Photoperiod regulates dietary preferences and energy metabolism in young developing Fischer 344 rats but not in same-age Wistar rats

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Togo Y, Otsuka T, Goto M, Furuse M, Yasuo S. Photoperiod regulates dietary preferences and energy metabolism in young developing Fischer 344 rats but not in same-age Wistar rats. Am J Physiol Endocrinol Metab 303: E777–E786, 2012. First published July 17, 2012; doi:10.1152/ajpendo.00209.2012.—The effects of photoperiod on dietary preference were examined using young growing Fischer 344 and Wistar rats, which are seasonal and nonseasonal breeders, respectively. Rats were provided a low-fat, high-carbohydrate diet (LFD: 66/10/24% energy as carbohydrate/fat/protein) and high-fat, low-carbohydrate diet (HFD: 21/55/24% energy as carbohydrate/fat/protein) simultaneously under long- (LD: 16 h light/day) and short-day (SD: 8 h light/day) conditions for 3 wk. Fischer 344 rats preferred the LFD to the HFD under the LD condition, whereas preference for both diets was equivalent under the SD condition. Consequently, their body weight and total energy intake exhibited 11–15 and 10–13% increases, respectively, under the LD condition. Calculation of energy intake from macronutrients revealed that rats under the LD condition consumed 20–24 and 9–13% higher energy of carbohydrates and proteins, respectively, than those under the SD condition. In contrast, Wistar rats preferred the LFD to the HFD irrespective of photoperiod and exhibited no photoperiodic changes in any parameters examined. Next, Fischer 344 rats were provided either the LFD or HFD for 3 wk under LD or SD conditions. Calorie intake was 10% higher in the rats fed the LFD than those fed the HFD under SD condition. However, rats under LD condition exhibited 5–10, 14, and 64% increases in body weight, epididymal fat mass, and plasma leptin levels, respectively, compared with those under the SD condition irrespective of dietary composition. In conclusion, photoperiod regulates feeding and energy metabolism in young growing Fischer 344 rats via the interactions with dietary macronutrient composition.

Energy intake; fat; carbohydrates; leptin; nutrition

Most organisms living in temperate zones primarily use the photoperiod to perceive seasonal changes in their environment. Photoperiodic regulation in energy metabolism is one of the crucial strategies used by animals to survive in nature. Photoperiodic changes in energy metabolism are also found in domestic and experimental animals such as Djungarian hamsters (2, 11), Fischer 344 rats (8–10), and sheep (17). In hamsters and Fischer 344 rats, body weight and food intake are reduced by exposure to short-day (SD) conditions (2, 8–10, 11). Since photoperiod affects the growth and production of edible organisms in natural conditions, the amount and kinds of food sources of animals vary throughout the year. Consequently, it is reasonable to speculate that the nutritional requirements or food preferences of animals are affected by the photoperiod as well. Indeed, in Sprague-Dawley rats, preference for sweet solutions is regulated by the interaction between dietary fat and photoperiod; voluntary sucrose intake increases in rats fed a high-fat diet compared with those fed a low-fat diet under SD photoperiods, whereas sucrose intake does not differ between rats fed high- and low-fat diets under long-day (LD) photoperiods (27). Seasonal changes in food intake and preference are also remarkable in patients with seasonal affective disorder (SAD), a syndrome characterized by recurrent bouts of depression that occur regularly in a specific season, especially winter. SAD patients typically exhibit increased appetite and carbohydrate cravings during the winter months (21).

The mechanisms underlying the photoperiodic regulation in body weight have been reported using Siberian hamsters. A number of studies suggest the involvement of the sensitivity of the hypothalamic energy center to the Ob gene product leptin, which is secreted by adipocytes and represents an important anorexigenic signal for the regulation of body weight (30). Since fat mass is positively correlated with serum leptin levels, LD-acclimated hamsters with greater fat mass exhibit higher concentrations of serum leptin than those in SD-acclimated hamsters (14, 31). Paradoxically, higher levels of anorexigenic leptin signals are paralleled with a higher food intake in LD-acclimated hamsters (14). These data imply the resistance to leptin signaling under the LD condition. Indeed, the leptin-induced losses of body weight and fat mass are higher under SD photoperiods than under LD ones in Djungarian hamsters (2, 14). At the molecular level, leptin sensitivity appears to be mediated by suppressor of cytokine signaling 3 (SOCS3), a leptin-inducible inhibitor of leptin signaling, in the arcuate nucleus of the hypothalamus (3, 4, 24, 31); SOCS3 expression increases under long photoperiods independent of body fat and serum leptin levels (31).

