Nutritional Design for Repletion of Liver and Muscle Glycogen during Endurance Exercise without Inhibiting Lipolysis

Shinichi SAITO and Masashige SUZUKI*1

Laboratory of Biochemistry of Exercise and Nutrition, Institute of Health and Sports Sciences, The University of Tsukuba, Niihari-gun, Ibaraki 305, Japan

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Summary The effects of fructose (F), a glycogen precursor, and arginine (A), a stimulator of insulin secretion, on glycogen stores in liver and skeletal muscle and lipolysis were studied in endurance exercising rats, in comparison with that of glucose (G) and A. The addition of citrate (C), which previously has been shown to stimulate liver and muscle glycogen repletion after exhaustive exercise, was also tested. Rats were meal-fed twice daily and underwent treadmill running 6 days a week for 3 weeks (experiment 1) or treadmill running for 3 days and the usual voluntary wheel running of 3 days a week for 5 weeks (experiment 2). On the final days of experiments, rats were given one of the following water solutions; a 3.3g F+0.5g A per kg bw, a 3.3g G+0.5g A per kg bw and water (experiment 1) and a 3.3g F+0.5g A+0.5g C per kg bw (experiment 2), at the 1.5h point during a 3h treadmill running course at 26 and 32.5 m/min up a 7° incline, respectively. There was a significant reduction in liver and soleus muscle glycogen during the period of exercise. The administration of either F+A or G+A during exercise was useful in preventing the further depletion of liver and muscle glycogen stores. The administration of G+A significantly suppressed an increased adipose tissue lipolysis caused by exercise, when compared with that of either F+A or water (experiment 1). The addition of C to F+A appeared to spare more soleus and liver glycogen stores during exercise, when compared with G+A (experiment 2).

Key Words endurance exercise, fructose, glucose, arginine, citrate, liver and muscle glycogen, serum IRI, serum free fatty acid, lipolysis, rat

1 斉藤 慎一, 鈴木 正成
* Reprint requests and correspondence should be addressed to M. Suzuki.

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The relationship between glycogen stores in liver and skeletal muscle and the endurance exercise has been well documented \((1, 2)\). Our recent investigations \((3, 4)\) have been conducted to study the nutritional composition of drink or meal which can lead to a rapid repletion of liver and skeletal muscle glycogen stores following exercise in rats.

It has been also reported \((5–7)\) that endurance performance can be increased by sparing muscle and liver glycogen by the use of free fatty acids \(\text{(FFA)}\) during exercise.

Glucose and many other carbohydrates which yield glucose molecules by hydrolysis are highly glycogenetic and useful for glycogen synthesis because they stimulate insulin secretion \((8)\). However, the administration of glucose is not always desirable with endurance sports, because glucose inhibits lipolysis which provides an important fuel, FFA, for contracting muscles \((9, 10)\). We have recently demonstrated \((11)\) that arginine, which is known to stimulate insulin secretion \textit{in vitro} \((12, 13)\) and \textit{in vivo} \((14, 15)\), given after an exhaustive exercise does not inhibit lipolysis. On the other hand, it has been reported that fructose has little effect on insulin secretion compared with glucose \((16, 17)\), and does not inhibit lipolysis \textit{in vivo} \((18)\).

The purpose of the present investigation is to show the effects of combined fructose and arginine given during a bout of endurance exercise on the repletion of glycogen stores in liver and skeletal muscle and lipolysis in rats. We also examined the additive effect of citrate to fructose and arginine on glycogen repletion in the liver and muscles during exercise.

**METHODS**

The experiments were conducted using 4-week old female JCL\textsuperscript{®}-Sprague-Dawley rats, weighing 65–75 g (40 rats in experiment 1 and 39 rats in experiment 2; Clea Japan Inc., Tokyo). The animals were housed five or six to a cage in a room maintained at 22 ± 1°C. The lights were kept on between 07:00–19:00h. They were meal-fed with stock chow \((\text{CE-2, Clea Japan Inc.})\) at 18:00–18:30h and 08:00–08:30h, and water was freely administered.

A running exercise between 21:00–21:20h was conducted for 6 days (experiment 1) or 3 days (experiment 2) per week on a motor driven treadmill up a 7° incline \((3)\). Running speed was gradually increased over periods of 3 weeks (experiment 1) or 5 weeks (experiment 2) until the rats were able to run at the rate of 26 or 32.5 m/min, respectively. In experiment 2, each rat was moved to an individual rodent activity cage and allowed to run voluntarily in wheels \((19)\) between 21:00–08:00h for 3 days per week during the 5 weeks training period. Daily voluntary running every week was 8,380 ± 800, 11,020 ± 1,100, 17,200 ± 1,200, 17,900 ± 1,180, and 16,000 ± 1,030 m/day \((\text{mean ± SEM})\). The weight of rats at the end of the training period was 153 ± 2 g \((\text{experiment 1})\) and 157 ± 2 g \((\text{experiment 2})\).

