Enhanced Glycogen Repletion in Liver and Skeletal Muscle with Citrate Orally Fed after Exhaustive Treadmill Running and Swimming

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Summary A possibility whether an oral feeding of citrate, which has been reported to inhibit phosphofructokinase in vitro, following exercise to exhaustion could increase the rate of glycogen repletion in liver and soleus muscle was tested in treadmill running trained (experiments 1 and 2) and swimming trained rats (experiment 3). An exhaustive running or swimming was loaded at the end of the experiments, resulting in a significant reduction in liver and soleus muscle glycogen stores. The feeding of 1.0 and 0.5 g of citrate per kg of body weight just after the exhaustive running could significantly increase the liver glycogen repletion during a 2-hr recovery period, but this was not observed in soleus muscle (experiment 1). As compared with a single feeding of 3.3 g of glucose per kg of body weight, a mixed feeding of 0.5 g of citrate and 3.0 g of glucose after an exhaustive running (experiment 2) and swimming (experiment 3) could significantly enhance the repletion of glycogen stores in both liver and soleus muscle. These results clearly indicate that the postexercise feeding of citrate can stimulate the glycogen repletion in liver and skeletal muscle during an early period of recovery.

Key Words treadmill running, swimming, exhaustive exercise, glycogen repletion, liver and skeletal muscle, citrate feeding, rat

The importance of liver and muscle glycogen stores to physical performance in both sprint and endurance exercises is generally recognized (1–3). In most of sport events two competitions are held in a day, morning and afternoon. Therefore a rapid recovery of liver and muscle glycogen stores, of which larger parts might be utilized with the morning competition, is needed to have a good result in the...
afternoon competition.

The replenishment of liver glycogen depleted with heavy exercise could be obtained easily (4), however, with muscle glycogen it has been reported to take over 24 (5), 48 (6), and more than 72 hr (7) in man.

*In vitro*, citrate has been known to inhibit the activity of phosphofructokinase [EC 2.7.1.11] in the liver (8, 9), muscle (10, 11), and other tissues (12). Thus, the ingestion of citrate could promote glycogenesis in animal tissues through inhibiting glycolysis during the recovery period. However, this possibility has not been elucidated yet.

The purpose of this study was to evaluate the effects of postexercise feeding of citrate on liver and muscle glycogen repletion.

**EXPERIMENTAL**

*Animal care.* Sprague-Dawley female rats of 4 weeks (experiment 1; 44 rats) and 5 weeks old (experiment 2; 24 rats), and male rats of 3 weeks old (experiment 3; 21 rats) were used. The rats were housed five or six per cage with controlled light (07.00-19.00 hr) and in a dark (19.00-07.00 hr) cycle. In experiments 1 and 2, rats were given a stock chow (CE-2, CLEA Japan Inc., Tokyo) *ad libitum*, and, in experiment 3, rats were meal-fed the stock chow twice a day (07.00-07.30 hr and 19.00-19.30 hr). Water was given freely in all experiments.

*Training program.* In experiments 1 and 2, rats did a running exercise between 18.00 and 22.00 hr everyday on a motor driven treadmill (13). Running speed and duration were gradually increased over 2 weeks (experiment 1) and 8 days (experiment 2) until the rats were able to run at 26 m/min up a 3° incline for 60 min/day (experiment 1) and at 32 m/min up a 8° incline for 45 min/day (experiment 2). In experiment 3, swimming exercise was used; 4-6 rats were placed together in a plastic barrel (53 cm diameter and 50 cm deep with 30-35°C water). Rats were tied with a load, corresponding to 2% of body weight, to their neck during swimming. The exercise started at 22.00 hr everyday and lasted for 30 min over 11 days.

*A final bout of exercise.* At the end of experiments 1 and 2, rats were fasted for 6 hr prior to a final bout of exercise. An exhaustive treadmill running started at 18.00 hr and lasted for 2 hr at 32 m/min up a 8° incline. In experiment 3, rats were fed from 19.00 to 19.30 hr and fasted for 2.5 hr prior to a final exercise. An exhaustive swimming started at 22.00 hr with a load, corresponding to 4% of body weight, tied to the rats’ neck. Mean swimming time was 2.5 hr.

