Mating behavior is controlled by acute changes in metabolic fuels

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Mating behavior is controlled by acute changes in metabolic fuels. Am J Physiol Regulatory Integrative Comp Physiol 282: R782–R790, 2002; 10.1152/ajpregu.00383.2001.—Mild food restriction for 48 h inhibits mating behavior in female musk shrews (Suncus murinus). However, mating behavior is restored after a 90-min feeding bout. In this series of experiments, we examined the role of metabolic fuels in this behavioral restoration. First, drugs reported to block glycolysis or fatty acid oxidation were given 2 h before mating. Both treatments inhibited mating in food-restricted females that were refed after treatment. Blood glucose levels were assessed in females that were fed ad libitum, food restricted, or food restricted and refed for 90 min. Food restriction significantly lowered blood glucose compared with ad libitum feeding or food restriction in combination with 90 min of refeeding. However, neither glucose nor fat alone could substitute for food and promote mating behavior in food-restricted females. In addition, analysis of ketone bodies and body composition in females demonstrated low or undetectable levels of these energy substrates. Our data suggest that musk shrews have relatively little stored energy. Therefore, female musk shrews rely on continuous food intake and monitor multiple cues acutely, including glucose availability and fatty acid oxidation. This ensures that mating does not occur when adequate energy is unavailable.

Successful reproduction requires energy, typically attained by ingesting food consisting of proteins, fats, and carbohydrates. These macromolecules are broken down into amino acids, glycerol, fats, and simple sugars. The brain almost exclusively uses glucose for energy production, while glucose and fatty acid oxidation are used in the periphery (18). In cases where glucose availability is inadequate (e.g., starvation and diabetes), ketone bodies can be used for neural energy. When food intake is limited, compensatory changes ensure that metabolic homeostasis is maintained (30). Initially, stored glycogen is broken down into glucose. With continued deprivation, the micromolecules in the liver are used to form glucose via gluconeogenesis. Next, fatty acid oxidation becomes the primary energy source in the periphery, and glucose or ketone bodies are shunted to the brain.

During metabolic challenges, energy can be conserved by inhibition of processes that are unnecessary for immediate survival. For example, restricted food availability or limitations of specific metabolic fuels inhibit sexual behavior (5, 14, 17, 20), hypothalamic gonadotropin-releasing hormone secretion (13), gonadotropin secretion (3, 4, 19), estrous cyclicity (22, 23, 28, 29), and steroidogenesis (17, 19) in a wide variety of species (reviewed in Ref. 37). This is a reversible mechanism that allows females to maximize reproductive efficacy and synchronize reproduction with nutritional resources. Although estrous cycles and ovarian steroid secretion in Syrian hamsters and rats are inhibited by food deprivation, treatments that inhibit only the oxidation of glucose block cycling but do not block lordosis (10, 27). Also, ovariectomized female Syrian hamsters treated with estradiol and progesterone require administration of glycolysis and fatty acid oxidation blockers to disrupt mating behavior (5, 14). In female rats, fatty acid oxidation cannot substitute for glycolysis, and inhibition of glycolysis blocks mating (10). This suggests that different metabolic cues control these two aspects of reproduction in different species.

In female musk shrews (Suncus murinus), ovulation is induced by mating. Food restriction to 60% of ad libitum intake for 48 h inhibits mating behavior in female shrews, even though endogenous ovarian hormone levels are not diminished (11, 33). Restoration of sexual behavior occurs rapidly after 90 min of access to unlimited food (33). Unlike other species in which hormone treatment after food restriction supports mating behavior (17), food-restricted female musk shrews will not mate, even when ovarian hormone is provided (33).

