Indonesian Journal of Science & Technology

Journal homepage: http://ejournal.upi.edu/index.php/ijost/

Fermented Grain Beverage Supplementation Following Exercise Promotes Glycogen Supercompensation in Rodent Skeletal Muscle and Liver

*Tsubasa Shibaguchi[1]†, Rie Ishizawa[1,2]†, Atsushi Tsuji[4], Yuya Yamazaki[4], Keizo Matsui[5] and Kazumi Masuda[1,3]**

[1]Venture Business Laboratory, Organization of Frontier Science and Innovation, Kanazawa University, Ishikawa 920-1192, Japan

[2]Waseda Institute of Sports Nutrition, Comprehensive Research Organization, Waseda University, Saitama 359-1192, Japan

[3]Faculty of Human Sciences, Kanazawa University, Ishikawa 920-1192, Japan

[4]Department of Chemistry and Food, Industrial Research Institute of Ishikawa, Ishikawa 920-8203, Japan

[5]Fukumitsuya Sake Brewery, Ishikawa 920-8638, Japan

*Correspondence: E-mail: masudak@staff.kanazawa-u.ac.jp

†T. Shibaguchi and R. Ishizawa contributed equally to this work

ABSTRACT

The purpose of this study was to investigate the effects of post-endurance exercise fermented grain beverage (FGB) supplementation on glycogen reaccumulation in rat skeletal muscle and liver. Twelve-hour fasted male Wistar rats, 10-week-old, performed five 30-min bouts of swimming separated by 5-min rest periods in order to deplete tissue glycogen storage. The rats were orally administrated either water, glucose, or FGB in 30, 60, 90, and 120 min after the exercise. Immediately and/or 240 min after the exercise, soleus and gastrocnemius muscles and liver were removed and analyzed. A large glycogen reaccumulation was observed in skeletal muscles and liver at 240 min after the exercise when either glucose or FGB were ingested. This response tended to be greater in FGB-treated than in glucose-treated animals, particularly in liver and gastrocnemius muscle. These results suggest that post-endurance exercise FGB supplementation enhances glycogen restoration in both skeletal muscle and liver.

ARTICLE INFO

Article History:
Submitted/Received 01 Dec 2016
First revised 03 Jan 2017
Accepted 30 Mar 2017
First available online 01 Apr 2017
Publication date 01 Apr 2017

Keyword:
Fermented Grain Beverage, Rodent Skeletal Muscle, Glycogen Supercompensation, Liver.

1. INTRODUCTION

Carbohydrates are a main energy source for the body and are reserved as glycogen primarily in skeletal muscle and liver. In general, the amounts of glycogen stored in these tissues are known to be one of the important factors to limit the endurance exercise performance (Bergström *et al.*, 1967). Therefore, the search for a highly effective strategy for promoting glycogen storages before exercise and/or recovery of depleted glycogen stores after exercise in skeletal muscle and liver is an important topic in the field of sports and exercise science.

In the area of sports and exercise science, numerous studies have been performed to clarify the mechanisms and methods for increasing glycogen storages in skeletal muscle and liver for many years. For example, a high-carbohydrate diet increases glycogen levels in both skeletal muscle and liver, and this response is enhanced when tissue glycogen contents are depleted by exercise, fasting, and/or a low-carbohydrate diet before the high-carbohydrate diet intake (Bergström *et al.*, 1967; Bergström & Hultman, 1966; Nilsson & Hultman, 1973). These evidences are based on the method called "carbohydrate loading" so far. In addition to this method, post-exercise co-ingestion of carbohydrates and other nutrients, such as amino acids (Morifuji *et al.*, 2010), citric acid (Saitoh *et al.*, 1983) and so on, enhances glycogen accumulation in skeletal muscle and/or liver compared with carbohydrate ingestion alone. However, it is still unclear what kind of intervention is most effective for glycogen stores in skeletal muscle and liver.

