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Female reproductive energetics in mantled howler monkeys (*Alouatta palliata*): A follow-up study

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Abstract

Objective: Reproduction entails several challenges to primate females, among which energetic costs are remarkable at certain stages of the reproductive cycle. Still, females may use behavioral and physiological strategies to cope with those challenges. We had previously reported covariation between female energetic condition through the reproductive cycle and time-budget adjustments in mantled howler monkeys (*Alouatta palliata*). Accordingly, we suggested that behavioral flexibility allowed coping with the energetic challenges of reproduction. Subsequent evidence from the same population, however, suggested otherwise, so we performed a follow-up study on the variation in female reproductive energetics based on a larger sample of females.

Methods: We studied 48 free-ranging adult females at Los Tuxtlas (Mexico). We assessed energy balance via urinary C-peptide concentrations (2717 urine samples), behavioral energy intake and expenditure (5728 sampling hours), and physiological energy expenditure via fecal triiodothyronine metabolites (fTH3; 3138 fecal samples).

Results: We found that energy balance varied among reproductive states: (a) cycling was a period of low C-peptide concentrations; (b) the highest C-peptide concentrations occurred during gestation; and (c) the beginning of lactation marked a notable decrease in C-peptide concentrations, which then improved at mid-lactation to again decline at lactation offset. These peaks and valleys in energy balance did not seem to be associated with variation in energy acquisition but were rather mirrored by activity levels and fTH3 during lactation.

Discussion: Energy balance was not preserved through the reproductive cycle, supporting previous contentions that the reproductive performance of female man-

tled howler monkeys may be energetically constrained. The contrast between these and results that we have previously reported, highlights the importance of conducting follow-up studies to continually improve our understanding of the reproductive energetics of primate females.

KEYWORDS

C-peptide, energy expenditure, energy intake, lactation, thyroid hormone

1 | INTRODUCTION

Internal conception, in utero development, and lactation confer female mammals a remarkable influence over their reproductive success (Hayseen & Orr, 2017). Conversely, female mammals devote more time and energy to reproduction than males and are also vulnerable to predation, disease, and death during certain stages of the reproductive cycle (Bronson, 1989; Gittleman & Thompson, 1988). Thus, behavioral and physiological traits that allow for coping with reproductive challenges should be under strong selection in females, and their study is crucial to understand the evolution of female reproductive strategies and life history.

Metabolization of energy reserves and modulation of both feeding behavior and activity levels may allow for coping with the energetic demands of reproduction (Emery Thompson, 2013). For instance, during the first few months post-partum, black howler monkeys (*Alouatta pigra*) and women rest less and feed for longer (Dias et al., 2011; Piperata & Dufour, 2007; Prentice & Prentice, 1988). In contrast, chacma baboons (*Papio ursinus*) spend more time resting (Barret et al., 2006) and gestating and lactating savannah baboons (*P. cynocephalus*) have low thyroid hormone concentrations, presumably reflecting energy sparing (Gesquiere et al., 2018). Lactating chacma baboons and white-faced capuchins (*Cebus imitator*) do not feed for longer than non-lactating females (McCabe & Fedigan, 2007). However, lactating white-faced capuchin females feed faster than other females (McCabe & Fedigan, 2007), although their physical condition is not related to reproductive state per se, but is rather influenced by the interaction of reproductive state, rank, and feeding competition: low-ranking, lactating females fallback on fat reserves during periods of high feeding competition (Bergstrom et al., 2020). Physical condition does not vary between lactating and non-lactating females in both mountain gorillas (*Gorilla beringei beringei*) and olive baboons (*P. anubis*; Grueter et al., 2014; Rosetta et al., 2011). In the latter, energy expenditure is unrelated to rank, but despite having higher energy acquisition than low-ranking females, high-ranking females lose more weight from lactation to the resumption of cycling (Rosetta et al., 2011).

While this diversity in physiological and behavioral responses to the energetic demands of reproduction could be associated with phylogenetic and allometric constraints (Martin, 1995, 2007), socioecological factors are also important: significant variation in physical condition according to reproductive state occurs when there is variation among females in the access to food resources (Bergstrom et al., 2020; Emery

Thompson et al., 2012; Grueter et al., 2014; McCabe et al., 2013; Rosetta et al., 2011). This could explain why the energy balance of mantled howler monkeys (*A. palliata*), a species in which females form dominance hierarchies but show little aggression during feeding (Jones, 1980), does not vary among reproductive states (Cano-Huertes et al., 2017).

