Muscle glycogen supercompensation is enhanced by prior creatine supplementation

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ABSTRACT
NELSON, A. G., D. A. ARNALL, J. KOKKONEN, R. DAY, and J. EVANS. Muscle glycogen supercompensation is enhanced by prior creatine supplementation. Med. Sci. Sports Exerc., Vol. 33, No. 7, 2001, pp. 1096–1100. Purpose: Recently, it was shown that glycogen supercompensation tended (P = 0.06) to be greater if creatine and glycogen were loaded simultaneously. Because the authors suggested that creatine loading increased cell volumes and, therefore, enhanced glycogen supercompensation, we decided to determine whether an enhanced glycogen supercompensation could be realized if the glycogen loading protocol was preceded by a 5-d creatine load. Methods: Twelve men (19–28 yr) performed two standard glycogen loading protocols interspersed with a standard creatine load of 20 g·d⁻¹ for 5 d. The vastus lateralis muscle was biopsied before and after each loading protocol. Results: The initial glycogen loading protocol showed a significant 4% increase (P < 0.05) in muscle glycogen (Δ 164 ± 87 mmol·kg⁻¹ d.m.), and no change (P > 0.05) in total muscle creatine. Biopsies pre- and post-creatine loading showed significant increases in total muscle creatine levels in both the left leg (Δ 141.1 ± 31.1 mmol·kg⁻¹ d.m.) and the right leg (Δ 36.6 ± 19.8 mmol·kg⁻¹ d.m.), with no change in either leg’s muscle glycogen content. After the final glycogen loading, a significant 53% increase in muscle glycogen (Δ 241 ± 150 mmol·kg⁻¹ d.m.) was detected. Finally, the postcreatine load total glycogen content (694 ± 156 mmol·kg⁻¹ d.m.) was significantly (P < 0.05) greater than the precreatine load total glycogen content (597 ± 142 mmol·kg⁻¹ d.m.). Conclusion: It is suggested that a muscle’s glycogen loading capacity is influenced by its initial levels of creatine and the accompanying alterations in cell volume. Key Words: ERGOGENIC AID, CARBOHYDRATE LOADING, MUSCLE CELL VOLUME

Nutritional ergogenic aids have long been used to enhance work and athletic performance. Among the many different dietary supplements whose ergogenic benefits have been investigated, dietary-induced increases in creatine and glycogen concentrations within a muscle cell have constantly demonstrated positive benefits. Creatine loading improves the performance of high-intensity short-duration exercise (see review 11), and glycogen loading is beneficial in the performance of moderate-intensity long-duration exercise (see reviews 3,9). In addition to their respective influences on work capacity, creatine and glycogen loading have other similar characteristics. Increased entry of either creatine or glycogen into a muscle cell changes the cell’s osmotic balance, which necessitates increases in intracellular water (14,19). Also, the entry of either glucose or creatine into the muscle cell is enhanced when blood insulin levels are high (6,16). Finally, the magnitudes of creatine and glycogen loads are enhanced if exercise is performed before the loading protocol (18).

Because creatine loading and glycogen loading are ergogenic aids for activities at different ends of the work intensity continuum, investigations examining their combined influence on muscle have not been readily forthcoming. Recent work by Robinson and associates (18), however, supports a reversal in this attitude. In an attempt to increase muscle creatine accumulation, Robinson et al. (18) had individuals ingest large doses of both creatine and glucose after a bout of exhaustive exercise. They found that their exercise/ingestion protocol resulted in greater total creatine levels. They attributed the increases in creatine to an exercise induced up-regulation of the activity and number of Na⁺-K⁺ pumps augmented by glucose’s enhancement of insulin mediated creatine uptake. An unexpected finding was a tendency (P = 0.06) for the creatine/glucose ingestions to induce greater muscle glycogen concentrations than the glucose-only ingestions. This trend toward greater glycogen accumulation was attributed to creatine loading increasing muscle cell volume, a proven accentuator of glycogen synthesis (13).
If the mechanism (i.e., increased glycogen supercompensation due to an increased cell volume subsequent to increased creatine storage) proposed by Robinson et al. (18) is a factor controlling the magnitude of glycogen supercompensation, then a person should see a greater glycogen supercompensation if they were to load creatine and glycogen serially. In other words, initial muscle creatine levels should impact the effectiveness of a standard glycogen loading protocol. On the other hand, cellular glycogen supercompensation appears to have an upper limit (6). Unfortunately, the definitive mechanism(s) responsible for this upper limit are unknown. Traditional thought, however, has linked the upper storage limit to cell volume regulatory mechanisms. In general, most cells experience a decrease in functional capacity and an inhibition in protein and glycogen degradation when cell volume becomes large (see review 12). Muscle cells in particular have a decreased contractile function when cell volumes are enlarged (17). Moreover, the research linking increased glycogen with cell volume expansion (13) only followed cellular glycogen incorporation for 90 min post cellular expansion. Thus, it may be inappropriate to extend the influence of this mechanism over the entire 3–5 d it usually takes muscle glycogen stores to reach maximum. Therefore, it is possible that if creatine and glycogen are serially loaded, the magnitude of the glycogen load might be lower due to a creatine-induced maximal cell volume.