Dietary preferences are affected by both genetic and environmental factors. In a previous study, 13 mouse strains were provided separate carbohydrate, fat, and protein diets; most strains consumed more calories from the fat diet, whereas a few strains consumed more calories from the carbohydrate diet (28). However, no studies on dietary macronutrient selection in response to photoperiodic changes in any mammalian species have been performed. Therefore, the aim of this study was to clarify whether food selectivity and consequent body weight gain and fat mass are affected by photoperiod in young growing Fischer 344 rats, which are seasonal breeders (8–10), and same-age Wistar rats, which are nonseasonal breeders. This study further clarified the interaction between dietary macronutrient composition and photoperiod using young growing Fischer 344 rats. The energy metabolism mechanisms in these animals were studied by analyzing plasma leptin and corticosterone concentrations as well as the expression of SOCS3 in the hypothalamus.
MATERIALS AND METHODS

Animals

Male Fischer 344 and Wistar rats (4 wk old) were obtained from Charles River Laboratories (Yokohama, Japan) and SLC (Shizuoka, Japan), respectively. Animals were housed individually in 39.5 × 22.5 × 18.5 cm polypropylene cages with stainless-steel wire tops. They were kept under SD conditions [SD: 8:16-h light-dark (8L16D), lights on from 0900 to 1700, ~50 lux] in light-tight boxes placed in a room at 25 ± 1°C for ≥1 wk before the experiment. They were initially kept under SD conditions in all experiments since inductive (SD to LD conditions) and suppressive (LD to SD conditions) pathways of photoperiod-regulated functions involve different cascades (34), and we focused on the inductive effects of LD conditions throughout the study. They were provided filtered tap water and standard laboratory diet [powdered MF purchased from Oriental Yeast (Tokyo, Japan)] ad libitum. Diet was provided in stainless-steel cases that prevented rats from scattering the powdered diet. All animal experiments in the present study were conducted in accordance with the Guidelines for Animal Experiments of the Faculty of Agriculture of Kyushu University as well as the Law (No. 105) and Notification (No. 6) of the Japanese Government. The experiments were approved specifically by the Animal Care and Use Committee of Kyushu University.

Diets

The low-fat high-carbohydrate diet (LFD) and high-fat low-carbohydrate diet (HFD) were prepared for both experiments 1 and 2; they were mixed using different amounts of ingredients (Table 1) (29) to modify the amounts of carbohydrates and fat. The LFD contained 9.87 and 66.42% of total energy as fat and carbohydrates, respectively; the HFD contained 55.33 and 20.96%, respectively.

Experiment 1: Effects of Photoperiod on Dietary Selection

After 1 wk of acclimatization to powdered MF diet under the SD condition, Fischer 344 rats were divided into two groups. One group [n = 5 (Fischer 344) and n = 6 (Wistar)] was transferred to the LD condition (LD: 16L8D, lights on from 0900 to 0100, ~50 lux), and the other group [n = 6 (both Fischer 344 and Wistar)] was maintained under the SD condition. At this point, the powdered MF diet was replaced with the LFD and HFD diets, which were provided simultaneously in each cage for self-selection. The body weight and food intake of each diet were measured every 2–3 days for 3 wk at the beginning of the light phase (3 times/wk). Thereafter, rats were subsequently fasted overnight (16 h) to remove the acute effects of meals just before the sampling time point on leptin signaling (23) and euthanized by isoflurane anesthesia at the middle of each light phase (1300 for SD and 1700 for LD). Their brains were dissected and euthanized by isoflurane anesthesia at the middle of each light phase (1300 for SD and 1700 for LD). Their brains and blood were collected and stored in the same manner as in experiment 1. Their epididymal fat depots were also weighed. Feed efficiency was calculated by dividing body weight gain (g) by total energy intake per animal (kcal).

Hormone Measurement

Total corticosterone in plasma was measured in duplicate using a corticosterone enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s protocol, except for the use of Steroid Displacement Reagent (2.5%; Enzo Life Sciences, Farmingdale, NY) in the plasma dilution step. The detection limit for the assay was 30 pg/ml. The intra- and interassay coefficients of variation were 2.8 and 8.9%, respectively.

