Consequences of Fatherhood in the Biparental California Mouse (*Peromyscus californicus*): Locomotor Performance, Metabolic Rate, and Organ Masses

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ABSTRACT

Although effects of motherhood on mothers have been well documented in mammals, the effects of fatherhood on fathers are not well known. We evaluated effects of being a father on key metabolic and performance measures in the California mouse, *Peromyscus californicus*. California mice are genetically monogamous in the wild, and fathers show similar parental behavior to mothers, with the exception of lactation. To investigate the impact of fatherhood on fathers, focal males were paired with an intact female (breeding males), a tubally ligated female (nonbreeding males), or another male (virgins). Starting 3–5 d after the birth of each breeding pair's first litter, males were tested for locomotor performance (maximum sprint speed, treadmill endurance), basal metabolic rate (BMR), and maximum oxygen consumption ($\dot{V}O_2$max). At the end of the 11-d test period, mice were euthanized, hematocrit was determined, and organs were weighed. Speed, endurance, and $\dot{V}O_2$max were significantly repeatable between two replicate measurement days but did not differ among groups, nor did BMR. Breeding males had significantly larger hind limb muscles than did nonbreeding males, whereas virgin males had heavier subcutaneous fat pads than did nonbreeding and breeding males. Several correlations were observed at the level of individual variation (residuals from ANCOVA models), including positive correlations for endurance with $\dot{V}O_2$max, $\dot{V}O_2$max with testes mass, and some of the digestion-related organs with each other. These results indicate that fatherhood may not have pronounced performance, metabolic, or morphological effects on fathers, at least under standard laboratory conditions and across a single breeding cycle.

Keywords: energetics, exercise, individual variation, metabolic rate, parental behavior, paternal care, performance, rodent.

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Introduction

Life-history trade-offs have been an important theme in evolutionary biology for more than 8 decades (Fisher 1930; Williams 1966; Reznick 1992; Roff 1992; Stearns 1992). Organisms have limited resources, so to maximize Darwinian fitness, an organism must balance investment in itself with investment in its offspring (Zera and Harshman 2001). Investing too heavily in one or the other, especially under energetically challenging conditions, could result in damage to the organism itself and/or to current or future reproductive efforts. Mammalian parenthood illustrates the importance of life-history trade-offs, as the heavy cost of raising young is often at odds with the parents' own survival.

Mammalian motherhood engenders a variety of well-documented effects on morphology, physiology, and behavior, as well as substantial energetic costs (e.g., Gittleman and Thompson 1988; Hammond 1997; Speakman 2008). What we know about the energetic costs comes primarily from work on rodents and human beings. Evidence from house mice suggests that metabolic rate is higher in pregnant and lactating females than in control counterparts (e.g., Speakman and McQueenie 1996). Mothers' metabolic rate after parturition correlates significantly with litter size but not average pup mass at weaning (Hammond et al. 1994; Johnson et al. 2001). Additionally, pregnant females, when given voluntary access to running wheels, run significantly less than virgin and ovariectomized females (Zhao et al. 2013).

In contrast to females, virtually nothing is known about physiological, morphological, behavioral, or energetic effects of fatherhood in species that exhibit paternal care. Male mammals often invest heavily in efforts to increase mating success (e.g., through territoriality, courtship, or combat) but rarely invest directly in rearing their offspring; paternal care occurs in only 5%–10% of mammalian taxa (Kleiman and Malcom 1981). Fatherhood in these taxa can potentially be quite costly. Depending on the species, males may care for young while females forage (Gubernick et al. 1993; Wright and Brown 2000). Fathers may also spend much time protecting, transporting, hud-

dling, grooming, nest building, and generally caring for (maintaining health and thermoregulation) the young in monogamous species (Brown 1985; Dewsbury 1985; Lonstein and De Vries 1999). Without the father present, offspring survival can decrease significantly (Wynne-Edwards and Lisk 1989; Gubernick et al. 1993; Wright and Brown 2000).

Under field conditions, fathers are likely to have increased activity levels and therefore increased energetic costs. In several biparental species, fatherhood can affect body mass and body composition (Ziegler et al. 2006; Campbell et al. 2009). For example, in California mice (*Peromyscus californicus*), males housed with primigravid females did not gain mass over the course of their mates' pregnancy, whereas age-matched males housed with a nonbreeding (tubally ligated) female showed significant increases in mass (Saltzman et al. 2015). Moreover, relative masses of several organs (adrenal gland, thymus, spleen) differed between California mouse fathers and nonfathers (de Jong et al. 2013; see also Harris et al. 2013). In prairie voles (*Microtus ochrogaster*), reductions in fat pad mass were reported in first-time fathers compared to nonfathers (Campbell et al. 2009).