An outline of the time schedule for the final days of the experiments is shown in
Fig. 1. Time schedules for the last days of the experiments 1 and 2. Rats were given one of the following 3 types of test solutions (T); fructose + arginine (F+A), glucose + arginine (G+A) or water alone (experiment 1), and F+A, G+A or fructose + arginine + citrate (F+A+C) (experiment 2), at the mid-point of 3 h treadmill running. *Some of the rats stopped exercising after the administration and recovered between 22:30–24:00 h. Rats were killed at the indicated times (†).

In experiment 1, 5 rats were killed before the start of running. The treadmill running began from 21:00 h, and, after 1.5 h of running, 5 rats were killed. The remaining rats were divided into 3 groups and orally given a 2.5 ml test solution containing either 3.3 g fructose and 0.5 g arginine (F+A), 3.3 g glucose and 0.5 g arginine (G+A) per kg body weight or water alone as same as previously described (3). After the fluid administration, these rats continued to run and 5 rats from each group were killed at 23:15 h and 24:00 h.

In experiment 2, all rats started to run from 21:00 h. After 1.5 h of running, 5 rats were killed. The remaining rats were divided into 3 groups and received one of 3 test solutions; F+A, G+A or the mixture of 3.3 g fructose, 0.5 g arginine and 0.5 g citrate (F+A+C) per kg body weight in the same manner as experiment 1. After the administration, rats from each group were divided into either exercising or recovering groups. The exercising rats continued to run, but the recovering rats stopped exercising afterwards. These rats were killed at 24:00 h.

All rats were killed by decapitation. Blood was withdrawn and serum was separated. Liver and soleus muscle were removed and immediately frozen in liquid nitrogen, and stored at −80°C until an assay of glycogen (20) could be done. Serum samples were determined for concentrations of glucose (21), FFA (22), glycerol (Glycerol and Neutral Fat UV Test, Boehringer Mannheim GmbH, W. Germany), and immunoreactive insulin (IRI) (a double antibody radioimmunoassay using the
Insulin Radioimmunoassay Kit, Commissariat A L’energie Atomique, France). In experiment 1, perirenal fat pads were obtained for *in vitro* lipolysis study as described previously (4). Pieces of perirenal fat pads, weighing 80–100 mg, were incubated with mild shaking at 37°C for 60 min in 2 ml of Krebs-Ringer bicarbonate buffer, containing 2% bovine serum albumin (Fraction V, essentially fatty acid free; Sigma Co., St. Louis), pH 7.4. (-)Epinephrine(+)-bitartarate (Sigma Co.) was either not added (basal lipolysis) or added (epinephrine-stimulated lipolysis) at 5 μg/ml in the incubation medium. After incubation, the reaction was stopped by cooling the flasks in ice water for 5 min, and free fatty acid concentrations in the incubation medium were determined (21).

Statistical analysis was done by the Student’s unpaired *t*-test.

**RESULTS**

**Experiment 1**

*Serum glucose, insulin, FFA and glycerol concentrations* (Fig. 2)

The F + A administration during exercise did not show the changes in serum glucose concentration. On the other hand, the G + A administration caused a significant rise in serum glucose concentration from 146 ± 5 to 194 ± 4 mg/100 ml (*p* < 0.05) in 45 min, which was followed by a gradual decrease toward the pre-administration values. Following water administration, a slight reduction in serum glucose concentration was observed during the latter half period of exercise. Consequently, the difference in serum glucose concentration 45 min after the administration was significant between G + A and either F + A (*p* < 0.05) or water (*p* < 0.01), and also significant at 24:00h between both G + A and water (*p* < 0.05).

Serum IRI showed a slight increase after either G + A or F + A administration, but a slight decrease after water administration, resulting in a significant difference between water and either F + A (*p* < 0.05) or G + A (*p* < 0.05) 45 min later. However, in all the experiments serum insulin level returned to a pre-administration level at 24:00h.

After either F + A or water administration, the serum FFA concentration showed a slight elevation during the latter half period of exercise. However, after G + A administration the serum FFA concentration showed a significant decrease (*p* < 0.05), which was followed by a significant elevation (*p* < 0.05). There were significant differences in serum FFA concentrations between G + A and either F + A (*p* < 0.01) or water (*p* < 0.01) 45 min after the administration.

