Physical activity of adult female rhesus monkeys (Macaca mulatta) across the menstrual cycle

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IN FEMALE RATS AND MICE, changes in activity have been reported across the 4- to 5-day estrous cycle, with an increase in activity at proestrus and estrous (3, 9, 19, 23, 34, 47, 54). Activity of female rats has been shown to decrease after ovariectomy (42, 51, 59) and to increase in response to administration of estradiol to ovariectomized rats (12, 44, 51, 59). Hamsters, ferrets, and cows have also been reported to show an increase in activity at the time of estrus (4, 14, 21, 26, 33). However, data collected in nonhuman primates (17, 28, 41) and humans (2, 7, 8, 11, 35, 48) has been much more variable. None of these studies has simultaneously measured ovarian function across the menstrual cycle and directly measured activity levels. Several studies in women (2, 7, 35) have reported increases in activity midcycle, but activity increases are also reported in the luteal phase in these studies. And two studies in women have found no change in activity across the cycle (11, 48), whereas one reported a decrease in activity at midcycle (8).

It would be important to have a clear understanding of any regularly occurring changes in activity across the menstrual cycle in women and female nonhuman primates when designing studies in the female to examine the effects of activity and fitness on many health parameters. Because regular physical activity has been reported to decrease the risk of a number of major chronic diseases (43), including cardiovascular disease and hypertension (6), diabetes (24), cancer (46), obesity (37), depression (37), and attention-deficit/hyperactivity disorders (1), an understanding of the link between reproductive hormones and physical activity levels in women would allow a better understanding of many different disease processes in females.

Studies examining physical activity and fitness have used a number of different strategies to document individual activity levels. Self-report and questionnaires have been the most common methods utilized to document the amount of activity a person undertakes (6, 11, 46, 56), but several studies (22, 30) have shown that self-report can be very inaccurate. Over the past decade the use of three-way accelerometers to quantify movement in all directions has become more common, as accelerometers have been miniaturized (10). Using this technology, two- to fourfold differences in activity have been reported in studies of children and adults (16, 29, 36). We have reported an eightfold difference in daily activity levels in sedentary vs. active monkeys (46), and fivefold differences in activity have been reported in various strains of mice (52).

To determine whether significant changes in activity occur over the menstrual cycle, we measured activity continuously across one menstrual cycle in seven adult female rhesus monkeys using three-way accelerometers programmed to collect data at 1-min intervals throughout the cycle. Daily blood samples were collected to track cyclic changes in the reproductive hormones estradiol and progesterone. We found that, although all females showed ovulatory, normal-length menstrual cycles with a preovulatory rise in plasma estradiol levels and a luteal phase rise in progesterone levels, physical activity did not change across the menstrual cycle. We also found that the range of activity occurring in these seven ovari-intact monkeys was similar to the range of activity measured in 15
long-term ovarioctomized monkeys. These findings provide strong evidence that physical activity levels are not influenced by gonadal steroid hormone levels in this primate species.

METHODS

Animals. Seven adult female rhesus monkeys (Macaca mulatta), 4–10 yr of age, weighing between 5.7 and 10.7 kg, were studied. The animals lived in social groups with two to three monkeys in pens (measuring 14 × 11 × 10 ft), which had perches at various heights and various toys available. Skylights provided natural lighting supplemented with artificial lighting from ~0730 to 1600 each day. Temperature was maintained at 24 ± 3°C. This experiment was conducted in December 2005 and January 2006, during which time dawn was at ~0730 and dusk at ~1715. Monkeys were fed Purina high-protein monkey chow (no. 5045; Ralston Purina, St. Louis, MO) supplemented with seeds, fresh fruit, and vegetables. Each day monkeys were trained to come to the front of the pen so that menses could be detected by swabbing their vaginal area with a cotton-tipped swab. All aspects of the study with these animals were reviewed and approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

