, such as arctic ground squirrels (*Urocitellus parryii*), select diets with intermediate PUFA content to optimize rather than maximize PUFA recruitment, with squirrels feeding on high or low PUFA diets being unable to hibernate as well in the wild [10,19]. Supporting these field data, individuals offered diets with varying concentration of PUFAs under experimental conditions achieved WAT PUFA levels close to those observed in the wild [10,15,16,20,21]. By contrast, individuals fed variably high or low PUFA diets (compared to natural ranges) did not decrease metabolic rates and did not display the energetic savings afforded to those with optimum levels [6,21,22]. The timing and duration of dietary shifts also played a role: captive ground squirrels that switched diets from high to low PUFA content a few weeks before hibernation, were able to increase torpor expression, whereas ground squirrels that switched from low to high PUFA content immediately before hibernation were unable to do so [6,20,23].

While cold-adapted hibernators optimize PUFA recruitment to maintain function and minimize lipid peroxidation under low T_a conditions, hibernators from warmer environments, e.g. living closer to the equator, can spare PUFA metabolism. It has been shown that tropical hibernators, like echidnas, store and metabolize MUFAs and SFAs rather than PUFAs to sustain hibernation [24].

Dwarf lemurs (family Cheirogaleidae), primate hibernators endemic to the island of Madagascar, also rely on MUFAs to sustain hibernation in the wild. One species in particular, the fat-tailed dwarf lemur (*Cheirogaleus medius*), inhabits the exceptionally seasonal dry deciduous forests of western Madagascar. Fat-tailed dwarf lemurs are primarily frugivorous and preferentially feed on sugary fruits in preparation for hibernation, which lasts approximately seven months a year [25–27]. Although foods consumed prior to hibernation are relatively low in lipids, dwarf lemurs can double their body mass and deposit large amounts of fat in their tails [28]. Two fatty acids comprise more than 80% of dwarf lemurs' WAT: oleic acid (18 : 1n9, MUFA, 63%) and palmitic acid (16 : 0, SFA, 21%). PUFAs account for less than 3% of WAT, with linoleic acid (18 : 2n6, 1.5%) present at a slightly greater percentage than α-linolenic acid (18 : 3n3, 1.1%) [29]. Given the low lipid and high sugar content of their diets, and the overrepresentation of oleic acid in WAT, it is thought that oleic acid is primarily converted via endogenous metabolism from fruit-based carbohydrates [29]. PUFAs, albeit at low abundance, may be necessary for hibernation, as ambient temperatures may occasionally drop to values lower than 10°C. However, it remains to be tested whether PUFA recruitment is proportional to their availability in natural foods or optimized for hibernation in the dry forests of Madagascar.

We studied WAT composition in captive fat-tailed dwarf lemurs subjected to different dietary and T_a conditions. At the Duke Lemur Center (DLC), dwarf lemurs undergo shallow daily torpor when maintained at stable warm temperature conditions (22–25°C) [30] but express hibernation—multi-day torpor bouts—when exposed to low ambient temperature (T_a at 10–15°C) and food restriction protocols [31,32].

Herein, we analysed the nutritional composition of a standard high-fat diet (HF) versus a modified high-sugar (HS) diet provisioned to dwarf lemurs during the fattening period prior to hibernation. Second, we investigated if dwarf lemurs fattened on HF or HS diets differed in their (WAT) fatty acid profiles pre-hibernation. Finally, we compared WAT profiles in dwarf lemurs at post-hibernation after exposure to warm (approx. 23.5°C) or low (10–15°C) T_a during hibernation. Our data on captive dwarf lemurs are then compared to data from their wild counterparts.

Under the hypothesis that dietary substrates and environmental conditions modulate the fatty acid composition of WAT, we predict that captive dwarf lemurs provisioned with HS diets during the fattening season will display a WAT profile more similar to those of their wild peers at comparable seasonal times than to dwarf lemurs provisioned a HF diet. We also predict that captive dwarf lemurs fed HS diets during fattening and exposed to cold conditions during hibernation will display WAT profiles more similar to wild dwarf lemurs after hibernation, relative to dwarf lemurs exposed to warm conditions during hibernation in captivity. Finally, if physiological reliance on PUFAs decreases as hibernators are exposed to warmer conditions, we predict that dwarf lemurs, as tropical hibernators, will maintain low levels of PUFAs in WAT, regardless of diet in captivity.

This study aims to understand mechanisms of fat recruitment and utilization in a tropical hibernator, and more generally, contribute to the discussion of how food quality and quantity may affect hibernation patterns in natural populations.

2. Methods

(a) Subjects and housing conditions

We selected 20 dwarf lemurs for this study (adult males, $n = 14$; adult females, $n = 5$; juvenile female, $n = 1$). At the DLC, dwarf lemurs are housed in rooms with thermal conditions around 24°C (between 22–25°C). Dwarf lemurs in our study are subjected to a reverse light cycle and photoperiodic shifts that

