Body Composition at Vaginal Opening in Mice as Influenced by Food Intake and Photoperiod: Tests of Critical Body Weight and Composition Hypotheses for Puberty Onset

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ABSTRACT

To determine if photoperiod and food intake influenced age and body composition at vaginal opening (VO), 80 mice were exposed to either 18L:6D (LL) or 6L:18D (SL) and fed either 3.64 g/mouse per day (HIGH) or 2.73 g/mouse per day (LOW) of Wayne Lab-Blox. Treatments began at weaning (21 to 23 days of age). Mice were killed at VO and analyzed for fat, water and protein content. Mice fed the HIGH ration were younger (P<0.01; 35.9 vs. 41.1 days) and heavier (P<0.001; 17.8 vs. 15.4 g) at VO than mice fed the LOW ration. Neither age nor weight at VO were affected by photoperiod. Fat content at VO was greater (P<0.001) for HIGH than LOW. Differences in fat percentage at VO between diet groups were greater for LL (9.7 vs. 7.0%) than for SL (9.1 vs. 8.6%), resulting in a photoperiod X ration interaction (P<0.01). Percent water and protein at VO were not significantly affected by photoperiod or diet. Within-group correlations between body weight and age at VO were not significant. Body water/body weight was inversely proportional to age at VO. This resulted from increased fat deposition in older animals of the LL-LOW group and increased percent protein in older animals of other groups. We concluded that dietary intake did not influence age at VO by affecting age at which a critical body weight or fat content were attained. A photoperiod of 18L:6D did not affect age at VO relative to 6L:18D.

INTRODUCTION

Restriction of food intake or lowering of energy density of the diet delays the onset of puberty in many species including the rat (Kennedy and Mitra, 1963; Glass et al., 1976, 1979; Frisch et al., 1977), bovine (Sorenson et al., 1959; Grass et al., 1982), pig (Zimmerman et al., 1960; Friend et al., 1976) and sheep (Allen and Lamming, 1961). Attainment of a "critical body weight" has been proposed as one determinant of age at puberty that could be involved in nutritional effects on puberty. Kennedy and Mitra (1963) concluded that puberty in rats is more dependent on weight than age since food restriction delayed first estrus, but did not affect weight at first estrus. Frisch and Revelle (1970) proposed that puberty attainment in women is triggered by a critical body weight as weight at menarche did not vary with age at menarche. The critical body weight hypothesis is probably not universally applicable since diet can affect weight as well as age at puberty in several species (Sorenson et al., 1959; Zimmerman et al., 1960; Allen and Lamming, 1961; Friend, 1976; Glass et al., 1976, 1979; Ronneklev et al., 1978; Grass et al., 1982) and weight at menarche in women has been found to vary with age (Frisch et al., 1971).

It has also been proposed (Frisch et al., 1973, 1977) that puberty occurs after an alteration in metabolic rate brought about by changes in body fatness. This theory is based on findings that percentage of the body estimated to be fat and water did not vary with age at menarche in girls (Frisch et al., 1973) and that amount of body water/body weight and body fat at first estrus did not differ between rats fed diets high or low in energy (Frisch et al., 1977). Kirtley and Maher (1979) found similar results using genetically obese rats. The "critical body fat" hypothesis has been questioned by Glass et al. (1979) who found that body fat/body...
weight at vaginal opening (VO) was greater for rats fed ad libitum as compared to food-restricted rats.

If puberty depends on attainment of a certain body composition, food intake could influence age at puberty by influencing age at which this critical composition is reached. Because photoperiod has been reported to alter growth rate and body weight (Peters et al., 1978; Hoffmann, 1979; Malhine et al., 1979; Schanbacher and Crouse, 1980), the effect of photoperiod on age at puberty for species which are not seasonal breeders (Malhine et al., 1979; Kamwanja and Hauser, 1983; Hansen et al., 1983) might be exerted in a manner similar to that of nutrition.

Since body composition at puberty has been measured directly only in the rat (Frisch et al., 1977; Wilen and Naftolin, 1978; Glass et al., 1979) an experiment was done to test whether body composition at puberty (vaginal opening) in laboratory mice was affected by level of food intake and photoperiod. We hypothesized that if puberty was dependent on attainment of a particular body composition, then composition at VO would not differ between treatment groups. Photoperiod was included as a treatment to determine if photoperiod affects age at VO in mice and if so, whether the effect is due to changes in growth rate.