Fifteen adult female rhesus monkeys (Macaca mulatta), 9–13 yr of age, weighing between 4.7 and 11.1 kg, and living in individual stainless steel cages (32 × 24 × 27 or 32 × 34 × 27 in.) in a temperature-controlled room (24 ± 2°C) with lights on for 12 h/day (0700–1900), were also studied (49). The monkeys had been ovarioctomized 1 yr prior to the study. The monkeys had been ovarioctomized 1 yr prior to the study. The monkeys were maintained on a high-fat diet [35% fat (58)]. Monkeys were fed ad libitum with high-protein monkey chow (no. 5045; Ralston Purina, St. Louis, MO) supplemented with seeds, fresh fruit, and vegetables. Each day monkeys were trained to come to the front of the pen so that menses could be detected by swabbing their vaginal area with a cotton-tipped swab. All aspects of the study with these animals were reviewed and approved by the Oregon National Primate Research Center Institutional Animal Care and Use Committee.

Activity measurement. The physical activity of each monkey was recorded continuously using triaxial Actical accelerometers [MiniMitter, Bend, OR (50)]. The Actical monitor detects acceleration in all directions by utilizing an omnidirectional sensor, and monitors were programmed to record activity counts per minute. The activity monitors were mounted in tight-fitting stainless steel boxes that were attached to loose-fitting metal collars (Primate Products, Immokalee, FL). Animals were sedated with Ketamine HCl (100 mg/ml im, Ketaject; Phoenix Pharmaceuticals, St. Joseph, MO) to initially put the collar and activity monitor on each monkey and then again every 45 days to download the activity data and reprogram the monitors.

Blood sample collection. Blood samples were collected in the morning every day throughout the study (±1 menstrual cycle) using previously published methods (58). Monkeys were trained to jump from their pens into a portable transport box and were then carried to a nearby sampling room and put in a specially designed bleed cage, which allowed the monkeys to present their legs for collection of a blood sample from the femoral vein. Samples (1.5 ml) were collected, allowed to clot at room temperature for ≥1 h, refrigerated overnight, and then centrifuged at 2,500 rpm for 15 min at 4°C, and serum was removed and stored in glass vials at −20°C until assays were performed. Hematocrits were measured weekly to ensure that they remained in the normal range.

Assays. Serum samples were assayed in the Endocrine Services Core Facility at the Oregon National Primate Research Center for estradiol and progesterone, as previously described (49). Estradiol and progesterone were measured using a Roche Elecsys 2010 clinical instrument and the assay reagents from Roche Diagnostics (Indianapolis, IN). For estradiol, assay sensitivity was 10 pg/ml and the interassay variability 7.5%, and for progesterone, assay sensitivity was 0.03 ng/ml and the interassay variability 3.6%.

Data analysis. Correlations between serum levels of reproductive hormones and levels of physical activity for each ovary-intact monkey were assessed at three phases of the menstrual cycle: the early follicular phase, the day of the preovulatory rise in estradiol, and the midluteal phase. The early follicular phase corresponded to days 1–5 of the menstrual cycle, with day 1 of the cycle being the first day of menses. The day of the preovulatory rise in estradiol was identified as the day when serum estradiol levels reached a maximum value. The midluteal phase corresponded to days 5–9 or 6–10 of the luteal phase, with the day of the preovulatory estradiol surge representing day 1 of the luteal phase.

Daily mean activity was calculated from midnight of 1 day to 1159 of the next day. Daytime activity was calculated from 0800 to 1600 each day, as these hours were consistently hours of daylight at this time of year. Nighttime activity was calculated from midnight to 0600 each day, as these hours were consistently hours of dark when no people were present in the facility. Maximum activity was the minute of each day when the maximum activity counts per minute were recorded.

Prior to all statistical analyses, normality and homogeneity of variance were tested. All data met the criteria for using parametric
A more detailed examination of activity also showed no significant change in daytime activity \((F_{2,4} = 0.463, P = 0.65)\), nighttime activity \((F_{2,4} = 1.53, P = 0.26)\), or maximum activity \((F_{2,4} = 1.98, P = 0.18)\), when measures made in the follicular phase, on the day of the preovulatory rise in estradiol, or in the luteal phase were compared (Table 1).