Plasma leptin concentration was measured using a YK051 Rat Leptin-HS ELISA kit (Yanaihara Institute, Shizuoka, Japan). Each standard and all samples were measured in duplicate. The detection limit for the assay was 18 pg/ml. The intra- and interassay coefficients of variation were 3.2–5.9 and 3.1–6.1%, respectively.

Real-Time PCR

The hypothalamus was punched out from the brain slices (2 mm) using a 2.2-mm-diameter needle. Total RNA was isolated using Isogen (Nippon Gene, Tokyo, Japan) from the hypothalamus, and cDNA synthesis was performed using 1 μg of total RNA using the PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Osaka, Japan) according to the manufacturer’s protocol. To analyze the expression levels of SOCS3, real-time PCR was performed using the MX3000P Real-Time QPCR System (Stratagene, Santa Clara, CA) with SYBR Premix Ex Taq (TaKaRa). GAPDH mRNA levels were used as internal controls. The primers for amplification were as follows: SOCS3: forward 5’-TTCGGGACTAGTAGGAAGGA-3’, reverse 5’-AGG-GCCCACTTAGTAGTT-3’ (product size: 123 bp); GAPDH: forward 5’-CCCCCAATGTATCCTTGTG-3’, reverse 5’-TAGCCAGGATGCCTTTAGT-3’ (product size: 118 bp). The PCR reaction was per-

Table 1. Composition of experimental diets expressed as percent by weight

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>LFD</th>
<th>HFD</th>
</tr>
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<tbody>
<tr>
<td>Corn starch</td>
<td>42.32</td>
<td>23.91</td>
</tr>
<tr>
<td>Granulated sugar</td>
<td>18.14</td>
<td>0</td>
</tr>
<tr>
<td>Milk casein</td>
<td>21.99</td>
<td>28.69</td>
</tr>
<tr>
<td>Vegetable shortening</td>
<td>1.61</td>
<td>26.6</td>
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<tr>
<td>Corn oil</td>
<td>2.46</td>
<td>3</td>
</tr>
<tr>
<td>Cellulose</td>
<td>9.16</td>
<td>11.95</td>
</tr>
<tr>
<td>AIN-93 vitamin mix*</td>
<td>0.81</td>
<td>1.06</td>
</tr>
<tr>
<td>AIN-93G mineral mix*</td>
<td>3.22</td>
<td>4.21</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.18</td>
<td>0.24</td>
</tr>
<tr>
<td>dl-Methionine</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>Energy density, kcal/g</td>
<td>3.71</td>
<td>4.84</td>
</tr>
<tr>
<td>Carbohydrate energy ratio, %</td>
<td>66.42</td>
<td>20.96</td>
</tr>
<tr>
<td>Fat energy ratio, %</td>
<td>9.87</td>
<td>55.33</td>
</tr>
<tr>
<td>Protein energy ratio, %</td>
<td>23.71</td>
<td>23.71</td>
</tr>
</tbody>
</table>

LFD, low-fat high-carbohydrate diet; HFD, high-fat low-carbohydrate diet.

*The vitamin and mineral mixes were obtained from KBT Oriental and contain 97 and 22% sucrose, respectively.
formed as follows: 1 cycle of pre-denaturation at 95°C for 30 s followed by 40 cycles of amplification, including denaturation for 5 s at 95°C and annealing at 60°C for 30 s. The mRNA expression levels were determined using the threshold cycles for amplification of unknown samples by comparing them with the standard curves generated by amplification of serial dilution standards with a known number of molecules (between $10^2$ and $10^8$ copies) of SOCS3 and GAPDH DNA. Calculated levels of SOCS3 and GAPDH templates ranged from $10^2$ to $10^5$ and from $10^6$ to $10^8$ copies, respectively. The specificity of PCR products was confirmed by analyzing the dissociation curves.

**Statistics**

For experiment 1, Student’s t-test was used to analyze the effects of photoperiod on plasma corticosterone and leptin concentrations, testicular weight and epididymal fat mass, and SOCS3 mRNA levels for each rat strain. To test the effects of photoperiod (i.e., SD vs. LD) and exposure duration (wk) on body weight gain, energy intake and food selectivity data were analyzed using two-way repeated-measures ANOVA, depending on the parameter considered. When significant main effects or significant interactions were detected ($P < 0.05$), post hoc comparisons were performed with Bonferroni multiple comparison tests. Values are expressed as means ± SE.