Fatherhood in biparental mammals is associated with hormonal changes that may influence energy balance. For example, testosterone concentrations decrease significantly during fatherhood (Wynne-Edwards 2001; Saltzman and Ziegler 2014; Bales and Saltzman 2015). Testosterone plays a major role in development and maintenance of many aspects of male physiology and morphology, such as potentially increasing energy utilization (Moore and Hopkins 2009), altering body composition (Blouin et al. 2008), and increasing physical activity (Ibebunjo et al. 2011). Few studies have characterized effects of fatherhood on glucocorticoid (cortisol and corticosterone) concentrations in males of biparental mammalian species; however, in several species, glucocorticoid levels increase during the mate's pregnancy and decrease back to baseline levels shortly after parturition (Wynne-Edwards 2001; Saltzman and Ziegler 2014). Glucocorticoids can increase energy mobilization (Sapolsky et al. 2000) and alter body composition (Sainsbury and Zhang 2012), in addition to having possible effects on activity levels (Malisch et al. 2007). Leptin levels have received little attention with respect to fatherhood but were significantly lower in fathers compared to unpaired male prairie voles (Campbell et al. 2009). Along with androgens and glucocorticoids, leptin is essential for energy balance. Much of the work on leptin has focused on its effects on increasing energy utilization (van Dijk 2001), but body composition can also be affected (Pelleymounter et al. 1995), as can activity levels (Girard et al. 2007; Meek et al. 2012). Other metabolically important hormones (e.g., thyroid hormones, adiponectin, progesterone, prolactin) might also influence paternal energetics (Saltzman and Ziegler 2014).

Changes in metabolically important hormones and/or in body mass might also affect locomotor performance, which in turn can be crucial for success in various behaviors (e.g., foraging, predator escape, territorial defense) under natural conditions (Careau and Garland 2012). Some aspects of locomotor performance correlate with body mass. If fathers lose a significant portion of their body mass postpartum, then locomotor performance could be affected as well. It is not known whether decreases in body mass in fathers of biparental mammals are completely accounted for by changes in body fat or whether visceral organs and/or skeletal muscle are also altered (Saltzman et al. 2015). Changes in heart, skeletal muscle, or lung mass and associated changes in cardiovascular or respiratory function might alter locomotor performance or maximal aerobic metabolic rate ($\dot{V}O_2max$). $\dot{V}O_2max$ is a strong determinant of endurance capacity (Joyner and Coyle 2008) and shows a weak positive correlation with home range size among species of mammals (Albuquerque et al. 2015), as well as a positive correlation with relative brain size (Raichlen and Gordon 2011). Given that testosterone often correlates positively with locomotor performance and can increase $\dot{V}O_2max$ (Caminiti et al. 2009), lower circulating testosterone levels in fathers might decrease performance.

Our experiment was designed to characterize the short-term effects of parenthood on performance, energetics, and morphology of fathers housed in a relatively benign environment. It was conducted in the California mouse, a genetically and socially monogamous, biparental, nocturnal rodent. This species' physiology, morphology, and behavior have been well characterized (Gubernick 1988; Trainor and Marler 2001; Chauke et al. 2011; Dlugosz et al. 2012). California mouse fathers enhance pup survival and development of their offspring in both natural and lab settings (Dudley 1974; Gubernick and Teferi 2000; Wright and Brown 2002) and engage in all of the same parental behaviors as mothers, except lactation (Gubernick and Alberts 1987). Thus, this species provides a good model for studying the physiological effects of paternal care. We characterized ecologically relevant measures of locomotor performance (maximal sprint speed, treadmill endurance, $\dot{V}O_2max$), energetics (basal metabolic rate [BMR]), morphology (body and organ masses), and an indicator of blood oxygen-carrying capacity (hematocrit) in first-time fathers during the early to mid-postpartum period, as compared with two types of nonreproductive males (housed with either a tubally ligated female or another male).

Methods

Subjects

All animals were healthy adults born and reared in our breeding colony at the University of California, Riverside. Mice were descended from animals purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia; Harris and Saltzman 2013). Animals were housed in 44 × 24 × 20-cm polycarbonate cages with aspen shavings. Food (Purina 5001 rodent diet) and water were available ad lib. Animals were kept on a 14L:10D lighting cycle (lights on at 0500 hours) with humidity at approximately 60%–70% and ambient temperature at approximately 20°–25°C. At the time of weaning (27–31 d of age), animals were ear punched for identification and housed in same-sex groups of four age-matched individuals.

After sexual maturity (age range = 83–126 d) males were placed into one of three social and reproductive conditions: paired with an intact female (breeding males; $n = 18$), paired

with a tubally ligated female (nonbreeding males; $n = 12$), and paired with an unrelated male from their original same-sex group (virgin males; $n = 14$). Nonbreeding males and virgin males were used to control for cohabitation with pups and with a female, respectively. Pairs that engaged in persistent aggression were removed from the study (one breeding and one nonbreeding). All procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* and approved by the University of California, Riverside, Institutional Animal Care and Use Committee.

