Diurnal Changes in Glycogen Stores in Liver and Skeletal Muscle of Rats in Relation to the Feed Timing of Sucrose

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Summary  Diurnal changes in tissue glycogen stores were determined in rats in relation to the feed timing of sucrose. Rats were daily meal-fed on a 35% sucrose diet at 20.00–21.00 and a basal diet at 08.00–09.00, or meal-fed on the same diets at the reversed time for 7 weeks. Half of the animals were allowed voluntary wheel-running between 21.00–08.00, but the remaining animals were restricted to exercise for 24 h. At the end of the feeding period, both groups of rats were killed at 4-h intervals. Glycogen stores in liver and soleus muscle of sedentary rats showed sharp increases over 8 h after the sucrose meal regardless of its feed timing. The increases continued for only 4 h in exercised rats given the sucrose diet at the evening meal time. In contrast, glycogen stores in these tissues did not show any noticeable increase after the basal diet regardless of its feed timing. Thus, the feeding of sucrose at the evening meal time seems to be more preferable than at the morning meal time in order to have higher glycogen stores in liver and skeletal muscle during the physically active phase of the day in rats. Diurnal changes of glycogen contents in heart and adipose tissue differed from those in liver and skeletal muscle.

Key Words  tissue glycogen store, diurnal change, sucrose feed timing, exercised rat

Glycogen stores in liver and skeletal muscle have been well recognized to influence physical performance. To accumulate much glycogen in these tissues prior to physical competitions, glycogen loading regimens (1–5) have been developed and preferentially used by top athletes engaging in endurance types of sports, such as marathon and nordic skis, etc. However, since the program of a glycogen loading is taken about 5–7 days prior to a competition, this nutritional treatment is hard to apply to athletes who have frequent games in a day or a few days during the several

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day competition period. Thus, it is important to develop nutritional programs which can stimulate the tissue glycogen accumulation in a short period, with peaks at around the competition hour (6, 7).

The present experiments were undertaken to search for the effective feed timing of sucrose on glycogen stores in liver and skeletal muscle. For the purposes, diurnal changes in tissue glycogen levels were determined in rats controlled using either the feed timing of a sucrose meal or the period of physical activity. The present results indicated that glycogen stores in both liver and skeletal muscle reached peak levels 8 h after the ingestion of the sucrose meal regardless of its feed timing.

EXPERIMENTAL

Two separate experiments were conducted with 5–6 weeks old male JCL-Sprague-Dawley rats (CLEA Japan Inc., Tokyo). A sucrose diet was prepared by adding sucrose to a basal diet (CE-7 powder, CLEA Japan Inc.) at a 35% (w/w) level (8), resulting in 74% carbohydrate level for the sucrose diet. Rats were individually housed in ordinary cages (sedentary rats) and rodent activity cages (exercised rats) in a room maintained at 23 ± 1°C and lightened between 07.00–19.00. They were meal-fed on the sucrose diet at 20.00–21.00 and the basal diet at 08.00–09.00 in experiment 1 (80 rats) and meal-fed on the two diets at the reversed time of experiment 2 (80 rats). Water was fed ad libitum. In both experiments, exercised groups of 40 rats were allowed to run voluntarily in wheels between 21.00–08.00, but sedentary groups of 40 rats were restricted to exercise for 24 h.

Body weight, food consumption and voluntary running activity were measured every day. After 7 weeks of feeding, 5–6 rats of both groups were killed by decapitation at 08.00, 12.00, 16.00, 20.00, 24.00 and 04.00 in experiments 1 and 2. Diurnal changes in concentrations of plasma glucose (9), free fatty acids (FFA) (10), and glycogen contents (11) in liver, soleus muscle, heart and perirenal adipose tissue were measured.

Data were statistically analyzed using Student’s t-test.

