Effect of Hypercaloric versus Isocaloric Lipid Diet Ration on Diurnal/Nocturnal Eating Pattern in Self-Selecting Rats

Josée Normand and Louise Thibault*

School of Dietetics and Human Nutrition, Macdonald Campus of McGill University, Ste Anne de Bellevue, Québec H9X 3V9, Canada

(Received August 17, 1992)

Summary Analyses of rats diurnal/nocturnal feeding behaviour were made according to the caloric density of the lipid ration. Adult male Sprague-Dawley rats were simultaneously offered three pure macronutrient diet rations: protein, carbohydrate, and lipid. The last was either kept isocaloric to protein and carbohydrate rations or was hypercaloric. Measurements of the food intake were recorded at 12-h intervals with respect to the dark phase and to the light phase of the circadian cycle (24 h). Rats fed the hypercaloric lipid diet ration showed a significantly higher total energy intake compared with animals fed the isocaloric lipid diet ration. Also, in the hypercaloric group, the percentage of energy ingested as protein was significantly higher during both the light and dark phases, whereas the percentage of energy ingested as carbohydrate was significantly lower during the light phase and the 24-h cycle when compared with that of the isocaloric group. These results indicate that the caloric density of the lipid diet ration has differential effects on the diurnal/nocturnal energy intake and macronutrient choice and suggest that these feeding patterns may modify the outcome of experiments using macronutrient diet rations with different caloric density.

Key Words: lipid, caloric density, diurnal/nocturnal, self-selecting, rats

In the rat, feeding is inhibited during the day and facilitated during the night. An enhanced lipolysis that provides free fatty acids from the adipose reserves indirectly modulates the diurnal inhibition of feeding, whereas enhanced nocturnal lipogenesis facilitates feeding [1, 2]. Conversely, rats allowed to select their diet from separate sources of carbohydrate, protein, and fat usually ingest predominant-
ly carbohydrate at the beginning of the dark phase, which selection then progressively decreases, and eat more protein and fat than carbohydrate during the light period [3, 4].

Since Richter's [5] pioneering work on rats allowed to select their own diets from pure nutrients, several studies have been conducted to understand the sensory and metabolic aspects of dietary selection of specific macronutrients. Among these studies, the macronutrient diet rations were kept isocaloric or not, depending upon the fat ration, which varied the most in this regard. The caloric density of the diet rations is probably one of the experimental variables relating to the inconsistencies that we noted upon reviewing results of experiments on diet selection. The goal of the present study was therefore to examine rats maintained on pure sources of macronutrients, carbohydrate, protein, and fat, using fat rations isocaloric or hypercaloric to the carbohydrate and protein rations. The specific objectives of this study were to determine whether energy intake and patterns of macronutrients selection vary in relation to the energy density of the fat ration and to analyze the patterns of intake in relation to the diurnal/nocturnal cycle.

MATERIALS AND METHODS

Animals and diets. Twenty-one adult male Sprague-Dawley rats (Charles River Laboratories, St-Constant, Canada) initially weighing 225-275 g were used in the experiment. The rats were individually housed in a room maintained at 23°C with an automatic light-dark cycle (light from 08:00 to 20:00). Food and tap water were supplied ad libitum. After a seven-day adaptation period to the environment and to a single casein diet, the animals were randomly divided on the basis of body weight. The two experimental groups were simultaneously presented with three diets: a high carbohydrate ration, a high protein ration, and a high lipid ration.

Table 1. Composition of diets (dry weight, g/100 g diet).*

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Single diet</th>
<th>Isocaloric Carbohydrate</th>
<th>Protein</th>
<th>Lipid</th>
<th>Hypercaloric Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>15</td>
<td>92.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextrin</td>
<td>55.9</td>
<td>77.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>0.5</td>
<td></td>
<td></td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Vegetable shortening</td>
<td>1.5</td>
<td></td>
<td></td>
<td>30.3</td>
<td>68.3</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>53.6</td>
<td>2</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>7.6</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>Chlorine chloride</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Total (g)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Energy density (kcal/g)</td>
<td>3.88</td>
<td>3.81</td>
<td>3.81</td>
<td>3.81</td>
<td>6.98</td>
</tr>
</tbody>
</table>

* Prepared in our laboratory using ingredients supplied by ICN Nutritional Biochemicals (Mississauga, Ontario, Canada).
One group consisting of 11 rats was given isocaloric diets while the other group of 10 rats was offered hypercaloric lipid ration in addition to carbohydrate and protein diets. The composition of the diets are described in Table 1.

During 2 weeks of ingestion of the different diets, macronutrient diet ration intake was measured at 08:00 (end of dark phase) and at 20:00 (end of light phase). Water intake as well as body weight were recorded every 2 days.

Statistical analysis. Results were analyzed using Student’s t-test to compare the consumption of food and water as well as body weight of the two experimental groups of animals.

