Resting Metabolic Rate Is Positively Correlated with Parental Care Behavior in a Dwarf Hamster

SABRINA CLAVIJO-BAQUET[1,2]*,
NICOLÁS CUMPLIDO[1],
AND FRANCISCO BOZINOVIC[2]

[1]Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla, Santiago, Chile
[2]Departamento de Ecología, Center of Applied Ecology & Sustainability (CAPES), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

ABSTRACT

Endotherms maintain high and constant body temperatures through the production and maintenance of metabolic heat. Defining the evolutionary history of these thermal adaptations and the selective factors responsible for the evolution of endothermy despite its high metabolic costs have been elusive and controversial topics in evolutionary biology. In this sense, several models have been proposed to explain the evolution of endothermy. Among them, the parental care model explains the increase in resting metabolic rate (RMR) by the action of natural selection favoring parental care. Thus, a positive relationship between parental care behavior and RMR is predicted. However, there appears to be no or little previous work experimentally testing this relationship. In the study presented here, RMR was increased through L-tyrosine injections and parental care behavior was measured. This treatment allowed us to test the relationship between RMR level and parental care behavior in a dwarf hamster. It was found that increased RMR enhanced male parental care. Specifically, male latency time, or the time until contacting and picking up their pups, decreased when RMR increased. This study demonstrates the positive relationship between RMR and the allocation of resources to parental care. This study supports the main assumption of Kotejas's parental care model and accepts Koteja's proposed explanation for the evolution of endothermy as a plausible hypothesis. *J. Exp. Zool. 325A:274–282, 2016.* © 2016 Wiley Periodicals, Inc.

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The endothermic traits shared by mammals and birds are among the most notable examples of evolutionary convergence among vertebrates. Specifically, continuous endothermy is the maintenance of high and constant body temperatures (T_b) through the production and conservation of metabolic heat (McNab, 2002). Therefore, the cost of living is greater for an endothermic organism than for an ectotherm. This fact is supported by the noted basal (BMR) or resting metabolic rates (RMR) of endotherms that can be 20 times higher than the metabolic rates of reptiles of similar body size (Koteja, '91; Ricklefs et al., '96). Defining the evolutionary history of these thermal adaptations and the selective factors responsible for the evolution of endothermy despite its high metabolic costs have been elusive and contro-

versial topics in evolutionary biology (Hayes and Garland, '95; Ruben, '95). In this sense, several theoretical models have been

proposed addressing the evolution of endothermy; this topic has been extensively reviewed in Hayes and Garland ('95). Among the models that have been proposed, Koteja's and Farmer's models propose that the evolution of these costly traits could be possible by means of parental care selection (Farmer, 2000; Koteja, 2000, 2004).

Koteja's parental care model (hereafter referred to as the parental care model) proposes that the evolution of endothermic traits (specifically RMR) occurred due to natural selection acting on parental care. Enhanced parental care, such as increasing the intensity of guarding or feeding young, requires an increase in locomotor activity (Koteja, 2000, 2004). This increase in sustained locomotor performance can only be maintained through increased daily energy expenditure (DEE) and energy assimilation capacity. In order to increase energy assimilation capacity, larger or more metabolically intense visceral organs, including the liver and kidneys, and a more extensive alimentary tract in general are required. The visceral organs and the alimentary tract are prominent factors influencing RMR; hence, increasing their size and/or metabolic intensity elevates RMR. Thus, the parental care model suggests that increased RMR was attained as a by-product of natural selection acting on parental care (Koteja 2000, 2004).

Although some previous studies have shown a positive relationship between metabolic rate and reproductive performance (Drent and Daan, '80), Koteja (2000) is the first author to propose an explanation for the origin of endothermy as a consequence of this relationship (Koteja, 2004). The parental care model requires, in order to be a plausible explanation for the evolution of endothermy, that individuals with higher RMR values also have larger visceral organs, are more active, and perform more parental care (either feeding or guarding young). In other words, this model relies on positive correlations between several physiological and behavioral traits. For example, a positive relationship between RMR and visceral organ size and between RMR and activity level is expected. Finally, the model predicts a positive relationship between RMR and allocation of resources to parental care (Koteja, 2000, 2004).

