EFFECTS OF REPRODUCTION ON MORPHOLOGY OF MALE CALIFORNIA MICE (PEROMYSCUS CALIFORNICUS)

By

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Abstract

In all mammalian species, females experience physiological and morphological changes during pregnancy and lactation. In the few mammalian species in which males provide care for their offspring, fathers, too, may undergo physiological and morphological shifts across reproduction; however, little is known about these changes. The California mouse, Peromyscus californicus, is one of the few rodents that exhibits biparental care of offspring: both males and females participate in rearing their young. Therefore, this species is a useful model for studying the effects of fatherhood on fathers. We studied two groups of male mice, fathers (N=8) and non-breeding (vasectomized) males (N=2). Fathers' parity ranged from 2 to 12 litters. Body mass and body composition of fathers and non-breeding males were determined every 4-5 days, starting 3-4 days after the birth of the fathers' pups. Data collection continued until day 12 after the birth of the next litter for fathers or until day 43 for vasectomized males. At each time point, mice were weighed and masses of fat, lean tissue, free water, and total water were determined by magnetic resonance imaging. Results will help improve our understanding of the demands placed on fathers during reproduction and how these demands might be modulated by fathers' age, parity, litter size, and sex of pups.

Acknowledgments

We thank the staff of the Spieth Vivarium and members of the Saltzman lab for maintenance of the animals. I would also like to thank Wendy Saltzman and Meng Zhao for their discussion and helpful comments on earlier drafts. This research was supported by NSF grant IOS 1256572.

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Introduction

The California mouse, *Peromyscus californicus*, is one of few mammalian species that exhibit monogamous mating and biparental care; males in only about 5-10% of mammals routinely participate in caring for their offspring (Kleiman and Malcom 1981; Woodroffe and Vincent 1994). In contrast, females in all mammalian species have an integral role in rearing young because they provide a home in their wombs for prenatal development (in most species) and provide nourishment to their offspring via post-partum lactation (Kleiman 1977). There may be direct and indirect costs associated with female parental care; providing care may directly affect energy expenditure and nutrient intake while indirectly causing females to invest less in their own physiology, leading, for example, to decreases in thermoregulation, immune function, and physical activity (Speakman 2008).

Little is known about how engaging in paternal care affects fathers' physiology directly and/or indirectly, but it is known that paternal care is important for offspring survival and development in at least some biparental species (Dudley 1974a). Paternal care is potentially energetically costly for fathers and may have evolved because it increases the odds of offspring surviving to adulthood, potentially leading to a life-history trade-off between offspring survival and the father's survival and future reproductive output (Speakman 2008). In some species of small mammals the trade-offs parents make by altering their own physiological processes are enough to balance out the increase in energetic costs, to the point where there is no net change in energy expenditure (Speakman 2008).

In many biparental mammals paternal care has been shown to cause hormonal changes in fathers that mimic those of their mates during pregnancy and the lactation period. For example, elevated prolactin levels in fathers have been found during their mate's gestation period and have been seen to increase postpartum in fathers during paternal care (Saltzman and Ziegler 2014). Other changes include high testosterone in expectant fathers, followed by a drop after parturition. These hormonal changes are not present in non-fathers and may have metabolic effects leading to morphological changes in fathers, such as fluctuation in body mass and body composition.

Male California mice (*Peromyscus californicus*) present a good model in which to study the morphological effects of paternal care because fathers of this species invest time and presumably energy to increase the survival of their litters (Cantoni and Brown 1977). During the lactation period, an energetically costly time for females, male California mice exhibit paternal behavior by helping to create and maintain the nesting area and providing warmth to the pups when they are not being tended to by their mother (Dudley 1974a; Gubernick and Teferi 2000). Fathers spend an equal amount of time nesting with the pups compared to mothers, and one parent will spend more time caring for the litter to compensate when the other is absent, which can be common in the wild when one parent has to forage for food (Dudley 1974b). In their natural environment, California mice may have to account for fluctuations in external temperature while rearing their offspring, which may energetically stress the fathers when providing paternal care during huddling. One theory is that the presence of paternal care allows mothers to forage for longer periods of time, thus increasing milk production. This in turn increases the milk supply available to pups, thereby enhancing their survival rate and increasing their rate of developmental weight gain (Dudley 1974a; Cantoni and Brown 1977). California mouse fathers have also been found to spend an equal amount of time grooming and maintaining close contact with their pups as mothers do, thus supporting the model of shared parental investment (Dudley 1974b; Gubernick and Alberts 1987).

In the case of the California mouse, it was found in a lab study that when the father was removed from litters of four or more pups and the mother was allowed to rear the litter alone while having to work to obtain food, the offspring survival rate drastically decreased. Some of the females stopped lactating after removal of their male mate, suggesting the cost associated with rearing pups alone was too high, preventing females from investing energy into lactation, an energetically expensive activity (Cantoni and Brown 1977; Gubernick and Teferi 2000). Another study found that when female California mice reared offspring without mates, their adult sons were less aggressive and less likely to win fights, behaviors that may play a role in protecting their future offspring (Becker et al. 2010). The presence of paternal care has

been shown to affect pup survival and development in various ways, but little is known about how paternal care affects fathers' body composition.

Mammalian mothers undergo many morphological changes and physical transitions, including pregnancy, body mass gain/loss, and parturition, and fathers in some biparental mammals undergo similar fluctuations in body mass. In the biparental common marmoset (*Callithrix jacchus*) and cotton-top tamarin (*Saguinus oedipus*), fathers experience weight gain during their female mate's pregnancy, especially in the weeks leading up to birth (Ziegler et al. 2006, Sanchez et al. 2008). Ziegler et al. (2006) suggest that male weight gain leading up to parturition in biparental species occurs in preparation for the energetic costs associated with rearing a litter. Biparental male prairie voles (*Microtus ochrogaster*) experience reduction in body weight and fat reserves when engaging in pair-bonding and paternal care post-partum, demonstrating a similar pattern to mothers (Campbell et al. 2009). However, Saltzman et al. (2015) found that male California mice exhibit low body mass when housed with pregnant females in comparison to males housed with tubally ligated females. In addition, two studies found that California mouse fathers saw an increase in body mass across their mate's pregnancy when housed with the previous litter's pups, whereas fathers housed with only a pregnant female did not (Harris et at. 2011; Saltzman et al. 2015). The increase in male body mass in the presence of pups supports the idea that there may be a potential energetic cost associated with paternal care, and that fathers prepare for this cost by increasing energy reserves and thus body mass.