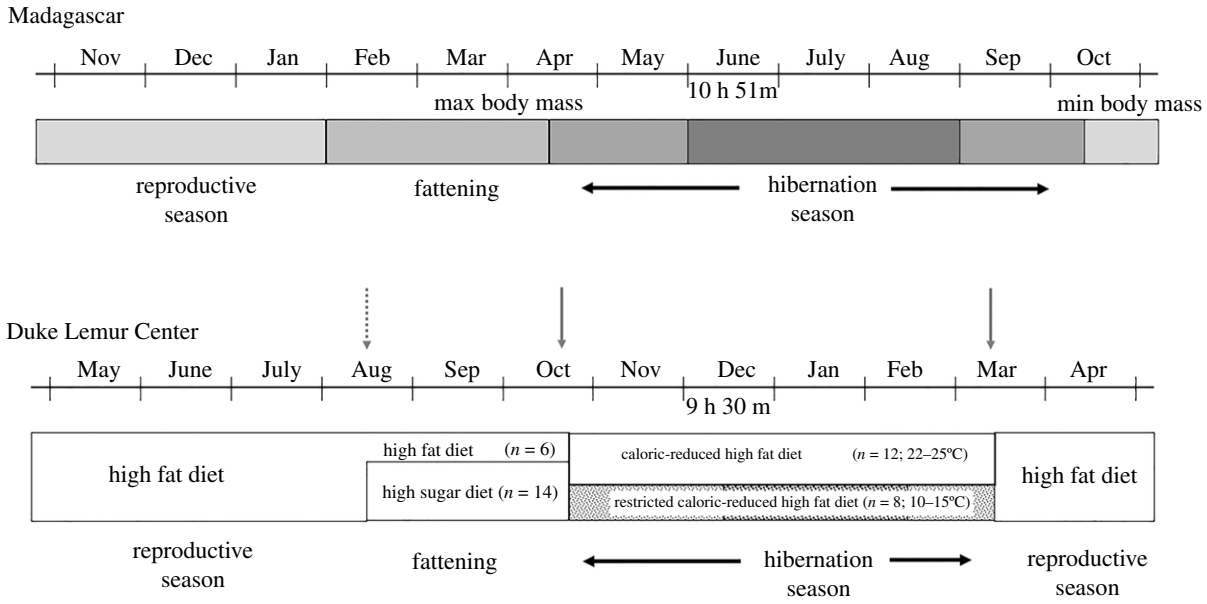

Figure 1. Dwarf lemur major events, in Madagascar and at the DLC. Dwarf lemurs were offered a high-fat or high-sugar diet during the fattening period at the DLC. All animals on the high-fat diet and a subset of dwarf lemurs in the high-sugar diet remained under warm temperature conditions year-round. The rest of the animals on the high-sugar diet were transferred to temperature-controlled rooms during the hibernation season. Dotted arrow indicates timing of fat sample collection in dwarf lemurs provided high-sugar diets only. Solid arrows indicate timing of fat sample collection for all dwarf lemurs in this study before and after hibernation.

approximate the natural seasonal variation in North Carolina, USA. (i.e. opposite to Madagascar). Daylight is adjusted by 15 to 30 min every other week, with the shortest days (9.5 h light) set between 2 December and 1 January, and longest days (14.5 h light) between 2 June and 17 June. Thus, major seasonal changes (e.g. fattening, 'hibernation' and reproduction) can be identified at the DLC, but at different months than in the wild (for more information about dwarf lemurs at the DLC see [31]). At the DLC, we define the period between November and March as 'hibernation' regardless of whether dwarf lemurs are housed under warm or cold temperature conditions. To facilitate comparisons between published data from Madagascar and our study at the DLC, we illustrate monthly schedules, dietary and temperature variation of experimental groups and in the wild in figure 1.

Our study subjects were categorized in one of three groups according to whether they were provided a HF or a HS diet during the fattening period (mid-August to mid-October) and whether they were housed under warm or cold conditions during hibernation (October to March). All groups received a standard, HF diet during the reproductive season (April to mid-August). Group 1 individuals ($n = 6$, 2 females, 4 males) were provided a standard HF diet during the fattening season and a caloric-reduced HF diet during the hibernation season. Group 1 lemurs were housed under warm temperature conditions year-round. Group 2 individuals ($n = 6$, all males) received a HS diet during the fattening season. Like Group 1, Group 2 individuals were offered a caloric-reduced HF diet during the hibernation season and housed under warm temperature conditions year-round. Group 3 individuals ($n = 8$, 4 males, 4 females) were provided a HS diet during the fattening season, and a caloric-reduced HF diet during the hibernation season. Unlike individuals from Group 2, however, dwarf lemurs in Group 3 were placed under food restriction and offered food according to their activity status during hibernation (for details on food restriction, see [31]). These lemurs were housed in cold temperature-controlled rooms (10 to 15°C) (figure 1).

Starting in mid-August until mid-March, every other week, dwarf lemurs were weighed (in grams) and tail base and middle tail circumferences were measured with a measuring tape (in cm). We calculated tail girth (a proxy for fattening in this species) as the average between tail base and middle base circumferences.

(b) Dietary conditions and sampling

Standard, HF diets per individual at the DLC contain approximately 12 g fruit/vegetable mix, 6 g of commercial monkey biscuit and 2 mealworms; standard caloric-reduced HF diets contain approximately 4 g fruit/vegetable mix, 2 g of monkey biscuit and no mealworms. For this study, we created a HS diet consisting of approximately 14–16 g fresh fruit, 2–4 g biscuit, 2 g dried fruit and occasional drizzle of honey. The total number of calories per portion between the standard HF diet and the HS diet was approximately the same (approx. 40 kcal). Both food regimens were readily eaten by the dwarf lemurs, and food bowls were empty upon removal from animal rooms every morning; however because several dwarf lemurs were housed socially (only 4 out of 16 dwarf lemurs of this study were housed solitarily) we were unable to determine whether some individuals ate more food than did others in the group, or if they prioritized specific items within their diets.

Between August 17 and September 29, 2020, we collected full portions of the control HF diets ($n = 9$) and full portions of the HS diets ($n = 9$) for nutritional analysis. All samples were sent to CVAS for analysis of crude protein, ethanol soluble sugars, simple sugars and fatty acid content (https://www.foragelab.com/Resources/Lab-Procedures/).

