Effect of the Classic 1-Week Glycogen-Loading Regimen on Fat-Loading in Rats and Humans

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Summary The purpose of this study was to elucidate the fat-loading effect of the classic 1-wk glycogen-loading regimen histologically in rats and physiologically in humans. In the rat and human studies, an exhaustive swimming exercise and cycle ergometer exercise were loaded on day 1 of a 6-d feeding period, respectively. Thereafter, both the rats and humans were divided into a glycogen-loading regimen consisting of a 3-d high-fat diet and a 3-d high-carbohydrate diet or a 6-d high-carbohydrate diet. After the feeding period in the human study, the human subjects performed a test exercise on day 7 using a cycle ergometer. In the rat study, the intramuscular triglyceride (IMTG) content was 69% greater \(^{p<0.05}\) after the glycogen-loading regimen than after the high-carbohydrate diet feeding on day 7. In the human study, the respiratory exchange ratios (RER) after the glycogen-loading regimen were 4.9–6% lower than those after the high-carbohydrate diet during the test exercise on day 7 \(^{p<0.05}\). Our findings suggest that the classical 1-wk glycogen-loading regimen maintained the storage and enhanced the utilization of energy sources during exercise in the skeletal muscle, and that it provides a fat-loading effect, in addition to the glycogen-loading effect, to the skeletal muscle.

Key Words fat-loading, glycogen-loading, IMTG, rat, human

Glycogen in skeletal muscles is an important energy source for exercise and its depletion during endurance exercise causes a decline in performance (1–4). Therefore, before endurance competitions, such as a marathon or triathlon, a 1-wk glycogen-loading regimen has classically been applied to load extra glycogen in skeletal muscles (2). The classic glycogen-loading regimen involves initial depletion of glycogen storage in the skeletal muscles by exhaustive exercise on day 1, followed by feeding on high-fat and high-protein diets between day 1 and 3, to up-regulate the activity of glycogen synthetase (5). Thereafter, a high-carbohydrate diet is initiated between day 4 and 6 to supercompensate the storage of glycogen in skeletal muscles by up to 1.5–2-fold more than that of the baseline level on day 1 (5).

Lipids are another significant energy source for endurance exercises (6) and are stored as triglycerides in the skeletal muscle fiber (intramuscular triglyceride, IMTG) (7–10), while IMTG is utilized effectively and plays a central role in providing energy during endurance exercise (11–15). Changes in its storage dynamics in response to the classic glycogen-loading regimen are not well understood.

We hypothesized that the high-fat diet during the first 3 d promotes IMTG storage, such that the classic 1-wk glycogen-loading regimen has a fat-loading effect in addition to its glycogen-loading effect. In the present study, we elucidated the fat-loading effect of the classic glycogen-loading regimen histologically in rats and also physiologically in humans.

MATERIALS AND METHODS

Rat study.

Animals and experimental design: The experimental protocols for animal handling (Fig. 1) were reviewed and approved by the Institutional Animal Care Committee of Tsurumi University School of Dental Medicine. Thirty-six male Wistar rats (6 wk of age, 200±4 g) were purchased from CLEA Japan, Inc. (Tokyo, Japan). Two or three rats were housed per cage with controlled light during 7:00–19:00 h and free access to water. For 7 days before the experimental period, all rats were accustomed to meal-feeding on 8.5 g of commercial rat chow (CE-2, CLEA Japan) twice a day, at 9:30 and 18:30.

On day 1 of the experimental period, an exhaustive swimming exercise was performed by 30 rats and the remaining six rats were sacrificed without exercise. Just after the exhaustive exercise, six rats were sacrificed and, the remaining 24 rats were divided at random into a glycogen-loading regimen group (12 rats) and a high-carbohydrate diet group (12 rats). Each rat in the high-carbohydrate diet group was fed 8.5 g of high-carbohydrate diet (protein : fat : carbohydrate = 25 : 5 : 70%, 330 kcal/100 g) twice a day from days 1 through 6.
Each rat in the glycogen-loading regimen group was fed 8.5 g of a high-fat diet (25:70:5%, 551 kcal/100 g) twice a day from days 1 to 3 and then 8.5 g of a high-carbohydrate diet from days 4 to 6 (6). Six rats from each group were sacrificed on days 4 and 7.

Swimming exercise: The swimming exercise was performed in a plastic barrel (Ø50×54.5 cm) filled with 32–37˚C water (50 cm deep). During the 5 d of the pre-experimental period, all rats swam with a lead weight corresponding to 1–2% of their body weights tied to their bodies (17). Then, the rats performed an exhaustive swimming exercise from 12:00 on day 1 in a plastic barrel with a water-stream generated by compressed air and with the same weight attached (17). On days 2, 3, 5, and 6, the rats swam for 10 min without the water-stream or weights.