Many of the previous studies on paternal care and its effects on fathers' morphology have used mammals that undergo postpartum ovulation, meaning the females are typically simultaneously pregnant and lactating; this makes it difficult to interpret any changes observed in fathers' body mass. Paternal experience may be another variable when analyzing changes in male body mass. Leading up the birth of their second litter, male prairie voles exhibit a lower body mass compared to the birth of their first litter, suggesting lower energy costs associated with providing care to later litters (Campbell et al. 2009); this contrasts with the previously mentioned findings of Saltzman et al. (2015) in California mice. The noted

studies all suggest that paternal care is energetically costly, but the conflicting data call for further analysis on how paternal care affects the body mass of male California mice.

Virtually nothing is known about changes in body composition (e.g., fat mass, lean mass) that underlie changes in body mass in fathers across their mate's reproductive cycle. However, Campbell et al. (2009) found that in male prairie voles leading up to the birth of their second litter, low body mass was associated with low fat mass. Therefore, we performed this study to clarify how body mass, fat mass and lean mass change in California mouse fathers across their mates' gestation and lactation period. Lean tissue mass includes anything in the body that is not fat or water, such as organs, muscle, and bones. Fat stores in mammals are useful for storing potential energy because they yield large amounts of energy during catabolism (Young 1976). Many animals that hibernate during cold periods increase their fat storage to ensure that they have enough energy to keep their body running during months without foraging. This pattern of weight gain has also been seen during times of reproductive stress and is not limited to hibernation patterns (Young 1976).

The potential energetic costs associated with paternal care and the mirrored weight gain seen in the common marmoset and cotton-top tamarin led to the development of our hypothesis: fathers will show increases in total body mass and fat mass leading up to parturition and a decrease postpartum due to paternal energy investment in rearing young. We collected data on the fat and lean tissue body composition of male California mice at 8 different time points across their female mate's reproductive cycle. We considered variables such as parity of the father, size/mass of the litter, and individual pup mass, which were not accounted for in the Saltzman et al. (2015) study. We believe litter size/mass may affect fathers' body mass because it has been reported that number of pups per litter correlates positively with body mass and fat mass in California mouse fathers (Zhao et al. 2017). Incorporating litter size and the parity/age of fathers as variables, we expect to see increasing body mass and fat mass in fathers with increased litter mass due to a possible increase in energy investment due to paternal care. We also expect to see an increase in the body mass of higher-parity fathers due to increased paternal experience.

Methods

Animals

We used male and female California mice, *Peromyscus californicus,* that were bred in our colony at University of California, Riverside (UCR) and descended from mice purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA). Mice were housed in polycarbonate cages (44 x 24 x 20 cm) with aspen shavings for bedding, cotton wool for nesting material, food (Purina 5001 Rodent Chow, LabDiet, Richmond, IN, USA), and water. Food and water were provided *ad libitum.* The colony lights were on a 14:10 light:dark cycle, with lights on at 05:00 h and lights off at 19:00 h. The room temperature and humidity were approximately 21˚C and 55%, respectively. Cages were checked daily and changed weekly, with minimal disturbance in between.

At 27-30 days after birth, prior to the birth of the next litter of pups, the young mice were ear-punched for identification and transferred from their natal cage into a group of 3-4 same-sex, age-matched, related/and or unrelated animals. At 80-137 days of age (young to mid-adulthood), virgin males and females were placed in opposite-sex pairs for breeding, with cage mates no more closely related than first cousins. Gestation in this species is approximately 32-35 days long, and conception typically occurs within 1-2 days after parturition (Gubernick 1988).

Experimental Design

Subjects were 10 male *P. californicus*, including 8 fathers and 2 non-breeding (vasectomized) controls (see below). At the beginning of data collection, fathers ranged in age from 5 to 19 months (12.3 ± 2.0 months, mean ± SE), and controls were 10.0 ± 0.0 months. Fathers had sired 1-11 litters (7.1 ± 2.5) prior to this experiment, and the control males had not sired any litters. Fathers had been paired with their female mate at 10.1 ± 1.5 months of age. Controls had been paired with a female mate at 3.5 ± 0.0 months

of age and were vasectomized 21 days after pairing. All males were housed with their mate for the entire duration of this study.

For fathers, data on body mass and body composition (see below) were collected every 4-5 days, starting 3-4 days after their female mate had given birth to a litter (Litter A). Data collection continued on this schedule until the fathers' next litter (Litter B) was born, after which data were collected at two additional time points. Therefore, the last data point for each father was 3-12 days (8.0 ± 0.8 days) after the birth of Litter B. For control males, data were similarly collected every 4-5 days for a period of 43 days.

Vasectomies

Control males were anesthetized using isoflurane, and vasectomies were performed using standard antiseptic procedure. A 1 cm ventral midline incision was made above the genital region. The vas deferens was sutured and cut, and all other reproductive structures were carefully placed back into the abdominal region. The incision was closed using dissolvable sutures (Monocryl Suture 4-0 FS-2, Ethicon, San Angelo, TX), and the outer skin layer was closed using tissue glue (Vetbond Tissue Adhesive 1469SB, St. Paul MN, USA).

For the first seven days after surgery, each control male was housed in a standard cage separated from its female mate by a steel mesh barrier, to prevent physical contact during post-operative recovery. Both food and water were provided *ad libitum* for the vasectomized males and their mates. Seven days after the vasectomies were performed, the steel mesh barriers were removed and the control males and females were reunited with no barriers to physical contact.