Recently, we have developed a new beverage derived from a certain grain by using a fermentation technique and called it fermented grain beverage (FGB). This beverage basically contains a high proportion of carbohydrates, amino acids, citric acid and so on, but the concentration of these components can be adjusted relatively easily by changing the production methods and fermentation conditions. Since the FGB compositely has the components effective for glycogen stores, supplementation of the FGB is expected to promote not only glycogen storage but also post-exercise glycogen restoration in skeletal muscle and liver. However, the bioactivity of FGB still remains unclear. Therefore, in the present study, we investigated the effects of post-exercise FGB supplementation on glycogen contents in skeletal muscle and liver.

2. MATERIALS AND METHODS

2.1. Animals

All experimental procedures performed in the current study were approved by the Ethics Committee on Animal Experimentation of Kanazawa University (Protocol #: AP-163772) and followed the Guide for the Care and Use of Laboratory Animals of the Physiological Society of Japan. Ten-week-old male Wistar rats (n = 15) were used for the present study. Animals were housed in an air-conditioned room with 12:12-h light-dark photoperiod. A standard solid chow (MF; Oriental Yeast, Tokyo, Japan) and water were provided *ad libitum*.

2.2. Experimental design

Following 1 week of acclimation, rats were randomly assigned to two primary groups: sedentary control and exercise. The exercise animals were further divided into four groups: immediate post-exercise, post-exercise water ingestion, post-exercise glucose ingestion, and post-exercise FGB ingestion. Animals in both control and exercise groups were fasted for 12 hours. After the fasting period, the rats in all exercise groups swam for 2.5 hours in five

DOI: http://dx.doi.org/10.17509/ijost.v2i1
p- ISSN 2528-1410 e- ISSN 2527-8045

30-min bouts separated by 5 minutes of rest in order to deplete skeletal muscle and liver glycogen. All rats swam in a barrel filled with water maintained at 35°C to a depth of 40 cm with a surface area of 1120 cm^2/rat. Water, glucose solution, or FGB were orally administrated to the rats in each post-exercise group by using a feeding tube at 30, 60, 90 and 120 minutes after the swimming exercise. FGB contained carbohydrates (glucose and fructose), amino acids, citric acid, and so on (data not shown). The dosage of glucose solution and FGB was 1 g carbohydrate/kg body weight (approx. 1.0–1.1 mL/rat) per dose (total carbohydrate intake = 4 g/kg body weight) (Sano *et al.*, 2012). The same volume of water was provided to the post-exercise water ingestion group.

2.3. Sampling

Immediately and 240 minutes after the exercise, soleus and gastrocnemius muscles of both hindlimbs and liver were dissected from the rats in exercised groups under anesthesia (sodium pentobarbital, 50 mg/kg body weight, *i.p.*). The muscles and liver of the control group were also dissected to show the baseline after fasting. The collected tissues were washed in ice-cold saline, cleaned of excess fat, nerve and connective tissue, and then weighted. The tissues were then rapidly frozen in liquid nitrogen and stored at -80°C until analyses.

2.4. Tissue glycogen contents

A portion of soleus and deep portion of gastrocnemius (GasD) muscles and the liver (~20 mg) was minced and homogenized in 10 (muscle) and 20 volume (liver) of ice-cold Glycogen Hydrolysis Buffer (BioVision, CA, USA) using an ultrasonic homogenizer (UR-21P; TOMY, Tokyo, Japan) on ice. The homogenate was then centrifuged at 12,000 rpm for 5 minutes at 4°C, and the supernatant was used for glycogen analysis. Glycogen contents of skeletal muscles and liver were determined using a Glycogen Colorimetric Assay Kit II (BioVision, CA, USA) according to the manufacture's protocol, and expressed as mg/g tissue weight. The amount of change in glycogen (ΔGlycogen) from the value of immediate post-exercise to that of 240-min post-exercise in each post-exercise group was also calculated.

2.5. Statistics

All values were expressed as mean ± standard error (SE). Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The level of significance was set at $P < 0.05$. All statistical analyses were performed using the EZR software (Kanda, 2013).