Histology: The rats were sacrificed by exsanguination under ether anesthesia. The right extensor carpi ulnaris muscle, one of the extensor muscles in the forearms, was removed, weighed, frozen in dry ice-acetone, and stored at −80˚C until histological analysis. Transverse sections of the middle portion of the extensor carpi ulnaris muscle were prepared at a 10 µm thickness with a cryostat. The sections were thaw-mounted on glass slides and air-dried for 60 min. The sections were stained with Oil red O to determine the IMTG content (20) or stained with the periodic acid-Schiff (PAS) reaction to assess the glycogen content (21). The staining images were visualized with a fluorescent or light microscope (BX61; Olympus Optical, Tokyo, Japan) and images were uploaded, using a digital camera (C9100; Hamamatsu Photonics, Hamamatsu, Japan, DP70; Olympus Optical), into a personal computer. The area of lipid droplets stained with Oil red O and the total intensity of PAS staining of the five and three rectangular regions from a rat, respectively, were averaged to obtain the mean value for each rat. This value was then further averaged to obtain the mean value for six rats.

Human study.

Subjects: Six healthy recreationally trained male volunteers [age, 21.5±5.3 y; height, 170.5±5.5 cm; weight, 67.8±7.1 kg; maximal oxygen uptake (VO2peak), 55.2±5.3 mL/kg/min] participated in this study and performed 2–3 h of exercise per day/5–6 d/wk. All subjects were previously well-informed about the purpose of the study and the experimental procedure, and each gave informed consent. The study protocol was approved by the Waseda University Ethics Committee.

Pretest: Before the experiment, subjects performed an incremental exercise test with a cycle ergometer (Monark 818E, Sweden) to exhaustion to determine peak oxygen uptake (VO2peak). 55.2±5.3 mL/kg/min participated in this study and performed 2–3 h of exercise per day/5–6 d/wk. All subjects were previously well-informed about the purpose of the study and the experimental procedure, and each gave informed consent. The study protocol was approved by the Waseda University Ethics Committee.
Simultaneous Glycogen- and Fat-Loading

For the following 3 d, they were given a high-carbohydrate diet (19%:15%:66%, 1,034 kcal) throughout the experimental period of 6 d (Fig. 2, Table 1) (22–24). The two trials were randomized and crossover designed with washout periods of 2 wk–2 mo. Each subject performed exhaustive exercise on day 1, and a test exercise on day 7 using a cycle ergometer.

Exercise: Subjects exercised for 90 min on the cycle ergometer at 60% VO2peak on day 1 of the experiment to deplete muscle glycogen stores of fast-twitch fiber and slow-twitch fiber. Glycogen decreases slowly in low-intensity exercise (25). 60% VO2peak in the middle of aerobic and anaerobic exercise was adopted in this experiment. And subjects also exercised daily for 2–3 h on days 2 through 6. The test cycling exercise on day 7 was 60 min at 50% VO2peak for 3 h after a breakfast (22%:15%:63%, 1,034 kcal) (26). They arrived at the laboratory 2 h before the test exercise and rested for 40 min at 24°C and 60% RH to allow collection of the resting expired gas sample during the final 10 min. The subjects ingested a 5% glucose solution (0.5 g/kg body weight) at 60 min and 30 min before the test exercise to inhibit release of free fatty acid (FFA) from the adipose tissues. Blood samples were collected at 60 min before and at 0, 30, and 60 min during the test exercise, and at 30 min after initiation of the recovery period. The gas samples were collected for 4 min each at 15–11 min before the test exercise and at 11–15, 26–30, 41–45, and 56–60 min during the test exercise.

Blood analysis: The blood samples were collected from fingertips into two micro-capillaries (60 μL each) with a Multi-Lancet S (Arkray Co. Ltd., Japan). The plasma was separated and stored at −80°C until used. The plasma glucose and FFA concentrations were analyzed using a Glucose CII Test Kit (Wako Pure Chemical
Industries, Ltd., Japan) and NEF A C Test Kit (Wako Pure Chemical Industries, Ltd.), respectively.

Respiratory gas analysis: The oxygen uptake (VO$_2$) and carbon dioxide emissions (VCO$_2$) in the expired gas were analyzed using a respiratory gas analyzer (AE-300S; Minato Medical Science, Osaka, Japan), and the respiratory exchange ratios (RER) were calculated by dividing the VCO$_2$ by VO$_2$.

Energy expenditure: The VO$_2$ and VCO$_2$ in the expired gas were analyzed using a respiratory gas analyzer, and energy expenditure was calculated by using the equation of Weir (27).

Statistical analysis. A Mann-Whitney U test was used to compare the median values between the high-carbohydrate diet and the glycogen-loading regimen groups. Tukey’s test was used to compare the mean values between two time points during the experimental period.

RESULTS

Rat study

Muscle glycogen. Figure 3A–F shows images of the extensor carpi ulnaris muscle of rats stained with PAS. The intensity of the PAS staining in the muscle fibers (glycogen content) was analyzed by image analysis software (Fig. 3G). The glycogen content in the skeletal muscle decreased by 36% between the pre- and post-exercise on day 1 (p<0.01). In the high-carbohydrate diet group, the glycogen content increased by 24% on day 4 (p<0.05) as compared with the pre-exercise level, and the level remained high on day 7 (p<0.05). In the glycogen-loading regimen group, by contrast, the muscle glycogen content returned to the pre-exercise on day 4, and then increased by 30% over the pre-exercise level on day 7 (p<0.01).