Research to date has not determine what the effects a maximized substrate store has upon the serial load of another substrate. Hence, the purpose of this study was to determine whether an increased total muscle creatine content achieved through a 5-d loading program would lead to greater glycogen supercompensation from a standard 3-d glycogen supercompensation protocol.

METHODS

Subjects

Twelve men (19–28 yr) volunteered to participate in the study. All subjects were physically active, but none were engaged in any type of structured physical training. In addition, none of the subjects had performed either a creatine or glycogen loading protocol for the previous 6 months. The experimental protocol was approved by the institutional review board, and each volunteer gave written and oral consent before performing any part of the experiment.

Protocol

Overview. See Figure 1 for a depiction of the study’s time line. The experiment consisted of two glycogen loading protocols interspersed with a creatine loading protocol. Before and after each of the glycogen and creatine loads, muscle biopsies where taken from either the right or left vastus lateralis muscle.

Glycogen loading no. 1. To determine the upper limit of a standard 3-d glycogen supercompensation protocol in a vastus lateralis with a normal creatine load, the right vastus lateralis of each subject underwent an initial glycogen depletion/loading protocol. To minimize subject discomfort and to prevent any confounding influence arising from performing multiple glycogen loading protocols within a short time period, the left leg was exempted from the initial glycogen loading protocol. After a biopsy of the right vastus lateralis (B1R), each subject performed a one-legged cycling maximal graded exercise test (GXT). Each GXT began at a work rate of 15 W (90 rev·min⁻¹ against a resistance of 1.62 N), and work rate increased 15 W every 2 min until the subject could no longer maintain the required pedal cadence. Upon completion of the GXT, the subject began one-legged cycling (in cadence) at 75% of the maximum workload achieved during the GXT. The subjects cycled until they were unable to maintain a pedal cadence of 90 rpm for 20 s. Once this fatigue point was reached (approximately 2.5 h for each person), the ergometer’s resistance was increased by 0.5 kg, and the subject was asked to pedal (one-legged) as fast as possible until he was no longer able to turn the cycle’s crank. The duration of this activity was usually 60 s. After this fatiguing exercise, the subject moved over to a knee-extension weight lifting machine. The load was set at 9.1 kg (20 lbs), and the subject performed multiple sets of single leg knee-extensions (90° range of motion) to failure. A 30-s rest period separated each set to failure, and this exercise continued until the subject could only perform 10 or less repetitions in a set. This ended the glycogen depletion routine.

Three days of glycogen loading followed the glycogen depletion. Within 10 min of the end of the glycogen depletion exercises, the subjects drank a minimum of 475 mL of a powdered sports drink mixed at double strength. The subjects then received the first of their three daily food allotments (nine caloric equivalent meals). Total caloric content of each daily food allotment exceeded the product of the subject’s body weight (lbs) × 15. The caloric ratio of CHO:fat:protein was approximately 80:10:10, which yielded a daily carbohydrate intake of approximately 6.6 g·kg⁻¹ body mass. Each of the three daily food allotments consisted of three caloric equivalent meals, each consumed in the presence of an investigator. In addition, the subjects refrained from ingesting any dietary supplements, alcohol, tea, coffee, other nonsupplied beverages, or any additional...
food during the 3 d. During the loading period, the subjects refrained from all strenuous and/or recreational physical activity. All of the subjects, however, were college students, which necessitated a small amount of walking between classes. These walks did not exceed 300 m at one time, and total continuous walking time never exceeded 5 min.

After the 3 d of glycogen loading, each subject underwent a second muscle biopsy. During this biopsy, muscle samples came from both the right (B2R) and left (B2L) vastus lateralis muscles. The incision for the second biopsy of the right leg was located approximately 1 cm distal to the previous incision.