**DISCUSSION**

Each monkey in this study had a normal-length, ovulatory menstrual cycle during the period when activity was being monitored. Despite clear changes in ovarian steroid hormones across the cycle, with a robust preovulatory rise in estradiol (shown in Fig. 4) and a luteal phase rise in progesterone, there was no change in activity across the menstrual cycle. To maximize our ability to detect a midcycle change in activity associated with the midcycle rise in estradiol, we tried several different analytical strategies. Besides looking at activity on the day of the maximal estradiol concentration (shown in Figs. 4 and 5), we also looked at mean activity on the day of the preovulatory rise in estradiol combined with the day after the maximal estradiol concentration, as we were concerned that there might be a lag time between when estradiol rises and when it might have an effect on neural systems regulating activity. However, using neither analytical approach, were we able to detect a change in activity associated with the midcycle rise in estradiol. In addition, when we looked across the

![Fig. 4. Mean serum estradiol levels (black bars) and mean levels of physical activity (hatched bars) at 3 phases of the menstrual cycle, the early follicular phase, the day of the preovulatory rise in estradiol, and the midluteal phase \((n = 7)\). *Significant difference from serum estradiol concentrations in the early follicular phase.](http://ajpendo.physiology.org/)

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**RESULTS**

Both the ovary-intact and ovariectomized monkeys showed large individual differences in physical activity. The mean activity levels for ovary-intact and ovariectomized monkeys were similar \((280.9 \pm 39.0\) and \(275.1 \pm 51.9\) counts/min, respectively; Fig. 1). The range of activity in ovary-intact monkeys \((162.1–428.7\) activity counts/min; Fig. 1). In both groups, activity measured initially was very similar to activity measured 1 mo later \((ovariectomized: r = 0.94, P < 0.001;\) Fig. 2A; ovary-intact: \(r = 0.96, P = 0.001;\) Fig. 2B). As we have previously reported in ovariectomized monkeys \((50\) the difference in activity between the less active and more active ovary-intact monkeys was apparent throughout the daylight hours (Fig. 3).

The ovary-intact monkeys showed no significant differences in daily mean physical activity across the three phases of the menstrual cycle \((F_{2,12} = 0.23, P = 0.80;\) Figs. 4 and 5). However, all monkeys experienced ovulatory menstrual cycles of 23–31 days in length, with a preovulatory rise in estradiol and a rise in progesterone \((4.04 \pm 0.53\) ng/ml) during the luteal phase. There was also no cyclic change in activity detected when data were averaged for the day of the maximal rise in estradiol and the following day \((F_{2,12} = 0.82, P = 0.46)\).

Serum estradiol concentrations varied significantly across the menstrual cycle \((F_{1,005.5,023} = 40.06, P = 0.001;\) Fig. 4), with a significant increase in estradiol from the early follicular phase \((41.4 \pm 2.9\) pg/ml) to the day of the preovulatory rise in estradiol \((245.6 \pm 37.3\) pg/ml, \(P = 0.002)\). There was no correlation between mean physical activity and serum estradiol concentrations either on day 5 of the follicular phase \((r = -0.19, P = 0.68)\), on the day of the preovulatory rise in estradiol \((r = -0.21, P = 0.66;\) Fig. 6), or during the day 7 of the luteal phase \((r = 0.003, P = 0.99)\).