Body Mass and Body Composition

At each time point, the body mass of fathers and control males was recorded to the nearest 0.01 g between 09:00 and 14:00 h. Immediately after weighing, data on body composition were collected using an Echo MRI Body Composition Analyzer E26-216-M (Echo Medical Systems, Houston, TX, USA). This procedure is non-invasive; males were placed in a plastic tube and inserted into the MRI machine without sedation or anesthesia for 78-90 seconds. The procedure provides data on masses of fat, lean tissue, free water, and total water for each animal. After each scan, fathers were returned to their home cage with their mate and pups, and control males were returned to their home cages with their mate. Fathers and control males were removed from their cage for no more than 15 minutes on each day that data collection occurred.

On postnatal day (PND) 13 or 14 (the mid-lactation period) and PND 23 or 24 (late lactation), each litter of pups was weighed as a whole to the nearest 0.01 g. The sex of each pup was recorded on day 23-24, and litters were permanently removed from their natal cages on PND 27-28.

Statistical Analysis

Data were analyzed using repeated-measures analyses of covariance (ANCOVA), Pearson's correlations, and regression analysis using SPSS. Age of males, litter size, parity, and total litter mass were used as covariates where appropriate; lean masses for fathers and controls were $\log_{10}$-transformed to improve normality. For traits measured twice, such as total litter mass, the two data points were compared using a paired t-test. P values <0.05 (2-tailed) were considered significant.

Results

Fathers' Body Mass and Body Composition over Time

We used ANCOVAs with father's age as a covariate, to determine whether fathers' body mass, fat mass, lean mass, percent fat mass, and percent lean mass varied across the eight time points of data collection (see below).

Body Mass

Males' body masses did not differ among the eight time points ($F(7,42) = 1.735$, $P = 0.127$; Fig. 1). There was not a significant main effect of reproductive group ($F(1,42) = 1.121$, $P = 0.331$) or a significant interaction between time and reproductive group ($F(7,42) = 0.881$, $P = 0.529$). We also ran ANCOVAs on males' body mass for time points 1-6 (Litter A), time points 7-8 (Litter B), and time points 6-8 (connecting time points between Litter A and Litter B) and did not find any significant main effect of time, main effect of reproductive group, or time x reproductive group interaction.

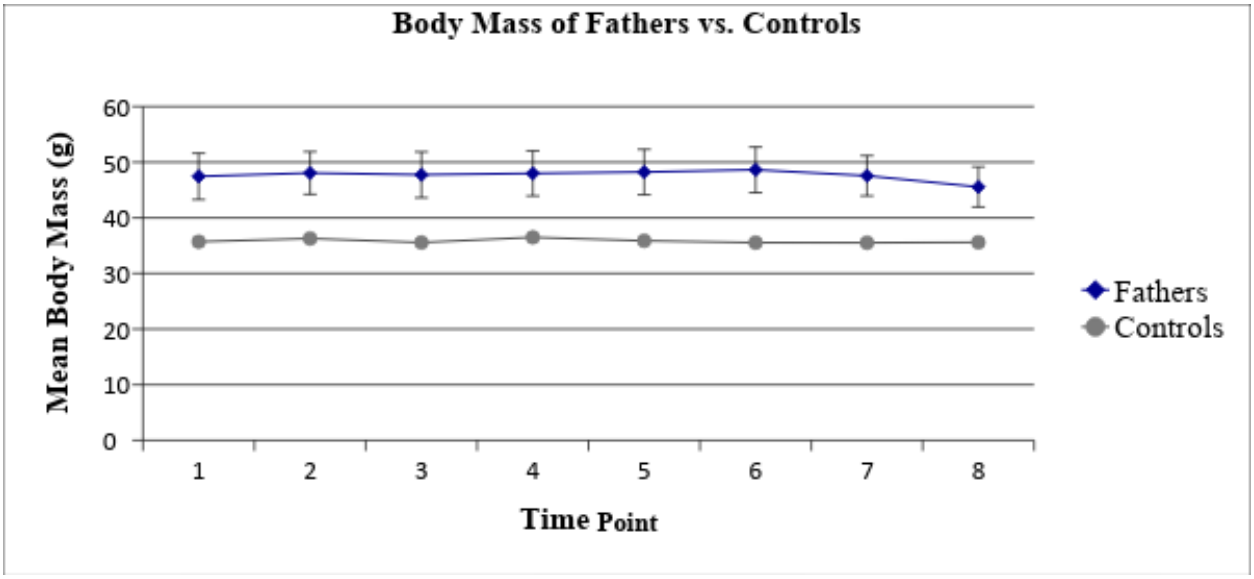

Fig. 1. Mean ± SE body mass. SEs are not shown for the Control group due to the small sample size (N=2). Each father's second litter (Litter B) was born between time points 6 and 7. Body mass did not differ significantly between groups or across time in fathers.

Fat Mass

Males' fat masses did not differ among the eight time points ($F(7,42) = 2.955$, $P= 0.103$; Fig. 2). There was not a significant main effect of reproductive group ($F(1,42) = 2.03$, $P= 0.204$) or a significant interaction between time and reproductive group ($F(7,42) =1.135$, $P = 0.036$). We also ran ANCOVAs on males' fat mass for time points 1-6 (Litter A), time points 7-8 (Litter B), and time points 6-8 and did not find any significant main effect of time, main effect of reproductive group, or time x reproductive group interaction.