**Creatine loading.** The day after the second muscle biopsy, each subject underwent a creatine loading protocol. Each subject daily ingested 5 g of a creatine monohydrate powder four times a day for a total daily consumption of 20 g. Four hours separated each dose/ingestion, and the subjects mixed each dose in 350–600 mL of either apple juice or sports drink. Creatine ingestion lasted for 5 straight days. As with the glycogen loading, the subjects refrained from strenuous and/or recreational physical activity.

The day after the 5th day of creatine loading, both the right (B3R) and left (B3L) vastus lateralis muscles were biopsied for either the third (R) or second (L) time. The incision for the biopsy of the right leg was located approximately 1 cm proximal to the first incision. The left leg biopsy was located approximately 1 cm distal to the first incision.

**Glycogen depletion/loading no. 2.** Immediately after the third set of muscle biopsies, the subjects’ left leg underwent a glycogen depletion/loading protocol to determine the upper limit of a standard 3-d glycogen supercompensation protocol in a vastus lateralis with a supranormal creatine level. The protocol followed for this second set of glycogen depletion exercises mirrored the protocol used in the first set, excepting this time for their convenience the subjects performed two-legged exercise. Upon completion of the depletion routine, the subjects were given the same diet provided previously (CHO:fat:protein, 80:10:10) for a total of 3 d. After the 3 d of glycogen loading, each subject underwent a fourth muscle biopsy session. During this biopsy, samples came from only the left vastus lateral mus-cle (B4L).

**Muscle biopsy technique.** Muscle biopsies (80–120 mg) were obtained with a Stille biopsy needle (5-mm OD) using the Bergström percutaneous biopsy method, as modified by Evans et al. (5). During each biopsy session, each incision site had two samples removed. The samples were frozen with Wallenberger tongs cooled to liquid N2 temperature, put in cryotubes, and placed in liquid N2. After the biopsy session, the muscle samples were lyophilized for 10–12 h and then stored at −85°C for later analysis.

**Analytical procedures.** Before analysis for either glycogen or total creatine, the dried muscle samples were teased apart under a dissection microscope to remove connective tissue, fat, and blood. Muscle glycogen concentrations were determined by the anthrone technique of Hassid and Abrahm (8). Total creatine content of the muscle samples was determined according to the procedures of Ennor and Rosenberg (4).

**Statistical analysis.** Glycogen data analysis utilized a two-way 2 × 2 (leg × treatment) repeated measures ANOVA that compared BR1, BR2, BL3, and BL4. Creatine data analysis utilized a two-way 2 × 2 (leg × treatment) repeated measures ANOVA that compared BR2, BR3, BL2, and BL3. Significance for both ANOVAs was set at P < 0.05. Post hoc analysis involved, where appropriate, the use of Tukey’s protected t-test.

**RESULTS**

Figure 2 depicts the muscle glycogen levels at the various biopsy time points. The statistical analysis yielded a significant (P = 0.006, ω² = 0.23) two-way interaction. The Tukey’s test showed that the B1R (right leg, pre-one leg glycogen load) and B3L (left leg, postcreatine load and pre-two leg glycogen load) were significantly less (P < 0.05) than B2R (right leg, post-one leg glycogen load & precreatine load), and B4L (left leg, post-two leg glycogen load). In addition, B2R was significantly less (P < 0.05) than B4L. In other words, the first glycogen loading protocol yielded a significant (P < 0.05) mean increase in muscle glycogen content of 164 ± 87 mmol·kg⁻¹ d.m. (41% increase). After creatine loading, the glycogen loading protocol yielded a significant (P < 0.05) mean increase in muscle glycogen content of 241 ± 150 mmol·kg⁻¹ d.m. (53% increase), and the second supercompensation yielded at larger glycogen content than the first.

Figure 3 depicts the total creatine levels at the various biopsy time points. The statistical analysis yielded only a significant (P < 0.0001, ω² = 0.29) treatment effect. In other words, B2R and B2L were significantly less (P < 0.05) than B3R and B3L, but there was no significant (P > 0.05) difference in mean total creatine content between the
right and left legs at either time point. Thus, creatine loading protocol yielded a significant ($P < 0.05$) mean increase in mean total muscle creatine content of $41.1 \pm 31.1$ mmol·kg$^{-1}$ d.m. (46% increase) in the left leg and $36.6 \pm 19.8$ mmol·kg$^{-1}$ d.m. (37% increase) in the right leg.