Experimental Design

Animals were weighed twice per week beginning when they were paired into their respective reproductive conditions. Intact females typically gave birth 31–45 d after pairing. Breeding males were given 3–5 d after parturition before the 11-d period of data collection commenced. Nonbreeding and virgin males were tested in conjunction with matched breeding males. All animals remained housed with their respective cage mates throughout the period of data collection, except where indicated below. For logistical reasons, males were processed in five separate cohorts, and cohort was used as a cofactor in statistical analyses (see below). However, all cohorts followed an identical procedural sequence (table 1).

Tubal Ligation

Females from the nonbreeding male group were tubally ligated so that mating and ovarian hormone production would occur but pregnancy would not (Harris and Saltzman 2013). Females were anesthetized using isoflurane, oviducts were ligated, and the incision was sealed with tissue glue. Females were then housed singly for 2 wk to recover before being paired with a male.

Maximal Sprint Speed

Locomotor performance (e.g., speed and stamina) is thought to be an important determinant of an animal's survival and reproduction in the wild (e.g., in terms of foraging or escaping from predators; Careau and Garland 2012). Maximal sprint speed is a common index of locomotor performance in terrestrial animals and is measured according to well-established procedures (Djawdan and Garland 1988). Briefly, males were placed on a racetrack (8 m long × 10 cm wide and with 30-cm-high walls) equipped with 12 sets of aligned photocells at 50-cm intervals. A rough rubber floor provided traction and ease of cleaning. At the beginning of each test, the mouse was placed near the start of the racetrack and encouraged to walk or run down it two to four times to become familiar with the track. Once testing began, the mouse was returned to the starting area. The photocells were activated and calibrated with a computer. The mouse was chased down the track, which triggered the photocells. This was performed on two consecutive days, five times per day, yielding a total of 10 trials per individual, from each of which we recorded the fastest 1.0-m interval. Trials were scored subjectively as poor, fair, OK, good, or excellent (see Swallow et al. 1998); trials scored less than OK were excluded from further consideration. The highest values from each day were used for analysis of repeatability. The single highest value for each mouse (assuming it had at least one trial scored as OK or better) was used as its maximal sprint speed (Djawdan and Garland 1988).

Maximal Exercise Endurance

Maximal endurance, like maximal sprint speed, is a common measure of exercise physiology in terrestrial animals (e.g., Djawdan 1993). Mice were placed in a clear Plexiglas chamber (6.5 cm × 12.5 cm × 44 cm) with an open bottom over a treadmill set to an incline of 25° (Kemi et al. 2002). An electrical grid with mild AC current (adjustable) at the back of the treadmill provided motivation to run. Males were trained over three consecutive days for 15 min, at 10, 14, and 18 m/min, respectively (Meek et al. 2009). On the 2 d of testing, males were placed on the treadmill as per the training regimen. The speed of the treadmill was brought up to 18 m/min for 15 min. From that point, the speed of the treadmill was increased by 4 m/min every 5 min until mice were exhausted, determined as when they could no longer maintain speed and remained on the electrical grid for more than 4 s (Lerman et al. 2002; Meek et al. 2009). Values for the 2 d were used to assess repeatability, and the higher value was used for analysis of maximal performance. Any noncooperating trials (scored as less than OK) were excluded from statistical analysis.

Maximal Aerobic Metabolic Rate

Maximal oxygen consumption ($\dot{V}O_2max$) is an index of the upper limit to the intensity of work that can be maintained aerobically for prolonged periods (Dohm et al. 1994; Joyner and Coyle 2008; Dlugosz et al. 2013). It was measured in a small

Table 1: Sequence of procedures over the 11 d of data collection in male California mice

	Day								
Time	1	2	3	4	5–7	8	9	10	11
Morning	Sprint speed	Sprint speed	…	BMR	Endurance training	Endurance test	Endurance test	…	Blood sampling
Afternoon	$\dot{V}O_2max$	$\dot{V}O_2max$	…	…	…	…	…	…	Organ masses

Note. Experimental groups were pair-housed breeding males, nonbreeding males (housed with a tubally ligated female), and virgin males (housed with another male). Timing of data collection in nonbreeding males and virgin males was time matched to that in breeding males. BMR = basal metabolic rate; $\dot{V}O_2max$ = maximal aerobic metabolic rate.

running-wheel respirometer (circumference: 51.8 cm; effective volume: 900 mL; Chappell and Dlugosz 2009). This device yields $\dot{V}O_2$max that does not differ significantly from values obtained with a motorized treadmill for California mice (Dlugosz et al. 2012; see also Dlugosz et al. 2013).

Mice were forced to run a short warm-up period, followed by a ramping up of speed approximately every 30 s until either the gas concentration did not change or the mouse could not keep up. Flow rates (2,400 mL/min) and gas concentrations were measured every second using Warthog LabHelper software (http://www.warthog.ucr.edu). Measurements were taken at approximately room temperature (20°–25°C). Excurrent air was subsampled (~150 mL/min), dried with soda lime and Drierite, and sent through an oxygen analyzer. Reference air was taken at the beginning and end of every trial, and a baseline was computed by linear regression.