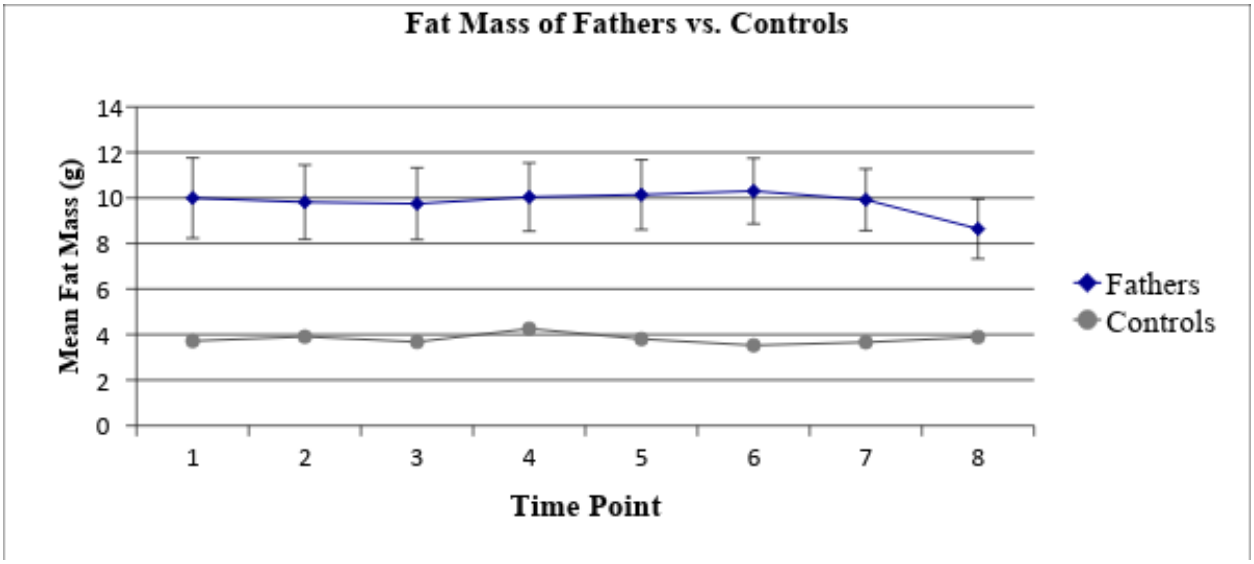

Fig. 2. Mean ± SE fat mass. SEs are not shown for the Control group due to the small sample size (N=2). Each father's second litter (Litter B) was born between time points 6 and 7. Fat mass did not differ significantly between groups or across time in fathers.

Lean Mass

Males' lean masses did not differ among the eight time points ($F(7,42) = 0.911$, $P = 0.507$; Fig. 3). There was no main effect of reproductive group ($F(1,42) = 0.517$, $P = 0.499$) or interaction between time and reproductive group ($F(7,42) = 0.854$, $P = 0.550$). We also ran ANCOVAs on males' lean mass

for time points 1-6 (Litter A), time points 7-8 (Litter B), and time points 6-8 and did not find any significant main effect of time, main effect of reproductive group, or interaction between time and reproductive group.

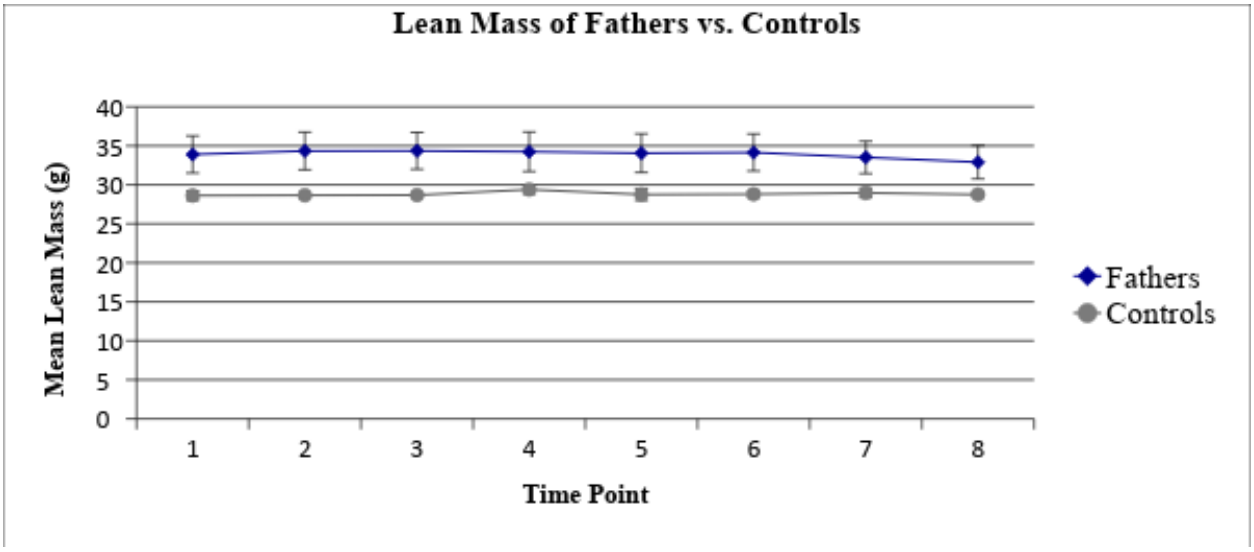

Fig. 3. Mean ± SE lean mass. SEs are not shown for the Control group due to the small sample size (N=6). Each father's second litter (Litter B) was born between time points 6 and 7. Lean mass did not differ significantly between groups or across time in fathers.

Percent Lean Mass

Males' percent lean masses did not differ among the eight time points ($F(7,42) = 1.328$, $P= 0.261$). There was not a significant main effect of reproductive group ($F(1,42) = 0.964$, $P= 0.47$) or a significant interaction between time and reproductive group ($F(7,42) = 1.985$, $P = 0.209$). We also ran ANCOVAs on males' percent lean mass for time points 1-6 (Litter A), time points 7-8 (Litter B), and time points 6-8 and did not find any significant main effect of time, main effect of reproductive group, or time x reproductive group interaction.

Percent Fat Mass

Males' percent fat masses did not differ among the eight time points ($F(7,42) = 2.084$, $P= 0.067$). There was not a significant main effect of reproductive group ($F(1,42) = 1.417$, $P= 0.279$) or a significant interaction between time and reproductive group ($F(7,42) = 0.969$, $P = 0.466$). We also ran ANCOVAs on males' percent fat mass for time points 1-6 (Litter A), time points 7-8 (Litter B), and time points 6-8 and did not find any significant main effect of time, main effect of reproductive group, or time x reproductive group interaction.

Body Mass of Fathers and Parity

We performed a regression to analyze the relationship between father's parity and father's body mass at the initial start of data collection (PND 3-9, $B = 1.082$, $P = 0.127$), and the last time point of data collection before Litter B was born (PND(27-32), $B = 1.500$, $P = 0.151$). No significant correlations were found for either time point.