The purpose of this study was to determine which metabolic fuel(s) is essential for mating behavior. The fact that behavioral restoration after refeeding is so...
rapid in musk shrews makes them an excellent model species in which to examine primary signals from food that affect the nervous system. We blocked glycolysis or fatty acid utilization during the refeeding period to determine which of these metabolic processes is necessary for restoration of mating behavior. Females were assigned to food intake conditions and treated with 2-deoxy-d-glucose (2-DG) to inhibit glycolysis or mercaptoacetate (MA) to block fatty acid oxidation. To determine whether these drugs were selectively affecting mating behavior, we measured a nonsexual exploratory behavior, scent marking. Next, blood glucose levels were measured in females subjected to various feeding conditions as well as females treated with different amounts of glucose to determine appropriate doses for a replacement study. Then food-restricted musk shrews were provided with glucose or vegetable shavings and paper towels for bedding. The animals were maintained on a 14:10-h light-dark cycle (lights on at 0600 and 1200). Females were placed in a clean Plexiglas box (39 × 19 × 10.5 cm) with a sexually experienced male. The pair was given ≥30 min to mate. Latencies to tail wag, receive mounts, receive intromissions (placed and misplaced), and receive an ejaculation were recorded. Because female musk shrews do not have a reflexive lordosis posture, the male must mount while walking behind the moving female and is often unable to attain an intromission in which the penis enters the vagina. These types of intromissions are referred to as “misplaced,” while those including vaginal contact are “placed.” The test ended when the female received five placed intromissions or an ejaculation or at the end of the 30-min test period (whichever occurred first). All testing was conducted by an observer uninformed as to the treatment of the animals.

**Methods**

**Animals**

Sexually and experimentally naive female musk shrews (30–45 days) were used for all studies. In this species, females are nearly adult weight at weaning (day 20) and are able to reproduce beginning at 24 days of age (unpublished data); thus females used for these studies were fertile adults. All animals were born and bred in our colony at the University of Virginia. Musk shrews do not have a vaginal, ovarian, or behavioral estrous cycle (6, 26). Thus it is not necessary to monitor cycles, and sexual receptivity can occur in nonpregnant shrews whenever they have sufficient contact with males (26). Animals were housed individually in a single-sex room in solid-bottom cages (28 × 17 × 12 cm) with pine wood shavings and paper towels for bedding. The animals were maintained on a 14:10-h light-dark cycle (lights on 0600 Eastern Standard Time). The ambient temperature was 23 ± 1°C. Food (Purina Cat Chow,Ralston-Purina, St. Louis, MO) and water were available ad libitum. All experiments were conducted in accordance with University of Virginia animal care and use guidelines.

**Food Monitoring Procedure**

Individual body weight and food intake were monitored daily for 4 days. Food was weighed and given each day between 1200 and 1400 (Eastern Standard Time). Females weighing 18–24 g were randomly assigned to one of three feeding groups: the AL group continued to be fed ad libitum for the next 48 h, the FR group was restricted to 60% of average individual food intake, as established during the baseline data-collection period, for the next 48 h, and the RF group was also restricted to 60% of ad libitum food intake for 48 h but was given access to food ad libitum 90 min before behavioral testing or blood glucose measurements. Previously, we showed that refeeding for 90 min restores mating behavior in females restricted in the manner described above (33).

**Mating Behavior Test**

All mating behavior tests were conducted between 0800 and 1200. Females were placed in a clean Plexiglas box (39 × 19 × 10.5 cm) with a sexually experienced male. The pair was given ≥30 min to mate. Latencies to tail wag, receive mounts, receive intromissions (placed and misplaced), and receive an ejaculation were recorded. Because female musk shrews do not have a reflexive lordosis posture, the male must mount while walking behind the moving female and is often unable to attain an intromission in which the penis enters the vagina. These types of intromissions are referred to as “misplaced,” while those including vaginal contact are “placed.” The test ended when the female received five placed intromissions or an ejaculation or at the end of the 30-min test period (whichever occurred first). All testing was conducted by an observer uninformed as to the treatment of the animals.

**Blood Glucose Measurements**

Females were anesthetized with halothane inhalant, and blood was collected from the tail vein. Blood glucose was measured using a blood glucose monitor (Glucometer Elite XL, Bayer, Elkhart, IN) and test strips. The glucose monitor was calibrated with a standard glucose solution after every 10 measurements. The coefficient of variation was 5.1–5.7%, with a detectability range of 20–600 mg/dl. All samples were within this range.