Oxygen consumption was calculated with Warthog LabAnalyst. Instantaneous corrections were used to account for the mixing and washout characteristics of the chamber (Bartholomew et al. 1981). $\dot{V}O_2$max (highest $\dot{V}O_2$ averaged over 1 min) was determined on each of 2 d to assess repeatability, and the higher of the two values for each animal was used for statistical analysis. No trials were scored as less than OK.

Basal Metabolic Rate

Basal metabolic rate (BMR), metabolic rate of an individual when inactive, performing no thermoregulatory work and not digesting any food, was measured as previously described (Dlugosz et al. 2012). Eight hours before testing, food was removed from the males' cages. After fasting, males were separated from their cage mates and placed in one of two small Plexiglas metabolic chambers (525 mL) with a small amount of bedding. The metabolic chambers were placed in a controlled environmental chamber set to 28°–30°C. BMR measurements were taken over an 8-h period during the males' inactive period (lights on). Subsampled excurrent air was dried (soda lime and Drierite) and sent through an oxygen analyzer as for $\dot{V}O_2$max (see above). Gas concentration, temperature, and flow rates (800 mL/min) were measured every 5 s, and 3-min reference readings were taken every 42 min. BMR was computed as the lowest $\dot{V}O_2$ averaged over 10-min intervals when $\dot{V}O_2$ was low and stable.

Organ Masses

Mice were euthanized with CO_2 for dissection according to previously used techniques (Chappell et al. 2007). The brain and all subcutaneous fat were removed and weighed. The following organs were removed, blotted dry, and weighed: ventricles of the heart, stomach, intestines, spleen, liver, kidneys, reproductive organs (including testes, epididymes, vas deferens), and right hind limb muscle (all muscles of the thigh and calf region). At a later date, stomach and intestines were thawed, cut open, cleaned of internal contents, blotted, and weighed: these values were used for analyses. In addition, reproductive organs were thawed, and the testes were weighed. Heart, liver, kidneys, and gut were weighed because they are thought to correlate with BMR (e.g., Chappell et al. 1999; Ksiazek and Konarzewski 2012); the heart and right hind limb muscle are expected to be related to locomotor performance; the spleen is linked to immune function (e.g., Ksiazek and Konarzewski 2012; Yang et al. 2013). Summed organ and/or muscle masses have also been linked with metabolism and energetics (e.g., Konarzewski and Diamond 1995; Russell and Chappell 2007).

Blood Collection and Hematocrit

Males were anesthetized with isoflurane, and blood was collected using heparinized microhematocrit capillary tubes inserted into the retro-orbital sinus (e.g., Chauke et al. 2011; Harris et al. 2011). Tubes were centrifuged at 4°C and 1,300 rpm for 12 min. Hematocrit was recorded, and plasma was removed and stored at −80°C for later use. Hematocrit is an indicator of blood oxygen-carrying capacity and is expected to correlate positively with $\dot{V}O_2$max (Kolb et al. 2010; Schuler et al. 2010).

Statistical Analysis

Repeatability of whole-animal performance measures was gauged with Pearson's correlations and paired *t*-tests. Analysis of covariance (ANCOVA) in SPSS 22.0 was used to compare traits among reproductive conditions (breeding males, nonbreeding males, and virgin males), with measurement cohort as a factor and age and body mass as covariates. Initially, we performed the overall *F*-test for group differences but eventually decided that it was somewhat more informative to compute all three a priori contrasts among the three groups. Although the three contrasts are not orthogonal (independent), each is of interest in one context or another, and as this is one of the first studies of its kind, we wanted to adopt a somewhat liberal approach to encourage further research.

Cohort and age were nuisance variables, so we do not report results for them. (Note that cohort effects may include seasonal variation as well as any unintended minor procedural differences that may have occurred among test sets.) For analysis of BMR, chamber was included as a nuisance factor. Organ masses, BMR, and $\dot{V}O_2$max were $\log_{10}$ transformed before analysis by ANCOVA, as was body mass when used as a covariate for analyses of $\log_{10}$-transformed measures. Residuals were checked for approximate normality and homogeneity among groups (reproductive conditions). We then used these residuals for analysis of correlations at the level of individual variation (e.g., Konarzewski and Diamond 1995; Chappell et al. 1999, 2007; Russell and Chappell 2007; Dlugosz et al. 2012). Within the breeding males only, we also performed regression analysis to determine whether litter size was a predictor of any trait, while controlling for age and body mass as covariates and including cohort as a factor.