3. Results

Glycogen concentration in soleus and GasD muscles, as well as liver, tended to decrease by nearly half of control immediately after the swimming exercise (**Figure 1**, $P > 0.05$). Subsequently, the amount of glycogen in these tissues exhibits a significant increase at 240 minutes after the exercise compared with the value of immediate post-exercise only when either glucose or FGB intake were administrated during post-exercise period ($P < 0.05$). Post-exercise FGB supplementation had a significant impact on glycogen reaccumulation following the exercise, particularly in the liver. The liver glycogen content in the post-exercise FGB ingestion group was significantly higher than that in all other groups ($P < 0.05$). Similar observations were noted in the GasD muscle, although there was no significant difference in the glycogen content between post-exercise glucose and FGB ingestion groups. In the soleus, however, the glycogen level in the post-exercise FGB ingestion group was significantly higher than that in only

DOI: http://dx.doi.org/10.17509/ijost.v2i1
p- ISSN 2528-1410 e- ISSN 2527-8045

immediate post-exercise and post-exercise water ingestion groups ($P < 0.05$).

There was no significant difference among three post-exercise ingestion groups in the value of ΔGlycogen in the soleus muscle **Figure 2**. However, the value of ΔGlycogen was significantly higher in the groups of post-exercise glucose ingestion in liver and post-exercise FGB ingestion in GasD and liver, compared with the group of post-exercise water ingestion ($P < 0.05$). Furthermore, the ΔGlycogen value in liver was also significantly greater in the post-exercise FGB than in the post-exercise glucose ingestion groups ($P < 0.05$).