Howler monkeys are arboreal Neotropical primates that live in social groups of variable size and composition (Di Fiore & Campbell, 2007). Their diet ranges from highly frugivorous to highly folivorous, depending on the availability of plant parts (Dias & Rangel-Negrín, 2015). This dietary flexibility is linked to several morphological, physiological, and behavioral adaptations, including large shearing molars to process leaves (Anthony & Kay, 1993), flexible digestive strategies (Espinosa-Gómez et al., 2013), the ability to neutralize plant secondary compounds (Espinosa-Gómez et al., 2018), variable diversity and activity of gut microbiota that buffers against fluctuations in energy and nutrient availability (Amato et al., 2014, 2015), and both long resting periods and lethargic pace during locomotion that favor energy-saving (Di Fiore & Campbell, 2007; Youlatos & Meldrum, 2011). Still, this dietary flexibility is constrained, as under highly folivorous diets individuals lose weight (Espinosa-Gómez et al., 2013). Additionally, behavioral flexibility of howler monkeys may be constrained by their energy-saving ecological strategy, as time-budgets are conserved across a wide range of contexts (Back & Bicca-Marques, 2019; Bicca-Marques, 2003; Cristóbal-Azkarate & Arroyo-Rodríguez, 2007) and physiological stress increases when individuals are more active (Dias et al., 2017; Dunn et al., 2013). Thus, howler monkeys are an interesting model to explore the interaction between metabolic function and behavioral flexibility.

We have previously documented that the behavior of female mantled howler monkeys varies through the reproductive cycle (Cano-Huertes et al., 2017): during the first half of lactation, females decrease their socialization time, and when more food is available, both gestating and cycling females spend more time feeding, and the latter also rest less. Given that energy balance (assessed via urinary C-peptide concentrations: Emery Thompson, 2016) did not vary among reproductive states, we concluded that behavioral adjustments allowed coping with the energetic demands of reproduction. Subsequent analysis of the same population, however, revealed that maternal energy balance varied non-linearly across lactation stages, being lower at both the beginning and the end of lactation than at mid-lactation (Dias et al., 2018). Additionally, high thyroid hormone concentrations (fecal triiodothyronine metabolites) in lactating females

suggested that they could be responding to energetic demands by increasing their basal metabolism, which should be correlated with behavioral adjustments (i.e., increased activity; Dias et al., 2017). Conversely, cycling females had the lowest thyroid hormone concentrations (i.e., downregulated basal metabolism; Dias et al., 2017), opposing the observed decrease in resting time in this state (Cano-Huertes et al., 2017). These conflicting results called for more research on the reproductive ecology of female mantled howler monkeys.

Here, we present the results of a follow-up study on the variation in the energy balance of female mantled howler monkeys based on a large sample of females. We hypothesized that, given that energetic demands vary through the reproductive cycle, females should exhibit behavioral and physiological mechanisms that allow for the maintenance of their energy balance among reproductive states. Specifically, we predicted that (a) female energy balance does not vary among reproductive states; and that during energetically costly states (beginning of lactation, end of lactation, and cycling) females (b) increase energy acquisition, and (c) decrease energy expenditure.

2 | METHODS

2.1 | Ethical note

Our study was noninvasive and adhered to the ASP Code of Best Practices in Field Primatology and to the ASP Principles for the Ethical Treatment of Nonhuman Primates. Research protocols were approved by the Secretaría de Medio Ambiente y Recursos Naturales of Mexico (SGPA/DGVS/04999/14) and complied with the legal requirements of the Mexican law (NOM-059-SEMARNAT-2010).

2.2 | Study sites and subjects

We conducted this study at the Los Tuxtlas Biosphere Reserve (Veracruz, Mexico; Figure 1). The climate in this region is hot and humid, with mean annual ambient temperature ranging from 24.1°C to 27.2°C and mean annual rainfall ranging from 1272 to 4201 mm (Gutiérrez-García & Ricker, 2011). Vegetation below 700 m a.s.l. was originally evergreen tropical rainforest, but due to human disturbance ongoing since the 1960's, it currently consists of a mosaic of original and secondary forests within a matrix of anthropogenic land covers, such as crops, pasturelands, and urban areas (Arroyo-Rodríguez et al., 2011; Castillo-Campos & Laborde, 2004). We studied females living in five forest fragments which have been the focus of several studies (Figure 1, Table S1). Specifically, we studied 48 adult females that could be easily identified via natural markings, such as patches of yellow fur in their tails and feet, scars, and facial features.

2.3 | Determination of female reproductive state

In a previous study of female energy balance, we considered only two lactation categories (0 to 8 months and 9 to 20 months postpartum; Cano-Huertes et al., 2017). Here, based on a larger number of sampled females, we followed the classification of lactation stages defined for the species (Balcells & Veà, 2009). Thus, for each study month we classified each female as: (a) cycling, when females were neither gestating nor lactating; (b) gestating, during the 186 days preceding a birth (Glander, 1980); (c) lactating 1, when they had an infant of 0 to 3 months of age (Balcells & Veà, 2009); (d) lactating 2, with an 4–8 months infant; (e) lactating 3, with an 9–14 months infant; and (f) lactating 4, with an 15–20 months juvenile. We based this classification on the observation of births and estimation of immature ages according to descriptions of physical and behavioral features (Balcells & Veà, 2009).