Comparisons Between Fathers and Controls

Initial body mass, fat mass, and lean mass for control (vasectomized) males and fathers were compared using independent t-tests. There was no significant difference between control males and fathers for initial body mass ($t = -1.546$, $P=0.146$) or initial lean mass ($t = -0.758$, $P = 0.462$). Initial fat mass differed slightly between fathers and control males ($t = -2.429$, $P=0.03$), however, as control males had a lower initial fat mass than fathers (Fig. 4).

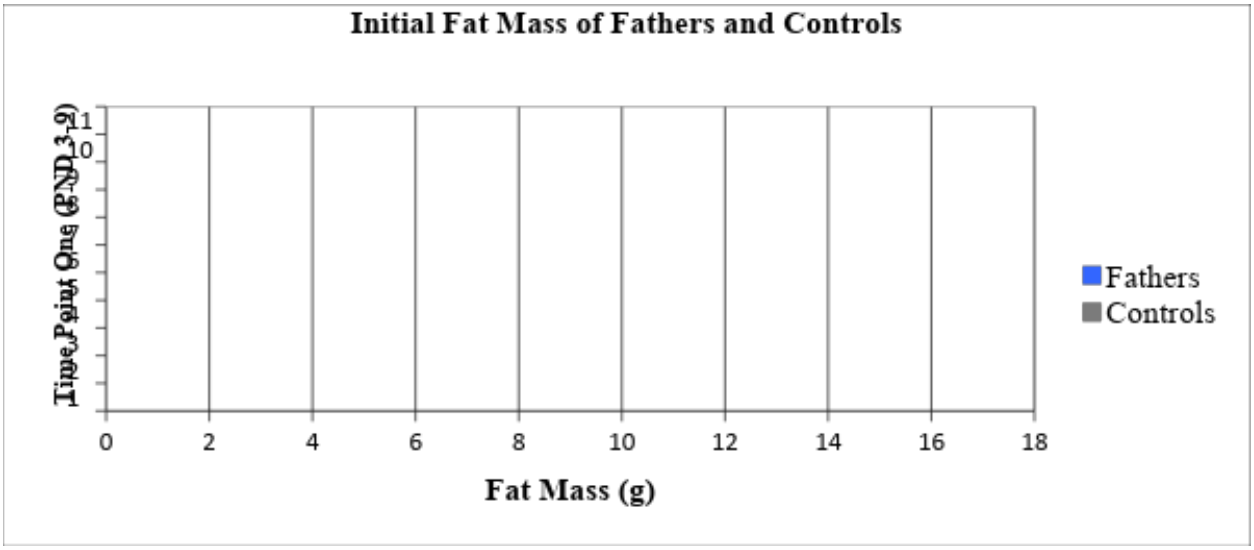

Fig. 4. Initial fat masses of individual fathers compared to those of control males, recorded on PND 3-9. An independent t-test revealed that control males had less initial fat than fathers did (t = -2.429, P = 0.03).

Total Litter Mass

Overall litter mass increased from day 13-14 to day 23-24 (t = -6.536, P = 0.000025). Regression analysis showed a significant effect of the father's parity (and/or the mother's, which was identical to the father's) on total litter mass (PND 13-14: B = 1.808, P = 0.0004; PND 23-24: B = 2.524, P = 0.0076; Fig. 5): as parity increased, the total litter mass increased as well. Total litter mass did not correlate either with the initial mass of fathers (PND 3-9) (Fig. 6) or with the father's last measured body mass before Litter B was born (PND 27-32); all values were R <0.524, P > 0.182. We also compared the total litter mass at PND 13-14 and 23-24 to the mass of the father at each of the six time points before Litter B was born and did not find any significant correlations.