**Materials and Methods**

**Animals and Treatments**

One-hundred female mice, from a randomly bred ICR strain, were reared in litters standardized at birth to 8 pups. At weaning (21 to 23 days of age) the mice were randomly assigned to 1 of 5 treatments. One group of 20 mice were killed at weaning by cervical dislocation to determine initial body composition. The remaining 80 were assigned in a 2 X 2 factorial design to one of two photoperiods, 18L:6D (LL) or 6L:18D (SL), and to one of two rations.

The high-fed group (HIGH) were fed ad libitum prior to weaning, at which time the two photoperiod groups were placed in separate light control rooms. Mice were kept in cages with 0 to 3 other mice, depending on when other mice began the experiment and experienced VO. Mice were weighed each day and checked for VO. They were killed at VO by cervical dislocation. Contents of the gastrointestinal tract and bladder were removed and the bodies stored at -20°C until analysis for water, protein and fat content. Bodies of the mice killed at weaning were treated in a similar manner.

**Analysis of Body Composition**

The corpses were quartered, put in aluminum weighing pans, weighed and kept at -70°C for approximately 10 min. Pans containing the mice were then placed on a heating rack (VirTis Co., Gardiner, NY; model 6215-0090, maximum temperature=32°C) and freeze-dried for 7 days in a VirTis lyophilizer (model 10-145 MR-BA). Amount of body water was calculated as the difference in weight before and after lyophilization. After freeze drying, mice were ground in a Micro-Mill (Technilab Instruments, Pequannock, NJ) and stored at -20°C until analysis of protein and fat content.

Duplicate aliquants of the ground mice (about 0.3 to 1 g) were digested by the micro-Kjeldahl method (AOAC, 1980) using a selenium catalyst. Samples were placed in 250-ml tubes with 15 ml reagent grade sulfuric acid, 1 to 2 g K2SO4 and 2 to 3 Hengar granules (Hengar Co., Philadelphia, PA) and digested in a block digester (Udy-Tecator, Herndon, VA) at 400°C until solutions became clear (2-3 h). After cooling, deionized water was added to a final volume of 100 ml. The diluted, digested samples were then assayed in duplicate for nitrogen content by the colorimetric procedure described by Weatherburn (1967) except that sample volumes were 10μl, final volumes were 3 ml and the reaction proceeded overnight at room temperature. Protein content was estimated as nitrogen content X 6.25. The within-assay coefficient of variation (CV) of the entire procedure averaged 2.9%. Recovery of nitrogen based on bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, MO; lot 101F-0334) was 95%. Between-assay CV based on 6 separate determinations of bovine serum albumin was 5.7%.

Fat content was determined by extracting samples ranging in dry weight from 0.3 to 4.5 g with diethyl ether (AOAC, 1980). After extraction for 18 h, samples were oven dried and weighed. Fat content was considered equal to the changes in weight after extraction. Duplicate determinations were done for only 25 mice because of lack of material. The within-assay CV for these mice averaged 1.1%.

**Statistical Analysis**

Data from one mouse in group SL-LOW was excluded because it was killed before VO at 84 days of age. Compositional data were not obtained from another animal in group LL-HIGH because of loss of sample. Water, protein and fat content were calculated as percentage of wet body weight for each mouse.

Differences in composition between mice killed at weaning and those killed at VO were tested by Dunnett’s procedure (Steel and Torrie, 1960). Effects
TABLE 1. Effect of photoperiod and ration on age, body weight and body composition at weaning and vaginal opening (VO).\(^a\)

<table>
<thead>
<tr>
<th>Time traits measured</th>
<th>Photoperiod</th>
<th>Ration</th>
<th>Age (days)</th>
<th>Weight (g)</th>
<th>Water (%)</th>
<th>Fat(^b) (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning</td>
<td>18L:6D</td>
<td>HIGH</td>
<td>35.3 ± 1.21</td>
<td>17.6 ± 0.43</td>
<td>70.1 ± 0.34</td>
<td>7.4 ± 0.36</td>
<td>20.1 ± 0.36</td>
</tr>
<tr>
<td>VO 18L:6D</td>
<td>LOW</td>
<td>40.5 ± 1.85</td>
<td>15.4 ± 0.27</td>
<td>69.5 ± 0.39</td>
<td>7.0 ± 0.41</td>
<td>20.0 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>VO 6L:18D</td>
<td>HIGH</td>
<td>36.4 ± 1.26</td>
<td>17.9 ± 0.54</td>
<td>68.7 ± 0.45</td>
<td>9.1 ± 0.38</td>
<td>19.8 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>VO 6L:18D</td>
<td>LOW</td>
<td>41.8 ± 2.41</td>
<td>15.6 ± 0.33</td>
<td>68.4 ± 0.60</td>
<td>8.6 ± 0.43</td>
<td>20.5 ± 0.41</td>
<td></td>
</tr>
</tbody>
</table>