For experiment 2, data were analyzed using two-way repeated-measures ANOVA to test the effects of photoperiod (i.e., SD vs. LD) and diet (i.e., LFD vs. HFD) as well as the interaction between them with respect to body weight, body weight gain, epididymal fat mass, energy intake, plasma corticosterone and leptin concentrations, and SOCS3 mRNA levels; regarding body weight gain and energy intake, the total amounts of these parameters during 2–3 wk were analyzed, since photoperiod affected the body weight from 9 days of exposure. When significant main effects or significant interactions were detected ($P < 0.05$), post hoc comparisons were performed with Bonferroni multiple comparison tests. Values are expressed as means ± SE.

**RESULTS**

**Experiment 1: Effects of Photoperiod on Dietary Selection in Fischer 344 and Wistar Rats**

**Effects of photoperiod on body weight gain and fat.** In Fischer 344 rats, body weight was significantly higher in rats maintained under the LD condition than those maintained under the SD condition (main effect of photoperiod, $P < 0.001$; Fig. 1A). Significant increases (11–15%) in the LD condition were detected after 17 days of the exposure ($P < 0.05$ for 17 days, $P < 0.01$ for 19 and 21 days; Fig. 1A). Body weight gain also exhibited a significant increase under the LD condition (main effect of photoperiod, $P < 0.001$; Fig. 1B), and significances were detected at 1–3 wk of the exposure ($P < 0.01$ for 1 wk (24% increase), $P < 0.001$ for 2 (27% increase) and 3 wk (42% increase); Fig. 1B). Epididymal fat weight divided by final body weight was significantly higher in rats maintained under the LD condition than those maintained under the SD condition (31% higher under LD condition, $P < 0.01$; Fig. 1C). In Wistar rats, the temporal changes in body weight were not significantly affected by the photoperiod (Fig. 1, D and E). Epididymal fat weight divided by body weight did not change significantly with photoperiod (Fig. 1F).

**Effects of photoperiod on dietary selection.** Fischer 344 rats maintained under the LD condition ate more of the LFD than the HFD (main effect of diet, $P < 0.001$), with a significant interaction between diet and time ($P < 0.05$); significant differences between LFD and HFD intake were detected at 2 (93% higher amount of LFD) and 3 wk (96% higher amount of LFD) ($P < 0.001$) (Fig. 2A). Caloric intake from the LFD was also higher than that from the HFD under the LD condition (main effect of diet, $P < 0.001$), with a significant interaction between diet and time ($P < 0.05$); a significant difference occurred at 3 wk (50% higher intake of LFD) ($P < 0.05$) (Fig. 2B). In contrast, the amounts and caloric intake of the LFD and HFD did not differ in rats maintained under the SD condition (Fig. 2, C and D).

Wistar rats ate much more LFD than HFD, irrespective of photoperiod, during the whole experimental period (main effect of diet, $P < 0.001$), with a significant interaction between diet and time ($P < 0.05$) (Fig. 2, E and G). As a result, caloric intake was derived mostly from the LFD in rats under both photoperiods (main effect of diet, $P < 0.001$; interaction between diet and time, $P < 0.05$; Fig. 2, F and H). Post hoc analysis revealed that there were significant differences of the amount (470–850% higher amount of LFD) and caloric intake (340–630% higher intake of LFD) between LFD and HFD groups during the whole experimental period ($P < 0.001$) (Fig. 2, E–H).

**Effects of photoperiod on total and macronutrient energy intake.** In Fischer 344 rats, total energy intake exhibited significant increases (10–13%) in rats maintained under the LD condition than those maintained under the SD condition (main effect of photoperiod, $P < 0.05$), with no interaction between photoperiod and diet (Fig. 3A). On the other hand, total energy intake did not differ significantly between photoperiodic conditions in Wistar rats (Fig. 3E).

Macronutrient energy intake was calculated on the basis of the carbohydrate, fat, and protein calories in the LFD and HFD. In Fischer 344 rats, carbohydrate and protein energy intake exhibited significant increases (carbohydrate: 20–24%; protein: 9–13%) in rats maintained under the LD condition than those maintained under the SD condition (main effect of photoperiod, $P < 0.05$), with no interaction between photoperiod and diet (Fig. 3, B and D), whereas fat energy intake did not change as a result of the photoperiod (Fig. 3C). In contrast, carbohydrate, fat, and protein energy intake did not significantly differ between photoperiodic conditions in Wistar rats (Fig. 3, F–H).