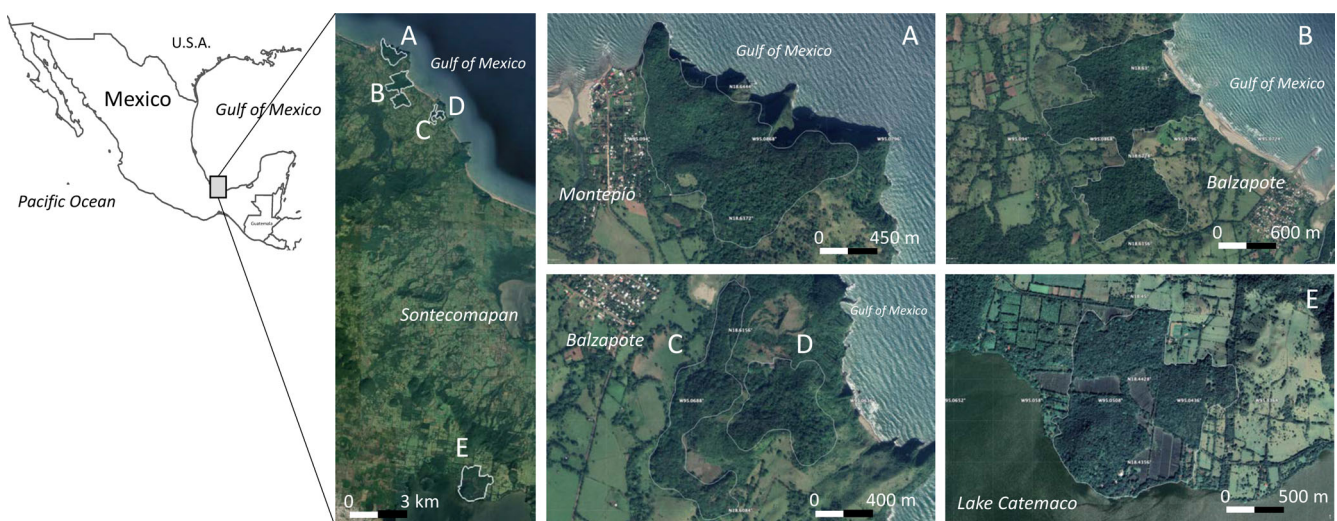


FIGURE 1 Location within Los Tuxtlas (Veracruz, Mexico) of the five forest fragments where we studied female mantled howler monkeys: Cerro del Borrego (a), Montepío 2 (b), Ripario (c), Balzapote (d), and La Flor de Catemaco (e)

This was done during and after data collection, in periodical visits to the groups up to 7 months after the end of the study (Table S2). If during a particular month a female was classified in more than one reproductive state (e.g., gestating and lactating 1), we analyzed only data from the reproductive state that spanned the most observation days in that month.

2.4 | Behavioral sampling

We used focal-animal sampling with 1-h continuous recording (Altmann, 1974) to study female time budgets and feeding behavior. We recorded the duration of the following behaviors: rest (sleep or static without interaction), feed (inspect food, bring food to mouth, chew, and swallow, move while feeding within a food patch), move (movement to a new area or tree), and socialize (social interactions). During feeding, we recorded the plant item that was consumed, classified as fruit (unripe or ripe), leave (young or mature), flower, or other item (e.g., bark). We also recorded the number of bites, defined as each occasion in which food was put into the mouth during a feeding episode (Reynoso-Cruz et al., 2016).

Behavioral sampling was performed between 2015 and 2018, with each group being studied in different moments and with a variable sampling effort (Table S1). Still, in each month we sampled female behavior for a minimum of 2.3 h, with a mean \pm SD of 10.1 ± 4.3 h of monthly sampling per female and a total of 5727.9 sampling hours during the study.

2.5 | Determination of urinary C-peptide and fecal thyroid hormone metabolite concentrations

We collected urine and fecal samples from females immediately after deposition whenever samples were unequivocally matched to subject identity. We collected fresh urine samples uncontaminated by soil, feces, or urine from other individuals by pipetting urine from a plastic sheet positioned underneath females during urination or directly from vegetation. We deposited urine samples in 5 ml snap-cap polypropylene microtubes. We collected fecal samples uncontaminated by urine from the forest floor and deposited them in polyethylene bags labeled with the identity of each individual. We kept samples in a cooler with frozen gel packs while in the field and stored them at the end of the day in a freezer at -20°C until processing. Time of collection and storage duration did not influence neither C-peptide nor fecal thyroid hormone concentrations (Figures S1 and S2).

We extracted fecal triiodothyronine metabolites (fTH3 hereafter) following a modification of the method described in Wasser et al. (2000). Briefly, we shook 0.6 g of homogenized, lyophilized, and pulverized feces for 20 h in 4.0-ml analytical-grade methanol. We centrifuged extracts (460 g for 30 min) and recovered the supernatant. After complete evaporation of the solvent in a water bath at 60°C for 20 h, pellets were reconstituted with 3-ml albumin buffer and used for fTH3 assays. We measured fTH3 with a chemiluminescent enzyme

immunoassay commercial kit (Total T3, Immulite, Siemens, CA) and an Immulite 1000 analyzer (Siemens, CA). fTH3 are reported as ng/g of dry feces.