**Body Composition Analysis**

This procedure has been modified from Leshner and Collier (16). Eight female shrews from each of the three feeding conditions (n = 24 total) were overdosed with pentobarbital sodium. Bodies were weighed, shaved, eviscerated and weighed again. Carcasses were dried in a 75–85°C oven for 2–5 days and finely homogenized. Lipid was extracted from a sample using petroleum ether, and the percentage of body fat and total grams of body fat were calculated. The lipid extraction was completed twice for each animal, and the average fat composition between the two trials was analyzed statistically.

**Ketone Body Assay**

This procedure has been previously described (16). Briefly, plasma samples from shrews were compared with a known standard curve and run in duplicate with samples from Syrian hamsters. Samples were deproteinated by sequential incubations in Ba(OH)2 and 0.575 N Zn(SO)4. They were centrifuged, and the supernatant was treated with NADH in phosphate buffer with β-dehydrogenase. They were incubated for 1 h and treated with 1 N HCl. On the next day, they were incubated with 0.3 N NaOH and then with 0.75% NAD, β-Dehydrogenase was added, and the fluorescence was read on a fluorometer.

**Effects of Glucoprivation on Mating Behavior**

**Dose response.** A dose-response study was conducted to identify a dose of 2-DG that would block glycolysis but not inhibit mating behavior in AL animals. Sexually naive female shrews (n = 57) were injected intraperitoneally with 0.09% sterile saline (0.1 mL/20 g body wt) or one of four doses of 2-DG dissolved in saline (350, 500, 700, or 1,400 mg/kg). To determine whether 2-DG treatment would affect acute food intake, the animals were placed in a clean cage with 3 g of food. The animals and food were weighed every 30 min before
behavioral testing. At 2 h after injection, the females were placed in a novel test cage with a male, and sexual behavior was recorded. The animal’s appearance and any signs of illness were recorded. Because shrews can show emesis (1, 35) if the drugs were making them very ill, this would be an obvious indicator.

**Single dose.** The lowest dose (350 mg/kg) of 2-DG tested was selected for this experiment, because this was the only dose tested that did not inhibit mating behavior. Sexually naive females (n = 59) were monitored for baseline food intake and assigned to AL, FR, and RF groups. At 0730–0830, one-half of the females in each group received an injection of 0.09% saline (0.1 ml/20 g) and the other one-half received 2-DG dissolved in saline (350 mg/kg). Females in the RF group received unlimited food for 90 min beginning 30 min after injection. At 2 h after treatment, females were tested for mating behavior.

### Effects of Lipoprivation on Mating Behavior

#### Dose response.
Sexually naive female musk shrews (n = 32) were injected intraperitoneally with 0.09% saline (0.1 ml/20 g body wt) or one of three doses of MA dissolved in saline (20, 50, or 80 mg/kg). At 2 h after the injection, females were placed in a clean test box with a sexually experienced male and tested for mating behavior. The animal’s appearance was noted, including any signs of illness. In addition, plasma ketone bodies and blood glucose were measured from food-restricted females given saline or MA.

**Single dose.** Although none of the doses tested had a significant effect on mating, there was a trend for fewer females to mate in the higher-dose groups. Thus the 20 mg/kg dose (our lowest dose) was used for this study. Our procedures were the same as those described for the 2-DG experiment, except saline and MA were given and 72 females were used.

### Effect of Metabolic Blockade on Scent-Marking Behavior

To determine whether 2-DG and MA had specific effects on sexual behavior, scent-marking tests were conducted. When female musk shrews were placed in a clean test box, they spend several minutes marking the cage with their scent glands (34). This is a robust and easily recognizable exploratory behavior indicative of the musk shrew’s response to a novel environment.

Baseline body weight and food intake data were collected (n = 24 females). We used only RF females for this experiment, because musk shrews in this group failed to show sexual behavior after receipt of metabolic inhibitors. Two days after food restriction, the animals were injected intraperitoneally with 0.09% saline (0.1 ml/20 g), 2-DG (350 mg/kg), or MA (20 mg/kg). At 30 min after injection, females were given unlimited access to food. At 2 h after drug treatment, females were placed in a clean test box of the same dimensions as the box used for mating behavior tests. Numbers of flank and neck marks and anogenital drags were recorded for 5 min.