Just before and after the exhaustive running and swimming exercise, 4 rats were sacrificed in any given experiment. In experiment 1, the remaining rats were divided into 3 groups and orally given 1 ml solution containing 1.0 g of citrate, 0.5 g of citrate or water per kg of body weight by an injection syringe, respectively. The amount of 1.0 g of citrate per kg of body weight used here was the same as used by Kuyper and Mattill (14). Four rats of each group were sacrificed 0.5, 1, and 2 hr after exhaustive running. In experiment 2, the remaining rats were divided into 2
groups and orally given 2 ml of isocaloric solution containing either 3.3 g of glucose or the mixture of 0.5 g of citrate and 3.0 g of glucose per kg of body weight, and 4 rats of each group were sacrificed 1 and 2 hr after exhaustive running. In experiment 3, the remaining rats were divided into 3 groups and orally given 2 ml of isocaloric solution containing either 3.3 g of glucose, 5.0 g of citrate or the mixture of 0.5 g of citrate and 3.0 g of glucose per kg of body weight, respectively. Four to 5 rats were sacrificed 2 hr after exhaustive swimming.

Rats were sacrificed by decapitation, and their liver and soleus muscle were rapidly dissected and weighed. The tissues were immediately frozen at \(-80^\circ\text{C}\) (experiments 1 and 2) or in liquid nitrogen (experiment 3) and stored at \(-80^\circ\text{C}\) until assay. Tissue glycogen was determined by the method of Lo et al. (15). Data were expressed as mg glycogen/g of wet tissue (experiments 1 and 2) and mg glycogen/g of dry tissue (experiment 3).

### RESULTS

**Effects of citrate feeding on glycogen repletion in liver and soleus muscle after a bout of exhaustive treadmill running (experiment 1; Fig. 1)**

The 2-hr treadmill running resulted in a significant reduction in liver (\(p<0.001\)) and soleus muscle (\(p<0.01\)) glycogen stores. Liver glycogen levels in water fed rats did not change during the 2-hr recovery period, as compared to their postexercise levels. In contrast, the rate of liver glycogen repletion was significantly faster in two groups of citrate fed rats than those in water fed rats during the 2-hr recovery period (\(p<0.05\)). Whereas, there was not found any marked difference in the rates of soleus

![Liver Glycogen](image1.png)

![Soleus Glycogen](image2.png)

**Fig. 1.** Effects of citrate feeding on glycogen repletion in liver and soleus muscle after a bout of exhaustive treadmill running (experiment 1). Each point and vertical line represent mean and standard error for 4 rats, respectively. \(\bigtriangleup\), 1.0 g citrate/kg of BW; \(\blacktriangle\), 0.5 g citrate/kg of BW; \(\bullet\), H\(_2\)O. *Significantly different from water feeding (\(p<0.05\)).
Fig. 2. Effects of the feeding of glucose or glucose with citrate on glycogen repletion in liver and soleus muscle after a bout of exhaustive treadmill running (experiment 2). Each point and vertical line represent mean and standard error for 4 rats, respectively. ●, 3.3 g glucose/kg of BW; △, 0.5 g citrate and 3.0 g glucose/kg of BW. *Significantly different from glucose feeding (p<0.05).

muscle glycogen repletion between the two groups of citrate fed rats and controls with the exception of a significance between 0.5 g of citrate and water during the 1st hr of the recovery period. The rate of soleus muscle glycogen repletion in 0.5 g of citrate fed rats was slightly faster than that in 1.0 g of citrate fed rats, but not significantly.

Effects of the feeding of glucose or glucose with citrate on glycogen repletion in liver and soleus muscle after a bout of exhaustive treadmill running (experiment 2; Fig. 2)

The 2-hr treadmill running resulted in a marked reduction in liver (p<0.01) and soleus muscle (p<0.001) glycogen stores in the same manner as experiment 1. There was not found any difference in the rates of liver glycogen repletion between glucose feeding and the mixed feeding of citrate and glucose during the 1st hr of the recovery period. Whereas, during the 2nd hr of the recovery period, the rate of liver glycogen repletion in rats fed citrate and glucose was significantly faster than that in rats fed glucose singly (p<0.05). Glycogen repletion patterns of soleus muscle were similar to those of liver, however, the difference between the single feeding of glucose and the mixed feeding of citrate and glucose was not significant during the recovery period.

