Reports

Availability of Metabolic Fuels Controls Estrous Cyclicity of Syrian Hamsters

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Food deprivation and weight loss inhibit ovulation and estrous behavior in Syrian hamsters. In the present experiments, lean hamsters were more susceptible to starvation-induced anestrus than fat hamsters. However, anestrus was not caused by changes in any dimension of body size per se, but instead by the availability of metabolic fuels. Simultaneous pharmacological blockade of both fatty acid oxidation and glycolysis inhibited reproduction, but, as long as one of these metabolic pathways could be used, estrous cycles continued. Thus, reproduction in female Syrian hamsters is sensitive to the general availability of oxidizable metabolic fuels.

A PROPOSED LINK BETWEEN FERTILITY and body weight has gained wide attention, largely because of reports of infertility in professional athletes, dancers, patients with eating disorders such as anorexia nervosa, and even recreational joggers and dieters who experience only moderate weight loss (*1*). Likewise, in domestic and laboratory animals, estrous cycles are disrupted and puberty is delayed by weight loss caused by food restriction or deprivation (*2, 3*). Although a critical body weight may be one prerequisite for normal reproduction, a useful model of the mechanisms determining reproductive status must account for factors other than body weight and composition. For example, puberty in rats can occur at significantly different body weights and fat-to-lean ratios, depending on previous food intake and exercise (*4*). Our results show that although lipid stores can buffer the effects of food deprivation on estrous cycles, reproduction is controlled by the general availability of metabolic fuels, rather than by any dimension of body size.

Female Syrian hamsters, *Mesocricetus auratus*, were used because they have a consistent 4-day estrous cycle that is highly sensitive to changes in energy availability (*5*). Food deprivation on days 1 and 2 of the estrous cycle blocks the next expected estrus, ovulation, and the postovulatory vaginal discharge in approximately 80% of females (*6*). This phasic starvation retards follicular development, decreases plasma estradiol, and inhibits the luteinizing hormone surge. We used phasic starvation to examine the link between energy balance and fertility.

To test the hypothesis that body fat stores can buffer the effects of starvation on estrous cyclicity, we subjected groups of hamsters (*7*) that differed in body weight, but not in age, to phasic starvation and monitored their estrous cycles. We created heavy, medium-sized, and light hamsters by varying the presentation of their diet (*8*). Hamsters were fed either powdered chow (powder), chow pellets of the same nutrient composition placed inside the cage ("pellets-in"), chow pellets in food hoppers outside the cage ("pellets-out"), or a high-fat diet (*9*) known to induce obesity in hamsters (high fat). The rank order of both body weight and fat content was high fat > powder > pellets-in > pellets-out (*10*). After two consecutive 4-day estrous cycles, half of the hamsters in each of the four groups were deprived of food on days 1 and 2 of the estrous cycle; food was available on days 3 and 4; sexual receptivity was determined just before lights-out on day 4; and vaginal discharge and ova in the oviducts were examined the next morning.

After phasic starvation, all hamsters in the two heaviest groups (powder and high fat) showed lordosis, vaginal discharge, and ovulation (Fig. 1). In the lightest group (pellets-out) none displayed lordosis, and only 10% showed vaginal discharge or tubal ova. The medium-sized group (pellets-in) had an intermediate incidence of lordosis, vaginal discharge, and ovulation. Uterine weight followed the same pattern. All hamsters that were not phasically starved showed lordosis, vaginal discharge, and ovulation.

These results show that the effect of phasic starvation on estrous cyclicity depends on the body weight before starvation. We hypothesized that the hamsters with higher body weights were protected from the effects of starvation on reproduction by their ability to use fatty acids mobilized from adipose tissues. To test this possibility we used methyl palmoxirate (MP), an inhibitor of fatty acid utilization, which blocks the transport of fatty acids into mitochondria (*11*).

After two 4-day estrous cycles, fat hamsters fed powdered chow for at least 5 weeks received one of the following treatments on days 1 and 2 of the estrous cycle: (i) MP (20 mg/kg) suspended in 0.5% methylcellulose twice a day by gavage, (ii) MP plus food deprivation, or (iii) the methylcellulose vehicle plus food deprivation. The groups did not differ significantly in body weight at the start of treatment. All hamsters were examined for estrous behavior and the vaginal discharge.

In these fat hamsters, food deprivation alone did not interrupt estrous cycles (Fig. 2), as shown in the first experiment (Fig. 1). Treatment with MP alone also did not affect estrous cycles in hamsters fed ad libitum. However, food deprivation interrupted the estrous cycles in 60% of hamsters treated concurrently with MP.