RESULTS

Weight gain, food consumption and voluntary running activity

Weight gain was significantly lower in exercised rats than in sedentary rats; sedentary vs. exercised = 258 ± 5 vs. 241 ± 6 g/7 weeks (mean ± SEM), p < 0.05 in experiment 1 and 245 ± 4 vs. 220 ± 5, p < 0.001 in experiment 2. However, food consumption during the feeding period was greater in exercised rats in experiments 1 and 2; sedentary vs. exercised = 3,649 ± 46 vs. 3,803 ± 43 kcal/rat/7 weeks, p < 0.05 in experiment 1 and 3,778 ± 51 vs. 4,091 ± 51, p < 0.001 in experiment 2. Voluntary running activity of exercised rats was 3–7 km/day in both experiments. Rats were highly active during a few hour either after the evening meal or before the morning meal.

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Table 1. Food consumption of sedentary and exercised rats at the final meal served before sacrifice (experiments 1 and 2).

|                  | Meal time | Kill time | Meal time | Kill time |
|------------------|-----------|-----------|-----------|-----------|
|                  | 20.00-21.00 | (h) | 08.00-09.00 | (h) |

**Sucrose diet**

| Group           | Basal diet (g) | Sucrose (g) | Basal diet (g) |
|-----------------|----------------|-------------|----------------|
| Sedentary       | 9.1 ± 0.8      | 4.9 ± 0.4   | 7.6 ± 0.9      |
| Exercised       | 11.1 ± 1.1     | 6.0 ± 0.6   | 10.9 ± 0.5     |
| Sedentary       | 10.4 ± 1.4     | 5.7 ± 0.8   | 8.6 ± 1.6      |
| Exercised       | 9.9 ± 1.9      | 5.3 ± 1.0   | 11.5 ± 0.8     |
| Sedentary       | 10.4 ± 0.7     | 5.6 ± 0.4   | 9.5 ± 1.4      |
| Exercised       | 9.6 ± 0.6      | 5.2 ± 0.3   | 10.2 ± 1.7     |

**Experiment 2**

| Group           | Basal diet (g) | Basal diet (g) | Sucrose (g) |
|-----------------|----------------|----------------|-------------|
| Sedentary       | 10.6 ± 1.1     | 10.1 ± 0.7     | 5.4 ± 0.4   |
| Exercised       | 9.6 ± 1.7      | 11.9 ± 1.2     | 6.4 ± 0.7   |
| Sedentary       | 7.6 ± 1.5      | 10.9 ± 1.5     | 5.9 ± 0.8   |
| Exercised       | 8.9 ± 1.5      | 11.2 ± 1.2     | 6.1 ± 0.6   |
| Sedentary       | 8.6 ± 1.1      | 11.3 ± 1.4     | 6.1 ± 0.7   |
| Exercised       | 8.5 ± 2.0      | 11.5 ± 0.3     | 6.2 ± 0.2   |

Values are means ± SEM for 5–6 rats of each group. *Sucrose diet was prepared by mixing a basal diet and sucrose in the ratio of 65 to 35% (w/w), and the consumption of the basal diet and sucrose was calculated, respectively.

Table 1 shows food consumption at the final meal served to rats before being sacrificed. In experiment 1, consumption of the sucrose diet was equal in both groups, but consumption of the basal diet fed at the morning meal time was higher in exercised rats than in sedentary rats. However, in experiment 2, both groups of rats consumed almost equal amounts of food.

**Diurnal changes in plasma glucose and FFA concentrations**

Feeding of the sucrose diet resulted in increased plasma glucose levels and decreased plasma FFA levels, regardless of its feeding timing (Fig. 1). On the other hand, the effect of the basal diet was not consistent on plasma glucose and FFA concentrations.
Fig. 1. Diurnal changes in concentrations of plasma FFA and glucose and in glycogen contents in liver, soleus muscle, heart and perirenal adipose tissue of sedentary and exercised rats meal-fed alternately on a basal diet and a 35% sucrose diet at different timing for 7 weeks. Each point and vertical bar represent mean and SEM for 5-6 rats, respectively. □, basal diet; ☐, sucrose diet; *, significant difference (p<0.05) between sedentary and exercised rats; ---○, sedentary rats; ----●, exercised rats.