**Effects of photoperiod on plasma hormones and leptin signaling genes in the hypothalamus.** In both rat strains, the plasma concentrations of corticosterone and leptin in the middle of the light phase after overnight (16 h) fasting did not change with the photoperiod (Fig. 4, A, B, D, and E). SOCS3 expression was not affected significantly by the photoperiod in either rat strain (Fig. 4, C and F).

**Experiment 2: Interaction Between Photoperiod and Dietary Fat/Carbohydrate Composition**

**Effects of photoperiod and dietary composition on body weight gain and fat mass.** Body weight gain was significantly higher in rats maintained under the LD condition than those maintained under the SD condition (main effect of photoperiod, $P < 0.001$; Fig. 5, A and B). Significant effects of photoperiod on body weight were observed after 9 days of the exposure to each photoperiodic condition (5–10% higher under LD condition; Fig. 5A). However, dietary fat/carbohydrate content did not significantly affect body weight gain (main effect of diet, $P > 0.05$; Fig. 5, A and B). There was no
significant interaction between photoperiod and diet in body weight gain. Epididymal fat weight was also significantly higher (14%) in rats maintained under the LD condition than those maintained under the SD condition (main effect of photoperiod, $P < 0.001$) but was not affected by dietary fat/carbohydrate content (main effect of diet, $P > 0.05$) (Fig. 5C). A significant interaction was not detected between photoperiod and diet in epididymal fat weight.

Effects of photoperiod and dietary composition on food/energy intake. Food intake and calculated total energy intake were significantly higher in rats maintained under the LD condition than those maintained under the SD condition (main effect of photoperiod, $P < 0.001$) but was not affected by dietary fat/carbohydrate content (main effect of diet, $P > 0.05$) (Fig. 5D). A significant interaction was not detected between photoperiod and diet in epididymal fat weight.

Effects of photoperiod and dietary composition on food/energy intake. Food intake and calculated total energy intake were significantly higher in rats maintained under the LD condition than those maintained under the SD condition (main effect of photoperiod, $P < 0.001$) but was not affected by dietary fat/carbohydrate content (main effect of diet, $P > 0.05$) (Fig. 5D). A significant interaction was not detected between photoperiod and diet in epididymal fat weight.

Fig. 1. Temporal changes in body weight and the final fat mass in Fischer 344 (A–C) and Wistar (D–F) rats maintained under long- (LD) and short-day (SD) conditions. A and D: temporal changes in body weight. B and E: weekly body weight gain. C and F: final epididymal fat mass normalized by body weight. Body weight on each day and body weight gain at each week were compared by Bonferroni multiple comparison tests ($^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. the respective LD value). Effects of photoperiod on fat mass were analyzed by Student’s $t$-test ($^{**}P < 0.01$). Values are means ± SE ($n = 5–6$).
there was no significant interaction between photoperiod and diet.

**SOCS3** mRNA expression exhibited a significant interaction between photoperiod and dietary fat/carbohydrate (**P**/H11021 0.01; Fig. 6C). Whereas dietary composition did not significantly affect **SOCS3** mRNA levels under the LD condition, the HFD lowered them significantly under the SD condition compared with the LFD (25% lower with HFD, **P**/H11021 0.05; Fig. 6C).

**DISCUSSION**

**Experiment 1**

Body weight and epididymal fat increased under the LD condition in young Fischer 344 rats (5–8 wk old), whereas they were not affected by the photoperiod in the same-age Wistar rats. The results in the Fischer 344 rats are concordant with those of previous studies (8–10, 25). Although Wistar rats are generally known to be insensitive to photoperiod, those exposed to LD condition for the longer period (190 days after weaning) exhibit significantly greater body weight than those exposed to short photoperiods (5). In this study, Wistar rats were exposed to LD or SD conditions for 3 wk, and body weight and epididymal fat mass were not changed with the photoperiods. Therefore, Fischer 344 and Wistar rats are useful models as photoperiodic and nonphotoperiodic strains, respectively, for studying food selectivity and the mechanisms underlying energy metabolism. Nota-
bly, the inhibitory effects of the SD condition on growing body weight are highly observed until 10–12 wk after weaning in Fischer 344 rats, although adult rats have responsiveness of body weight to the SD condition (25). Metabolism and growth of young Fischer 344 rats are also sensitive to photoperiod in terms of molecular levels, as shown by genome-wide analysis using hypothalamus of young Fischer 344 rats (22). Similarly, young animals exhibit a high sensitivity to photoperiod in multiple species, e.g., cotton rats (12). Thus, it is noted that the focus of our study is the metabolic properties during the young growing period but not during adulthood.