(c) WAT collection and analysis

We collected WAT samples from the dwarf lemurs' tails at three strategic time points: pre-fattening (August 2020; Groups 2 & 3); pre-hibernation (October 2020; all groups); and post-hibernation (March 2021; all groups) (electronic supplementary material, table S1). We successfully collected WAT samples from all lemurs in the pre-fattening and pre-hibernation seasons; however, we could not obtain fat samples from 5 dwarf lemurs post-hibernation due to complete depletion of fat deposits (electronic supplementary material, table S1). Fat aspirates were

Table 1. Nutritional composition of DLC diets; DM = dry matter, FA = fatty acid, HF = high fat, HS = high sugar.

nutrient	HF diet ($n = 9$) % DM	HS diet ($n = 9$) % DM	signif.[a]	HF diet ($n = 9$) % FA	HS diet ($n = 9$) % FA	signif.[a]
ES sugar	24.5	52.4	****			
crude protein	15.8	7.4	****			
fructose	7.75	21.45	****			
glucose	5.51	17.41	****			
sucrose	4.54	5.52	n.s.			
total fatty acid	4.55	1.72	****			
14 : 0	0.07	0.02	****	1.44	1.17	n.s.
16 : 0	0.88	0.36	****	19.51	21.79	**
16 : 1	0.07	0.03	****	1.55	1.83	*
18 : 0	0.3	0.11	****	6.65	6.53	n.s.
18 : 1n7	0.08	0.04	****	1.85	2.19	n.s.
18 : 1n9	1.41	0.48	****	30.83	27.33	****
18 : 2n6	1.5	0.55	**	32.99	31.59	n.s.
18 : 3n3	0.11	0.07	**	2.51	4.39	***

[a]Mann–Whitney test *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, n.s., not significant.

collected from the tail, using an 18- or 20-gauge spinal needle while dwarf lemurs were under anesthesia (Ketamine 10 mg kg^{-1} and Midazolam 0.3 mg kg^{-1}). Aspirates were stored in sterile, plastic tubes and immediately frozen at −80°C. Samples were sent to Eurofins SF Analytical DBA Craft Technologies for analysis on fatty acid composition.

(d) Statistical tests

Regarding diet composition, we compared macronutrient abundance of HF and HS diets (e.g. sugar and lipid content) by conducting non-parametric Mann–Whitney tests. Regarding the metrics of fattening, we compared body mass and tail girth in the pre-hibernation season between the three study groups by computing a Kruskal–Wallis test and Dunn's multiple comparison tests. We compared pre- and post-hibernation measurements of body mass and tail girth within study groups separately by computing three Wilcoxon matched-pairs signed-rank tests. These tests were all conducted in GraphPad Prism (v. 9.1.2).

Regarding WAT composition, we compared fatty acid profiles relative to diet, season and ambient temperature using a suite of analysis. In general, we prioritized linear mixed models (LMMs) to account for repeated sampling of individual lemurs; we used non-parametric tests for comparisons when individual lemurs were represented only once. We ran LMMs using the glmmADMB v. 0.8.3.3 package [33] and R v. 4.0.2 software [34] in Rstudio v. 1.3.959 [35], and non-parametric tests using GraphPad Prism.

We first compared WAT profiles in the subset of dwarf lemurs fed HS diets (Groups 2 and 3) collected in the pre-fattening season (August) and in the pre-hibernation season (October) using LMMs. In these models, individual fatty acids were entered as the response variable, season (two categories: pre-fattening or pre-hibernation) was entered as the explanatory variable, and individual lemur was entered as a random term. We next compared the WAT profiles at the pre-hibernation sampling point in dwarf lemurs fed the HF (Group 1) versus HS (Groups 2 and 3) diets using Mann–Whitney tests.

Because dwarf lemurs fed HF (Group 1) versus HS (Groups 2 and 3) diets exhibited different WAT profiles prior to hibernation, we tested for seasonal effects before and after hibernation within the HF and HS groups separately. For both HS and HF groups, we ran LMMs in which individual fatty acids were entered as the response variable, season (two categories: pre-hibernation or post-hibernation) was entered as explanatory variable, and individual lemur was included as a random term.

Lastly, we ascertained if ambient temperature differentially affected fatty acid metabolism during the hibernation season in dwarf lemurs fed HS diets. From the samples collected post-hibernation, we conducted Mann–Whitney tests of individual fatty acids between WAT from dwarf lemurs maintained at warm temperature or cold temperature during hibernation season (GraphPad Prism v. 9.1.2).

3. Results

(a) Differences in nutritional content of high-sugar versus high-fat diets

High sugar (HS) diets contained significantly more moisture than did HF diets (approx. 6% more) because of the high-water content of fresh fruits. HS diets also contained about double the sugar, but half the protein and fatty acids than did HF diets (table 1; electronic supplementary material, table S2). Within the sugar components, fructose and glucose were significantly increased in HS versus HF diets (table 1). The relative proportions of different fatty acids were comparable between diets; however, because lipid content was reduced overall in HS diets (1.7% versus 4.5%), fatty acids were significantly decreased when plotted as percentage of dry matter (table 1; electronic supplementary material, table S3).