The fact that MP treatment did not interrupt estrous cycles in hamsters fed the high-carbohydrate chow ad libitum raises the possibility that glucose or other fuels from the diet might have permitted normal reproductive function. To test this possibility, we examined the effects of MP and an inhibitor of glucose utilization, both alone and together, on hamster reproduction. We used 2-deoxy-D-glucose (2DG), a glucose analog

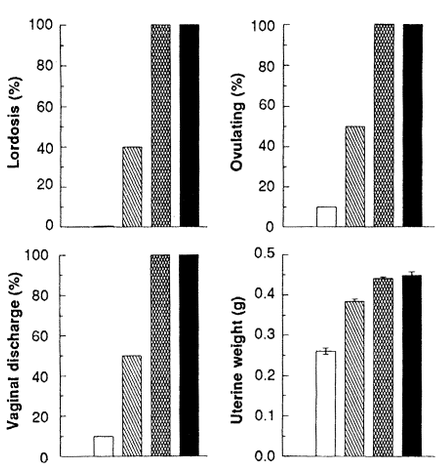

Fig. 1. Effect of food deprivation on the percentage of female hamsters that showed lordosis, postovulatory discharge, and ovulation, and on uterine weight. The hamsters had been fed one of four diets for at least 5 weeks: pellets-out (open bar) (n = 9), pellets-in (diagonal lines) (n = 10), powder (crosshatched bar) (n = 7), and high fat (solid bar) (n = 7). There were significant differences in the frequency of hamsters that showed lordosis [$G(3) = 31.326$, $P < 0.005$], the postovulatory discharge [$G(3) = 18.904$, $P < 0.005$], and tubal ova [$G(3) = 22.803$, $P < 0.005$], and in uterine weight [$F(3,29) = 11.829$, $P < 0.001$].

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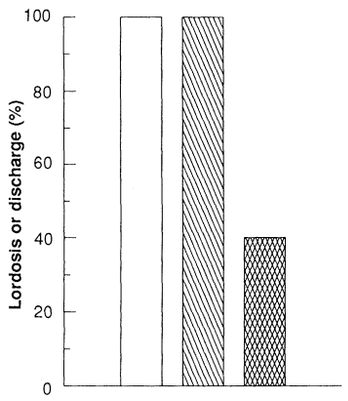

Fig. 2. Effect of MP on estrous cycles of fat hamsters. Hamsters that had been fed powdered chow for at least 5 weeks received one of the following treatments on days 1 and 2 of the estrous cycle: (i) food-deprived (open bar) (n = 9), (ii) treated with MP (diagonal lines) (n = 4), or (iii) both MP-treated and food-deprived (crosshatched bar) (n = 10). Differences in the frequency of hamsters showing either lordosis or the postovulatory discharge were significant [$G(2) = 12.942$, $P < 0.005$].

that blocks glycolysis in hamsters (*12*). Chow-fed (pellets-in) hamsters that weighed 116.9 ± 3.4 g and had two consecutive 4-day estrous cycles were divided into five groups that did not differ significantly in mean body weight. They were treated on days 1 and 2 of the estrous cycle as follows: (i) untreated, food restricted so that the caloric intake matched that of group iv, (ii) treated with MP (20 mg/kg) four times a day, (iii) injected intraperitoneally with 750 mg of 2DG per kilogram of body weight in 0.9% saline four times a day, (iv) treated with both MP and 2DG, or (v) food deprived. Any group that did not receive MP or 2DG was treated with an equivalent volume by body weight of the appropriate vehicle. A previous pilot study showed that treatment with 2DG and MP slightly decreased food intake (*13*). Therefore the food intake of group i was restricted to match the food intake of group iv (2DG plus MP). Furthermore, groups i and iv were fed 1 ml of a 25% glucose solution by gavage twice daily to ensure that their caloric intake was similar to that of the other groups.

In hamsters fed ad libitum (pellets-in), treatment with MP alone or 2DG alone did not block estrous cycles, but treatment with both drugs concurrently significantly decreased the percentage of hamsters that showed normal estrous cycles (Fig. 3). Anestrus in the MP plus 2DG-treated hamsters cannot be attributed to the small drop in food intake of this group, because normal estrous cycles were seen in 87% of the untreated hamsters that had been food restricted so that their caloric intake matched that of the hamsters treated with MP plus 2DG. The mean body weight of the MP plus 2DG group was not lower than that of the food-restricted group on any day of the estrous cycle.

These results support the hypothesis that changes in reproductive status are signaled by changes in the general availability of metabolic fuels, rather than by the presence or absence of any one specific fuel. The fact that the chow-fed (pellets-in) hamsters showed normal estrous cycles when either fatty acid oxidation or glycolysis was inhibited suggests that neither fatty acids nor glucose is critical for normal reproductive function. This suggestion is also supported by the finding that food-deprived hamsters (which are assumed to have a decreased availability of glucose) continue to show normal estrous cycles as long as fatty acids are available for mobilization from adipose tissue (Figs. 1 and 2). Furthermore, hamsters continue to show normal estrous cycles when fed only a glucose solution (*6*) or pure vegetable shortening, supporting the idea that the availability of either glucose or fatty acids alone is sufficient for continued reproductive function (*14*).