### Effects of Fuel Manipulation and Glucose Administration on Blood Glucose

**Feeding status and blood glucose.** Sexually naive females (n = 10) were tested in all three feeding conditions. The order of feeding condition assignment was randomized. Baseline weight and food intake data were collected and initial groups were assigned (AL, FR, and RF) as described previously. All blood glucose measurements were taken between 0930 and 1100. After blood collection, females were returned to their cage and given food ad libitum. After 3 days, baseline food intake and body weight data collection resumed for 4 days, and then females were randomly assigned to a different group.

#### Glucose ingestion and blood glucose.
Baseline body weight and food intake data were collected as described above (n = 12 animals). After 4 days, nine females were food restricted to 60% of ad libitum intake. The remaining three females were fed ad libitum for the next 2 days. At 0700, 2 days after food restriction, water bottles were removed from the females’ cages. At 0900, AL females received water and FR females were given water (n = 3) or glucose dissolved in water (200 and 500 g/l, n = 3 in each group) in their water bottles. Ninety minutes after the bottles were returned, the amount of fluid consumed was quantified, and blood glucose was measured using the methods described above.

#### Glucose injection and blood glucose.
Baseline body weight and food intake data were collected as described above (n = 36 animals). After 4 days, 30 females were food restricted to 60% of ad libitum intake. The other six females were fed ad libitum for the next 48 h. After 2 days, the FR females received no injection, an intraperitoneal injection of 0.09% saline (0.1 ml/20 g), or an intraperitoneal injection of one of two doses of glucose dissolved in saline (500 or 1,400 mg/kg). Blood glucose was measured 45 or 90 min after treatment. This created six treatment groups (n = 6 per group).

### Effects of Glucose Administration on Mating Behavior

#### Glucose ingestion.
After baseline body weight and food intake data were collected, females (n = 24) were assigned to the AL (n = 8) or the FR (n = 16) feeding condition. To ensure consumption of adequate glucose solution on the day of testing, water bottles were removed from all cages at 0700. After 90 min, water bottles were returned: AL females were given water to drink, while FR females were given water or glucose dissolved in water (200 g/l). After an additional 90 min, females were tested for mating behavior. Females did not require prior experience with glucose solution; during pilot studies, we noted that all females drink this novel solution during a 90-min period.

#### Glucose injection.
After baseline body weight and food intake data were collected, females (n = 40) were assigned to the AL (n = 10) or FR (n = 30) feeding condition. The AL females were given an intraperitoneal injection of saline. The FR females were given 0.09% saline (0.1 ml/20 g) or one of two doses of glucose dissolved in saline (500 or 1,400 mg/kg). Ninety minutes after treatment, females were tested for mating behavior.

### Effects of Fat Administration on Mating Behavior

Females (n = 16) were given vegetable shortening for 48 h to familiarize them with this novel diet. During pilot studies, we found that musk shrews, on first exposure to vegetable shortening, would ingest little or no fat over a 90-min period. However, when fat was their only food source, females ingested amounts of shortening that had caloric values greater than or equal to calories provided in cat chow. When the animals were returned to a cat chow diet, baseline body weight and food intake data were collected as described above. Females were assigned to the AL (n = 4) or the FR (n = 12) feeding condition. On the day of testing, eight FR females were given vegetable shortening before testing. After 90 min of treatment, females were tested for mating behavior.
Statistical Analysis

The latencies to perform various mating behaviors were compared using a two-way ANOVA. Factors were feeding condition and drug treatment. Student-Newman-Keuls test was used for post hoc comparisons where appropriate. The percentage of females that mated was analyzed using a $3 \times 2$ $\chi^2$ test or a Fisher’s exact test. Blood glucose concentrations were compared using repeated-measures ANOVA. The frequency of scent-marking behaviors, blood glucose concentrations from FR females treated with saline or glucose, caloric value and grams of food consumed during the refeeding period, and body composition data were compared using a one-way ANOVA. Data were considered significantly different if $P < 0.05$.