IMTG. Figure 4A–F shows images of the extensor carpi ulnaris muscle of rats stained with Oil red O. The area of lipid droplets stained with Oil red O in the muscle fibers (IMTG) was analyzed by image analysis software (Fig. 4G). IMTG decreased by 30% between pre- and post-exercise on day 1 (p<0.05). In the high-carbohydrate diet group, IMTG remained quite stable between days 1 and 7. However, in the glycogen-loading regimen group, IMTG increased by 48% on day 4 as compared with the pre-exercise level (p<0.01), and slightly decreased between days 4 and 7. IMTGs were 42 and 69% greater on day 4 (p<0.05) and day 7 (p<0.01), respectively, in the glycogen-loading regimen group than in the high-carbohydrate diet group.

Human study

Plasma glucose. Figure 5A shows changes in the plasma glucose concentration of subjects in the high-carbohydrate diet and glycogen-loading regimen groups. Plasma glucose concentrations were not significantly different at any time point between the two groups. The concentration levels remained quite stable at all time points.

Plasma free fatty acid. Figure 5B shows changes in the plasma FFA concentration of subjects in the high-carbohydrate diet and glycogen-loading regimen groups. Plasma FFA concentrations did not show a significant difference between the two groups.

Respiratory exchange ratio. Figure 6 shows changes in respiratory exchange ratio (RER) for 5 min at rest and at 10 min intervals during 60 min of test exercise in the human study. Each column and vertical bar represent the mean and SD for 6 subjects, respectively. Significant difference: glycogen-loading regimen vs high-carbohydrate diet, *p<0.05.
in the RER of subjects in the high-carbohydrate diet and glycogen-loading regimen groups. The RER was not significantly different between the two groups at rest. RERs in the glycogen-loading regimen group were 4.9–6% lower than those in the high-carbohydrate diet group at 11–15, 26–30, and 41–45 min during the test exercise (p<0.05), but not at 56–60 min.

Energy expenditure. Figure 7 shows changes in the energy expenditure of subjects in the high-carbohydrate diet and glycogen-loading regimen groups. The energy expenditure was not significantly different between the two groups.

DISCUSSION

In the rat study, the glycogen content in the glycogen-loading regimen and high-carbohydrate diet feeding groups on day 7 was significantly higher than the pre-exercise levels on day 1, indicating that both diets provide a glycogen loading effect to the skeletal muscle in rats. Previous studies demonstrated the glycogen-loading effect of the classic 1-wk glycogen-loading regimen in rat skeletal muscle based on a biochemical analysis (16). In the present study, we confirmed the previous result based on a histological analysis. In the present human study, muscle glycogen content was not monitored. However, in previous studies, the classic 1-wk glycogen-loading regimen showed a muscle glycogen-loading effect in humans (2,22). Thus, we consider that the 1-wk glycogen-loading regimen employed in the present human study could increase the muscle glycogen content.

In the present rat study, the IMTG content in the glycogen-loading regimen group on days 4 and 7 was higher than the content in pre-exercise day 1 and in the high-carbohydrate diet group on those same days. This result suggests that the glycogen-loading regimen stimulates the storage of IMTG in addition to the storage of glycogen in skeletal muscle. Our findings are consistent with previous studies in rats (16,19) and humans (26). In the present human study, the energy expenditure was not significantly different between the two groups, and the RER was lower in the glycogen-loading regimen than in the high-carbohydrate diet group. The concentration of plasma FFA did not show a significant difference between the two groups. Glucose ingestion before exercise is reported to inhibit the release of FFA from adipose tissue (28). Our findings suggest that there is higher oxidation of IMTG in the glycogen-loading regimen group than in the high-carbohydrate diet groups, which is consistent with previous human studies (22, 24, 26, 29, 30). Collectively, the results of the present rat and human studies suggest that the classic 1-wk glycogen-loading regimen maintains the storage, promotes the usage of IMTG, provides a fat-loading effect (via the storage and the usage of IMTG), and loads higher levels of glycogen to skeletal muscles.

Previous human studies have shown that the fat-loading effect is essentially dependent on the fat content of the diet, i.e., a 40–68% high-fat diet creates a fat-loading effect in the skeletal muscle (22, 24, 31–33). In the present studies, the high-fat diet in rats (protein : fat : carbohydrate=25 : 7 : 5% of calories) and humans (22 : 72 : 6%) did result in increased fat-loading in the skeletal muscle.

To enhance the storage of lipids in skeletal muscle, FFA uptake and TG synthesis can be enhanced by stimulating lipoprotein lipase (LPL) activity in the skeletal muscle (34). Increased fat in the diet increases LPL activity in skeletal muscle and increases the storage of IMTG (32). By contrast, a high-carbohydrate diet over a period of just 3 d reduces LPL activity in the skeletal muscle (35). To enhance the fat-loading effect of the glycogen-loading regimen, it may be useful to add compounds which could stimulate muscle LPL activity during the high-fat diet period within the first 3 d of the classic glycogen-loading regimen to increase the incorporation of plasma TG into skeletal muscle.

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