Regarding the relationship between basal metabolism and visceral organ size, previous work has shown that mice with higher BMR values also had larger visceral organs (Konarzewski and Diamond, '95; Książek et al., 2004; Burton et al., 2011); in this study, individuals were derived from artificially selected mouse lines with high and low basal metabolism. Furthermore, some authors have indicated that there is a correlation between metabolic rate and reproductive performance (i.e., litter size and milk production) (Johnson et al., 2001a, 2001b; Kam et al., 2003; Krol et al., 2003; Boratynski and Koteja, 2009). In this sense, Sadowska et al. (2013) have shown that females from high BMR mouse lines also invest more in parental care, measured as a pup growth rate. However, to our knowledge, neither experimental nor correlational studies have found or sought evidence sup-

porting this model by testing the relationship between RMR and parental care behavior. This may be because RMR and parental care behavior are extremely labile and vary within and among several species of mammals and birds (Cruz-Neto and Bozinovic, 2004; Bozinovic, 2007; Bozinovic et al., 2007, 2009; Swanson and Bozinovic, 2011). The variability in RMR and parental care behavior makes explaining the origin of endothermy difficult when trying to assume a relationship between these traits. Consequently, the aim of this study was to experimentally test the relationship between parental care behavior and RMR, which is one the main assumptions of the parental care model for the origin of endothermy. This relationship was tested using pups and males of the hamster *Phodopus campbelli*, a species that exhibits biparental care behavior.

MATERIAL AND METHODS

Study Model

P. campbelli is a rodent species from Asia (Wynne-Edwards, '95), and males of this species exhibit parental care, which has been described extensively (Wynne-Edwards, '95, 2003). *P. campbelli* is an excellent model to test the relationship between RMR and parental care behavior because it is possible to manipulate males without interfering with females and their brood. Manipulating the metabolism of males instead of females ensures that RMR changes are transparent, and confounding factors such as lactation are avoided. In addition, this species has one of the shortest life cycles among mammals (Wynne-Edwards, '95); this allows for the study of several reproductive periods of the same couple in a relatively short period of time. *P. campbelli* offspring are altricial, being born without hair, closed eyes, and incapable of thermoregulation until 18 days after birth. Offspring development is divided into six age classes each with duration of 3 days. Throughout development, thermoregulatory ability is improved (Newkirk et al., '95). Specifically, (1) during the first 3 days, pups are naked, pink, and lose heat passively; (2) from day 4–6, the fur starts to form, weak thermogenesis begins, and pups have limited locomotion within the nest; (3) from day 7–9, the fur is almost complete and pups actively huddle; (4) from day 10–12, pups exhibit limited ability to maintain their core body temperature in the absence their parents; (5) from day 13–15, pups begin to exit the nest to explore the environment; and (6) from day 16–18, pups are independent (Newkirk et al., '95). The parental care carried out by both parents varies throughout the pup's development. For instance, the frequency that pups are left alone in the nest increases with offspring development (Wynne-Edwards, '95).