Nominal statistical significance was judged at $P < 0.05$ (two tailed). In total, this study involved 204 *P* values (not including nuisance variables and replacing the single *P* values from the

Table 2: Results of ANCOVA comparing locomotor performance and metabolic rate of male California mice housed in three experimental groups

							Male reproductive condition					
							Breeding		Nonbreeding		Virgin	
Trait	F_{group}	df_{group}	P_{group}	$F_{body\ mass}$	$df_{body\ mass}$	$P_{body\ mass}$	Mean	SE	Mean	SE	Mean	SE
Maximal sprint speed (m/s)	1.83	2, 35	.176	.001	1, 36	.977	2.11	.19	1.76	.19	2.15	.16
Treadmill endurance (min)	1.45	2, 35	.248	.04	1, 36	.844	26.76	2.06	24.20	1.98	28.31	1.70
Log_{10} $\dot{V}O_2$max (mL O_2/h)	2.20	2, 33	.126	38.52	1, 34	5e-7	.754	.015	.729	.016	.714	.014
Log_{10} BMR (mL O_2/h)	.28	2, 34	.755	11.29	1, 35	.002	−.266	.023	−.251	.024	−.271	.020

Note. Values indicated for means are estimated marginal means and associated standard errors (SE). For analysis of maximal oxygen consumption ($\dot{V}O_2$max) and basal metabolic rate (BMR), body mass was log_{10} transformed. All analyses included cohort as a factor and age as a covariate. Results for cohort and age are not shown because these were considered nuisance variables. Breeding, $n = 18$; nonbreeding, $n = 12$; virgin, $n = 12–14$.

ANCOVAs reported in tables 2 and 3 with the three a priori contrasts for each trait), 30 of which had nominal P values <0.05 (see "Results" and table S1, available online). These tests include a substantial amount of nonindependence from each other, both because the traits under study are in some cases correlated and because many of the tests are interrelated (e.g., the a priori contrasts computed for all three groups). To adjust for conducting multiple related tests, we used the adaptive false discovery rate procedure as implemented in PROC MULTTEST in SAS 9.4. Based on this procedure, the 16 smallest P values would have adjusted values <0.05, the largest having a nominal P value of 0.0041. All P values reported in the text and online supplementary material are raw values, not adjusted for multiple comparisons.

Table 3: Results of ANCOVA comparing log_{10}-transformed organ masses (g) and hematocrit (%; not logged) of male California mice housed in three experimental groups

							Male reproductive condition					
							Breeding		Nonbreeding		Virgin	
Trait	F_{group}	df_{group}	P_{group}	$F_{body\ mass}$	$df_{body\ mass}$	$P_{body\ mass}$	Mean	SE	Mean	SE	Mean	SE
Brain	1.44	2, 33	.252	.00	1, 34	.983	−.060	.010	−.068	.010	−.082	.008
Stomach	.46	2, 34	.634	9.47	1, 35	.004	−.201	.017	−.213	.019	−.223	.014
Intestines	.46	2, 33	.635	14.62	1, 34	.001	.344	.032	.310	.036	.324	.026
Liver	1.17	2, 35	.321	113.31	1, 36	2e-12	.181	.015	.207	.016	.191	.013
Right hind limb muscle	3.27	2, 33	.051	98.86	1, 34	2e-11	.211	.009	.185	.011	.192	.008
Subcutaneous fat pads	5.23	2, 35	.010	16.91	1, 36	2e-4	.192	.043	.140	.044	.328	.037
Heart ventricles	.09	2, 35	.914	43.16	1, 36	1e-7	−.840	.018	−.831	.019	−.833	.016
Pancreas	.63	2, 35	.537	7.03	1, 36	.012	−1.167	.036	−1.210	.038	−1.203	.031
Mean kidney	.39	2, 34	.680	52.05	1, 35	2e-8	−.670	.023	−.654	.024	−.648	.021
Reproductive organs	.96	2, 35	.395	18.70	1, 36	1e-4	−.012	.035	.014	.036	.054	.030
Mean testis	.13	2, 35	.881	4.57	1, 36	.040	−.772	.041	−.787	.043	−.799	.036
Hematocrit	1.33	2, 32	.278	1.17	1, 32	.288	43.57	1.65	46.24	1.87	46.42	1.50

Note. Values indicated for means are estimated marginal means and associated standard errors (SE). For all organs (but not hematocrit), body mass was log transformed. All analyses included cohort as a factor and age as a covariate. Results for cohort and age are not shown because they were considered nuisance variables. Breeding, $n = 17–18$; nonbreeding, $n = 11–12$; virgin, $n = 12–14$. Reproductive organs include testes and all other male reproductive tissue except the baculum.

Results

To examine changes in body mass that occurred within individual males, we used all of the measures of body mass (about 10 per male) that were recorded between the time of pairing and the last mass recorded before parturition (mice were not weighed on the day of birth) and computed a least squares linear regression of body mass on measurement day individually for each male. We then analyzed the slope of this regression as the dependent variable in an ANCOVA that included age at pairing as a covariate and cohort as a factor. We found no statistically significant differences among the three groups, whether considering the overall F-test ($F_{2,36} = 0.84$, $P = 0.441$) or the three a priori contrasts (all $P > 0.229$). We also then analyzed the mean body mass for all values before parturition and again found no significant differences among groups ($F_{2,36} = 0.87$, $P = 0.429$) or for the three a priori contrasts (all $P > 0.202$). Finally, we analyzed the mean body mass during the measurement trials (excluding the one taken after fasting for BMR) and again found no group differences ($F_{2,36} = 0.87$, $P = 0.427$; all P values for a priori contrasts >0.194).