(b) Differences in body mass and tail girth across groups

Dwarf lemurs fed HS diets achieved comparable body mass and tail girth to dwarf lemurs fed HF diets before hibernation (Dunn multiple comparisons tests, p = n.s.) (figure 2). After hibernation, all dwarf lemurs lost significant body mass,

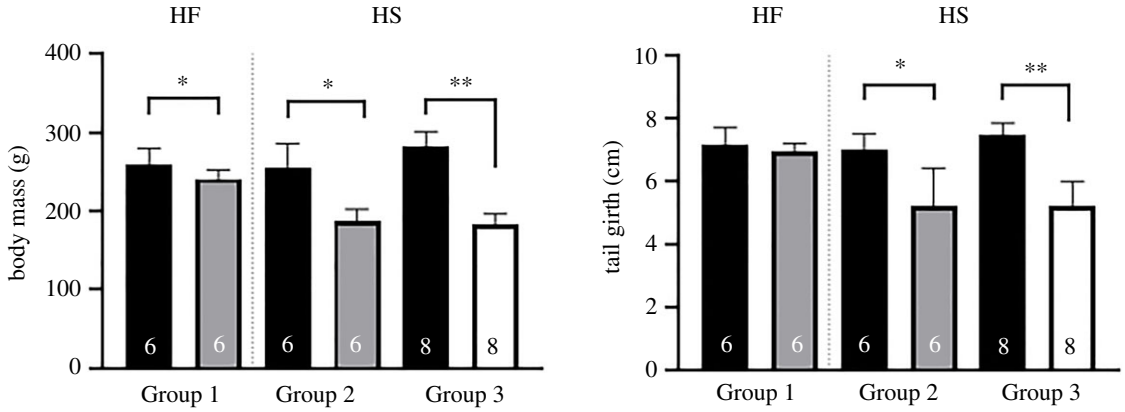

Figure 2. Body mass (left) and Tail girth (right) from dwarf lemurs fed high-fat (HF) and high-sugar (HS) diets before and after hibernation. Pairwise comparisons of matched samples pre and post-hibernation. Pre-hibernation, black bars; post-hibernation-warm temperature conditions, grey bars; post-hibernation-cold temperature conditions, clear bars. Mean and bars indicating 95% CI, sample sizes inside bars. Wilcoxon matched-pairs signed rank test *$p < 0.05$, **$p < 0.01$.

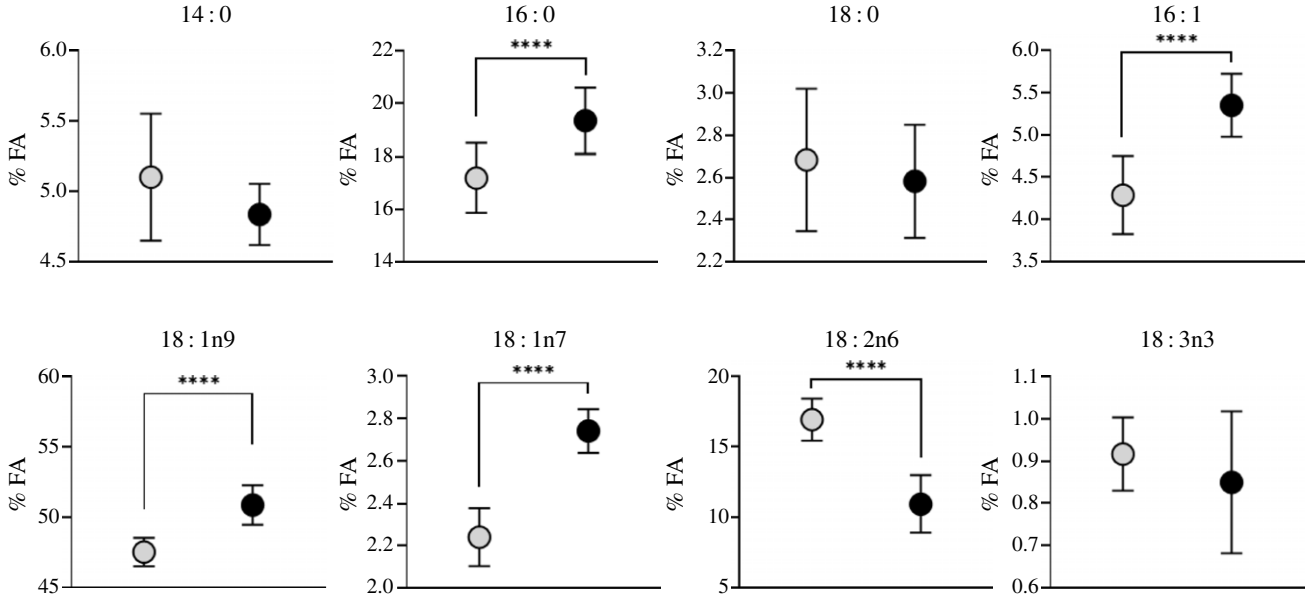

Figure 3. WAT fatty acid composition from fat samples collected pre-fattening before HS diet implementation (light grey symbols) and after diet implementation at the pre-hibernation sampling (black symbols). Mean and 95% CI. Data presented as percentages of total fatty acids FA. Statistics from LMMs described in the text, ****$p < 0.0001$.

but only dwarf lemurs that had been provisioned HS diets significantly reduced tail girth (figure 2; electronic supplementary material, table S4).

(c) Differences in WAT profiles across conditions

(i) Did WAT profiles differ before and after HS diet implementation?