Experimental Design

Pairs were housed in boxes (25 × 25 cm) and provided with sawdust to nest. They were subjected to a summer photoperiod (14L: 10D), fed with rat formula and sunflower seeds ad libitum, and water was available in inverted cylinders. We performed three
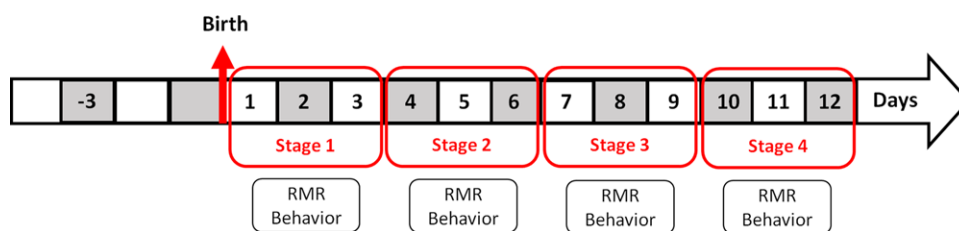
Figure 1. Temporal depiction of treatments including behavioral and metabolic records. Treatment injections (gray boxes) started three days before female parturition and continued every 2 days. Behavioral and metabolic records were recorded once per developmental stage (red boxes).

randomly ordered treatments for each male, namely, (1) a control without manipulation, (2) a treatment control where males were injected with saline solution, and (3) a treatment where males were injected with a solution of L-tyrosine (120 μg/100 g). L-tyrosine is known to increase the metabolic rate in mammals (Belza and Jessen, 2005; Belza et al., 2007). We used L-tyrosine instead of other conventional compounds (e.g., thyroxine) to avoid influencing reproduction and behavior in general (Anderson and Schanberg, '75; Goldsmith and Nicholls, '92). Injections were initiated 3 days before parturition and continued every 2 days until day 15 after female parturition (Fig. 1). During the entire experimental period, the males received the same amount of L-tyrosine. The first four pup developmental stages were selected as the experimental period because during this time pups are more dependent on both parents. Consequently, we maximized our efforts during the stages when the males and litters are most sensitive. After weaning, the parents were enclosed to breed again for one of the two remaining treatments. Following the second treatment, the third treatment was performed for each male following the same procedures as described above.

RMR and behavior were recorded once per developmental stage (see Fig. 1). Males were weighed before all metabolic records. To avoid perturbations in behavioral and metabolic records when measurements and injections were performed on the same day, injections were applied after metabolic measurements and 5 hr before behavioral tests. Finally, one behavioral and one metabolic record were obtained per developmental stage of each treatment for all males. Initially, the experimental design included a total of 15 males; however, we were not able to perform all treatments and all measurements for all of the males due to various reasons (e.g., male death, female did not become pregnant, etc.). Subsequently, the observations are not independent and the sample size varies with the analysis. To account for this, effective sample size (N_e) was estimated for each analysis (see the Results section) (Zuur et al., 2009).

Respirometry
Male RMR was measured through O_2 consumption in an open respirometry system equipped with a FC-10a O_2 analyzer (Sable

Systems, Henderson, Nevada, USA). Males were placed in metabolic Plexiglas chambers (1000 mL) at noon during their resting period and for 3 hr in a controlled temperature cabinet (Sable Systems, Henderson, Nevada, USA) at 30 $\pm$ 0.5°C. The chambers received air that passed through CO_2/water absorbent granules (Baralyme and Drierite, respectively) at 800 mL/min from a MFS2 variable pump and a mass flow system (Sable Systems (Lighton, 2008)). The excurrent air passed again through columns of Baralyme and Drierite before passing through the O_2-analyzer (Sable System, Henderson, Nevada, USA), which was calibrated with a known mix of oxygen (20%) and nitrogen (80%) certified by chromatography (Indura, Casilla, Chile). Air was sampled every 5 sec by the O_2 analyzer. The mass flow meter of the O_2 analyzer was calibrated monthly with a volumetric (bubble) flow meter. Due to the fact that water vapor and CO_2 were scrubbed before entering the O_2 analyzer, oxygen consumption was calculated as Withers ('77): $V_{O2} = [FR*60*(F_iO_2 - F_e O_2)]/(1 - F_i O_2)$, where FR is the flow rate in mL/min, and F_i and F_e are the fractional concentrations of O_2 entering and leaving the metabolic chamber, respectively. Outputs from the oxygen analyzer (%) and the flow meter were digitalized using a Universal Interface II (Sable Systems, Henderson, Nevada, USA) and recorded on a personal computer using EXPEDATA data acquisition software (Sable Systems, Henderson, Nevada, USA). We averaged the O_2 concentration of the excurrent air stream over a 10-min period after steady state was reached.