When the highest value recorded on each of the two measurement days (fig. 1) was used, sprint speed was repeatable between days ($r = 0.466$, $N = 35$, two-tailed $P = 0.005$) and did not differ on average between days (paired $t = -0.196$, $P = 0.846$). Maximal sprint speed (highest value ever recorded) did not differ significantly among groups and did not vary with body mass (table 2). When mass was removed from the model, maximal sprint again did not differ among reproductive conditions ($F_{2,36} = 1.87$, $P = 0.168$).

Treadmill endurance running time (fig. 1) was repeatable between days ($r = 0.692$, $N = 40$, two-tailed $P < 0.0001$) but averaged significantly lower on trial 2 (paired $t = -2.105$, $P = 0.042$). Reproductive condition did not have a significant effect on males' maximal locomotor endurance, and endurance was unrelated to body mass (table 2). Endurance also did not differ significantly among reproductive conditions when mass was removed from the model ($F_{2,36} = 1.48$, $P = 0.241$).

The measurements of maximal aerobic capacity were highly repeatable between days ($r = 0.947$, $N = 44$, two-tailed $P \ll 0.0001$) and did not differ between days (paired $t = -0.501$, $P = 0.619$). However, $\dot{V}O_2$max was correlated with body mass ($r = 0.609$ and 0.619 for the two trial days), and body mass itself was highly repeatable ($r = 0.988$). Therefore, following previous studies (e.g., Hayes and Chappell 1990; Dohm et al. 2001), we also computed residuals from linear regressions of $\dot{V}O_2$max on body mass separately for the 2 d and found that residual values (fig. 1) were still highly repeatable ($r = 0.904$, $N = 44$, two-tailed $P \ll 0.0001$).

$\dot{V}O_2$max (fig. 2; table 2) covaried positively with body mass but did not differ significantly among reproductive conditions (table 2). Similarly, BMR varied with body mass but did not differ among breeding, virgin, and nonbreeding males (table 2).

Breeding males (fig. 3) had significantly larger hind limb muscle masses (estimated marginal mean [g] for $\log_{10}$ hind

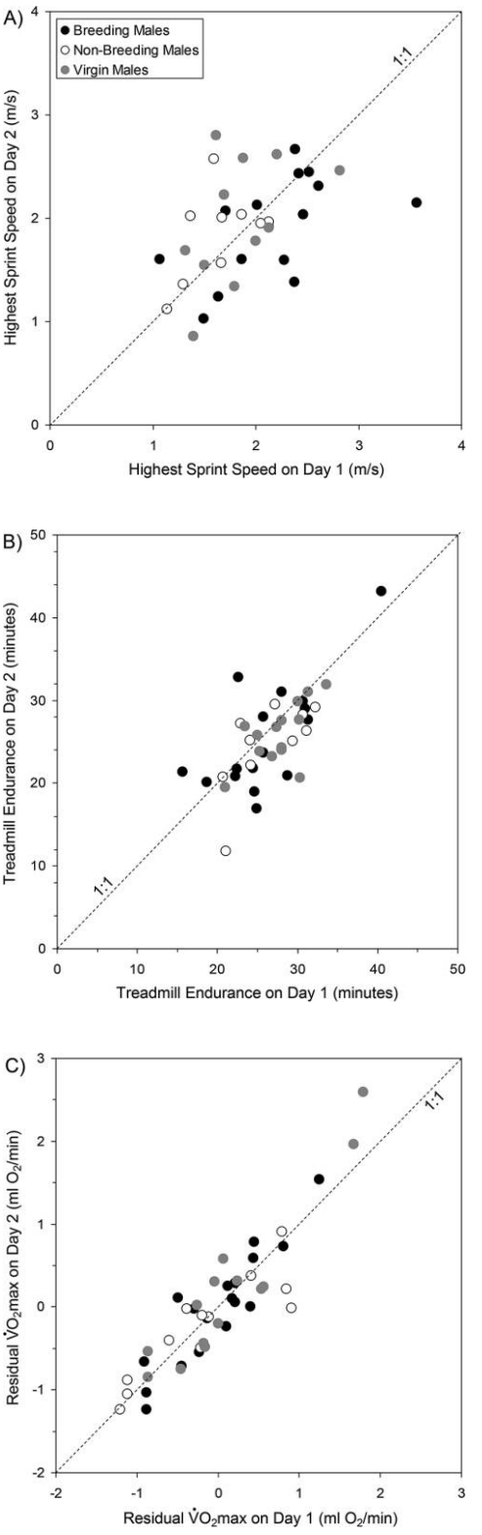

Figure 1. Statistically significant repeatability of sprint speed (A; $r = 0.466$), treadmill endurance (B; $r = 0.692$), and residual maximal oxygen consumption ($\dot{V}O_2$max; C; $r = 0.904$). See text for further statistical analyses.