We determined urinary C-peptide concentrations via radioimmunoassay (Human C-Peptide, Merck Millipore, Billerica, MA) and a gamma counter (Cobra Model E5005, Packard, Inc., Downers Grove, IL). We standardized C-peptide measurements by dividing C-peptide concentrations by creatinine concentrations (measured via enzyme immunoassays with an auto analyzer: Gallery, Thermo Fisher Scientific, Waltham, MA). C-peptide levels are therefore reported as pg/mg of creatinine.

We performed analytical validations of all assays as described in Rangel-Negrín et al. (2014); (Table S3). The protocols that we used to extract and quantify C-peptide and fTH3 have been previously biologically validated to detect insulin and thyroid function, respectively, in mantled howler monkeys (Cano-Huertes et al., 2017; Dias et al., 2017). We analyzed a total of 2717 urine and 3138 fecal samples, with a mean \pm SD of 56.6 ± 30.9 urine samples and 65.4 ± 32.6

TABLE 1 Variables analyzed in this study pertaining to variation in the behavior and physiology throughout the reproductive cycle in 48 female mantled howler monkeys studied at Los Tuxtlas

Variable	Description/attributes in this study
Female reproductive state	Cycling (116 female/months), gestating (116), lactating 1 (82), lactating 2 (81), lactating 3 (108), lactating 4 (73)
Female energy balance	Urinary C-peptide concentrations. C-peptide [insulin] lowers glucose levels and stimulates energy storage. It is positively related to energy balance (Emery Thompson, 2016). Mean \pm SD = 6043 ± 4510 pg/mg ($N = 2717$ urine samples)
Fruit consumption	Time spent eating fruits, which are the main source of sugars and fats for howler monkeys (Righini et al., 2017). Mean \pm SD = 2318 ± 1668 s ($59 \pm 35\%$ of total feeding time; $N = 5727.9$ behavioral sampling hours)
Young leaf consumption	Time spent eating young leaves, which are the main source of protein for howler monkeys (Righini et al., 2017). Mean \pm SD = 1571 ± 1301 s ($40 \pm 15\%$ of total feeding time; $N = 5727.9$ behavioral sampling hours)
Bite rate	Number of bites per unit of time, which represents a reliable measure of food intake in mantled howler monkeys (Reynoso-Cruz et al., 2016). Mean \pm SD = 9.3 ± 4.6 bites/min ($N = 704$ female/month records)
Activity	Time (s) spent feeding, moving, and socializing divided by time resting (Dias et al., 2017). Mean \pm SD = 0.33 ± 0.19 ($N = 704$ female/month records)
fTH3	Fecal thyroid (T3) hormone metabolites. Thyroid hormones are involved in the regulation of basal metabolism, such that higher fTH3 indicate higher energy expenditure (Dias et al., 2017). Mean \pm SD = 90.9 ± 56.9 ng/g ($N = 3138$ fecal samples)

fecal samples analyzed per female, and 4.8 ± 3.3 urine samples and 5.5 ± 3.6 fecal samples analyzed per female per month.

2.6 | Data organization and statistical analysis

We defined measures of energy balance, energy acquisition, and energy expenditure that have been previously used to study howler monkey energetics (Dias et al., 2017; Rangel-Negrín et al., 2018). Specifically, we used monthly values per female of (a) C-peptide concentrations as a measure of energy balance; (b) time spent consuming fruits, time spent consuming young leaves (the main foods of mantled howler monkeys: Dias & Rangel-Negrín, 2015), and bite rate (number of bites per unit of time) as measures of energy intake; and (c) an activity index and fTH3 as measures of energy expenditure (Table 1; Figure S3). We explored the relationships between female energy balance (urinary C-peptide concentrations) and both measures of energy intake (fruit consumption and bite rate) and energy expenditure (activity and fecal thyroid hormone metabolites) with a linear mixed model using female identity as a random variable.