Behavioral Records
Behavior was recorded at night between 10 PM and 1 AM using red light. The records were made with males alone in the breeding box. Displacement tests were conducted; 1 L was taken with tweezers and put it in a corner of the box. Then, the following behavioral variables were measured in males: pup contact, pup pickup, and pup retrieval latencies. This test was conducted five times per night for each male. This test has been performed extensively in rodent studies to assess changes in parental care behavior (Girard et al., 2002; Schradin and Pillay, 2003); previous work includes tests with *P. campbelli* (Wynne-Edwards, 2003; Vella et al., 2005; Hume and Wynne-Edwards, 2006). All

tests conducted were in accordance with the Animal Care Guidelines of the Pontificia Universidad Católica de Chile. The work was conducted under the ethics permit DFCB-042/2010 from the Biological Sciences Animal Ethics Committee.

Data Analysis

All variables were log-transformed to achieve normality, and latency values from the five tests per each night were averaged. Thus, one value representing male performance was derived per male during each developmental stage and for each treatment. It must be noted that all treatments were performed for each male; therefore, observations are not independent (Fig. 1). The effect of nonindependence was incorporated in our models by controlling RMR and latencies responses by male. Then, linear mixed models and generalized least squares (GLS) were calculated in order to test the effect of treatment on RMR and to analyze the relationship between RMR and parental care behavior. By using GLS and mixed models, we were able to include a variance structure (e.g., fixed variance structure) associated with each male. GLS is essentially a weighted linear regression, so if a variance structure is not included in the model, data are fitted to a linear regression (Zuur et al., 2009). Mixed models have a fixed part that explains the response variable as a function of the explanatory variables (as a linear regression). Additionally, they also have a random part, which contains components that allow for heterogeneity, nested data (random effects), temporal correlation, spatial correlation, or a combination of these factors (Zuur et al., 2009). For example, with mixed models it was possible to estimate a random slope, intercept, or both, for each male. Only a random intercept model was fit because this type of model is comparable with a block design. However, instead of associating the error with each male, we obtained an average intercept for the whole population of males and an intercept for each individual. Therefore, when the model of best fit has mixed effects, it is possible to observe each male behavior response in response to RMR. Following this, a full model including all predictor variables and their interactions was tested; this model incorporated different variance structures by selecting the best variance structure that fit the data and then selecting relevant variables for the model. Model selection was done using Akaike information criterion (AIC) (Akaike, '71, '74), and model validation was done following Zuur's criteria (Zuur et al., 2009). A graphical approach was used for the model validation in order to search for patterns in the residuals that could be due to a violation of assumptions. When a pattern in the residuals was observed, new error structures were tested until no irregularity or deviation was found. When AIC values could not be distinguished between competing models (ΔAIC $<$ 2), the log likelihood ratio test was performed.

In particular, to test for the effect of the treatment on RMR, we performed a mixed model for each developmental stage controlling for male and including a random effect for male (random intercept). We included treatment as a factor and body mass as a covariate; the interaction between treatment and body mass was also included. After selecting the model that best explained RMR for each developmental stage (see above), RMR differences among treatments were tested using post hoc comparisons (the Tukey test) (Pinheiro and Bates, 2006). For latency, the full model including RMR, developmental stage, body mass, treatment, and all interaction terms was first calculated. To eliminate confounding factors related with treatment that could be responsible for behavioral changes, treatment was included as a nominal variable. If the best model only included treatment variables (C, control; CT, treatment control, T, Tyrosine), then the behavior changes would only be due to the treatment's side effects. Contrary, if the best model included RMR, changes in behavior would mainly be due to RMR changes. Analyses were performed using "nlme" and "lme4" libraries of the R Statistical Package (Bates et al., 2013; Pinheiro et al., 2009). The variable pup retrieval latency was not included in our analyses because we did not have enough observations to build GLS or mixed models.