Dwarf lemurs fed HS diets during the fattening season had different WAT fatty acid compositions between the pre-fattening and pre-hibernation seasons (figure 3; electronic supplementary material, table S5). Specifically, the proportions of the SFA, palmitic (16 : 0, $z = -4.96$, $p < 0.0001$) and all monounsaturated fatty acids (MUFA), such as palmitoleic (16 : 1, $z = -9.84$, $p < 0.0001$), oleic (18 : 1n9, $z = -4.85$, $p < 0.0001$) and vaccenic acid (18 : 1n7, $z = -8.36$, $p < 0.0001$), significantly increased during the fattening season, whereas the proportion of one PUFA, linoleic acid (18 : 2n6, $z = 8.39$, $p < 0.0001$) significantly decreased during fattening (figure 3).

(ii) Did WAT profiles differ between dwarf lemurs provisioned HF versus HS diets at pre-hibernation?

At the pre-hibernation sampling point, WAT from dwarf lemurs provisioned HS versus HF diets significantly differed in 6 out of 8 fatty acids: Dwarf lemurs fed HS diets displayed significantly greater percentages of one SFA (myristic, 14 : 0), and all three MUFAs, but had significantly lower percentages of the two PUFAs (table 2).

(iii) Did WAT profiles change during hibernation?

WAT composition changed in dwarf lemurs sampled at pre- and post-hibernation periods, but the magnitude of these changes differed depending on the respective fatty acid, the diet and T_a conditions during hibernation. Dwarf lemurs fed HF diets during fattening (Group 1) showed significantly lower percentages of two SFAs (palmitic, 16 : 0, $z = 6.55$, $p < 0.0001$; stearic, 18 : 0, $z = 3.4$, $p = 0.00067$) and one PUFA (α-linolenic, 18 : 3n3, $z = 5.05$, $p < 0.0001$) post-hibernation versus pre-hibernation. Another PUFA (linoleic, 18 : 2n6, $z = -3.74$, $p = 0.00018$) was significantly increased in post

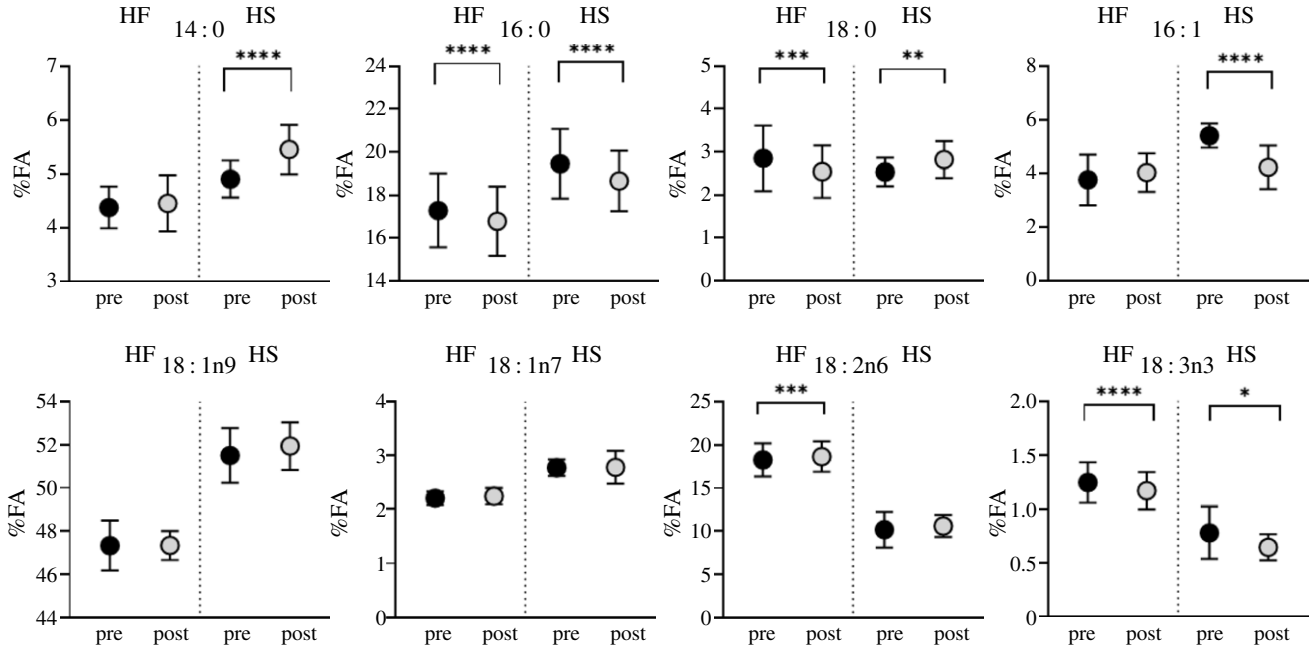

Figure 4. WAT fatty acid composition from dwarf lemurs fed high-fat (HF) and high-sugar diets (HS) sampled before and after hibernation. Pre-hibernation: black-filled symbols; post-hibernation: grey-filled symbols; data presented as percentages of total fatty acids FA, mean and 95% CI. Statistics from LMMs described in the text, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Table 2. WAT fatty acid composition from dwarf lemurs provisioned high-sugar diet (HS) or high-fat diet (HF) at pre-hibernation sampling. Results from Mann–Whitney tests, mean and s.d. Data presented as percentages of total fatty acids.