Under the LD condition, Fischer 344 rats consumed more of the LFD than the HFD; meanwhile, those under the SD condition consumed equivalent amounts of both the LFD and HFD. As a result, total calorie intake was significantly greater under the LD condition, in which both body weight and fat mass increased. These data demonstrate that photoperiod influences dietary self-selection in response to a photoperiod-stimulated requirement for calorie intake. Interestingly, when calorie intake from macronutrients was calculated in the present study, carbohydrate intake was much higher under the LD condition, whereas fat energy intake was approximately constant (100 kcal/wk) in both photoperiods. Preferences for carbohydrate-rich diets are also reported in young growing LOU rats, which prefer carbohydrates to fat and protein compared with middle-aged and old rats (32). These preferences are due presumably to the low efficiency of high-fat diets for

Fig. 3. Temporal changes in total and macronutrient energy intake per week in Fischer 344 (A–D) and Wistar (E–H) rats maintained under LD and SD conditions. A and B: total energy intake (kcal/wk). B and F: carbohydrate energy intake (kcal/wk). C and G: fat energy intake (kcal/wk). D and H: protein energy intake (kcal/wk). Effects of photoperiod were analyzed by 2-way ANOVA, and significant main effects of photoperiod were detected in total, carbohydrate, and protein energy intakes in Fischer 344 rats (P < 0.05). Values are means ± SE (n = 5–6).
body weight gain; a previous study shows that low-carbohydrate high-fat diets reduce body weight gain and insulin-like growth factor I and growth hormone levels in plasma compared with those provided standard laboratory chow (6). Therefore, Fischer 344 rats housed under the LD condition might have eaten the LFD voluntarily to increase their growth in response to the stimulatory effects of the long photoperiod. Meanwhile, exposure to the SD condition resulted in rats balancing LFD and HFD consumption to optimize their growth. These changes in dietary preferences would also be important in natural conditions in which photoperiod gradually changes, since body weight gain in Fischer 344 rats and Siberian hamsters responds strongly to the gradually increasing/decreasing photoperiod (7, 8). In addition, cold ambient temperature also fluctuates with seasons, and cold ambient temperature increases fat preferences in Wistar rats (1). Thus, photoperiod and temperature may synergistically regulate the dietary preferences in natural conditions.

In contrast to Fischer 344 rats, Wistar rats preferred the LFD to the HFD irrespective of photoperiod. A previous study showed that Wistar rats provided a LFD exhibit reduced body weight gain but increased epididymal fat (%total body weight) compared with those provided standard laboratory chow (6). In addition, previous studies suggest the strong preference of LFD by Wistar rats since these rats prefer protein and carbohydrates to fat among self-selected diets (i.e., carbohydrates, fat, and protein) (16). Similarly, when nonphotoperiodic Sprague-Dawley rats are allowed to self-select from pure macronutrient diets, most consume a diet rich in carbohydrates (26). Thus, preference for LFD appears to be one of the properties of nonphotoperiodic rats. Combining the results of the Wistar and Fischer 344 rats, Fischer 344 rats housed under the LD condition exhibited the phenotype of Wistar rats, whereas exposure of Fischer 344 rats to the SD condition induced specific effects on food preferences. A previous study showed that the exposure of Fischer 344 rats to the SD condition suppresses the
expression of type 2 deiodinase, a thyroid hormone-activating enzyme, in the mediobasal hypothalamus, whereas it is constitutively high irrespective of photoperiods in Wistar rats (33). The thyroid hormone signaling in hypothalamus may be involved in the photoperiodically regulated food preferences, since hyperthyroidism increases the carbohydrate consumption in humans (20).

The plasma concentrations of leptin and corticosterone and mRNA levels of SOCS3 in the hypothalamus examined in the middle of the light phase after overnight fasting did not change with the photoperiod in either Fischer 344 or in Wistar rats. These data are reasonable for Wistar rats, since photoperiod did not alter their body weight, body fat mass, energy intake, or food selectivity. However, in Fischer 344 rats, exposure to the LD condition increased epididymal fat, which secretes leptin into the plasma. Although the reasons for this discrepancy remain unclear, the fasting time (16 h) prior to the plasma sampling might have been too long to accurately detect differences. The results regarding SOCS3 mRNA levels in the hypothalamus might also be a consequence of overfasting or the secondary effects of leptin. Alternately, these parameters might have diurnal variations that are regulated by photoperiod and/or fasting (19, 23), and the sampling time point in this study might not detect the differences occurred at different time points.