RESULTS

Body mass was included in the RMR best-fit model for all developmental stages (S-Table 1 in the Supporting Information), and it had a significant effect (stage 1, $F_{1, 16} = 15.76$, $P < 0.01$; stage 2, $F_{1, 18} = 11.60$, $P < 0.01$; stage 3, $F_{1, 18} = 44.61$, $P < 0.001$; stage 4, $F_{1, 19} = 33.14$, $P < 0.001$). In addition, treatment was included in the RMR best-fit model for developmental stage 1, and a significant increase in RMR was found due to the injection of L-tyrosine (stage 1, $F_{2, 16} = 8.46$, $P < 0.01$; Fig. 2). Nevertheless, we did not find a significant effect of L-tyrosine on the RMR level for later developmental stages since the treatment variable was not included in the best-fit model (S-Table 1 in the Supporting Information). The body mass-corrected RMR for the first developmental stage was 49.35 ± 3.32 (mean and standard error [SE]) for the C treatment, 55.55 ± 4.17 for the CT treatment, and 69.61 ± 5.00 ml O^2 hr^{-1} for the T treatment ($N_e = 26.7$) (Fig. 2).

Regarding parental care behavior, contact latency best fit was achieved by a mixed model with a random intercept (by male) and including RMR, developmental stage, and the interaction of these variables (Table 1). For pickup latency, the best-fit model also included RMR, stage, treatment, and the interaction between RMR and stage (Table 2). However, this model did not include any variance structure associated with male (see the Methods section). RMR and stage had a negative effect on pickup latency, whereas CT and T had a positive effect on parental care behavior (Table 2). Because treatment was a nominal variable, one state did not have an estimated coefficient (here control, C) and was used only as a contrast for the other treatments (CT and T). As the control treatment and tyrosine had positive estimated coefficients, the individuals belonging to these treatments had larger pickup latencies than control individuals (Table 2). Overall for
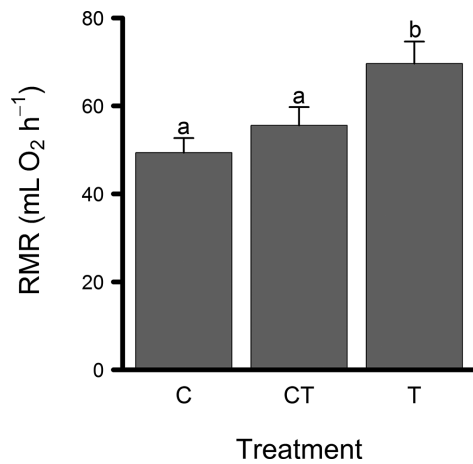
Figure 2. RMR body sized corrected during the first developmental stage. (C) Control, (CT) saline solution, and (T) L-tyrosine treatment, $P < 0.05$. Treatments with different letters indicate statistically significant differences between group means ($P < 0.05$); groups sharing the same letters are statistically equivalent ($P > 0.05$). Notice that analysis was performed with the log-transformed variables, but the results are shown with the raw variables ($N_e = 26.7$). Error bars denote the standard error.

Table 1. Summary of the model that best fit pup contact latency data.

Variable	Parameter estimated	t Value	P
Intercept	11.24	3.389	<0.001
RMR	−1.84	−2.244	<0.05
Stage	−2.52	−2.100	<0.05
RMR × stage	0.56	1.884	<0.05

Estimated parameters with their associated *P* values are shown for all variables in the model (RMR, Stage, and the interaction term between them). A mixed model with random intercept for each male was generated.

Table 2. Summary of the model that best fit pup pickup latency data.