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**TABLE 1**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean Activity (counts/min)</th>
<th>Mean Estradiol (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular</td>
<td>245.6 ± 37.3</td>
<td>82.1 ± 5.3</td>
</tr>
<tr>
<td>Ovulation</td>
<td>238.5 ± 29.3</td>
<td>138.0 ± 6.8</td>
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<tr>
<td>Luteal</td>
<td>275.1 ± 51.9</td>
<td>37.3 ± 6.5</td>
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</tbody>
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**TABLE 2**

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**FIGURES**

- **Fig. 3.** There was a 2.6-fold difference in physical activity between the most sedentary monkey (A) and the most active monkey (B). This difference was most notable during the daylight hours (between 0700 and 1800).
- **Fig. 4.** Mean serum estradiol levels (black bars) and mean levels of physical activity (hatched bars) at 3 phases of the menstrual cycle, the early follicular phase, the day of the preovulatory rise in estradiol, and the midluteal phase \((n = 7)\). *Significant difference from serum estradiol concentrations in the early follicular phase.**
estradiol (serum estradiol concentrations measured on the day of the preovulatory rise in Fig. 6). There was no correlation between the level of physical activity and groups and the ovariectomized monkeys were eating a high-fat diet and were housed alone, may have contributed to differences in the range of activity that we found.

Our findings support two previous studies in women that did not find a change in activity across the menstrual cycle (11, 48). Both of these studies monitored activity using a unidirectional accelerometer, in one case a pedometer and in the other case an actometer worn on the wrist. However, in both studies, hormonal changes in reproductive hormones were assessed indirectly by measuring changes in body temperature. Nevertheless, both studies attempted to quantify both the activity and the hormonal changes during the cycle in an accurate, nonbiased manner. The two studies that have shown midcycle rises in activity in women (2, 35) have assessed either activity or stage of the menstrual cycle by self-report. They also reported secondary rises in activity in the late luteal phase, a time when estradiol would be low rather than elevated as at midcycle. Because the rodent literature strongly suggests that estradiol increases the midcycle estradiol in rodents, the increase in activity in the luteal phase would seem to be activity alterations resulting from causes other than high circulating levels of estradiol.

There are other circumstances under which the regulation of activity in rodents is divergent from the regulation of activity in primates. Of note, undernutrition or fasting in rodents leads to an increase in activity (10, 15, 31, 45), whereas undernutrition in both nonhuman primates and humans leads to a decrease in activity (13, 18, 20, 25, 40). The differential regulation of physical activity in response to calorie reduction has been hypothesized to be dependent on whether an animal has sufficient stored energy to make it through a time of famine metabolizing stored energy (and slowing activity would protect their energy stores) or whether their stored energy is low, and thus survival would be dependent on finding food and increasing activity would facilitate foraging (38, 39).

Table 1. Daytime, nighttime, and maximum activity across the menstrual cycle of 7 rhesus monkeys

<table>
<thead>
<tr>
<th>Activity Measure</th>
<th>Follicular Phase</th>
<th>Day of the Preovulatory Estradiol Peak</th>
<th>Luteal Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime activity</td>
<td>597.99±95.4</td>
<td>570.40±61.2</td>
<td>624.81±67.1</td>
</tr>
<tr>
<td>Nighttime activity</td>
<td>43.33±9.5</td>
<td>21.8±5.9</td>
<td>33.62±7.6</td>
</tr>
<tr>
<td>Maximum activity</td>
<td>4,490±416</td>
<td>5,362±697</td>
<td>4,812±657</td>
</tr>
</tbody>
</table>

Values are means ± SE and in counts/min.

Fig. 6. There was no correlation between the level of physical activity and serum estradiol concentrations measured on the day of the preovulatory rise in estradiol ($r = -0.21, P = 0.66$).
With the growing body of evidence that level of physical activity has a very positive effect on many aspects of health (37, 43, 46), it seems likely that there will be a substantial interest in understanding the mechanisms by which activity influences various physiological processes. To get a clear picture of the mechanisms underlying the influence of activity on processes as diverse as cardiovascular regulation, oncological processes, osteoporosis, and mental health, it will be important to study both males and females. The findings of the current study suggest that such studies will be facilitated in female primates, as it will not be necessary to account for changes in activity across the menstrual cycle.

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GRANTS

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REFERENCES


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