RESULTS

Energy intake

Energy intake of rats fed hypercaloric and isocaloric lipid diet rations is shown in Fig. 1. Even though the two groups showed the same day-to-day pattern of energy intake, the hypercaloric group tended to exhibit a greater energy consumption. As shown in Fig. 2, in both groups of animals, the mean energy intake was significantly higher \((p<0.001)\) during the 12-h dark than during the 12-h light phase. Although no significant differences were observed between the mean energy intake of rats fed hypercaloric and isocaloric lipid diet rations within 12-h light or 12-h dark phases, the isocaloric group displayed a significantly lower \((p<0.05)\) energy intake than the hypercaloric one on a 24-h basis.

Vol. 14, No. 1, 1993
Mean intakes of the three macronutrients (grams of carbohydrate, protein and lipid) during the 12-h dark phase, the 12-h light phase, and 24-h cycle are presented in Fig. 3. In both groups of animals, intakes of the three macronutrients were significantly higher during the 12-h dark phase than during the 12-h light phase (Isocaloric group: carbohydrate and protein, p<0.001; lipid, p<0.01. Hypercaloric group: carbohydrate, protein and lipid, p<0.001). Although not statistically significant, rats from the isocaloric group consumed 28% more carbohydrate than rats from the hypercaloric group during the 12-h light phase. The protein intake of rats fed the isocaloric lipid diet ration was significantly lower than that of rats fed the hypercaloric lipid diet ration during both light and dark phases (p<0.05) and 24-h cycle (p<0.001). No significant difference between the two experimental groups was found regarding their lipid consumption.

The percentage of total energy taken as carbohydrate (%E-carbohydrate), protein (%E-protein), and lipid (%E-lipid) is presented in Fig. 4. In both groups of animals, a significant difference was observed in the percentage of total energy represented by the three macronutrients between the 12-h light and the 12-h dark phases. Rats fed the isocaloric lipid diet ration consumed a significantly higher %E-carbohydrate during the 12-h light phase (p<0.05) and the 24-h cycle (p<0.01) in comparison to those fed the hypercaloric lipid diet ration. On the other hand, rats fed the hypercaloric lipid diet ration consumed more %E-protein than animals fed the isocaloric diet ration during both the dark (p<0.05) and the light (p<0.01) phases, as well as over the 24-h cycle (p<0.001). The 12-h light phase was marked by the isocaloric group of rats consuming 27% more energy as lipid than the hypercaloric group.
Fig. 3. Mean intakes of carbohydrate, protein, and lipid during the 12-h dark and 12-h light phases, and 24-h cycle. Values are expressed as means± SEM. * means significantly different from hypercaloric group at $p<0.05$; **, $p<0.01$. ++ means significantly different from the corresponding 12-h phase value at $p<0.01$; +++, $p<0.001$. ■ isocaloric; □□□□ hypercaloric.
Fig. 4. Mean percentage of total energy taken as carbohydrate, protein, and lipid during the 12-h dark and 12-h light phases, and 24-h cycle. Values are expressed as means±SEM. • means significantly different from hypercaloric group at $p<0.05$; **, $p<0.01$; ***, $p<0.001$. □ isocaloric; □□□ hypercaloric.
Water intake

There was no significant difference in water intake between the two experimental groups of rats. In fact, rats fed the hypercaloric lipid diet ration consumed about 11.6% more water than those fed the isocaloric lipid diet ration (Hypercaloric: 86.4±3.8 ml/2 days; Isocaloric: 77.4±3.5 ml/2 days).

Body weight

Variations in body weight of animals are presented in Fig. 5. A gradual increase in body weight of the two groups of rats was observed throughout the experimental period. Although not statistically significant (p >0.05) the increase in body weight tended to be more pronounced in rats fed the hypercaloric lipid diet ration than in those fed the isocaloric diet one. Indeed, the hypercaloric group had an average weight gain of 2.8±1.0 g/day in comparison to 1.7±0.8 g/day for the isocaloric group. At the end of the experiment, body weight of rats fed isocaloric lipid diet ration and those fed hypercaloric lipid diet ration were established at 331.1±12.5 g and 346.6±14.1 g, respectively.

DISCUSSION

The present results demonstrate that the use of a high-caloric density lipid ration modifies energy intake and macronutrient selection when compared with an isocaloric lipid ration. Rats fed the hypercaloric lipid diet ration exhibited a greater mean caloric consumption on a 24-h basis than rats fed the isocaloric lipid ration. The most dramatic alterations in macronutrient intake by the use of the hypercaloric lipid ration were the promotion of protein intake during both 12-h dark
and light phases and the depression of carbohydrate intake during the 12-h light phase and on a 24-h basis.

The use of separate sources of the three macronutrients and substitution of an isocaloric fat ration for a high-caloric fat ration in another study led to a decrease in percent fat and protein intakes and an increase in carbohydrate intake [6]. The discrepancy between the results of the latter study and the present one regarding fat intake may be due to the type of fat employed (vegetable fat and safflower oil in [6] vs. vegetable fat and soybean in our study). Indeed, simply altering the type of fat available led to modifications in fat intake [7]. The present results, however, extend the previous findings [6, 7] showing that rats fed a high fat ration selectively increase their protein intake during the light and dark phases but decrease their intake of carbohydrate during the light phase only when compared with rats offered an isocaloric-fat ration.