Variable	Parameter estimated	t Value	P
Intercept	14.98	3.561	<0.001
RMR	−2.90	−2.801	<0.01
Stage	−5.64	−2.755	<0.01
CT	0.82	3.251	<0.01
T	0.69	2.647	<0.05
RMR × Stage	1.37	2.736	<0.01

Abbreviations: CT, treatment control; T, tyrosine treatment.
Estimated parameters with their associated *P* values are shown for all variables in the model (RMR, Stage, the interaction term between them, and treatment as a factor).
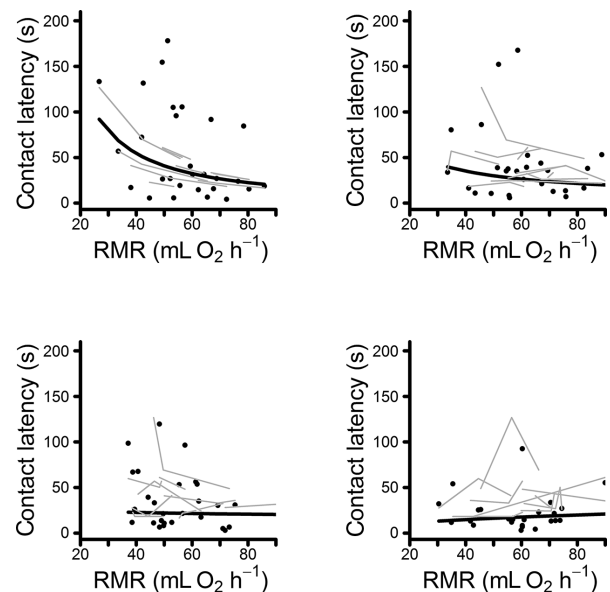A general linear model without a variance structure was generated.

Figure 3. Relationship between pup contact latency and RMR for each developmental stage. The black line represents the predicted contact latency for the whole population of males, whereas gray lines show contact latencies for each individual. Notice that the best model for contact latency is a mixed model with a random intercept for each male. Upper panels show the first (left) and second (right) developmental stages. Bottom panels show the third (left) and fourth (right) developmental stages ($N_e = 36.6$).

pickup latency, we found a treatment side effect that was opposite to the effect of metabolism, latency increased depending on treatment. All of the analyzed models and model selections for both behavioral variables are shown in the Supporting Information (S-Tables 2–5 in the Supporting Information).

As mentioned in the Methods section, when the random intercept mixed model was fitted, two sets of intercepts were estimated: one for the whole population of males (black line) and then single intercepts for each individual (gray line) (Fig. 3). All males followed the same pattern during the first developmental stage, whereas during the second to the fourth developmental stages, males did not respond consistently (Fig. 3). To summarize, for both parental care behavior variables (i.e., contact

and pickup) RMR increased parental care whereas latency behavior decreased; this was especially apparent during the first developmental stage (Figs. 3 and 4). In other words, as predicted by Koteja's parental care model for the origin of endothermy, we observed that when RMR increased due to L-tyrosine injection, parental care behavior was enhanced. However, for all other