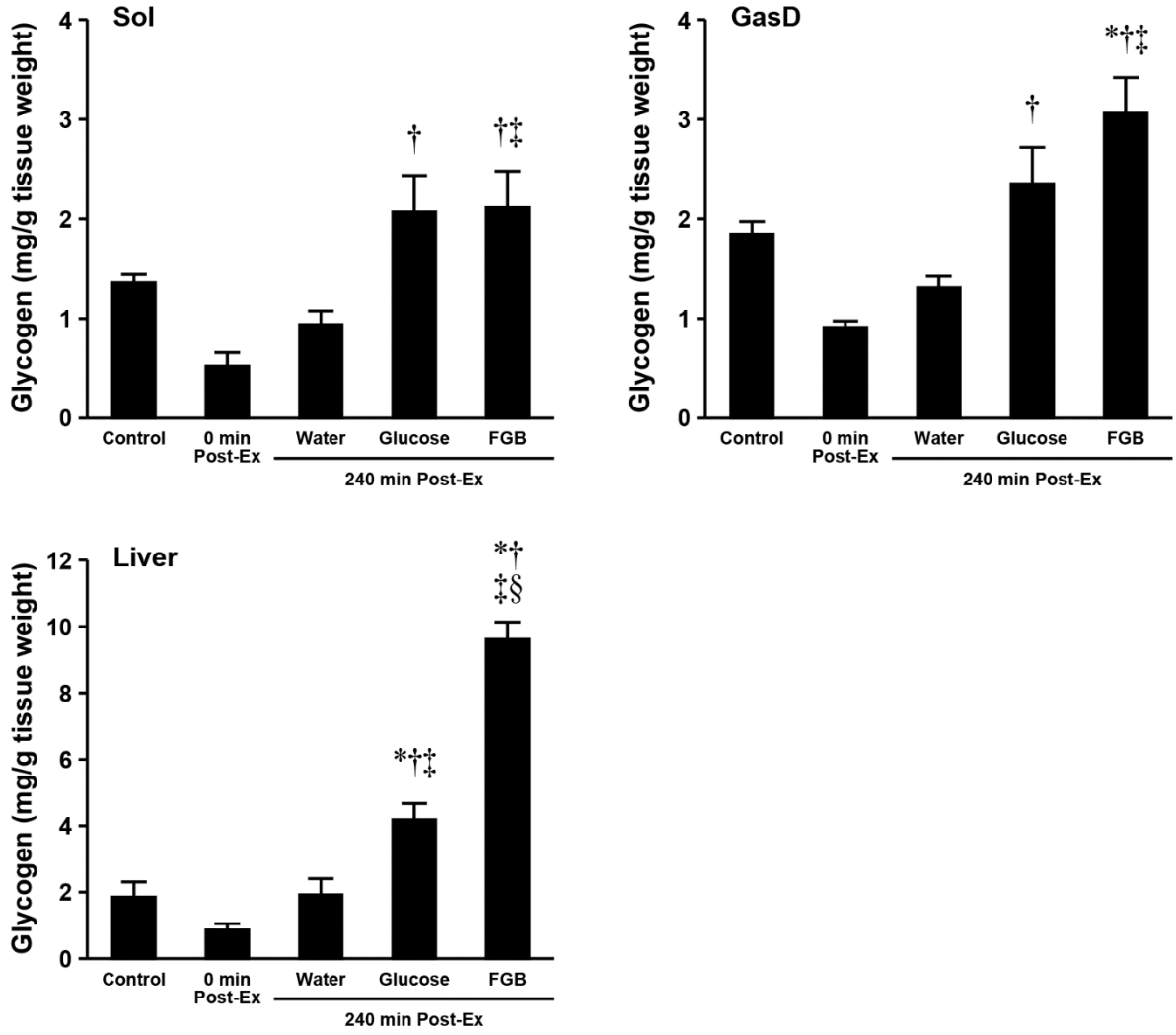

Figure 1. Effects of post-exercise (Post-Ex) fermented grain beverage (FGB) supplementation on glycogen restoration in soleus (Sol) and deep portion of gastrocnemius (GasD) muscles and liver. Values are means ± SE. n = 3/group. *,†, ‡, §Significantly different from control, immediate post-exercise, post-exercise water ingestion, and post-exercise glucose ingestion groups, respectively ($P < 0.05$).