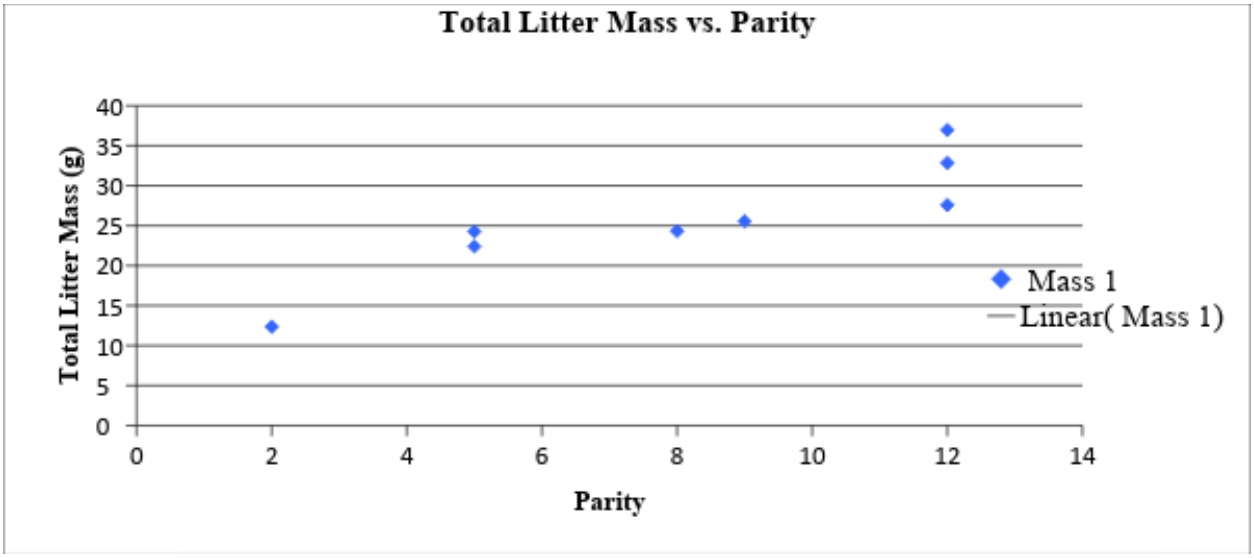

Fig. 5A

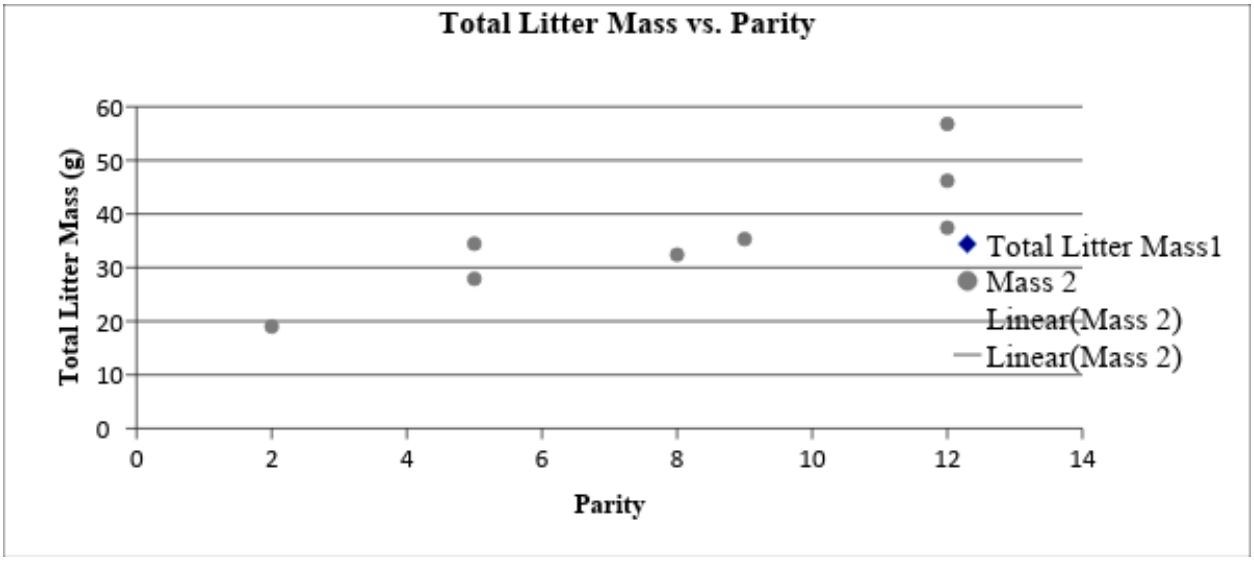

Fig. 5B

Fig. 5. Regression of total mass of Litter A, measured on postnatal day 13-14 (Mass 1, Fig. 5A) and postnatal day 23-24 (Mass 2, Fig. 5B), on father's parity. Both Mass 1 (B = 1.8078, P = 0.0004), and Mass 2 (B = 2.5241, P=0.0076) increased with increasing parity. The number of pups per litter also tended to rise with increasing parity, but the mean mass of individual pups did not (see Fig. 7 and 8).

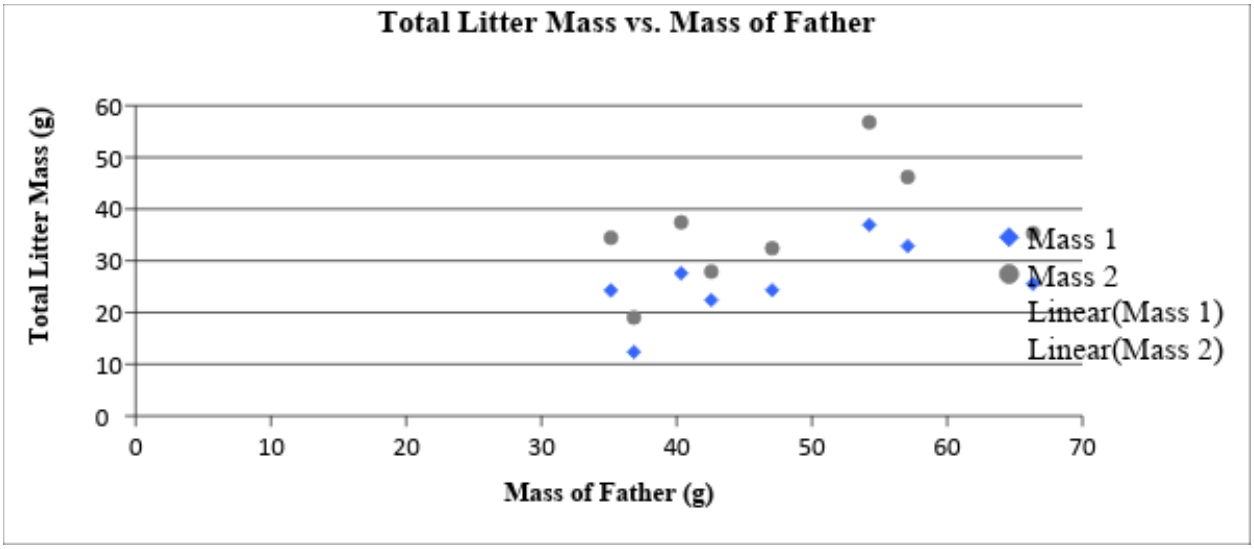

Fig. 6. Correlation between father's body mass at time point 1 (3-9 days after the birth of litter A) and total litter mass, measured on postnatal day 13-14 (Mass 1) or 23-24 (Mass 2). Neither Mass 1 ($P = 0.190$, $R^2 = 0.2976$) nor Mass 2 ($P = 0.182$, $R^2 = 0.2750$) changed significantly with increasing mass of fathers.

Mean Pup Mass

We calculated the mean mass of each pup at PND 13-14 and 23-24 by dividing total litter mass by the number of pups in the litter. Regression analyses did not find significant effects of parity on mean pup mass (PND 13-14: $B = 0.268$, $P = 0.150$; PND 23-24: $B = 0.050$, $P = 0.807$; Fig. 7). We did, however, find a positive association between parity of the father (and/or the mother, which was identical to the father's) and the number of pups born per litter (Fig. 8); however, we were unable to analyze this association statistically due to the low variation in litter sizes (1-3 pups). Finally, we compared mean pup masses with the father's initial mass (PND 3-9) and the father's last body mass before Litter B was born (PND 27-32). All values were $R<0.025$ and $P>0.853$; no significant correlations were found.