Figure 4. Relationship between pickup latency and RMR for each developmental stage. Notice that the best model for pickup latency was a generalized linear model without a variance structure associated with male. Upper panels show the first (left) and second (right) developmental stages. Bottom panels show the third (left) and fourth (right) developmental stages ($N_e = 71.2$).
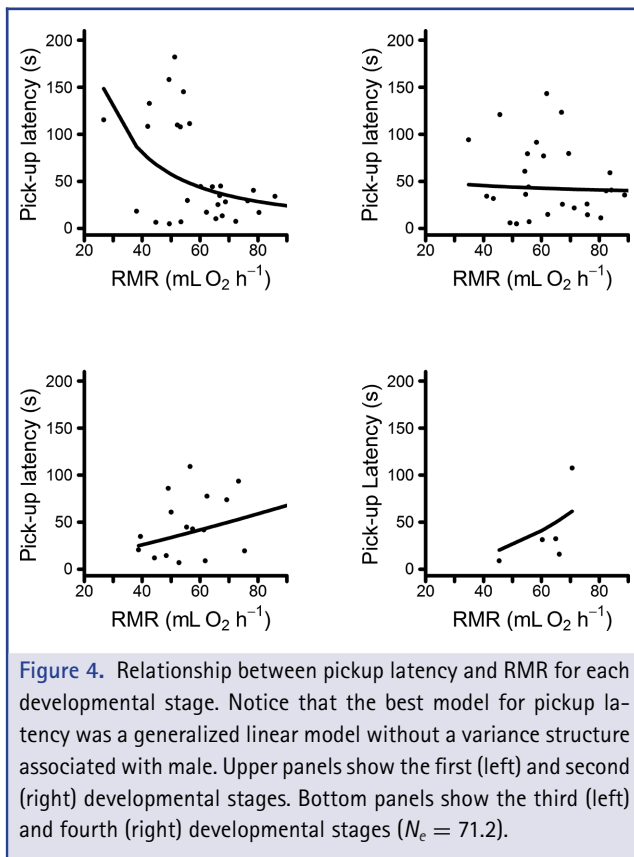
developmental stages (2-4), this trend either disappeared or was reversed (Figs. 3 and 4).

DISCUSSION

A positive relationship between the magnitude of RMR and parental care behavior was found for Campbell's dwarf hamster *P. campbelli*. Specifically, there was a decrease in latencies to pup contact and pup pickup as RMR increased (Figs. 3 and 4). Although only males were manipulated in this study, previous work with this species has shown that without male parents −which implies biparental care− pup development suffers (Wynne-Edwards, '95). Therefore, given that a positive relationship between RMR and parental care behavior was found here, the main assumption of the parental care model was experimentally tested and corroborated (Koteja, 2000). Though it must be noted that the authors do not seek to argue that this hypothesis is more plausible than others proposed to explain the evolution of endothermy, however the data presented here indicate that the parental care model is indeed a valid hypothesis.

The effect of treatment on RMR was significant only during the first developmental stage, most likely because the L-tyrosine treatment used in this study is more conservative than other treatments for increasing RMR. Furthermore, an effect of ac-

climation may be responsible for the absence of RMR increase during the last developmental stages. Additionally, males were not fasted prior to metabolic measurements, and then the heat increment of feeding (HIF) could affect RMR results. If males under tyrosine treatment systematically eat more than other treatment males and they were measured always after feeding, then the RMR increase could be overestimated. However, in a rodent species with a high food retention time (17 hr), HIF peak is below 15% of total V_{O_2} production (Nespolo et al. 2003). Then, we could assume that for a hamster species with retention food time of 9 h, trhe HIF increment could not explained the 41% of V_{O_2} increment observed in RMR results (see Fig. 2). Moreover, HIF could also increase RMR variation in all males with the consequent loss of statistical power, making difficult to find significant treatment effects in the later developmental stages.

During the first developmental stage, a decrease in male pickup latencies as RMR increased indicates that parental care is enhanced when RMR increases. Additionally, the contact latency mixed model with random intercepts for each male illustrated that all males responded equally during the first developmental stage (Fig. 3). Pup contact and pup pickup latencies increased during the third and fourth stages (Figs. 3 and 4). Nevertheless, during these stages an array of behaviors was observed because not all males responded in the same way. Also, during later stages males responded heterogeneously; this fact along with the absence of treatment effects on RMR at later developmental stages supports the hypothesis that males were acclimated to the L-tyrosine treatment.