Our data do not support the notion that there is a critical body weight or composition necessary for reproduction. For example, the hamsters treated with both 2DG and MP did not lose weight, yet 83% of them were anestrous (Fig. 3). Furthermore, 60% of hamsters that were deprived of food and treated with MP became anestrous despite their high body weight (Fig. 2). Other investigators also have argued that changes in reproductive status are not brought about by changes in any one dimension of body size per se (*4, 15*).

There is precedence for the control of behavior by the general availability of metabolic fuels. In rats, greater increases in food intake were seen in response to blockade of both glucose and fatty acid utilization than to blockade of either metabolic pathway alone (*16*). In some ways this may be analogous to the mechanism by which metabolic fuels influence estrous cycles: changes in behavior are signaled by the general availability of fuels, rather than by one critical substrate. Paradoxically, treatment with 2DG and MP either alone or together does not increase food intake in hamsters (*17*).

Reproductive responsiveness to metabolic fuels may be part of a general adaptation to changes in energy supply and demand. In many mammals, the response to energetic challenges is to forgo reproduction in favor of activities that increase the chances of individual survival (*3*). The availability of oxidizable metabolic fuels may signal peripheral or central mechanisms that either increase food intake, delay reproduction, or both, until sufficient energy supplies are available for successful pregnancy and lactation.

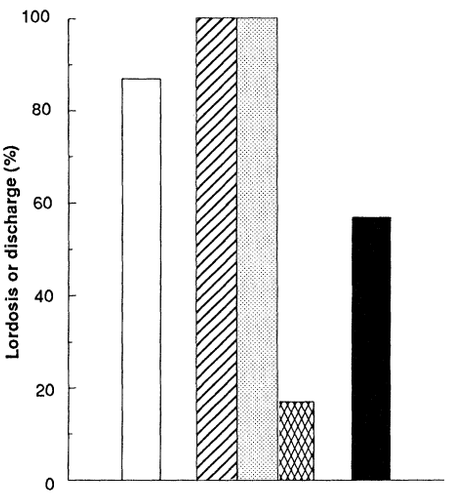

Fig. 3. Effect of inhibition of glucose and fatty acid utilization on hamster estrous cycles. Hamsters fed chow (pellets-in) were treated as follows on days 1 and 2 of the estrous cycle: (i) untreated, food restricted so that their food intake matched that of group iv (open bar) (n = 7); (ii) MP, treated with MP (diagonal lines) (n = 4); (iii) 2DG, treated with 2DG (shaded bar) (n = 5); (iv) MP plus 2DG, treated with both MP and 2DG (crosshatched bar) (n = 7); and (v) food-deprived (solid bar) (n = 7). There were significant differences in the frequency of hamsters that showed either lordosis or the postovulatory discharge [$G(4) = 17.1552$, $P < 0.005$]. There were no significant differences among groups i, ii, and iii, nor between groups iv and v.

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7. Adult Syrian hamsters, Lak:LVG, 85 to 95 g in body weight, purchased from Charles River Breeding Laboratories, Wilmington, MA, were housed in a room maintained at 21° ± 2°C on a 16:8 light: dark cycle with lights on at 0700 hours.
8. Purina Laboratory Rodent Chow, no. 5001, 3.4 kcal/g, 28% protein, 60% carbohydrate, and 12% fat.
9. Two parts Purina Laboratory Rodent Chow, no. 5001 and one part Sweet Life brand vegetable shortening; 5.3 kcal/g; 12% protein, 26% carbohydrate, 62% fat (by calories); G. N. Wade, *Physiol. Behav.* **29**, 701 (1982).
10. Body weights (mean ± SEM): High fat, 155.6 ± 4.6 g; powder, 142.6 ± 2.7 g; pellets-in, 114.4

± 2.4 g; and pellets-out, 100.7 ± 1.9 g. Parametrial fat pad weights: high fat, 5.6 ± 1.8 g; powder, 4.7 ± 0.5 g; pellets-in, 1.4 ± 0.2 g; and pellets-out, 0.8 ± 0.2 g.
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18. We thank M. I. Friedman and L. P. Morin who laid the conceptual groundwork for this research; J. D. Blaustein and G. J. DeVries for helpful comments on this manuscript; J. Alexander, R. Mergendahl, and M. H. Brown for technical assistance; and C. Bowden of McNeil Pharmaceuticals for the methyl palmoxirate. Supported by NIH research grants NS 10873-17 and AM 32976-06, National Institute of Mental Health Research Scientist Development Award MH 00321-09, and by NSF research grant BNS 8719361.