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In both groups of rats in experiments 1 and 2, liver, soleus muscle and adipose tissue showed marked increases in glycogen store after the ingestion of the sucrose diet (Fig. 1). However, such noticeable increases in glycogen stores were not observed after the ingestion of the basal diet except for the soleus muscle in experiment 1. Voluntary exercise inhibited the sucrose diet-induced glycogen repletion in liver and soleus muscle during 24.00–04.00. Contrarily, it was interesting to find that adipose tissue glycogen repletion after the ingestion of the sucrose diet was considerably enhanced with voluntary exercise.

Glycogen stores in heart were not increased with the ingestion of the basal and sucrose diets in sedentary rats of experiments 1 and 2. During the voluntary running period, heart glycogen stores were considerably higher in exercised rats than in sedentary rats in both experiments.

**DISCUSSION**

Glycogen stores in liver, skeletal muscle, heart and adipose tissue of rats have been reported to show diurnal changes in which phase and magnitude could be influenced by feeding patterns (12–19). Our laboratory work (20) indicated that meal feeding at the initial 2 h of the dark period increased glycogen stores in the above tissues and also strongly influenced the diurnal changes in the tissue glycogen stores in rats. The animals in these studies, however, were fed ad libitum or meal-fed once a day, which is a far different feeding pattern from that of humans who usually take two or three meals in a day. Therefore, in the present study the feeding pattern of two meals a day was employed in order to get more applicable nutritional informations on tissue glycogen repletion for athletes.

To obtain high glycogen storages in liver and skeletal muscle at around the competition hour, the present results seem to indicate that sucrose might be more preferable to be taken at the breakfast time of the competition day rather than at the dinner time of the preceding day.

In experiment 2, the amounts of final food consumed by both groups of rats were larger in the sucrose diet than in the basal diet. This might partly be concerned with a greater glycogen accumulation into tissues with the sucrose diet than the basal diet. On the other hand, in experiment 1, the final food consumption was almost equal for the two diets in both groups, thus, it seems that sucrose might be responsible for a stimulated glycogen accumulation into tissues after the ingestion of the sucrose diet.

In the present study, glycogen stores in heart showed different diurnal changes from those in liver and soleus muscle. Interestingly, exercised rats had higher glycogen stores at any time point examined than sedentary rats, regardless of the timing of sucrose meal feeding. In addition, in exercised rats of experiments 1 and 2, heart glycogen stores during the period of exercise were larger than those during the period of restricted physical activity. The present results agree with previous works...
from our laboratory (20) and others (21, 22) who have reported higher glycogen contents at the dark-time than at the light-time during the 24-h feeding cycle. This might be related with findings that fatty acids are oxidized in preference to carbohydrates in heart at both low (23–25) and high (25–27) levels of work. As a consequence, not only glycogen sparing but also glycogenesis enhancement with the elevated concentrations of stimulants for glycogen synthetase (28), such as glucose-6-phosphate, citrate, etc., might have occurred in heart.

Perirenal adipose tissues of exercised rats meal-fed the sucrose diet in the morning showed a sharp increase in glycogen stores and maintained significantly higher glycogen levels during the physically inactive period than those of the relating sedentary rats. Since there was no difference in the sucrose meal consumption between the two groups, the sucrose meal-induced glycogenesis enhancement in the adipose tissues of exercised rats might be due to the direct effects of exercise before the final sucrose meal and/or to adaptation to the chronic voluntary exercise. On the other hand, perirenal adipose tissues of sedentary rats meal-fed the sucrose diet in the morning showed elevated glycogen stores during physically inactive periods. This was not observed in sedentary rats meal-fed the sucrose diet at the evening meal time. Lipogenesis activity and glycogen storage levels have been reported to be positively related with adipose tissue of rats (12). Thus, sucrose consumption at the timing preceding to physically inactive period would enhance body fat accumulation during the phase of low energy expenditure.

In conclusion, the present data have shown that the glycogen peaks in liver and skeletal muscle could be obtained about 8 h after the sucrose ingestion in rats. Furthermore, as we have indicated in the previous studies (8, 29, 30), the present findings also suggest the importance of the feed timing of nutrients, in relation to their nutritional effects on metabolisms and physiological conditions of animals.

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