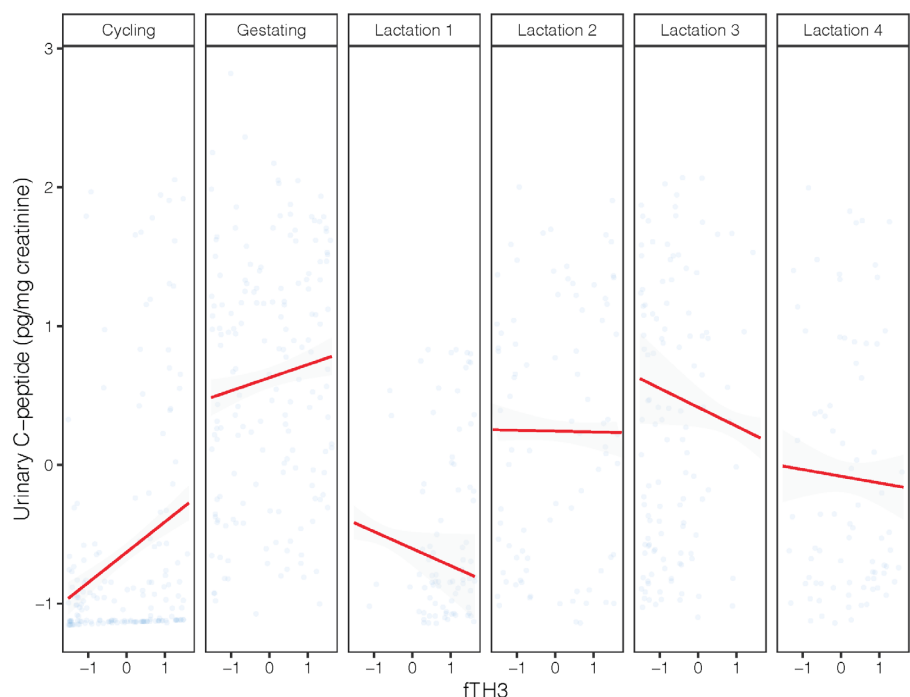
To test our predictions, we used mixed models in which female reproductive state was the fixed predictor and both female identity and group identity were random predictors to account for the repeated sampling of females through time and for intergroup variation in female responses to reproductive challenges, respectively. We used: (a) linear mixed models (i.e., Gaussian distribution) to analyze variation in urinary C-peptide concentrations, bite rate, and fTH3; (b) generalized linear mixed models with beta distribution to analyze the proportion of time spent consuming fruits, the proportion of time spent consuming young leaves, and the activity index. We standardized C-peptide and fTH3 values (i.e., subtracted the mean and divided the result by the *SD* of the original values) and squared-root transformed bite rate to improve model fit, as assessed via distribution of residuals and quantile–quantile plots to verify normality, and of residuals plotted against predicted values to assess homogeneity. To determine the influence of the random factor in models, we compared complete models (i.e., including both fixed and random factors) with null models including only the random factor with likelihood ratio tests. In all cases model comparisons were significant ($p < 0.01$), suggesting that the random factor had a limited influence on results. All analyses were performed with R 4.0.0 (R Core Team, 2020).

TABLE 2 Mixed model results of the variation in urinary C-peptide concentrations according to energy intake and energy expenditure in 48 female mantled howler monkeys (*Alouatta palliata*) studied at Los Tuxtlas

Predictor	Estimate	SE	t	p	95% C.I.	
					Lower	Upper
Fruit consumption	−0.04	0.037	−1.1	>0.05	−0.113	0.032
Bite rate	0.01	0.037	0.4	>0.05	−0.057	0.086
Activity	−0.03	0.037	−0.7	>0.05	−0.101	0.045
fTH3 ^a	−0.45	0.038	−2.8	0.013	−0.105	−0.045

^aFecal thyroid hormone metabolite concentrations.

FIGURE 2 The influence of fecal thyroid hormone metabolites (fTH3) on urinary C-peptide concentrations (proxy for energy balance) according to reproductive state in female mantled howler monkeys. Variables were centered before analysis. Areas shaded in gray depict the 95% confidence intervals



3 | RESULTS

3.1 | Relationships among energetic measures

Only fTH3 concentrations had a significant negative relationship with energy balance (Table 2). If female reproductive state is included as an interaction factor in this model, nonsignificant results remain for all factors (fruit consumption $\chi^2_6 = 3.9$, $p = 0.678$; bite rate $\chi^2_6 = 3.6$, $p = 0.717$; activity $\chi^2_6 = 5.1$, $p = 0.531$), except for the interaction between fTH3 and female reproductive state ($\chi^2_6 = 26.6$, $p < 0.001$; Figure 2). This result is linked to significant differences between Lactating females and both Cycling and Gestating females, except for Lactation 2 versus Gestating females (all post-hoc Tukey comparisons $p < 0.05$). Overall, the relationship between fTH3 and C-peptide was negative for females in Lactation 1, 3, and 4, and positive for Cycling and Gestating females.

3.2 | Energy balance of females through the reproductive cycle

Female energy balance, assessed via urinary C-peptide concentrations, varied among reproductive states ($\chi^2_5 = 216$, $p < 0.001$), being the highest during gestation and the lowest during cycling (Table 3 & Figure 3a). Specifically: cycling and lactating 1 females had lower C-peptide concentrations than females in all other states and gestating females had higher C-peptide concentrations than females in other states, except for lactating 3 females ($p < 0.05$ for all pairwise Tukey comparisons).