RESULTS

Glucose Is Necessary for Mating Behavior

When 2-DG (350 mg/kg) was administered before refeeding, mating behavior was blocked (Fig. 1). Mating occurred in significantly fewer RF females treated with 2-DG than RF females treated with saline or AL females receiving saline or 2-DG ($P < 0.05$). Feeding condition produced significant differences in the latencies to rump present, receive mounts, and receive placed intromissions, with the FR females taking significantly longer than the AL or RF females [$F(2, 55) = 9.97, 5.07, 3.15$, respectively, $P < 0.05$]. When analysis was restricted to females that displayed sexual behavior, no differences in latencies to perform these behaviors were noted. In addition, the dose-response data show that 2-DG at 350 mg/kg had no effect on the number of AL females that display mating behavior (Fig. 2). Interestingly, the two highest doses of 2-DG used for the dose-response study significantly impaired mating behavior in shrews fed ad libitum ($P < 0.05$) and promoted emesis. 2-DG at 700 mg/kg caused vomiting in 28.6% ($n = 4$) of treated females, and 60% of females ($n = 6$) treated with 2-DG at 1,400 mg/kg displayed emesis. None of the doses of 2-DG had a significant effect on food intake.

Fatty Acid Oxidation Is Necessary for Mating Behavior

When MA was administered before refeeding, mating behavior was inhibited (Fig. 3). Mating occurred in significantly fewer RF females treated with MA than in RF females treated with saline and AL females treated with MA ($P < 0.05$). Feeding condition affected the latencies to rump present, receive mounts, receive placed intromissions, and receive an ejaculation [$F(2, 63) = 10.26, 3.29, 3.45, 4.45,$ and 3.49, respectively, $P < 0.05$]. Post hoc comparisons revealed significantly longer latencies to perform these behaviors in FR females than in AL females, and the RF females were not significantly different from either group. When data analysis was limited to females that mated, the FR and RF females had longer latencies to rump present than AL females [$F(2, 35) = 5.39, P < 0.01$], but there were no differences in latencies to engage in any other behaviors. There was no effect of drug treatment on latencies to perform any behaviors. In the dose-response study, there were no differences among the groups in the numbers of females that mated, but the two higher doses (50 and 80 mg/kg) reduced the number of females that mated to 50%. In contrast to the 2-DG study, none of the treated females vomited. Interestingly, none of the shrews had measurable concentrations of plasma ketone bodies. Ketone concentration was <0.1 mM in musk shrews; in food-restricted Syrian hamsters run in the same assay, the concentration of ketone bodies averaged 3.2 mM. However, this dose of MA significantly elevated blood glucose concentrations compared with saline-treated FR controls: 85.3 ± 10 and 60.8 ± 6 mg/dl, respectively.

There were no differences in scent-marking behavior among animals treated with saline, MA, or 2-DG [$F(2, 15) = 0.308, P = 0.74$; total marks]. In addition, the numbers of scent marks recorded in this study are similar to those found in ad libitum-fed musk shrews (34; unpublished data).

Food Restriction Lowers and Refeeding or Glucose Administration Elevates Blood Glucose

Blood glucose concentrations were significantly lower in FR females than in AL and RF females [$F(2, 18) = 7.27, P < 0.01$]. Glucose ingestion by FR females significantly elevated blood glucose to that of AL females (87.0 ± 10.0, 67.0 ± 1.0, 81.0 ± 1.0, and 93.0 ± 5.0 mg/dl in AL, FR, and FR + 200 g/l glucose and FR + 500 g/l glucose, respectively, $P < 0.05$). Glucose treatment via injection elevated blood glucose levels in FR females to within the range noted in AL females.
(84.7 ± 3.4 mg/dl for low dose and 131.4 ± 14.3 and 70.8 ± 4.4 mg/dl for high dose at 45 and 90 min, respectively, in FR compared with 90.4 ± 7.5 mg/dl in AL). Blood glucose concentrations were significantly different in FR females treated with saline or 500 or 1,400 mg/kg of glucose \([F(5, 31) = 7.17, P < 0.0001]\). Post hoc comparisons revealed that this difference was due to significantly higher blood glucose concentrations in the females treated with 1,400 mg/kg of glucose and tested 45 min after treatment. A trend was noted for lower blood glucose in untreated FR females and females given 1,400 mg/kg of glucose measured after 90 min than in AL saline-treated controls \((P = 0.09\) and 0.07, respectively). It is possible that this trend is due to “stress”-related hormone release associated with injections. Unfortunately, we have no way to test this hypothesis.