	HF			HS			
fatty acid	mean %	s.d.	*n*	mean %	s.d.	*n*	*p*
14 : 0	4.38	0.37	6	4.9	0.44	14	0.0408*
16 : 0	17.29	1.64	6	19.46	2.11	14	0.0757 n.s.
16 : 1	3.76	0.9	6	5.41	0.59	14	0.0023**
18 : 0	2.85	0.73	6	2.53	0.43	14	0.3625 n.s.
18 : 1n7	2.21	0.12	6	2.77	0.2	14	<0.0001****
18 : 1n9	47.33	1.1	6	51.5	1.65	14	0.0023**
18 : 2n6	18.25	1.82	6	10.17	2.69	14	0.0002***
18 : 3n3	1.24	0.18	6	0.78	0.32	14	0.0016**

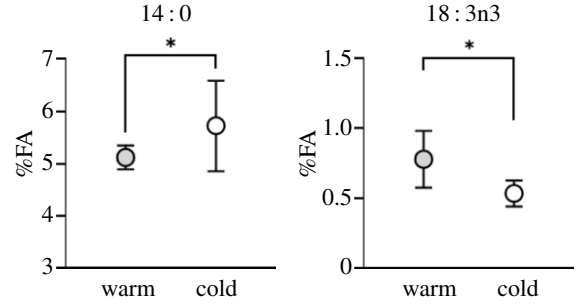

Figure 5. Fatty acids (SFA, myristic 14 : 0) and (PUFA, α-linolenic 18 : 3n3) that differed significantly in WAT from dwarf lemurs fed HS diets and subjected to warm or cold T_a during hibernation. Statistics from Mann–Whitney tests described in the text, $*p < 0.05$, mean and 95% CI.

versus pre-hibernation (figure 4). Dwarf lemurs fed HS diets during fattening (Groups 2 & 3) displayed a significant increase in two SFAs (myristic, 14 : 0, $z = 6.61$, $p < 0.0001$; stearic, 18 : 0, $z = 2.69$, $p = 0.0071$) from pre to post-hibernation. Contrarily, proportions of one SFA (palmitic, 16 : 0, $z = -4.19$, $p < 0.0001$), one MUFA (palmitoleic, 16 : 1, $z = -4.81$, $p < 0.0001$) and one PUFA (α-linolenic, 18 : 3n3, $z = -2.09$, $p = 0.037$) were significantly lower in post- versus pre-hibernation (figure 4). Other fatty acids were metabolized proportionately to their availability (figure 4; electronic supplementary material, table S5).

Finally, WAT profiles from dwarf lemurs fed HS diets but housed under warm (Group 2) or cold (Group 3) conditions during hibernation showed significant differences in two fatty acids post-hibernation: whereas myristic (14 : 0, Mann–Whitney test, $p = 0.0317$) was significantly increased in dwarf lemurs under cold conditions, α-linolenic (18 : 3n3, Mann–Whitney test, $p = 0.0317$) was significantly decreased in dwarf lemurs under cold conditions (figure 5).

When we group fatty acids as SFAs, MUFAs or PUFAs, the major difference in fat recruitment between dwarf lemurs subjected to HF versus HS diets is the greater

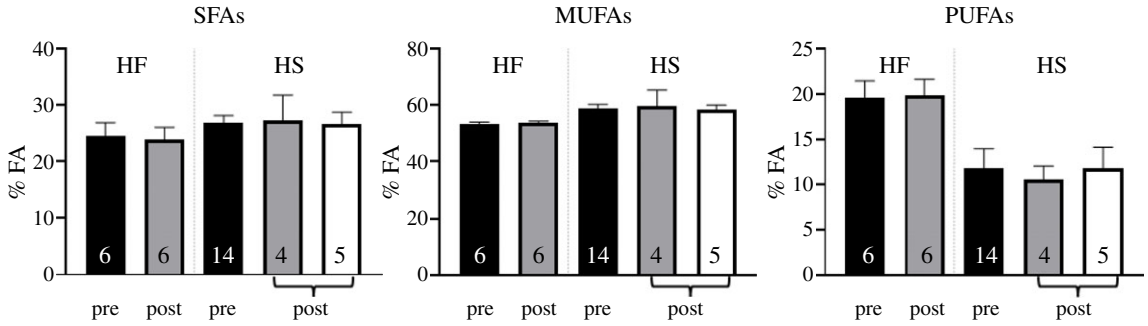

Figure 6. Percentages of combined saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in WAT from dwarf lemurs fed high-fat (HF) and high-sugar diets (HS) sampled before and after hibernation. Pre-hibernation: black bars; post-hibernation warm T_a: grey bars; post-hibernation cold T_a: clear bars; mean and 95% CI. Sample sizes indicated within bars.

Table 3. Fatty acid composition of wild foods in Madagascar, and HF and HS diets at the Duke Lemur Center; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids.

fatty acid	food items Madagascar[a]	HS diet DLC	HF diet DLC
14 : 0	0.02	0.02	0.07
16 : 0	0.68	0.36	0.88
16 : 1	0.01	0.03	0.07
18 : 0	0.12	0.11	0.3
18 : 1n9	0.46	0.475	1.41
18 : 2n6	0.51	0.55	1.5
18 : 3n3	0.40	0.07	0.11
SFA	0.82	0.49	1.25
MUFA	0.47	0.56	1.58
PUFA	0.91	0.63	1.64
UFA	1.31	1.18	3.22

[a]Estimated values from [29], percentage total dry matter.

proportion of PUFAs present in the WAT of dwarf lemurs provisioned HF diet (figure 6).

(d) Differences in wild foods, DLC diets and WAT composition between wild and captive dwarf lemurs

The fatty acid composition of wild foods in Madagascar, published previously in the literature, was more similar to that of HS diets than to HF diets (table 3). Wild foods and HS diets were comparable in the SFAs myristic (14 : 0) and stearic (18 : 0), MUFAs such as oleic (18 : 1n9) and palmitoleic (16 : 1) and PUFAs like linoleic (18 : 2n6). HS diets, however, showed lower content of the SFA palmitic (16 : 0) and the PUFA α-linolenic (18 : 3n3) compared to wild foods. With one exception (α-linolenic, 18 : 3n3), HF diets at the DLC show higher percentages of all analysed fatty acids than HS diets and Madagascar foods (table 3).