3.3 | Behavioral and fTH3 variation among reproductive states

Energy intake did not vary among females in different reproductive states (fruit intake: $\chi^2_5 = 4.9$, $p = 0.427$; young leaf intake: $\chi^2_5 = 5.5$,

TABLE 3 Mixed model results of the variation in urinary C-peptide concentrations according to reproductive state in 48 female mantled howler monkeys (*Alouatta palliata*) studied at Los Tuxtlas

State ^a	Estimate	SE	t	p	95% C.I.	
					Lower	Upper
Gestating	1.30	0.101	12.9	<0.001	1.10	1.49
Lactating 1	0.19	0.121	1.6	0.118	-0.05	0.43
Lactating 2	0.93	0.125	7.5	<0.001	0.68	1.17
Lactating 3	1.00	0.114	8.8	<0.001	0.77	1.22
Lactating 4	0.63	0.126	5.0	<0.001	0.38	0.87

^aComparisons against the cycling state.

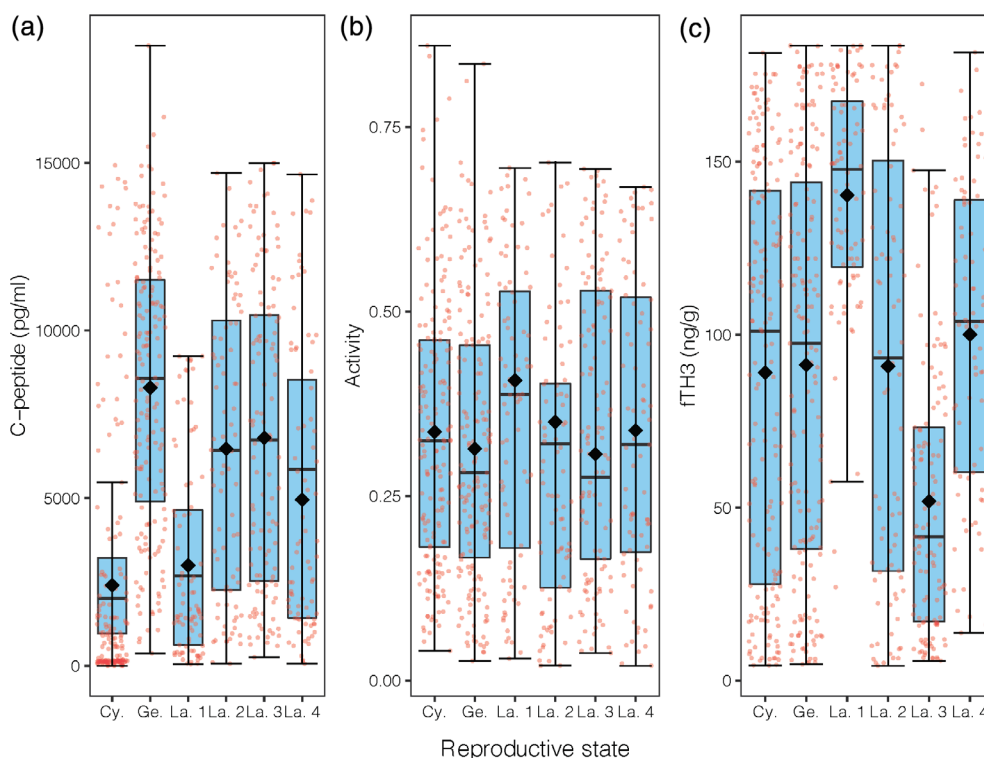


FIGURE 3 Variation among female reproductive states in urinary C-peptide concentrations (a), activity (b), and fecal triiodothyronine metabolites (fTH3; c). Thick lines inside the boxes are the medians; black diamonds are the means; box limits are the 25th and 75th percentiles; whiskers indicate 1.5*interquartile ranges; data points are red circles

TABLE 4 Mixed model results of the variation in energy intake according to reproductive state in 48 female mantled howler monkeys (*Alouatta palliata*) studied at Los Tuxtlas

Model	State ^a	Estimate	SE	Statistic ^b	p	95% C.I.	
						Lower	Upper
Fruit consumption	Gestating	-0.15	0.146	-1.0	0.311	-0.43	0.14
	Lactating 1	-0.02	0.177	-0.1	0.892	-0.37	0.32
	Lactating 2	-0.13	0.179	-0.7	0.479	-0.48	0.22
	Lactating 3	0.16	0.160	1.0	0.321	-0.15	0.47
	Lactating 4	0.10	0.182	0.5	0.591	-0.26	0.45
Young leaf consumption	Gestating	0.17	0.147	1.2	0.244	-0.12	0.46
	Lactating 1	0.04	0.178	0.2	0.820	-0.31	0.39
	Lactating 2	0.15	0.181	0.8	0.411	-0.21	0.50
	Lactating 3	-0.15	0.161	-0.9	0.343	-0.47	0.16
	Lactating 4	-0.09	0.182	-0.5	0.628	-0.45	0.27
Bite rate	Gestating	0.01	0.084	0.1	0.937	-0.16	0.17
	Lactating 1	0.10	0.103	1.0	0.310	-0.10	0.31
	Lactating 2	0.01	0.103	0.0	0.961	-0.20	0.21
	Lactating 3	0.03	0.093	0.4	0.713	-0.15	0.21
	Lactating 4	0.02	0.105	0.2	0.813	-0.18	0.23

^aComparisons against the cycling state.