### Glucose or Fat Replacement Alone Is Insufficient to Promote Mating Behavior

Glucose administration to FR females did not restore mating behavior, regardless of route of administration \((P < 0.05\) compared with AL females). When glucose was injected, 75% of AL females mated compared with ≤33.3% of FR females, regardless of treatment \((P < 0.05)\). In addition, because the 1,400 mg/kg dose of glucose raised blood glucose to AL levels after 45 min, five females treated with this dose were given a mating behavior test 45 min after treatment, but none of them mated. When females were given glucose to drink, the same effect was noted. Almost all AL females mated \((87.5\%)\) compared with ≤37.5% of FR females, regardless of glucose treatment \((P < 0.05)\). Similarly, ingestion of vegetable shortening did not reinstate mating behavior in FR females. All AL females mated, and significantly fewer FR musk shrews were receptive \((≤37.5\%),\) regardless of fat ingestion \((P < 0.05)\). It is important to note that the caloric intake from cat chow, glucose, and vegetable shortening was the same for the 90-min refeeding period (Table 1).

### Food Restriction Decreases Body Fat

Body composition analysis revealed that AL female musk shrews have an average body fat composition of 14.9%. Food restriction for 48 h significantly decreased the percentage of body fat to 9.1%. The AL group had a significantly higher fat content and percentage of body fat than FR and RF females (Fig. 4; \(P < 0.05\)). There were no significant differences in the other aspects of body composition (percent water or percent free-fat dry weight) among the feeding conditions \((P > 0.05)\).
mating behavior. Inhibition of glucose or free fatty acid oxidation alone blocked the restoration of mating that normally occurs with refeeding. At these doses of metabolic inhibitors, increased glucose oxidation cannot compensate for inhibition of free fatty acid oxidation and vice versa. These effects do not appear to be due to general malaise, as indicated by normal results on scent-marking tests and no obvious signs of illness. Food restriction significantly decreased circulating glucose concentrations, and acute 2-DG treatment blocked mating behavior in shrews fed ad libitum. Thus normal glucose utilization and blood glucose concentrations appear to be required for mating. Yet glucose treatments that elevated blood glucose in FR females to the level of AL females failed to restore mating behavior. This implies that while glucose is necessary, it alone is insufficient to counteract the effects of food restriction on reproduction in this species. Similarly, mating was inhibited by MA in refeed females, yet ingestion of pure vegetable shortening failed to elicit mating behavior. However, the caloric content of the vegetable shortening consumed was significantly less than the calories available in cat chow; thus it is possible that more calories from fat are required to influence mating. Another explanation for these data is that the mechanisms that control mating behavior in musk shrews are sensitive to separate signals generated from glucose and free fatty acid oxidation. Alternatively, behavior in musk shrews might depend on total energy availability, but metabolic demands could be so large that oxidation of glucose and free fatty acids is required to supply energy. Finally, individual metabolic fuels may fail to restore behavior because of deficiencies in essential amino acids and/or vitamins.

Much of the research on metabolic fuel and mating behavior interactions has been conducted in Syrian hamsters; thus it is useful to compare our data with current knowledge. In contrast to shrews, female Syrian hamsters must be fasted for 48 h to affect mating behavior. Even with this level of metabolic challenge, mating is not abolished; rather, lordosis duration is decreased (5). The ability of a mating interaction with reduced lordosis duration to produce a viable pregnancy has not been tested; therefore, the duration of lordosis in food-deprived Syrian hamsters might be sufficient for successful reproduction. Female musk shrews, on the other hand, when restricted to 60% of their ad libitum intake for 48 h, show severe reductions in the display of mating behavior. In ovarioctomized, steroid-primed Syrian hamsters, glycolysis and fatty acid oxidation must be simultaneously inhibited for 24–48 h to disrupt lordosis behavior (5, 14). In musk shrews, acute blockade of glycolysis or fatty acid oxidation alone inhibits mating behavior. Finally, the doses of metabolic inhibitors necessary to inhibit lordosis duration in Syrian hamsters are 750 mg/kg of 2-DG in combination with 25 mg/kg of methyl palmitate (14). In female musk shrews, much lower doses are sufficient. A single injection of 350 mg/kg of 2-DG blocks reinstatement of behavior after refeeding, and a single injection of 700 mg/kg significantly inhibits behavior in AL females. Taken together, these data suggest that musk shrews are more sensitive to acute availability of metabolic fuels than are Syrian hamsters.