DOI: http://dx.doi.org/10.17509/ijost.v2i1
p- ISSN 2528-1410 e- ISSN 2527-8045

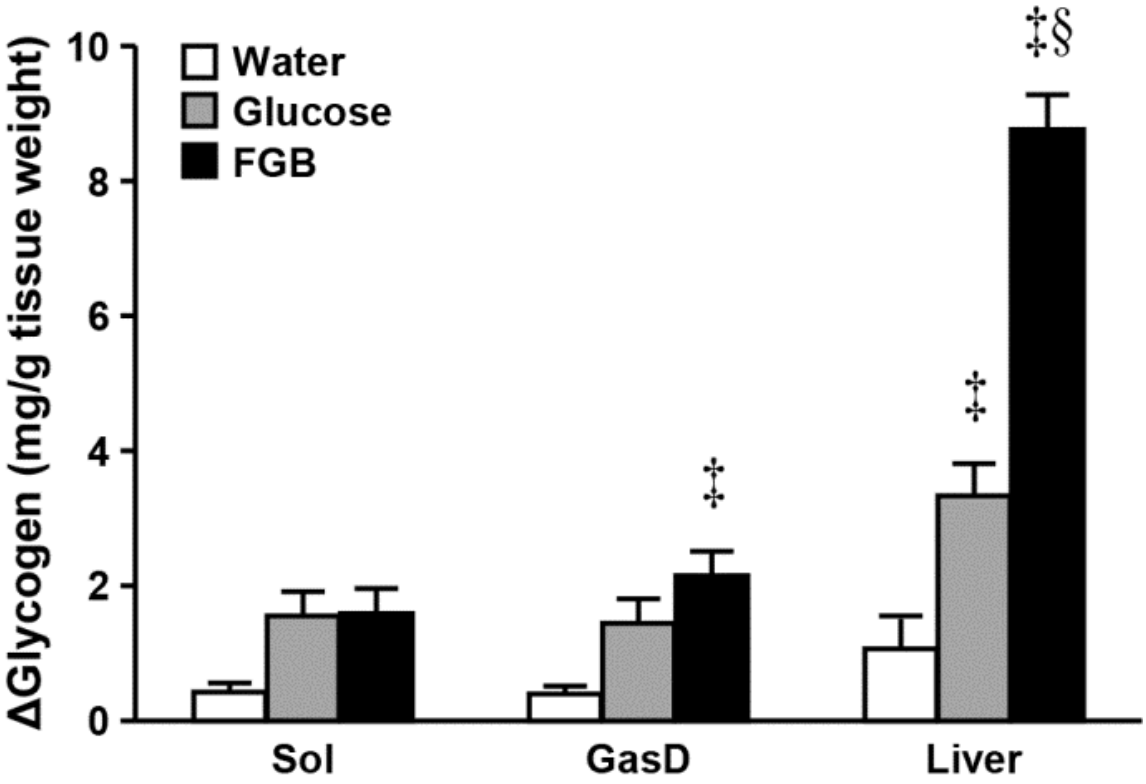

Figure 2. The amount of change in glycogen in skeletal muscles and liver during post-exercise period. ΔGlycogen is expressed as the amount of change from the value of immediate post-exercise to that of 240 min post-exercise in each post-exercise group. Values are means ± SE. n = 3/group. See Figure 1 for other abbreviations. ‡, §Significantly different from post-exercise water and glucose ingestion groups, respectively ($P < 0.05$).

2. DISCUSSION

We previously demonstrated that a single oral administration of FGB (1 g carbohydrate/kg body weight) to overnight-fasted rats caused a significant increase in the amount of glycogen storages in liver 2 hours after the administration when compared to the same volume ingestion of either water or glucose (unpublished observation). In agreement with our previous results, the present study showed that post-exercise FGB ingestion resulted in a significantly larger increase in glycogen accumulation in liver than either post-exercise water or glucose ingestion (**Figures 1 and 2**). These findings indicate that FGB supplementation facilitates not only glycogen storage but also post-exercise glycogen restoration in liver. In general, fructose is preferentially metabolized in the liver (McGuinness & Cherrington, 2003). It was also reported that post-exercise co-ingestion of glucose and fructose promoted liver glycogen repletion compared with glucose ingestion in human (Fuchs *et al.*, 2016). Moreover, Saitoh *et al.* reported that co-ingestion of glucose and citric acid after exercise induced a faster recovery from depleted glycogen levels in both skeletal muscle and liver than ingestion of glucose alone, which might result from the inhibitory effect of citric acid on the activity of phosphofructokinase, a late-limiting enzyme of glycolysis (Saitoh *et al.*, 1983). Therefore, post-exercise FGB supplementation-associated promotion of glycogen restoration in liver observed in the present study may be attributed to at least fructose and citric acid derived from the FGB.

DOI: http://dx.doi.org/10.17509/ijost.v2i1
p- ISSN 2528-1410 e- ISSN 2527-8045

In contrast to our previous findings, only animals ingested FGB after exercise exhibited a significantly large value of absolute content and variation of glycogen in GasD muscle compared with the control and post-exercise water animals, respectively (**Figures 1 and 2**). These results suggest that FGB supplementation accelerates post-exercise glycogen restoration in skeletal muscle. It has been well known that carbohydrate intake after glycogen depletion induced by exercise courses a greater extent of glycogen accumulation than carbohydrate intake alone (Bergström & Hultman, 1966). The possible mechanisms of this phenomenon are believed to be due to a promotion of glucose uptake in skeletal muscle via the activation of insulin-dependent and -independent pathways by exercise (Rose & Richter, 2005; Sakamoto & Holman, 2008). Some kinds of amino acids, such as leucine and isoleucine, also have the ability to stimulate glucose uptake in skeletal muscle and muscle cells by an insulin-independent mechanism (Doi *et al.*, 2003; Nishitani *et al.*, 2002). Moreover, Morifuji *et al.* reported that that post-exercise co-ingestion of carbohydrate and amino acids significantly promotes the recovery of skeletal muscle glycogen levels than glucose alone probably through both insulin-dependent and -independent mechanisms (Morifuji *et al.*, 2010). As described above, post-exercise co-ingestion of glucose and citric acid also stimulates skeletal muscle glycogen restoration after exercise. Thus, all the aforementioned findings suggest that both exercise and components of FGB contributed to a larger increase in glycogen contents in GasD muscle in the present study.