^bStatistics are χ^2 for fruit and young leaf models (beta distribution) and t for bite rate (Gaussian distribution).

TABLE 5 Mixed model results of the variation in energy expenditure according to reproductive state in 48 female mantled howler monkeys (*Alouatta palliata*) studied at Los Tuxtlas

Model	State ^a	Estimate	SE	t	p	95% C.I.	
						Lower	Upper
Activity	Gestating	-0.12	0.092	-1.3	0.179	-0.30	0.06
	Lactating 1	0.06	0.110	0.6	0.580	-0.16	0.28
	Lactating 2	-0.29	0.114	-2.6	0.011	-0.51	-0.07
	Lactating 3	0.02	0.101	0.2	0.866	-0.18	0.21
	Lactating 4	-0.06	0.114	-0.5	0.588	-0.29	0.16
fTH3	Gestating	-0.16	0.106	-1.5	0.133	-0.37	0.05
	Lactating 1	0.72	0.127	5.7	<0.001	0.47	0.98
	Lactating 2	-0.13	0.132	-1.0	0.332	-0.39	0.13
	Lactating 3	-0.79	0.121	-6.5	<0.001	-1.03	-0.55
	Lactating 4	0.11	0.133	0.8	0.409	-0.15	0.37

^aComparisons the against cycling state.

$p = 0.355$; bite rate: $\chi^2_5 = 1$, $p = 0.942$; Table 4). Activity varied among states ($\chi^2_5 = 11$, $p = 0.05$; Table 5 & Figure 3b), with (a) lactating 1 females being more active than both gestating and lactating 3 females, (b) cycling females more active than gestating and lactating 3 females; and (c) lactating 2 more active than lactating 3 females ($p < 0.05$ for all pairwise Tukey comparisons). The concentrations of fTH3 also varied among reproductive states ($\chi^2_5 = 147$, $p < 0.001$; Table 5 & Figure 3c), with lactating 1 and lactating 3 females having higher and lower values than all other states, respectively ($p < 0.05$ for all pairwise Tukey comparisons).

4 | DISCUSSION

We had previously reported that the energy balance of female mantled howler monkeys did not vary according to reproductive state (Cano-Huertes et al., 2017). Given several inconsistencies between this result and subsequent analyses of female behavior and physiology on the same population, we performed a follow-up study based on a larger sample. Contrary to what we expected, energy balance does vary through the female reproductive cycle: it increases as females progress from cycling to gestation, decreases at lactation onset, again

increases at mid-lactation, and decreases by the end of lactation. This variation is not associated with changes in feeding behavior but during lactation it mirrors activity and fTH3 concentrations: at low energy balance stages, females were more active and had higher fTH3 concentrations. Thus, mantled howler monkey females do not maintain their energy balance through reproductive states, and low energetic condition may be linked to high energy expenditure.

The beginning of the lactation period is highly demanding. Besides the metabolic costs of milk synthesis and mammary gland activity (Prentice & Prentice, 1988), similarly to other primate mothers (Wright, 1990) howler monkey mothers carry and transport their young infants continuously (Balcells & Veà, 2009). Additionally, as infants are particularly vulnerable to predation, infanticide, and aggression from other group members during this period (Clarke, 1990; Crockett, 2003), mothers must devote time to vigilance at the cost of other activities (Barret et al., 2006; Treves et al., 2003). Accordingly, females were more active and had higher fTH3 concentrations at lactation stage 1 than at other times. This contrasts with observations in other species, in which females either increase energy intake or decrease energy expenditure to offset energetic demands (Barret et al., 2006; Dias et al., 2011; McCabe & Fedigan, 2007; Piperata & Dufour, 2007). Thus, given the comparatively higher energy balance of females during gestation and mid-lactation, the beginning of the lactation stage is probably a period of transient energetic stress for female mantled howler monkeys.

Following the notable drop in female energy balance observed from gestation to lactation onset, C-peptide concentrations increased during mid-lactation. This is a probable consequence of the increasing independence of infants from their mothers for moving and feeding (Arroyo-Rodríguez et al., 2007; Balcells & Veà, 2009; Clarke, 1990), thus reducing the energetic burden of carrying and milk production. For instance, at La Flor de Catemaco, the percentage of time mothers spend carrying their infants decreases from 74% at the lactating 1 stage to 39% at lactating 2, and 15% at lactating 3 (Dias et al., 2018). The increase in C-peptide concentrations was mirrored by a decrease in activity levels and fTH3, suggesting a stronger influence of energy expenditure than of energy acquisition mechanisms on the regulation of female energy balance in this species. This is consistent with evidence that, whereas energy intake is rather constant despite fluctuations in the availability and consumption of different food types (Amato & Garber, 2014; Righini et al., 2017), when fruit availability decreases mantled howler monkeys spend more time moving, a likely consequence of the more uneven spatiotemporal distribution of fruits compared to leaves (Dunn et al., 2013).