Despite that an unconventional treatment was used to experimentally increase metabolism, our statistical analysis supports the notion that the observed behavioral changes were due to RMR increase. The model selection used here allowed for the distinction between treatment effects that were unrelated to RMR and those that were related to RMR per se. For example, the best fit for contact latency was the model that included RMR and developmental stage, and this model did not included treatment as a relevant factor explaining behavior. Furthermore, contact latency decrease was due to RMR increase in addition to the expected effect of development stage (Table 1). Although treatment and RMR are collinear factors and we could not affirm the magnitude of the RMR effect on contact latency, we can argue that RMR had some direct effect on it since when comparing models including only RMR or treatment, the RMR model showed the best fit (see S-Table 3 in the Supporting Information). For pickup latency, RMR, stage, and treatment were the variables that best explained behavioral changes (Table 2). Therefore, it was possible to observe that behavior latencies were affected by both RMR and treatment. However, RMR had a negative effect on latency; pickup latencies decreased as RMR increased. On the contrary, treatment had a positive effect on behavioral latency, showing that the males experiencing treatment side effects −unrelated to RMR− were slow to pick up their pups. In this

case, we performed post hoc comparisons for treatment levels, and we found that pickup latency for treatment (T) and control of treatment (CT) did not differ significantly ($z = -0.483$, $P = 0.629$) whereas pickup latency for control differed significantly from T and CT ($z = 2.647$, $P < 0.01$; $z = 3.251$, $P < 0.05$). This means that the observed side effect of treatment on pickup latency was due to males manipulation, making manipulated males slower than control ones. For all this, we point out that the relationship evidenced in this study between RMR and parental care is not an ambiguous or spurious correlation due to collinearity with treatment.

Regarding the mechanism responsible for enhanced male parental care due to RMR increase, our results are consistent with the parental care model proposed by Koteja (2000), who postulates that parents with a higher level of RMR may exhibit a higher DEE (and likely larger internal organs as well). Consequently, individuals involved in parental care need to increase their time allocated to foraging, thus increasing their activity level. Therefore, when noticing that the pup is out of the nest, more active parents will make contact and pick up the pup faster than less active parents. While activity level was not measured, it was evident that males under the effect of L-tyrosine were more active than control males. Unfortunately, DEE could not be measured for males because they were sharing their box with a female; further analyses are necessary to determine the specific relationship between DEE and parental care.

As mentioned above, we cannot state that the parental care model is the most plausible available explanation for the evolution of endothermy; nevertheless, we argue that this model is a valid hypothesis, as its underlying assumption was experimentally supported in the study herein presented. Further analyses are required to determine the relationship between enhanced parental care—due to RMR increase—and fitness. Hypothetically, enhanced parental care will improve offspring survival, whereas an increase in RMR will imply higher energetic costs for parents (Nagy, '87; McNab, 2002; Speakman et al., 2004). Also, greater activity levels and foraging rate may entail higher predation risk (Gilliam and Fraser, '87; Anholt and Werner, '95). Therefore, to determine the fitness consequences for the parental care model, it is necessary to consider both offspring benefits and parent costs, balancing both fitness components at the same time (Angilletta and Sears, 2003; Angilletta, 2009). In this sense, here we demonstrate the link between RMR and parental care behavior during initial pup development of *P. campbelli*. Although observations at the first stage of development might seem insufficient support for understanding what drives the evolution of the endothermy, it is seen that substantial selective pressure on pup survival allows to select expensive traits. For example, our species model has one of the shortest life cycles of mammals; nevertheless, a costly biparental care system was selected (Wynne-Edwards, '95), suggesting that increasing survival of pups may have had an effect on fitness anyway. Unfortunately,

fully understanding the relationship between the costs and benefits of endothermy and the selective pressures that might have conducted to this condition warrant more studies.

Finally, the parental care model posits that natural selection favored enhanced parental care and increased DEE with BMR increasing as a by-product (Koteja, 2000). In this study, the functional link between parental care behavior and metabolic rate is demonstrated, however, through the inverse relationship of manipulating RMR and measuring the behavioral response. Future experiments will need to test the phenotypic link between parental care and metabolism through either manipulations of parental care behavior and measurements of RMR response or by manipulating both parental care and RMR to demonstrate the universal nature of this relationship as posited by the parental care model.

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