Another major difference between Syrian hamsters and musk shrews is the timing of the effects of food limitation on behavior. For example, in Syrian hamsters, food deprivation or complete metabolic fuel blockade for 24–48 h decreases lordosis duration, and refeeding for ≥6 h is necessary to reverse this effect (5, 14). In musk shrews, however, the effects of 48 h of food restriction are reversed after only 90 min of refeeding (33). In addition, a single injection of a low dose of 2-DG or MA blocks mating behavior in FR shrews, and at higher doses, a single injection of 2-DG inhibits mating in AL shrews. This suggests that, in shrews, moment-to-moment oxidation of glucose and free fatty acids generates a signal that controls reproduction. One possible reason for this difference could be that adult female Syrian hamsters weigh 110–130 g and have as much as 40% body fat when fed their normal chow diet.
ad libitum, and thus reproduction in Syrian hamsters can be supported during fasting by mobilization of free fatty acids from this source (37). In addition, Syrian hamsters typically hoard food in their underground burrows, which provides an extra source of energy. It is possible that Syrian hamsters are relatively insensitive to acute limitations on food, because they typically have more stored energy available in fat as well as additional food in their hoard. Adult female musk shrews, in contrast, weigh 20–25 g, have <15% body fat when fed an ad libitum chow diet, and do not hoard food. It appears that musk shrews do not have significant fat stores that can act as a buffer from acute changes in available nutrition. In addition, these stores are rapidly depleted by only a mild food restriction. Musk shrews also lack ketone bodies, which are used in other species as an alternative substrate for ATP synthesis in times of low food availability. Instead, they appear to rely on metabolic cues that are available closer to the time that mating actually takes place. Thus it is instructive to classify Syrian hamsters as “disassociative” maters, since they can uncouple mating behavior from such immediate fluctuations in metabolic fuel availability. Musk shrews, in contrast, are “associative,” since acute metabolic fuel availability and mating behavior are tightly coupled.

The mechanism by which food limitation causes behavioral deficits is likely different between rodents and musk shrews. For example, food deprivation and metabolic fuel blockade in rodents decrease circulating estradiol levels (29), decrease sensitivity to exogenous steroid hormones (5, 20), and decrease the number of estrogen receptor-immunoreactive (ER-ir) neurons in the ventromedial nucleus of the hypothalamus (VMN), which is an area of the brain associated with female mating behavior in rodents (17, 20). In contrast, 48 h of food restriction in shrews has no detectable effect on circulating testosterone or cortisol levels (33) or the number of ER-ir or androgen receptor-immunoreactive cells in any area of the brain examined, including the medial preoptic area and the VMN (32; unpublished observations). In addition, administration of a supraphysiological dose of testosterone, the major steroid required for mating in female musk shrews (25), is unable to restore mating in food-restricted females (33). It is possible that, in rodents, the time course necessary to induce changes in the number of ER-ir cells in the VMN is such that behavior is unaffected by acute changes in metabolic fuel availability but is inhibited by manipulations that are severe enough to alter production of estrogen receptors in estrogen-sensitive neurons associated with sexual behavior. Musk shrews, on the other hand, respond rapidly to acute changes in food availability (33) and to individual metabolic fuels. This suggests that shrews sense changes in metabolic fuels and alter their behavior before detectable changes in plasma steroid concentrations or numbers of immunoreactive steroid-responsive cells as opposed to altering behavior only after food restriction initiates a series of downstream physiological changes.