Serum glycerol concentration significantly rose after either F + A (*p* < 0.05) or water (*p* < 0.05) administration from 0.24 ± 0.06 to 0.41 ± 0.03 or 0.46 ± 0.01 mmol/liter, respectively. Those increased glycerol levels fell afterwards. With G + A, serum glycerol concentration remained unchanged throughout all the periods of exercise. The difference in the serum glycerol level 45 min after G + A and water administration was significant (*p* < 0.05), but no difference was found at 24:00h between both groups.

Fig. 2. Changes in serum concentrations of glucose (A), IRI (B), free fatty acids (C) and glycerol (D) (experiment 1). Each point and vertical line represents the values of mean and SEM for 5 rats, respectively. ◯, administration of test solution. □, pre-exercise, pre-administration or water; △, F+A; □, G+A. ⋆⋆⋆, ⋆⋆⋆⋆Significant difference between the values of the two adjacent points (*p<0.05, **p<0.01, ⋆⋆⋆p<0.001). a,b,c,dSignificant difference between the values marked with same letter (*b,p<0.05, c,d,p<0.01, *p<0.001).

Lipolytic activity of perirenal fat pad (Fig. 3)

Basal lipolytic activity significantly increased during exercise, and was not affected by F+A or water administration. However, the G+A administration caused a marked reduction (p<0.05) in lipolytic activity, resulting in a significant difference between the values with G+A and either F+A (p<0.01) or water (p<0.01) 45 min after the administration. At 24:00h, the lipolytic activity showed comparable values in each group. Epinephrine-stimulated lipolytic activity was only slightly affected by administrations of these solutions, as shown in Fig. 3.
Fig. 3. Changes in the basal (A) and epinephrine-stimulated lipolysis (B) (experiment 1). Each point and vertical line represents the values of mean and SEM for 5 rats, respectively. ○, pre-exercise, pre-administration or water; △, F+A; □, G+A. Other legends are the same as for Fig. 2.

Fig. 4. Changes in glycogen contents in liver (A) and soleus muscles (B) (experiment 1). Each point and vertical line represents the values of mean and SEM for 5 rats, respectively. ○, pre-exercise, pre-administration or water; △, F+A; □, G+A. Other legends are the same as for Fig. 2.

**Glycogen stores in liver and soleus muscle (Fig. 4)**

Liver glycogen stores showed a significant reduction from 30±2 to 4±1 mg/g wet tissue (p<0.001) after water administration during the latter exercise period. However, after the administration of either F+A or G+A, liver glycogen reduction was smaller than after water administration. A marked difference in liver glycogen...
stores was observed between G+A and water 45 min \( (p<0.05) \) and 90 min \( (p<0.01) \) after the administration and also between F+A and water \( (p<0.01) \) 90 min later. These were no significant difference in liver glycogen stores between G+A and F+A administration.

Soleus glycogen stores showed a significant reduction from \( 1.2 \pm 0.1 \) to \( 0.8 \pm 0 \) mg/g wet tissue \( (p<0.05) \) after water administration, but remained unchanged during the last 45 min of exercise. Following F+A administration soleus muscle glycogen stores remained unchanged, but showed a slight repletion 90 min later. On the other hand, soleus muscle glycogen stores after G+A administration

![Graphs of serum concentrations](image)

**Fig. 5.** Changes in serum concentrations of glucose (A), IRI (B), free fatty acids (C) and glycerol (D) in the exercising rats (continuous line) or the recovering rats (broken line), as defined in the text (experiment 2). Each point and vertical line represents the values of mean and SEM for 5-7 rats, respectively. \( \bigcirc \), pre-administration; \( \triangle \), F+A, exercise; \( \square \), G+A, exercise; \( \diamond \), F+A+C, exercise; \( \Delta \), F+A, recovery; \( \blacksquare \), G+A, recovery; \( \blacklozenge \), F+A+C, recovery. Other legends are the same as for Fig. 2.

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showed a significant repletion \((p<0.05)\), but remained constant thereafter. There were significant differences between \(G+A\) and either \(F+A\) \((p<0.05)\) or water \((p<0.01)\) 45 min after the administration, and between either \(G+A\) \((p<0.01)\) or \(F+A\) \((p<0.05)\) and water 90 min later.

**Experiment 2**

*Serum glucose, insulin, FFA and glycerol concentrations* (Fig. 5)

Serum glucose concentrations in all the exercising rats showed a slight decrease during the latter half period of exercise. On the other hand, in the recovering rats, serum glucose concentration after \(F+A\) \((p<0.05)\) and \(F+A+C\) \((p<0.05)\) administration showed a significant decrease, but there was no significant change after \(G+A\) administration.