Effects of the feeding of glucose, citrate or glucose with citrate on glycogen repletion in liver and soleus muscle after a bout of exhaustive swimming (experiment 3; Fig. 3)

Both liver and soleus muscle glycogen stores showed a significant reduction with the 2.5-hr exhaustive swimming (p<0.05). Glycogen repletion during the
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Fig. 3. Effects of the feeding of glucose, citrate or glucose with citrate on glycogen repletion in liver and soleus muscle after a bout of exhaustive swimming (experiment 3). Each point and vertical line represent mean and standard error for 4-5 rats, respectively. ○—△, 3.3 g glucose/kg of BW; □—○, 5.0 g citrate/kg of BW; ○—△, 0.5 g citrate and 3.0 g glucose/kg of BW. * Significantly different from glucose or citrate feeding (p<0.05).

recovery period was significantly enhanced both in liver (p<0.05) and soleus muscle (p<0.05) with the mixed feeding of citrate and glucose as compared with the feeding of glucose or citrate alone. There was found no difference in the glycogen repletion between the single feeding of glucose and citrate.

DISCUSSION

Glycogen synthesis in liver and skeletal muscle depends on the activity of key enzyme, glycogen synthetase, which is positively regulated by glucose-6-phosphate (16). In vitro, citrate has been known to inhibit phosphofructokinase activity of rat liver (8, 9) and muscle (10, 11), and also activate mammalian hexokinase isoenzyme I (17), resulting in the elevation of glucose-6-phosphate concentrations in liver and muscle. Furthermore, in vitro, citrate can stimulate glycogen synthetase (18) and inhibit glucose-6-phosphatase (19) in rat liver. Other carboxylic acids than citrate have been also reported to stimulate glycogen synthetase (18). This suggests that orally-fed citrate may be able to indirectly enhance the glycogen repletion while being metabolized through the tricarboxylic acid cycle. In addition, citrate can trap cellular Ca²⁺ (20) which has been known to decrease glycogen synthesis but increase glycogen degradation (21). Such a complex-Ca²⁺ formation by citrate may help glycogen storing in rat tissues. These seem to favorably explain...
our present results that citrate could increase in vivo the rate of glycogen store repletion in liver and skeletal muscle, whenever citrate was orally administered singly or together with glucose immediately after exhaustive treadmill running and swimming.

Glycogenesis is well known to be stimulated by insulin (16). Maske (22) reported that glucose perfusion into pancreas increased the insulin release and citrate concentration in the pancreas. Thus, orally-fed citrate could elevate citrate concentration in the pancreas and stimulated insulin secretion, and consequently enhance glycogenesis in tissues.

Although the feeding of 0.5 g of citrate per kg body weight to exercise-exhausted rats could facilitate both liver and soleus muscle glycogen repletion in experiment 1, such an effect of citrate could not be detected when 5.0 g of citrate per kg body weight was similarly administered in experiment 3. The reason(s) that produced the discrepancy is not apparent, but several differences in experimental conditions between experiments 1 and 3 might be concerned; ad libitum-feeding vs. meal-feeding, treadmill running vs. swimming, different extents of fatigue and tissue glycogen disappearance with exhaustive exercise just before the citrate feeding, etc.

In relation to the last parameter, it has been well known that glycogen synthesis is inhibited by glycogen stored in the tissue (23). When glycogen stores of liver and soleus muscle of exercise-exhausted rats were compared between these two experiments on the basis of wet tissue weight, the residual glycogen levels immediately after the exhaustive exercise were found to be 12-fold and 4-fold higher in the liver and soleus muscle, respectively, in experiment 3 than in experiment 1. Thus, the greater tissue glycogen residues in the rats of experiment 3 might weaken the effect of citrate (5.0 g/kg BW) on tissue glycogen repletion after an exhaustive exercise. Another possibility may be that citrate could facilitate tissue glycogen repletion at the dose levels less than 5.0 g per kg of body weight in rats.

In the present study, glycogen repletion after an exhaustive exercise seemed to be more rapid in liver than in soleus muscle, but its reason is not clear. One possibility is that the liver might be affected more directly with orally administered citrate as compared with soleus muscle. Henneman and Shoemaker (24), in postabsorptive resting dogs, and Nielsen and Thomsen (25), in men during the recovery after an exhaustive exercise, observed an output of citrate across the leg and, contrarily, a simultaneous uptake of citrate from plasma across the splanchnic vascular bed. Thus, there may be some differences in the manner of citrate uptake between liver and skeletal muscle. Although the activation of liver glycogen synthetase by citrate has been pointed out (18), there has not been shown such evidence in skeletal muscle.

In conclusion, the present investigation clearly shows that an orally administered citrate can facilitate the repletion of liver and muscle glycogen which are depleted by an exhaustive endurance exercise in rats.

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