Another novel finding is that female musk shrews rely on acute fatty acid oxidation for successful mating. In addition, musk shrews oxidize fatty acids in their liver to a greater extent than most other mammals of similar size, and they use fatty acids as the primary source of energy for the kidney (21). To our knowledge, this is the first study to examine the relationship between fatty acid oxidation and mating behavior in this species. These data show that, in musk shrews, signals generated by a decrease in fatty acid oxidation alone inhibit reproduction, unlike other species that have been studied to date (30). These findings might be related to the fact that wild and laboratory shrews depend on fat for a larger portion of their caloric intake. In fact, Syrian hamsters forced to switch from utilization of glucose to free fatty acid oxidation experience suppression of estrous cycles in response to lipoprivic drug treatments (28). Musk shrews normally feed on insects; the average insect has 22–45% fat (38). Therefore, in the wild, we speculate that shrews probably attain much of their usable energy by oxidizing free fatty acids. In contrast, only 3–5% of the insect body is composed of sugars. Thus it is unlikely that appreciable amounts of sugar would be obtained from an insect diet (38). Musk shrews in our colony eat cat chow, which has a composition of 31.5% protein, 11.0% fat, 4.5% fiber, and 12% moisture. Rodent chow is made up of 23% protein, 6.5% fat, 4.0% fiber, and 8.0% ash. These two diets differ significantly in the amount of protein and fat. In addition, the rodent and cat chow diets contain 55.5 and 41% carbohydrate, respectively.

There is precedent for the idea that the importance of a metabolic signal depends on the degree to which the organism relies on these fuels. Friedman and colleagues (9) first demonstrated that increased food intake induced by inhibition of free fatty acid oxidation is greater in rats that have been preadapting to a high-fat diet. Similarly, hyperphagia in diabetic rats is normalized by feeding these rats a high-fat diet. Presumably, diabetic rats are more dependent on fuel fats and, thus, do not overeat when fed a high-fat diet (24). The relationship between the dependence on fuels and the strength of the physiological signal has now been demonstrated for food intake in rats and for reproduction in Syrian hamsters and musk shrews.

The female musk shrew responsivity to acute metabolic cues may reflect its evolutionary history and ecology. In the wild, musk shrews are not likely to have ad libitum access to food. In fact, food availability and fat stores are probably similar in wild musk shrews and in our food-restricted musk shrews (<10% body fat). Given that our food restriction paradigm led to a 33% reduction in body fat within 2 days, it is likely that if musk shrews have some stored energy, it is rapidly metabolized when food availability is limited. In wild musk shrews, pregnancy rates are correlated with rainfall (2, 12). The pattern of these data suggests that rainfall promotes insect populations, which leads to greater reproductive success (2). We speculate that, even in the wild,
female musk shrews rely heavily on food availability to support successful reproduction.

**Perspectives**

These findings are important, because they detail the relationship between metabolic fuels and reproduction in a new animal model, which has several specializations making it highly attractive for this work. Musk shrews are found in subtropical and tropical regions and have a less-pronounced breeding season (2, 12) than rodents that reside in the Temperate Zone, which are often used for this work. We would argue that they rely more on food availability, because seasonal cues such as changes in photoperiod do not vary to the extremes experienced by mammals that reside in the Temperate Zone. In addition, these animals may lack a body fat “buffer” and, as a consequence, are exquisitely sensitive to alterations in food availability. Thus these mammals may be opportunistic in their mating behavior, copulating when food supplies are plentiful and not mating when food reserves drop. This opportunism is also reflected in their lack of an estrous cycle and ability to mate within minutes any time they contact a male (26). These features provide new insights into the relationship between energy and reproduction.

Reproductive efficacy is a concern for humans and the agricultural industry. The relationship between food availability and fertility has been well established in humans (7, 31) and in a few animal models (reviewed in Ref. 8), but the contribution of specific metabolic fuels to this phenomenon remains unknown. By understanding the role of oxidizable metabolic fuels in reproduction, we will be able to improve reproductive function in women and animals experiencing infertility due to undernutrition.

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