In the present study, however, the positive effects of post-exercise FGB ingestion on glycogen storages were relatively low in soleus than in GasD and liver (**Figures 1 and 2**). We have no clear explanation of such phenomena at present. Further studies are needed to clarify this issue.

3. CONCLUSION

This study demonstrated that post-exercise FGB intake facilitated glycogen restoration in both skeletal muscle and liver. To our knowledge, this is the first study showing the effects of FGB supplementation on glycogen restoration after exercise in skeletal muscle and liver. Our data strongly suggest that FGB supplementation after exercise will be an effective method for increasing tissue glycogen.

4. ACKNOWLEDGEMENTS

This research was supported by a funding support for the promotion of new business activities from Small and Medium Enterprise Agency Ministry of Economy, Trade and Industry (K. Matsui). We appreciate Mr. Ryuichi Miyazaki for his technical assistance.

5. AUTHORS' NOTE

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article. Authors confirmed that the data and the paper are free of plagiarism.

DOI: http://dx.doi.org/10.17509/ijost.v2i1
p- ISSN 2528-1410 e- ISSN 2527-8045

6. REFERENCES

Bergström, J. & Hultman, E. (1966). Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature*, *210*(5033), 309-310.

Bergström, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and physical performance. *Acta physiologica scandinavica*, *71*(2), 140-150.

Doi, M., Yamaoka, I., Fukunaga, T., & Nakayama, M. (2003). Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochemical and biophysical research communications*, *312*(4), 1111-1117.

Fuchs, C. J., Gonzalez, J. T., Beelen, M., Cermak, N. M., Smith, F. E., Thelwall, P. E., Taylor, R., Trenell, M. I., Stevenson, E. J., & van Loon, L. J. (2016). Sucrose ingestion after exhaustive exercise accelerates liver, but not muscle glycogen repletion compared with glucose ingestion in trained athletes. *Journal of applied physiology*, *120*(11), 1328-1334.

Kanda, Y. (2013). Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone marrow transplantation*, *48*(3), 452-458.

McGuinness, O. P. & Cherrington, A. D. (2003). Effects of fructose on hepatic glucose metabolism. *Current opinion in clinical nutrition and metabolic care*, *6*(4), 441-448.

Morifuji, M., Kanda, A., Koga, J., Kawanaka, K., & Higuchi, M. (2010). Post-exercise carbohydrate plus whey protein hydrolysates supplementation increases skeletal muscle glycogen level in rats. *Amino acids*, *38*(4), 1109-1115.

Nilsson, L. H. & Hultman, E. (1973). Liver glycogen in man – The effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. *Scandinavian journal of clinical and laboratory investigation*, *32*(4), 325-330.

Nishitani, S., Matsumura, T., Fujitani, S., Sonaka, I., Miura, Y., & Yagasaki, K. (2002). Leucine promotes glucose uptake in skeletal muscles of rats. *Biochemical and biophysical research communications*, *299*(5), 693-696.

Rose, A. J. & Richter, E. A. (2005). Skeletal muscle glucose uptake during exercise: how is it regulated?. *Physiology*, *20*(4), 260-270.

Saitoh, S., Yoshitake, Y., & Suzuki M. (1983). Enhanced glycogen repletion in liver and skeletal muscle with citrate orally fed after exhaustive treadmill running and swimming. *Journal of nutritional science and vitaminology*, *29*(1), 45-52.

Sakamoto, K. & Holman, G. D. (2008). Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. *American journal of physiology-endocrinology and metabolism*, *295*(1), E29-E37.

DOI: http://dx.doi.org/10.17509/ijost.v2i1
p- ISSN 2528-1410 e- ISSN 2527-8045