In rodents fed ad libitum, the circadian rhythm of feeding behavior is determined primarily by the light-dark schedule. Being nocturnal animals, rats ingest nearly 70 to 80% of their total 24-h food intake during the dark phase [8]. Our results reveal a normal circadian pattern of feeding considering that around 31% of the total daily energy intake of the two experimental groups was consumed during the 12-h light period and around 69% during the 12-h dark period. The present work also showed variations in pattern of macronutrient intake in regard to the light-dark cycle. A study done in rats offered three pure macronutrient diets using isocaloric carbohydrate (dextrin, cornstarch, and sucrose) and protein (casein) diets and a hypercaloric lipid diet (lard) showed clear differences in macronutrient intake between the 12-h dark and 12-h light phases expressed as a preference for carbohydrate relative to protein during the night, while lipid and protein were preferred during the day [3]. Considering our results, the use of isocaloric macronutrients rations induced a preference for carbohydrate relative to protein and fat during both light and dark phases, whereas a protein and carbohydrate preference relative to fat was found during the day and the night in rats fed the hypercaloric lipid ration. Differences in the composition of the carbohydrate and lipid diet ration between our study and the latter may have led to these different diurnal nocturnal feeding patterns.

A higher total caloric intake with the use of single composite high-fat diets has been reported [9–11]. This is in agreement with the present work showing a higher consumption of total energy for the rats fed the hypercaloric lipid diet. However, the study of Ramirez [12] comparing three high-fat diets (42, 58, and 63% of calories derived from fat) showed that only the 42% fat diet (containing also 41% carbohydrate) promoted a higher caloric intake in rats. On the other hand, Oscai et al. [13] failed to show a difference in caloric intake with the use of three high-fat diets (42, 50, and 60% of calories derived from fat). In the present study, the concentration of the lipid-rich ration was 41.3% for the isocaloric group and 88.3% for the hypercaloric group of animals; the latter showed a higher total caloric consumption than the former. The discrepancy between our results and
those of Oscai et al. and Ramirez may be attributed to the use of dietary selection in the present experiment versus their use of a single diet that the animals needed to ingest completely in order to fulfill particular nutrient requirements.

It is known that in rats food intake is adjusted when the energy density of the diet is altered [14, 15]. In a classic experiment on dogs, Cowgill [16] reported that animals regulate their daily energy intake by caloric adjustment (i.e., by eating fewer grams of the high caloric ration) to maintain the same number of calories consumed per day. Further studies done on rats [14, 17] supported the concept of energy regulation by subjecting the animals to diets of different caloric density; as a result, rats maintained their daily energy intake by either increasing the frequency and/or the size of their meals of the lower density or decreasing their meals of high caloric density. In the present work, the rats did compensate for the caloric density of the lipid diet rations such that the lipid intake as well as the percentage of total energy taken as lipid was similar when either an isocaloric or a hypercaloric lipid ration was offered. In order to make this compensation, rats from the isocaloric group had to ingest more of the lipid diet ration and, by the way, more alphacel (cellulose), which was used to dilute energy of the isocaloric lipid diet ration. In parallel to this high-fiber consumption rats fed isocaloric diet rations had a lower energy intake mainly attributable to a decreased protein intake. In a human study conducted by Jenkins [18], fiber such as cellulose has been shown to reduce the activity of pancreatic trypsin in protein digestion. It has also been reported that fiber ingested in high quantities interferes with the rate of absorption of protein [19, 20]. The protein digestibility as well as the protein efficiency ratio is diminished, while protein requirements are increased, in response to added cellulose in the diet [20]. In addition to the effects of fiber on macronutrient intake, reduced activity of amylase and lipase were reported [18, 21], implying a possible role of fiber in carbohydrate and lipid intakes reported in the present study.

The sensory characteristics of diets such as its smell, taste or texture may play a major role in nutrient intake and regulation [9, 22, 23]. A variety of those properties contributes directly to the high palatability of the diet rations [24]. Forbes [23] suggested that the hedonic properties of high-fat diets are more important than the metabolic effects in stimulating intake. In the present study, the hypercaloric lipid diet ration was distinguished by its stronger smell compared to the isocaloric lipid ration. However, no difference was observed in the intake of lipid between the two experimental groups despite the apparent differences in sensory characteristics between the two lipid diet rations.

The present results showed that the feeding patterns of macronutrient selection are altered by the composition of the lipid ration. Further, they suggest re-examination of the discrepancies in the literature on the control of food intake and diet selection in light of the energy density and composition of the diet rations utilized.

Vol. 14, No. 1, 1993
The authors gratefully acknowledge Yuan Hua Wen and Nathalie Brassard for their technical assistance. This research was supported by the Natural Sciences and Engineering Research Council of Canada.

REFERENCES