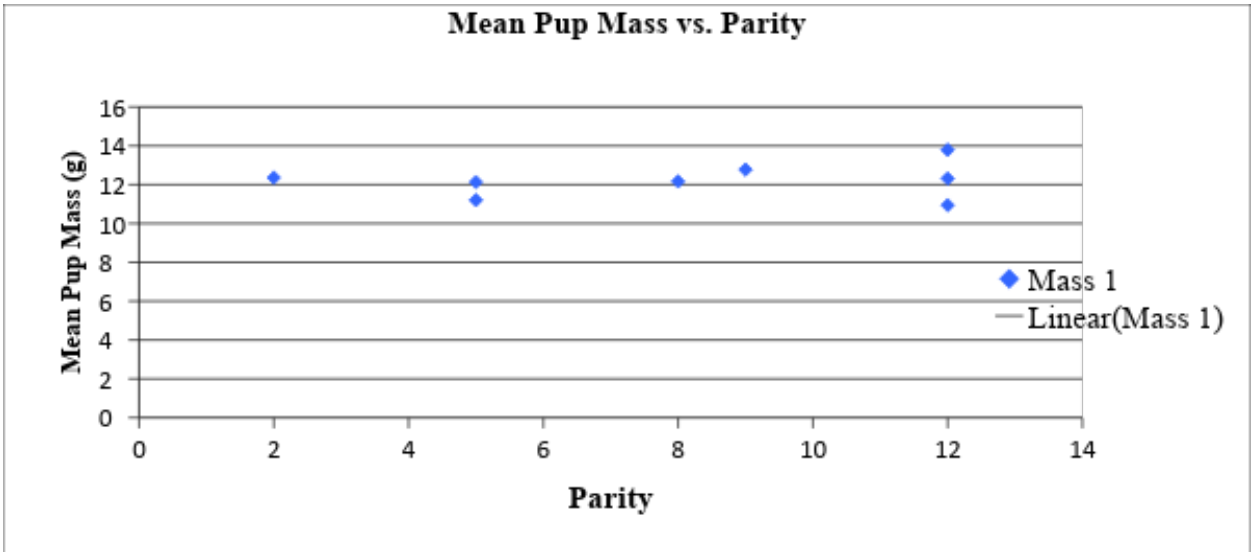

Fig. 7A

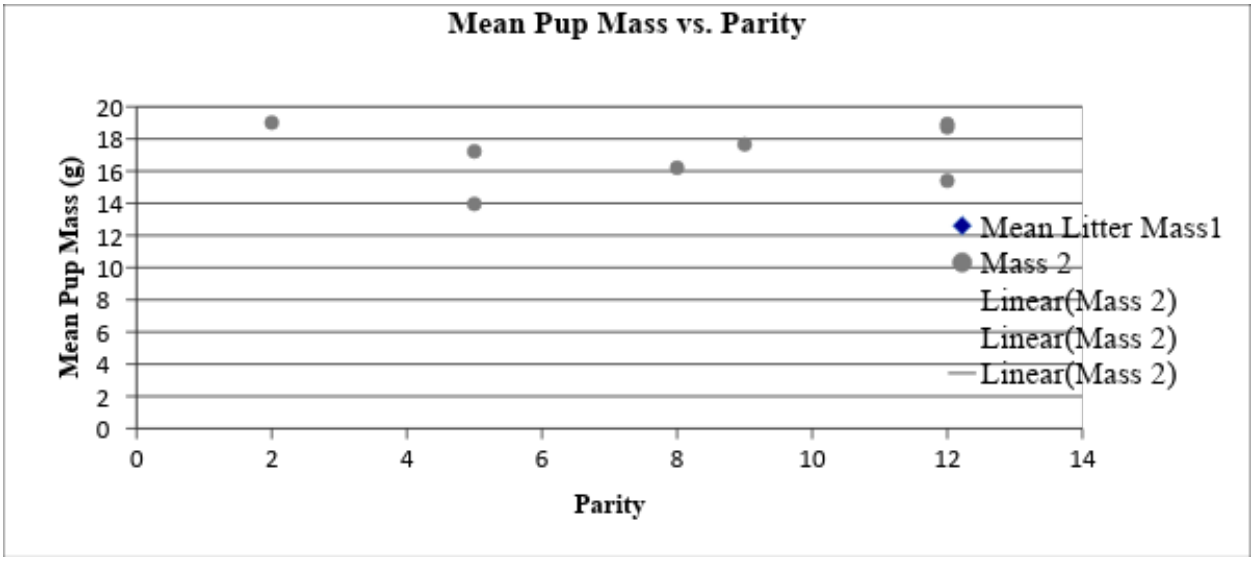

Fig. 7B

Fig. 7. Regression of mean pup mass of Litter A, measured on postnatal day 13-14 (Mass 1, Fig. 7A) and postnatal day 23-24 (Mass 2, Fig. 7B) on father's parity. Neither Mass 1 (B = 0.268, P = 0.150) nor Mass 2 (B = 0.50, P = 0.807) changed with increasing parity. Note that two fathers with parity of 12 had mean pup masses of approximately 19 g and therefore do not show up separately on the graph.