Photoperiod means

| 18L:6D | 37.9 | 6.5 | 69.0 | 8.3 | 19.8 |
| 6L:18D | 39.0 | 16.8| 68.6 | 8.9 | 20.1 |

Ration means

| HIGH | 35.9 | 17.8\(^**\) | 68.6 | 9.4\(^**\) | 19.7 |
| LOW  | 41.1 | 15.4 | 69.0 | 7.8 | 20.2 |

\(^a\)Means and standard errors.

\(^b\)Photoperiod x ration effect (P<0.01).

\(^c\)Differs from mice killed at weaning (P<0.10).

\(^d\)Differs from mice killed at weaning (P<0.05).

\(^e\)Differs from mice killed at weaning (P<0.01).

\(^f\)P<0.01.

\(^{**}\)P<0.001.
of photoperiod, ration and their interaction were determined by least-squares analysis of variance. Because the effect of photoperiod might depend on length of exposure to photoperiod, weight gain was calculated from 23 to 29 days of age and from 7 days before VO (−7) to VO. Weight gain from 23 days to VO was also analyzed with age at VO as a covariate to determine effects of photoperiod and ration independent of age at VO. Log transformation of the data for age at VO was necessary because of heterogeneity of variance. Log transformation did not correct heterogeneity of variance for weight at VO so Wilcoxon’s method (Steel and Torrie, 1960) was used to test for effects of photoperiod and ration on this trait. Various correlations were calculated, tested for heterogeneity of correlation and pooled when heterogeneity was not significant.

RESULTS

Means of traits measured at VO are presented in Table 1 and cumulative frequency distributions of age at VO are shown in Fig. 1. HIGH-fed mice were younger (P<0.01) and heavier (P<0.001) at VO than LOW-fed animals but neither photoperiod nor photoperiod × ration significantly affected these traits. Percent water and percent protein were similar (P>0.10) for all groups killed at VO. Percent fat at VO was less for LOW than HIGH, especially for mice exposed to LL, resulting in ration (P<0.001) and photoperiod × ration (P<0.01) effects. Based on comparisons with mice killed at weaning, percent water decreased from weaning to VO for LL-HIGH and SL-LOW groups (P<0.10) but not for other groups. Percent protein at weaning and at VO were similar (P>0.10) for all groups while relative fatness increased from weaning to VO (P<0.10 to P<0.01) for all groups except LL-LOW. Weight gain was greater (P<0.001) for HIGH than LOW regardless of when measured (Table 2). There were no effects of photoperiod or photoperiod × ration.

Correlation coefficients of age at VO with...
TABLE 2. Effect of photoperiod and ration on growth rate from 23 days of age to vaginal opening (VO).a

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>Ration</th>
<th>Body weight at 23 days of age (g)</th>
<th>Weight gain (g/day) from:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>23 to 29 days of age</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>−7 days to VO b</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>23 days to VO c</td>
<td></td>
</tr>
<tr>
<td>18L:6D</td>
<td>HIGH</td>
<td>9.3 ± 0.41</td>
<td>0.86 ± 0.046</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.63 ± 0.069</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.67 ± 0.027</td>
<td></td>
</tr>
<tr>
<td>18L:6D</td>
<td>LOW</td>
<td>9.0 ± 0.39</td>
<td>0.54 ± 0.043</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.31 ± 0.039</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.46 ± 0.026</td>
<td></td>
</tr>
<tr>
<td>6L:18D</td>
<td>HIGH</td>
<td>9.3 ± 0.44</td>
<td>0.83 ± 0.060</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.54 ± 0.065</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.66 ± 0.026</td>
<td></td>
</tr>
<tr>
<td>6L:18D</td>
<td>LOW</td>
<td>8.8 ± 0.36</td>
<td>0.55 ± 0.039</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.34 ± 0.053</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.51 ± 0.027</td>
<td></td>
</tr>
</tbody>
</table>