Serum IR1 level was not affected by any type of fluid in either the exercising or the recovering rats.

Serum FFA concentrations were slightly elevated in all the exercising rats. On the other hand, in the recovering rats, serum FFA concentrations fell significantly \((p<0.05)\) after \(G+A\) administration but remained unchanged after either \(F+A\) or \(F+A+C\) administration, resulting in a significant difference between \(G+A\) and either \(F+A\) \((p<0.05)\) or \(F+A+C\) \((p<0.01)\).

Serum glycerol concentrations also did not significantly change in the exercising rats. However, in the recovering rats, serum glycerol concentrations decreased significantly after administrations of \(G+A\) \((p<0.05)\) and \(F+A+C\) \((p<0.01)\).

![Graphs showing changes in glycogen contents in liver (A) and soleus muscle (B) in the exercising (continuous line) or the recovering rats (broken line), as defined in the text (experiment 2).](image)

Fig. 6. Changes in glycogen contents in liver (A) and soleus muscle (B) in the exercising (continuous line) or the recovering rats (broken line), as defined in the text (experiment 2). Each point and vertical line represents the values of mean and SEM for 5–7 rats, respectively. ○, pre-administration; △, \(F+A\), exercise; □, \(G+A\), exercise; ◊, \(F+A+C\), exercise; ●, \(F+A\), recovery; ■, \(G+A\), recovery; ●, \(F+A+C\), recovery. Other legends are the same as for Fig. 2.
Glycogen stores in liver and soleus muscle (Fig. 6)

Liver glycogen stores in the exercising rats remained unchanged during the latter half period of exercise. On the other hand, in the recovering rats, liver glycogen stores showed a significant increase after either F+A ($p<0.05$) or F+A+C ($p<0.05$) administration. But this increase was not observed after G+A administration. Consequently, there was a significant difference in liver glycogen content between G+A and F+A+C groups ($p<0.05$).

Soleus muscle glycogen stores in the exercising rats remained constant after all the fluid administrations, but at the end of the observation a significant difference between G+A and F+A+C groups ($p<0.05$) was discovered. On the other hand, in the recovering rats, soleus muscle glycogen contents after all the types of solutions were administered showed a marked ($p<0.001$) increase. This increase was more pronounced in G+A than in F+A groups, resulting in a significant difference between these two groups ($p<0.05$).

DISCUSSION

The present study demonstrated that an exercise-induced reduction of glycogen stores in liver and skeletal muscle can be prevented with the administration of F+A as well as G+A. In addition, the F+A administration appeared not to have any inhibitory effect on exercise-induced lipolysis when compared with that after G+A administration. These results suggest that F+A administration during exercise is useful not only for sparing glycogen in the liver and muscle but also in supplying serum FFA to the exercising muscle.

In the present study, the effect of G+A and F+A administration was almost comparable on serum insulin levels during exercise (Fig. 2B) or during either exercise or recovery (Fig. 5B). These results suggest that stimulating the effects of glycogen synthesis by insulin might occur at almost the same extent after both G+A and F+A administration.

The results from the recovering rats (Fig. 6, A and B) showed that F+A administration appeared slightly more effective for liver glycogen repletion than G+A administration, while the latter seemed more effective for soleus muscle glycogen repletion than the former. Cori (23) and Bergström et al. (24) have reported that in rats and humans fructose can be more effectively used as a precursor for liver glycogen than glucose. However, it has also been pointed out (25) that fructose utilization by muscles is very small and administered fructose is mostly converted to glucose or its metabolites by liver, and are then released into the blood circulation. Therefore, the above difference observed in the muscle glycogen repletion in the recovering rats between G+A and F+A may partly be related to the slow supply of glycogen precursor to muscles after the administration of fructose.

The addition of citrate to F+A appeared to be more effective for sparing the liver and soleus muscle glycogen stores than does F+A, both during and after Vol. 32, No. 4, 1986
exercise. The similar effect of orally administered citrate on tissue glycogen repletion in exercising rats was observed previously (3).

In conclusion, the present study shows that the depletion of liver and skeletal muscle glycogen stores caused by endurance exercise has been prevented by the administration of F+A and G+A during the course of exercise, and that increased lipolysis during exercise was not so inhibited after F+A as compared to that after G+A. In addition, our results suggested that the glycogen sparing by F+A administration would be improved by the addition of citrate.

REFERENCES

GLYCOGEN REPLETION DURING EXERCISE