Before hibernation, WAT profiles of DLC dwarf lemurs fed HS versus HF diets during fattening were more similar to those of wild dwarf lemurs (stearic acid, 18 : 0, being the exception) (table 4). Overall, wild dwarf lemurs showed a greater percentage of the MUFA oleic (18 : 1n9) and a lower percentage of the PUFA linoleic (18 : 2n6) in their WAT before hibernation than DLC dwarf lemurs.

4. Discussion

(a) Changes in the 'wild' direction

Overall, the HS diets introduced in this study, compared to standard HF diets, changed the nutrients available to dwarf lemurs during the fattening period, the composition of fatty acids stored in their tails prior to hibernation and the patterns of lipid depletion during hibernation in a manner that better resembled known patterns from wild dwarf lemurs in Madagascar. At a macroscopic level, captive dwarf lemurs fed both HF and HS diets achieved comparable levels of body mass and tail fattening before hibernation. Yet, dwarf lemurs fed HF diets maintained substantial tail girth even after hibernation, whereas dwarf lemurs provisioned HS diets fully depleted tail fat depots after 4.5 months, a trend that was accentuated in dwarf lemurs that hibernated under cold conditions. Expectedly, significant depletion of fat stores also mirrored the wild condition, as wild dwarf lemurs in Madagascar emerge from hibernation at about half of their body mass. The body mass of captive dwarf lemurs fed HS diets and housed in cold rooms varied between 150 g and 300 g, which is comparable to seasonal body mass fluctuation of fat-tailed dwarf lemurs in western Madagascar, between 130 g and more than 250 g [28].

Increasing fruit availability also shifted the nutritional composition of diets fed to captive dwarf lemurs to better match the composition of wild foods foraged during the fattening season in Madagascar. In turn, the WAT profiles of captive dwarf lemurs provisioned HS diets more closely resembled the WAT profiles from their wild counterparts before and after hibernation, showing expected seasonal signatures. Like wild dwarf lemurs during the pre-hibernation period, captive dwarf lemurs fed HS diets showed higher levels of MUFAs (e.g. oleic, palmitoleic) and SFAs (e.g. palmitic) in WAT compared to dwarf lemurs fed HF diets. The increase in the percentage of these fatty acids in WAT occurred despite an overall reduction of lipids in the HS diet in favour of sugar content. Thus, we confirm previous assertions that dwarf lemurs endogenously convert fatty acids, primarily oleic acid, from sugar substrates obtained from foraged fruits [29].

Table 4. WAT fatty acid composition in wild dwarf lemurs (Mada) before and after hibernation, and in captive dwarf lemurs fed high-fat (HF) or high-sugar (HS) and subjected to warm or cold T_a during hibernation; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; EFA: essential fatty acids (equivalent to PUFA: polyunsaturated fatty acids); UFA: unsaturated fatty acids.

fatty acid	pre-Hib Mada[a]	post-Hib Mada[a]	pre-Hib HS diet	post-Hib HS_Cold	post-Hib HS_Warm	pre-Hib HF_Warm	post-Hib HF_Warm
14 : 0	5.6	7.8	4.8	5.7	5.1	4.4	4.5
16 : 0	21.4	17.6	19.4	18.0	19.5	17.3	16.8
16 : 1	5.1	3.0	5.4	3.9	4.6	3.8	4.0
18 : 0	3.5	5.6	2.6	3.0	2.7	2.9	2.5
18 : 1n9	62.1	63.2	50.9	51.7	52.2	47.3	47.3
18 : 2n6	1.3	1.9	10.9	11.2	9.8	18.3	18.6
18 : 3n3	1.04	0.97	0.85	0.54	0.78	1.24	1.17
SFA	30.5	31.0	26.8	26.7	27.3	24.5	23.8
MUFA	67.2	66.2	59.6	59.1	60.3	53.8	54.2
EFA	2.3	2.8	12.8	13.2	11.5	20.7	21.1
UFA	69.6	69.0	72.3	72.3	71.8	74.6	75.3

[a]From [29].

Unlike wild dwarf lemurs, captive dwarf lemurs fed the HS diet showed almost 10 times the amount of one PUFA, linoleic acid, in WAT before hibernation. This result was somewhat surprising given that the availability of this PUFA was comparably low in both HS diets and wild foods. By contrast, another PUFA, α-linolenic acid, was found at low but comparable levels in WAT from all captive and wild dwarf lemurs. Fat metabolism observed in wild dwarf lemurs during hibernation, e.g. preferential depletion of palmitic, palmitoleic and α-linolenic acids, was very similar to that shown by captive dwarf lemurs fed HS diets.

(b) How do DLC dwarf lemurs' WAT profiles fit within the temperate-tropical hibernator continuum?

Cold-adapted hibernators select dietary items to optimize PUFA abundance in WAT before hibernation [14,36]. For instance, arctic ground squirrels, hibernating at low T_a, feed on foods like seeds and berries with relatively high PUFA content [37]. During hibernation, SFAs are preferentially metabolized, whereas MUFAs are secondarily metabolized and PUFAs are spared [36]. A similar pattern is shown by marmots, which also rely on herbivorous foods, including leaves, flowers and stems, to deposit fats in preparation for hibernation, and also preferentially metabolize saturated fats during hibernation. But whereas linoleic acid is preferentially retained (i.e. metabolized at lower rates than other fatty acids), α-linolenic acid is preferentially used during hibernation [38]. Although total PUFA content in WAT is comparable between wild arctic ground squirrels and marmots, linoleic acid is more abundant in the former and α-linolenic in the latter.