Experiment 2

Similar to the results of experiment 1, body weight and epididymal fat were higher in young Fischer 344 rats under the LD condition than under the SD condition. On the other hand, body weight gain was not affected by dietary composition under either photoperiod, although energy intake was greater in the rats provided the LFD compared with those provided the HFD under the SD condition. Consequently, the efficiency of food energy conversion to body weight gain was lower in rats provided the LFD under the SD condition; that is, energy metabolism was affected by the

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Fig. 5. Temporal changes in body weight (A), weekly body weight gain (B), food intake (g/wk; D), and total energy intake (kcal/wk; E) as well as final fat mass normalized by body weight (C) and feed efficiency (F) in Fischer 344 rats fed low- or high-fat diet under LD (LD-low and LD-high) or SD conditions (SD-low and SD-high). Effects of photoperiod and diet were analyzed by 2-way ANOVA (**P < 0.001), followed by Bonferroni multiple comparison tests (significance indicated by different letters). Values are means ± SE (n = 6).
interaction between photoperiod and dietary macronutrient composition.

Leptin secreted from white adipose tissue is a major regulator of body weight and has inhibitory effects on food intake (24). In this study, plasma leptin concentrations in the middle of the light phase after 8 h of fasting were significantly higher in Fischer 344 rats under the LD condition than those under the SD condition. This result is concordant with the greater epididymal fat under the LD condition. Experiment 2 employed 8-h fasting before plasma sampling since experiment 1 with 16-h fasting could not detect the differences in leptin levels in Fischer 344 rats despite higher mass of fat under the LD condition. Although these data suggest that food and energy intake are suppressed under the LD condition, they were higher in the LD rats than the SD rats. This discrepancy may be due to the upregulation of leptin resistance under the LD condition. Therefore, we examined the hypothalamic expression levels of SOCS3, which is related to photoperiod-driven leptin resistance (3, 4, 31). However, SOCS3 expression in the middle of the light phase did not exhibit significant changes with respect to photoperiod. In addition, rats under the LD condition exhibited lower levels of corticosterone, which is reported to increase body weight and leptin levels (13). Since leptin signaling system and plasma corticosterone levels have diurnal variations regulated by photoperiod and/or fasting (19, 23), more frequent sampling time point throughout 24 h is needed to clarify the precise mechanisms. Nevertheless, it is of note that SOCS3 expression was higher with HFD feeding under the SD condition but not under the LD condition. This appears to indicate a specific interaction between the SD and HFD, since HFDs generally increase SOCS3 expression in the arcuate nucleus, as observed in C57BL/6J mice (18). Furthermore, in our study, total energy intake was lower and feed efficiency higher with HFD feeding only under the SD condition. These specific alterations of energy homeostasis as a result of the HFD diet under the SD condition may be attributable to the altered SOCS3 expression.

Conclusion

The dietary preferences of young growing Fischer 344 rats were modulated by photoperiod, presumably in response to the altered energy requirements from specific macronutrients. Dietary carbohydrate content is likely to be a major determinant in these preferences, whereas fat content can be balanced between photoperiodic conditions. These differences were not observed in nonphotoperiodic Wistar rats. When Fischer 344 rats were forced to eat either the LFD or HFD, related gene expression in the hypothalamus (i.e., SOCS3), and feed efficiency were regulated by the interaction between photoperiod and dietary composition. These data imply that seasonal mammals can select required nutrition according to environmental changes; this may be related to the seasonal cycle of nutrition consumption in humans (15, 20). Our data are also valuable for improving efficient animal production with the use of seasonal nutrition balance to induce maximum growth in respective seasons.
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DISCLOSURES

The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

Y.T., T.O., M.G., and S.Y. performed the experiments; Y.T., T.O., M.G., and S.Y. analyzed the data; Y.T., M.G., and S.Y. interpreted the results of the experiments; Y.T. prepared the figures; Y.T. and S.Y. drafted the manuscript; Y.T., T.O., M.G., and S.Y. approved the final version of the manuscript; Y.T., T.O., M.G., M.F., and S.Y. approved the final version of the manuscript; M.F. edited and revised the manuscript; S.Y. did the conception and design of the research.

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