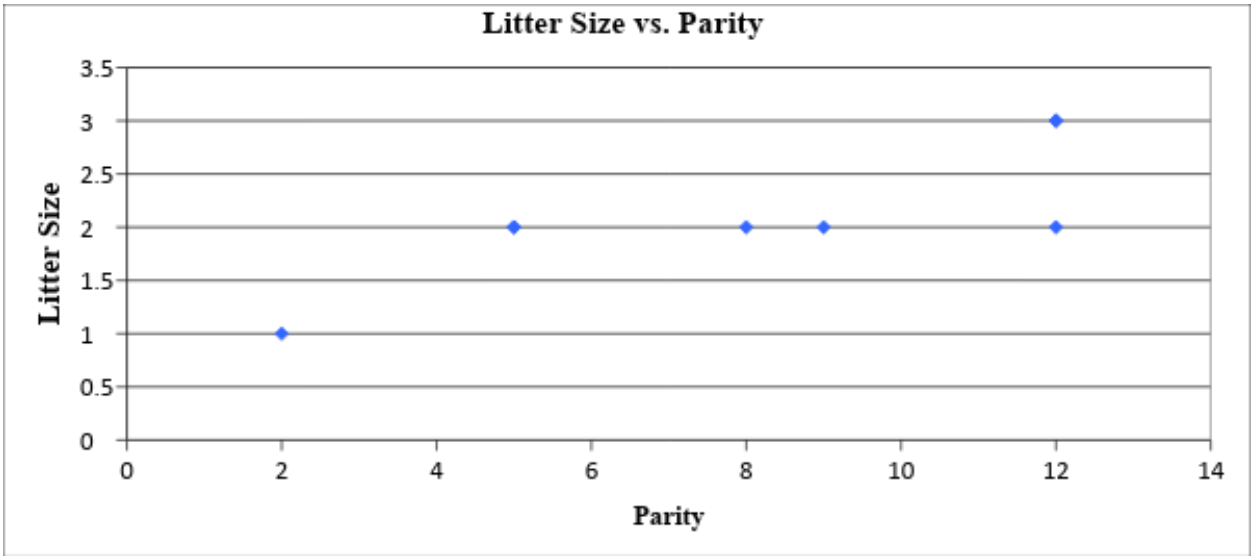

Fig. 8. The number of pups per litter (litter size) in relationship to the father's parity (and/or the mother's, which was identical to the father's). The total number of pups born tended to rise with increasing parity. Note that two fathers with parity of 5 had litters with 2 pups, and two fathers with parity of 12 had litters of 3 pups. Only one data point is discernible for each of these cases.

Discussion

In previous studies it was found that fathers in some biparental rodent and primate species show an increase in body mass leading up the birth of their offspring, and following parturition fathers experience a decrease in body mass (Ziegler et al. 2006, Sanchez et al. 2008, Campbell et al. 2009). Contrary to previous studies, we found no evidence that biparental California mouse fathers show an increase or decrease in body mass, fat mass, percent fat mass, lean mass, or percent lean mass across their female mate's reproductive timeline. However, the initial fat mass of fathers was greater than that of the vasectomized control males. Studies show that fathers undergo a series of hormonal changes that non-fathers do not, which may explain the differences we found between control non-fathers' and fathers' fat masses (Saltzman and Ziegler 2014). The differences in fat masses between control males and fathers could also be mediated by their differences in age; fathers were on average 12 months old compared to

the control males, who were 10 months old. Wolden-Hanson (2010) found that brown Norway rats show increases in total body mass and fat mass with increasing age. There was not a significant difference between fathers and controls for initial body mass or lean mass. It should be noted that we had only 2 control males and 8 fathers; therefore it is difficult to conclude the significance of our findings due to the small number of control animals.

One possible explanation for not observing a significant fluctuation in fathers' body mass across their female mate's reproductive timeline is the absence of external stressors that may be seen in their natural environment. All animals used in this study were housed in polycarbonate cages in a temperature-controlled room with *ad libitum* food and water. California mouse fathers exhibit increased acts of paternal care, such as time spent grooming pups and nesting with pups, in the absence of their female mate (Dudley 1974b). In this experiment fathers were never left alone with the pups and thus did not have to be the sole provider of parental care, which eliminated a demand they may face in the wild. In our temperature-controlled room that housed the animals, fathers were not stressed by rearing pups under extreme temperatures. In a previous study performed in our lab it was found that fathers living under challenging conditions - mesh climbing towers leading up to water and to food, which was restricted every three days - had greater fat masses and body masses than virgin males, non-breeding males, and fathers who were housed in standard cages, suggesting that stressors affect body composition in fathers (Zhao et al. 2018). Thus, we conclude that external factors from the environment of the California mouse may influence changes in fathers' body mass via increased energy expenditure during paternal care.

We investigated how parity (the total number of litters sired) may affect the body mass of fathers and found no significant effect, despite previous findings showing that the body mass of prairie vole fathers decreases with subsequent litters (Campbell et al. 2009). Harris et al. (2011) had similar findings to Campbell et al. 2009; they found that fathers' body mass spiked at the time of the weaning of their first litter and then decreased after the birth of their second litter. Our study was designed to exclude first-time fathers and only included fathers whose parity ranged between 2 and 12. Leaving out the first litter that

fathers sired could explain why our results are not consistent with Campbell et al. (2009) and Harris et al. (2011). Future studies should include first-time fathers to analyze the full morphological changes that male California mice undergo when caring for offspring.

Surprisingly, we did not find similar results to a study done recently in our lab showing that the number of pups per litter correlates positively with father's body mass and fat mass (Zhao et al. 2017). We weighed the total litter at two different time points, during mid-lactation (PND 13-14) and late-lactation period (PND 23-24). Though the total litter mass increased across the lactation period, it did not correlate with the father's body or fat mass as previously found. Likewise mean pup mass was not correlated with the father's body or fat mass. These findings suggest that the body and fat mass of fathers might not affect offspring morphological development, at least their body mass. However, paternal care is important in the development of California mouse offspring, and the absence of fathers has been linked to a slower rate of developmental weight gain (Cantoni and Brown 1997, Dudley 1974a). It could be that the body mass of fathers does not mediate any specific morphological development of pups, but that the mere presence of fathers and their paternal care is what is most important in predicting offspring body mass.

Fathers' (and/or mothers') parity had a positive effect on total litter mass. To determine the source of this effect, we also evaluated the relationship between fathers' parity and mean pup mass, but found no correlation. On the other hand, number of pups per litter tended to increase with increasing parity, although we were not able to analyze these data statistically due to the low variation in litter size. These findings suggest that an increase in the number of pups born, rather than increased mass per pup, mediated the increase in total litter mass with increasing parity. However, we are unsure whether the increase in total litter mass was mediated by an increase in father's parity, mother's parity, or the age of the mother and/or father. These factors, which were highly correlated, were not separated and independently tested, and further studies are needed to determine the underlying cause of the increase in total litter mass found in this study.

In summary, the results from this experiment did not show any significant changes in the body mass or body composition of California mouse fathers across their female mate's reproductive timeline. Our findings suggest that paternal care does not impose a significant increase in energy expenditure on fathers under standard laboratory conditions, but it is unclear how our findings relate to California mouse fathers rearing pups in their natural environment. Moreover, our study included body composition data of fathers across only two sequential litters, and the effects of paternal care on body composition may be apparent when analyzing fathers' body composition beginning at their first litter following through multiple rounds of reproduction.

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