In echidnas, tropical hibernators, fatty acid content in adipose tissue approximates that of the diets. Unlike arctic or temperate herbivorous rodents, however, echidnas are insectivorous and naturally feed on high MUFA diets. The three main fatty acids in WAT are oleic acid (approx. 60%), palmitic acid (approx. 17%) and linoleic acid (approx. 5%) [24]. Both oleic and palmitoleic acids (MUFAs) are preferentially metabolized during hibernation while other fatty acids proportionally increase during hibernation (i.e. they undergo a lower rate of depletion). [24] argued that although the amount of essential fatty acids (PUFAs) in WAT is much lower than that of arctic/temperate hibernators, echidnas metabolize MUFAs even when ambient temperatures are below 10°C. Perhaps, high levels of the MUFA oleic acid compensate for lower PUFA content, so that even low levels of PUFA are sufficient for echidnas to hibernate even at relatively low temperatures [24].

Pre-hibernating dwarf lemurs in the wild and fed high-sugar diets in captivity store considerable amounts of the MUFA oleic acid (50–62%) and palmitic acid (19–21%) in WAT. During hibernation, dwarf lemurs spare SFAs other than palmitic acid, preferentially metabolize MUFAs and α-linolenic acid (PUFA) and maintain levels of linoleic acid virtually unchanged after hibernation [29].

Thus, dwarf lemurs' WAT fatty acid profiles fall within ranges known from other tropical hibernators. However, dwarf lemurs endogenously convert oleic acid from fruit-based sugar substrates, unlike other tropical hibernators like echidnas, that obtain MUFAs directly from their wild foods.

(c) The PUFA mystery

Captive dwarf lemurs under 'renaturalized' (i.e. close to natural) conditions (i.e. fed high-sugar, low-lipid diets during fattening and housed in cold-temperature rooms to facilitate hibernation) had WAT profiles and metabolic signatures that closely resembled those seen in wild dwarf lemurs, with one exception: linoleic acid. Linoleic acid was the third most abundant fatty acid in the captive dwarf lemurs' WAT at 11%, after oleic acid (approx. 50%) and palmitic acid (approx. 19%). This percentage was almost ten times higher than in wild dwarf lemurs' WAT. On the other hand, the percentage of α-linolenic acid was similar in captive and wild dwarf lemurs, despite dietary variation of this PUFA in Madagascar's foods and DLC diets.

Previous analysis of WAT from DLC dwarf lemurs fed high-fat diets and hibernating under cold conditions showed that α-linolenic acid was preferentially metabolized during (early) hibernation whereas linoleic acid was used according to its availability (electronic supplementary material, table S6). This pattern was further observed in this study, specifically in the high-sugar cold group. Importantly, the role of α-linolenic acid in tropical and temperate hibernators is poorly understood. There are studies suggesting that this PUFA, and some of its derivatives, may play an important role in thermogenic function during hibernation [39]. In addition to their individual roles, the ratio between linoleic and α-linolenic acids of approximately 1 : 1 may be physiologically relevant and optimized prior to hibernation [40]. There is growing evidence, primarily derived from biomedical research, that an imbalance in the ratio omega-6 (e.g. linoleic) to omega-3 (e.g. α-linolenic) can be related to a range of health problems in humans, including propensities for cardiovascular disease [41].

In Madagascar, dwarf lemurs maintain a relatively low percentage of PUFAs in WAT, around 2% and at approximately 1 : 1 ratio (linoleic/α-linolenic), despite risks of potential exposure to relatively low T_a during hibernation. Notably, T_a in the dry deciduous forest of western Madagascar can fluctuate daily from approximately 5°C to more than 30°C (though lemurs can passively rewarm during the daily increase in T_a) [26]. It is possible that cold exposure is too sporadic in dwarf lemurs, allowing much lower optimum levels of PUFAs compared to temperate hibernators.

We show that at a macroscopic level, dwarf lemurs subjected to near natural diets depleted all fat deposits during hibernation season, regardless of T_a conditions. At a cellular level, dwarf lemurs feeding on high-sugar diets and hibernating under cold temperature conditions best approximated the patterns of fat metabolism experienced by their wild counterparts. As wild populations have been subjected to selection to optimize feeding and metabolic strategies in their natural environments, renaturalizing captive populations may be warranted to keep them healthy (e.g. lowering oxidative stress). Those conditions may also be the best chance to investigate underlying mechanisms of metabolic extremes in primates.

Ethics. Research protocols used in this study were approved by the Duke University Institutional Animal Care and Use Committee, under protocols A263-17-12 and A213-20-11. These protocols followed guidelines established by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Data accessibility. Primary data compiled in an Excel file, including nutritional composition of diets, fatty acid composition of dwarf lemurs' WAT, and body mass and tail girth used in analysis, are available as electronic supplementary material [42].

Authors' contributions. M.B.B.: conceptualization, data curation, formal analysis, investigation, methodology, supervision, writing—original draft, writing—review and editing; L.K.G.: formal analysis, investigation, methodology, writing—original draft, writing—review and editing; L.N.E.: methodology, resources, writing—review and editing; B.S.: methodology, resources, writing—review and editing; M.D.: methodology, writing—review and editing; C.O.: methodology, writing—review and editing; P.H.K.: investigation, writing—review and editing; J.F.: investigation, writing—review and editing; E.E.E.: funding acquisition, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare that we have no competing interests.

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