Photoperiod means

18L:6D       | 9.2    | 0.70 | 0.47 | 0.57
6L:18D       | 9.1    | 0.69 | 0.44 | 0.59

Ration means

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th>Daily weight gain, 23 to 29 days</th>
<th>Daily weight gain, −7 days to VO</th>
<th>Daily weight gain, 23 days to VO</th>
<th>Weight, VO</th>
<th>Percent water, VO</th>
<th>Percent fat, VO</th>
<th>Percent protein, VO</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIGH</td>
<td>9.3</td>
<td>0.85 b</td>
<td>0.59 b</td>
<td>0.67 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW</td>
<td>8.9</td>
<td>0.54</td>
<td>0.32</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Means and standard errors except for data in last column which are least-squares means and standard errors.

bFrom 7 days before VO to VO.

cAdjusted for age at VO by covariance analysis.

*P<0.001.

weight and changes in weight are presented in Table 3. Animals that were heavier at 23 days of age tended to attain VO at earlier ages. Weight gain from 23–29 days of age was not significantly related to age at VO. Mice older at VO had smaller weight gains in the 7 days prior to VO and from 23 days to VO. There were no significant correlations between age and weight at VO. Animals heavier at 23 days of age were also heavier at VO (pooled r=0.48).

In all groups, mice that were younger at VO tended to be higher in percent water (Table 3). There was heterogeneity of correlation (P<0.05) in the relationship of age at VO with percent protein and fat at VO. In the LL-LOW group, mice that were older at VO had a higher percent body fat, while percent protein was not related to age at VO. For other groups,

TABLE 3. Correlations between age at vaginal opening (VO) and other traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Group a</th>
<th>Pooled coefficient of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, 23 days</td>
<td>LL-HIGH</td>
<td>LL-LOW</td>
</tr>
<tr>
<td>Daily weight gain, 23 to 29 days</td>
<td>-0.31 *</td>
<td>-0.35</td>
</tr>
<tr>
<td>Daily weight gain, −7 days to VO</td>
<td>-0.24</td>
<td>-0.73 **</td>
</tr>
<tr>
<td>Daily weight gain, 23 days to VO</td>
<td>-0.79 **</td>
<td>-0.80 **</td>
</tr>
<tr>
<td>Weight, VO</td>
<td>0.11</td>
<td>-0.07</td>
</tr>
<tr>
<td>Percent water, VO</td>
<td>-0.32</td>
<td>-0.70 **</td>
</tr>
<tr>
<td>Percent fat, VO</td>
<td>-0.11</td>
<td>0.56</td>
</tr>
<tr>
<td>Percent protein, VO</td>
<td>0.36</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

*aLL=18 h light/day, SL=6 h light/day, HIGH=3.64 g feed/day, LOW=2.73 g feed/day.

bThere was heterogeneity of correlation (P<0.05).

*P<0.05.

**P<0.001.

†P<0.10.
animals older at VO had greater relative amounts of protein, but percent fat did not vary significantly with age.

**DISCUSSION**

Restricting dietary intake of mice delayed VO, indicating that as in many other species, puberty in mice is influenced by food intake. Our results do not support the theory that dietary intake altered age at VO by influencing the age at which a critical body weight or fatness was achieved. If this was true, weight and fatness at VO would not be affected by treatment. In actuality, HIGH-fed mice were heavier and fatter at VO than LOW-fed mice. The effect of ration on fatness at VO was especially true for mice exposed to LL. These results are in agreement with food-restriction experiments with other species (Zimmerman et al., 1960; Allen and Lamming, 1961; Friend, 1976; Ronneklev et al., 1978; Glass et al., 1979).

Comparisons of changes in body composition from weaning to VO provides additional evidence that VO does not occur at a certain fat composition. If the critical body fat hypothesis is true, groups depositing fat rapidly should reach puberty sooner than groups in which temporal changes in percent fat are small. Age at VO, however, was similar for LL-LOW and SL-LOW groups even though there was no change in percent fat from weaning to VO in the LL-LOW group. Furthermore, while rate of gain, weight at 23 days of age and weight at VO were similar for LL-LOW and SL-LOW groups, the former group was less fat at VO.

Although neither light nor feeding regimen significantly affected percent water at VO, differences between groups were related to differences in percent fat—the LL-LOW group, which was least fat, had the greatest relative amounts of water at VO. This was to be expected because the pooled correlation coefficient between percent fat and water was -0.78. Therefore, it is unlikely puberty was triggered by attainment of a critical hydration of the body.