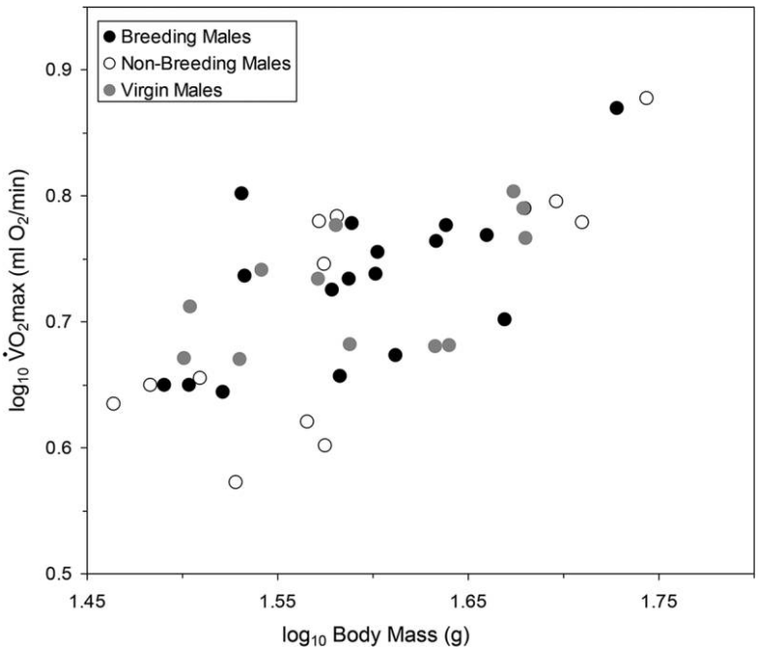

Figure 2. Log_{10}-transformed maximal oxygen consumption ($\dot{V}O_2max$) during forced exercise was strongly related to log body mass ($P < 0.001$) but did not differ statistically among groups ($P = 0.126$).

limb muscle mass = 0.211; 95% confidence interval = 0.192–0.230) than did nonbreeding males (0.185; 0.164–0.207; a priori contrast $P = 0.018$) but not significantly larger than virgin males (0.192; 0.176–0.209; $P = 0.163$). Virgin males (estimated marginal mean [g] for log_{10} fat pad mass = 0.328; 95% confidence interval = 0.252–0.403) had significantly heavier subcutaneous fat pads than nonbreeding males (0.140; 0.050–0.230; $P = 0.0028$ for a priori contrast) or fathers (0.192; 0.105–0.278; $P = 0.0254$ for a priori contrast; table 3).

No other organ masses differed among reproductive conditions when age corrected and mass corrected (table 3). Hematocrit did not differ significantly among reproductive conditions whether (table 3) or not ($F_{2,33} = 1.12$, $P = 0.337$) body mass was included as a covariate.

At the level of individual variation (residuals from the ANCOVA models presented in tables 2, 3), positive correlations were observed for sprint speed with endurance, endurance with $\dot{V}O_2max$, $\dot{V}O_2max$ with testes mass, and some of the digestion-related organs with each other (stomach with intestine, pancreas, and kidney; table S1). Considering only the breeding males, litter size (which ranged from two to four) was a significant positive predictor of stomach mass ($P = 0.019$; adjusting for body mass) but not of any other trait.

Discussion

We are interested in the general hypothesis that parenthood can influence morphological, physiological, and/or behavioral phenotypes of fathers. In this study, we tested whether fatherhood affected locomotor performance, metabolic rate, and morphology in the biparental California mouse. Contrary to our expectations, of all the performance and metabolic variables and organ masses measured, only hind limb muscle mass and subcutaneous fat pad mass (both adjusted for variation in body mass) differed statistically among males from the three groups.

Performance of various types of locomotion is essential to almost all behaviors, including components of paternal care. For that reason, we investigated sprint speed and locomotor endurance as measures of locomotor performance, as well as maximal rate of oxygen consumption ($\dot{V}O_2max$) as a primary determinant of aerobic locomotor endurance (Joyner and Coyle 2008; Dlugosz et al. 2012). For a small rodent such as *Peromyscus californicus*, sprint speed is presumably important when avoiding or escaping predators, whereas endurance may be necessary during such prolonged activities as foraging or patrolling the home range or territory (Djawdan and Garland 1988; Djawdan 1993). We expected that fathers would have reduced locomotor abilities and $\dot{V}O_2max$ compared to nonbreeding males and virgins for three reasons. First, if males devoted energetic resources toward caring for offspring, then they might have less energy available to maintain or repair muscle. Second, previous studies have shown breeding male California mice to have reduced circulating androgen levels (Trainor et al. 2003), which could adversely affect muscle performance. Third, a previous study found reduced body mass in males housed with breeding (primigravid) females as compared with nonbreeding males (Saltzman et al. 2015), suggesting that reproductive status affects