Female energy balance was the lowest during cycling. If the cessation of milk provisioning (weaning) is consistently synchronized with peaks in food availability, the lower energy balance of cycling females could correspond to a higher likelihood of being in this stage during subsequent periods of low food abundance. However, (a) this reproductive strategy is usually observed in small-sized primate species living in highly seasonal habitats (Janson & Verdolin, 2005), (b) reproductive seasonality among mantled

howler monkeys living at Los Tuxtlas is usually considered low (Cano-Huertes et al., 2017; Estrada, 1982; Rangel-Negrín et al., 2018; but see Cristóbal-Azkarate et al., 2017), and (c), in our sample, females at the weaning stage (lactating 4) were sampled in all months. If mantled howler monkeys fallback on energy reserves to subsidize the energetic demands of lactation, it is possible that those reserves have been depleted at lactation offset and females are in a negative energy balance state (Emery Thompson et al., 2012). This possibility is consistent with the observation of long periods of non-conceptive cycling (Glander, 1980; Milton, 1982) and of a link between the occurrence of conception and both low glucocorticoid and high thyroid hormone concentrations (Rangel-Negrín et al., 2018).

Condition-dependent conception, which is common in human and non-human primates, implies that females begin gestation with positive energy balance (Brockman & van Schaik, 2005; Valeggia & Ellison, 2009). Increases in insulin levels (proxied via C-peptide) during gestation, together with a reduction in activity, facilitate energy storage. This could be fundamental for coping with the costs of lactation although, as seen here, it is not sufficient to prevent a significant drop in female condition. The high energy balance of gestating females was not associated with adjustments in energy acquisition. Given that female mantled howler monkeys do not seem to compete directly for food, which in other species underlies variation in energetic condition (Bergstrom et al., 2020; Rosetta et al., 2011), it is unlikely that the high C-peptide concentrations of gestating females result from priority of access to food. However, females are more likely to lead group movements toward feeding trees when gestating than at other reproductive states (Ceccarelli et al., 2020). This raises the intriguing possibility that, as observed in other primate females (e.g., *Cebus capucinus*: Boinski, 1993; *Propithecus* and *Eulemur*: Erhart & Overdorff, 1999), leadership could yield foraging advantages that improve energetic condition. It remains for future research to examine this possibility as well as its underlying physiological mechanism, including, for instance, variation across reproductive states in appetite regulation via the actions of leptin and ghrelin (Emery Thompson, 2017; Havel, 2001).

In sum, the energy balance of female mantled howler monkeys varied among reproductive states: (a) cycling was a period of low energy balance; (b) highest energy balance occurred during gestation; and (c) the beginning of lactation marked another notable decrease in energy balance, which then improved at mid-lactation to again decline at lactation offset. These peaks and valleys in energetic condition did not seem to be associated with variation in energy acquisition but were rather mirrored by activity levels and thyroid hormone levels during lactation. Thus, energetic condition was not preserved through the reproductive cycle, supporting previous contentions that the reproductive performance of mantled howler monkey females may be energetically constrained. Finally, the contrast between these and results that we have previously reported (Cano-Huertes et al., 2017), highlight the importance of conducting follow-up studies to continually improve our understanding of the reproductive energetics of primate females.

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AUTHOR CONTRIBUTIONS

Ariadna Negrín: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing-original draft. **Alejandro Coyohua-Fuentes:** Data curation; investigation; methodology; visualization. **Amalia de la Torre Herrera:** Data curation; investigation; visualization. **Beatriz Cano-Huertes:** Data curation; formal analysis; investigation; visualization. **Eduardo Reynoso Cruz:** Data curation; investigation; visualization. **Enrico Ceccarelli:** Data curation; investigation; visualization. **Eugenia Eréndira Gómez Espinosa:** Data curation; investigation; visualization. **David Roberto Chavira-Ramírez:** Data curation; formal analysis; investigation; methodology; resources; supervision; visualization. **Diana Moreno Espinoza:** Data curation; formal analysis; investigation; visualization. **Domingo Canales Espinosa:** Funding acquisition; project administration; resources; visualization. **Natalia Maya Lastra:** Data curation; investigation; visualization. **Pamela Cruz Miros:** Data curation; investigation; visualization. **Samuel Cañadas Santiago:** Data curation; investigation; visualization. **Sara Garau:** Data curation; investigation; visualization. **Pedro Américo Duarte Dias:** Conceptualization; data curation; formal analysis; funding acquisition; methodology; project administration; resources; supervision; validation; writing-original draft.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available from the corresponding author at a reasonable request.

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