It is uncertain whether ration influenced age at VO by affecting age at which a critical percent protein was reached. This could be true as feed consumption has been reported to affect percent of the body composed of protein (Stanier and Mount, 1972; Meyer and Bradford, 1974) and because percent protein at VO was similar for all groups. However, there was no change in percent protein between weaning and VO. The major change in composition during this time for most groups was an increase in fat deposition at the expense of water. Therefore, the similarity between treatment groups in percent protein may reflect the small change in percent protein between weaning and VO.

If puberty within a group of mice treated alike occurred at a critical weight or composition, the correlation of age at VO with weight or composition at VO should be near zero. This was true for weight at VO (Table 3). Furthermore, animals that were heavier at 23 days of age tended to attain VO earlier than lighter mice, in agreement with observations of Engle and Rosasco (1927) and Monteiro and Falconer (1966). Yet, the correlation between growth rate from 23–29 days of age and age at VO was not significant. A negative correlation between these traits would be expected if attainment of a certain weight was related to puberty. When rate of gain was calculated relative to VO, it was negatively related to age at VO, probably because older mice gain at a slower rate than younger mice (Gunsett et al., 1981) and daily feed available per unit body weight decreased as mice became older and heavier.

Within groups, VO did not occur at a common body composition. Mice that were older at VO were lower in percent water and higher in percent fat (LL-LOW or protein (other groups). Wilen and Naftolin (1978) found similar changes in percent water and fat with age at first estrus in rats, but observed no relationship between age at first estrus and percent protein. Frisch et al. (1977) did not observe significant changes in body composition with age at first estrus.

The photoperiodic regimens used did not significantly influence age at VO. Similarly, Kirchhoff (1937) observed no effect of constant darkness, 1L:23D photoperiod or constant red light on age at first estrus of mice. Therefore, puberty in this species may not be modulated by photoperiod. Alternatively, because differences in photoperiod began between 21 and 23 days of age and VO occurred as early as 29 days, photoperiodic differences may not have commenced early enough to elicit an effect. There is an indication that age at VO may have been affected by photoperiod in those animals oldest at VO and therefore exposed the longest to photoperiodic differences. For animals fed LOW diets, the proportion that
were pubertal at a given age was similar for LL and SL groups until about 35 days of age (Fig. 1); afterwards mice exposed to LL became pubertal more rapidly than mice exposed to SL. The interaction between photoperiod and ration on fat percentage indicated that differences in fatness due to ration were greater for mice exposed to LL. These results may reflect photoperiodic and dietary regulation of hormones involved in lipid metabolism. The fact that percent fatness increased with age at VO in LL-LOW while percent protein increased in other groups may also have reflected differences between groups in the milieu of metabolic hormones.

Vaginal opening was used as the measure of puberty. Vaginal opening and first estrus (Vandenbergh et al., 1972) and first estrus based on smears and based on mating behavior (Kennedy, 1973) are not always coincident in mice. Because of this and because the degree of asynchrony between VO and first estrus can vary with treatment (Vandenbergh et al., 1972), our results may only pertain to onset of VO and not other pubertal events. In rats, except for the findings of Kennedy and Mitra (1963), differences in body weight between nutrition groups were similar when measured at VO or first estrus (Frisch et al., 1975; Glass et al., 1976; Wilen and Naftolin, 1978; Kirtley and Maher, 1979). Body composition has not been measured at both VO and first estrus in one experiment.

Our experiment indicated that dietary intake did not affect age at VO in the mouse by affecting age at which a critical body weight or level of fatness was reached. In this respect, the mouse appears to be similar to the rat (Glass et al., 1976, 1979; Wilen and Naftolin, 1978). Alternative mechanisms for the effect of nutrition on puberty in the mouse could include direct effects of diet on reproductive hormones (see Glass and Swerdloff, 1980 for review). If this is true, correlations between prepubertal weight and age at puberty could reflect an individual's nutritional status, rather than attainment of a critical body weight or composition. Photoperiod did not affect age at VO but did interact with ration to influence composition at VO.

ACKNOWLEDGMENTS

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and by NRS Awards 5–732–HD07007–03 and 5–732–HD07007–05 from the Public Health Service. The authors thank C. Stodd, H. Grimik and D. Hansen for their excellent technical assistance and advice and S. Kading and J. Busby for typing this manuscript. This is Meat and Animal Science Paper No. 824.

REFERENCES