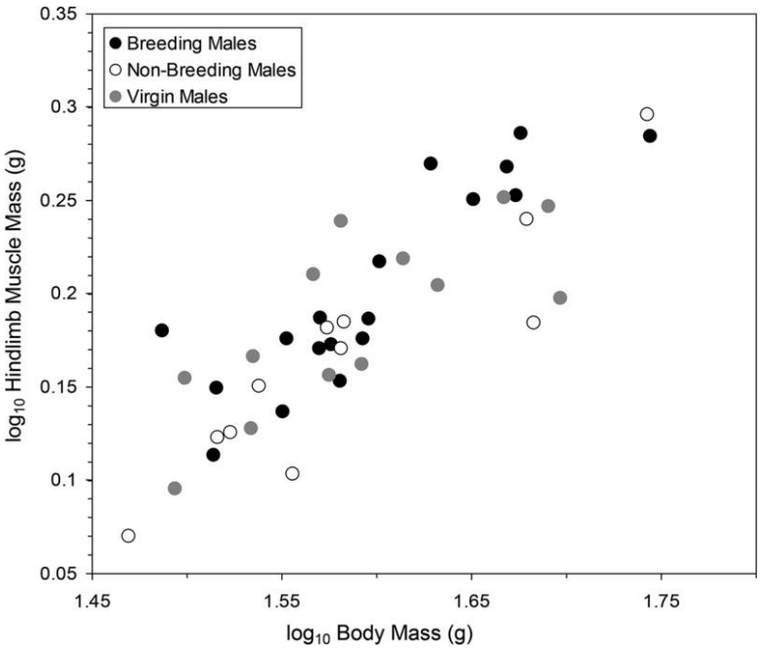

Figure 3. Log_{10}-transformed right hind limb muscle mass differed among groups ($P = 0.051$), adjusting for log body mass ($P \ll 0.001$) by ANCOVA (age and cohort were also included in the statistical model). Breeding males had significantly larger hind limb muscle masses than did nonbreeding males but not significantly larger than did virgin males (see text).

body condition in male California mice. Our predictions were not upheld by results of this study, as we found no statistically significant differences in performance among males maintained in the three reproductive conditions (table 2).

The absence of differences in speed, endurance, and $\dot{V}O_2$max in this study might be explained in several ways. First, unlike the previous study (Saltzman et al. 2015), we did not find differences among groups in body mass. Second, given how often males were tested (table 1), it is possible that stress or some sort of carryover effects overshadowed any effect fatherhood may have had on speed, endurance, or $\dot{V}O_2$max. In any case, the lack of differences in organismal performance abilities is consistent with our finding of few differences in the sizes (adjusted for body mass) of performance-relevant internal organs or hematocrit (table 3), although fathers and nonbreeding males had smaller fat pads than virgins and fathers had larger hind limb muscles than nonbreeding males (and virgins).

Basal metabolic rate reflects the sum of all processes that require energy in an endotherm under conditions of minimal energy use (e.g., resting, fasting, and thermoneutrality). Changes in body composition or in endocrine function are expected to alter BMR (White and Seymour 2005; Ksiazek and Konarzewski 2012). Although we did find group differences for subcutaneous fat pad mass (table 3; fig. 3), we did not find a parallel difference in BMR, perhaps because these fat pads are a rather small fraction of total body mass.

At the level of individual variation (residuals from the ANCOVA models shown in tables 2, 3), the strongest correlation we found was between $\dot{V}O_2$max and mass of the testes ($r = 0.461$, $P = 0.002$). Although we did not measure circulating hormone levels in this study, this result is consistent with experimental manipulations in humans showing that testosterone can increase $\dot{V}O_2$max (Caminiti et al. 2009).

Several caveats should be kept in mind when interpreting the results of this study. First, conditions of ad lib. food and water, coupled with small cages that do not require any exercise per se, may ameliorate any energetic stress related to fatherhood. Second, it is possible that the time frame of this study was not long enough to allow differences to emerge among the reproductive groups. We are currently addressing this possibility in a longitudinal study examining possible effects of fatherhood across multiple successive reproductive bouts. Third, repeated handling of animals and brief separations from their cage mates might have influenced paternal behavior and/or pup development. This seems unlikely, however, based on previous work in this species (de Jong et al. 2013; Harris et al. 2013).

In summary, we found little evidence that first-time fathers in a biparental rodent species experience important alterations in their morphology, physiology, or locomotor abilities. In other words, fatherhood does not appear to be costly in California mice, at least in terms of running performance, aerobic metabolism, and organ morphology. However, we caution that these results may not hold for fathers after caring for multiple litters or under natural conditions that are likely much more energetically (or psychologically) stressful than lab housing; possibilities are being addressed in current studies.

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