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Bright Light Induction of Strong (Type 0) Resetting of the Human Circadian Pacemaker

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The response of the human circadian pacemaker to light was measured in 45 resetting trials. Each trial consisted of an initial endogenous circadian phase assessment, a three-cycle stimulus which included 5 hours of bright light per cycle, and a final phase assessment. The stimulus induced strong (type 0) resetting, with responses highly dependent on the initial circadian phase of light exposure. The magnitude and direction of the phase shifts were modulated by the timing of exposure to ordinary room light, previously thought to be undetectable by the human pacemaker. The data indicate that the sensitivity of the human circadian pacemaker to light is far greater than previously recognized and have important implications for the therapeutic use of light in the management of disorders of circadian regulation.

THIRTY YEARS AGO, A PHASE RESPONSE curve (PRC) to light was first described (*1*), revealing the mechanism by which pacemakers driving circadian rhythms are synchronized (entrained) to the 24-hour day (*2*). Since then, PRC's to light have been described in nearly all eukaryotes studied except humans; it was believed that social contacts, rather than the light-dark cycle, synchronized the human circadian system to the 24-hour day (*3*). Subsequent studies demonstrated that the circadian system of normal subjects could be entrained by a 24-hour cycle of ordinary indoor room light and complete darkness (*4, 5*).

Evening exposure to bright light has been found to rapidly shift the phase of the endogenous component of the body temperature and cortisol cycles, even when the timing of the sleep-wake cycle was held constant (*6*). That experiment indicated that light could have a direct biological effect on the human circadian pacemaker, rather than an indirect synchronizing effect via its influence on the timing of sleep. We now report that the timing of light exposure has a critical effect on the magnitude and direction of the human circadian phase-resetting response to light. The human circadian pacemaker, which is more responsive to light than was previously postulated, can be reset to any desired phase by scheduled exposure to light for 2 to 3 days.

Typically, free-running activity rhythms of animals living for several weeks in constant darkness are interrupted by the presentation of brief light stimuli to evaluate the circadian resetting response of a pacemaker to light (*2*). However, in human subjects the free-running behavioral rest-activity cycle is an unreliable indicator of endogenous circadian phase and thus cannot be used to assess phase resetting (*7*). Hence, we have used the constant routine (CR) method to assess endogenous circadian phase (ECP), using the endogenous component of the body temperature cycle as a marker of the output of the human circadian pacemaker. Our CR procedure (*6*) is an extension and refinement of that of Mills (*8*), in which subjects are studied under constant environmental and behavioral conditions to unmask the endogenous component of the body temperature cycle by either eliminating, or distributing uniformly across the circadian cycle, physiologic responses to environmental and behavioral stimuli that can otherwise obscure it. Using the CR, we have found that repeated sequential estimates of the endogenous circadian phase at which the body temperature minimum (ECP_{min}) occurs (*9*) are highly correlated (Pearson's correlation coefficient, 0.998; $P < 0.001$), indicating that the CR is a reliable, reproducible procedure that has no measureable phase-shifting effect on the pacemaker. Therefore, we used the CR to assess the ECP_{min} both before and after the administration of a light stimulus in order to evaluate the phase-resetting effect of that stimulus (Fig. 1).

We applied a stimulus consisting of three cycles of exposure to a daily illuminance pattern that included bright light, ordinary indoor room light, and darkness (*10*) across the full range of initial circadian temperature phases (ϕ_i) (*11*). In order to determine the resetting response at these different phases, we began each resetting trial with an assessment of the initial pre-intervention ECP_{min} (t_1), exposed the subjects to the light stimulus, and then reassessed the final post-intervention ECP_{min} (t_2) (Fig. 1) (*11*). We conducted 45 resetting trials in 14 healthy young male subjects, aged 18 to 24 years (*12*), the data obtained represent a total of 420 subject-days of laboratory recording.

Exposure to our three-cycle light stimulus induced the largest phase shifts ($\Delta\phi > 8$ hours) when the light stimulus was centered around the initial ECP_{min} (t_1) (Fig. 2A). Since the ECP_{min} ordinarily occurs about 2 to 3 hours before the habitual wake time, centering the light stimulus around the ECP_{min} required inversion of the sleep-wake schedule (*13*); however, in 14 control trials in which the daily 5-hour episodes of bright light exposure were replaced with exposure either to room light (12 trials) or to darkness (2 trials), we found that such sleep-wake schedule inversion alone did not induce such large phase shifts (Fig. 2B) (*6*). This indicates that the observed phase shifts were induced primarily by the light exposure rather than by the displacement of sleep or activity.

To confirm that such light-induced phase shifts of the endogenous circadian temperature cycle accurately reflect phase shifts of the circadian pacemaker, we analyzed other established indicators of circadian rhythmicity before and after each of the resetting

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