**DISCUSSION**

Recently, it has been shown that simultaneously ingesting creatine and glucose after a single bout of exhaustive exercise results in a greater total muscle glycogen concentration as well as a greater total muscle creatine content. Robinson et al. (18) attributed the enhanced glycogen content to a creatine induced increase in cell volume. If increased cell volume is the mechanism behind the enhanced glycogen storage, then a previously creatine loaded muscle should also experience a greater glycogen load. Hence, the main purpose of this study was to document the impact of existing total muscle creatine levels upon the muscle’s capacity to supercompensate glycogen concentrations.

Similar to the findings of Robinson et al. (18), this study showed a synergy between creatine loading and glycogen supercompensation. In this case, performing a glycogen loading protocol after completing a creatine load resulted in a glycogen content ~10% greater than the expected glycogen load. Because increased entry of creatine can cause an increase in cell volume due to increased intracellular water (19) and increased cell volumes accentuate glycogen synthesis (13), this study appears to substantiate the supposition of Robinson et al. (18) (i.e., enhanced glycogen content is consequence of a creatine induced increase in cell volume).

Even though this study found a significantly greater glycogen concentration at the end of 3 d of carbohydrate loading, these results should not be interpreted as proof that a prior creatine load increases a muscle’s capacity to store glycogen. According to recent experimental evidence, the upper limit of glycogen storage in human muscle is approximately 1000 mmol·kg$^{-1}$ d.m (6). Because the postcreatine load glycogen supercompensation in this study ranged between 484 and 899 mmol·kg$^{-1}$ d.m., it is doubtful that any of the subjects reached their upper limit of glycogen storage. Similarly, although both Robinson et al. (18) and Low et al. (13) found greater glycogen loads, neither established whether the muscle cells’ glycogen content was at maximum. Therefore, based on the available data, a more accurate description of the synergy between creatine loading and glycogen loading is that creatine loading influences glycogen synthesis rate rather than maximal storage capacity. Clearly, studies following glycogen supercompensation until the subjects reach and maintain maximal levels are needed to help better understand creatine loading’s influence upon glycogen supercompensation.

In addition to showing a favorable influence of creatine loading upon glycogen loading, this study provides some insight into the relationship between initial muscle glycogen levels and subsequent creatine loading. Harris et al. (7) noticed an apparent exercise induced augmentation of creatine loading. They surmised that the exercise augmentation was due to improved blood flow. Robinson et al. (18), however, discounted the importance of improved blood flow and attributed the exercise increased creatine uptake to an exercise-induced up-regulation of Na$^+$/K$^+$ pumps. On the other hand, a factor not considered by either Harris et al. (7) or Robinson et al. (18) was the possibility of initial muscle glycogen levels at the initiation of creatine loading influencing total creatine accumulation. Also, cell volume might control the upper limits of any substrate accumulation. If so, then the exercise-induced increase total creatine accumulation noted by Harris et al. (7) and Robinson et al. (18) could have been due to a reduction in cell volume coincident with an exercise induced decrease in muscle glycogen. This uncertainty was one reason for the single leg glycogen depletion at the onset of this study. The creatine levels, however, of B3R (glycogen loaded muscle) and B3L (normal glycogen levels) were the same. Therefore, initial glycogen levels probably have no impact on a muscle’s capacity to creatine load.

Due to the strong correlation between high glycogen stores and the performance enhancement of moderate-intensity long-duration exercise (3,9), do the results of this investigation have any important ramifications for these athletes? Without any performance data, an absolute assessment of performance benefits cannot be made. Previous research, however, suggests a minimal impact on performance from sequentially loading creatine and glycogen. In 1993, Balsom et al. (2) found a decreased performance in a 6-km run over undulating terrain following creatine loading. These researchers attributed the lower performance to a creatine-loading-induced increase in the athletes’ body weight. Although the muscle glycogen concentration of the athletes in the Balsom et al. (2) study is unknown, it is doubtful that the modest increase in glycogen concentration (~10%) seen with sequential creatine loading and glycogen is sufficient to overcome the accompanying weight gain. Therefore, sequentially loading creatine and glycogen just before the performance of any weight-sensitive activity...
could be counter-productive. On the other hand, a positive benefit from sequential loading creatine and glycogen might be realized by athletes who engaged in a high-intensity glycogen-depleting contact sport, such as ice hockey. Individual performances in ice hockey have been related to muscle glycogen content (1), and the performance of high-intensity intermittent work can be improved by creatine loading (15). Because a single game of ice hockey can deplete glycogen stores by 60% (10), performance on successive days will diminish unless glycogen can be replenished quickly. Moreover, in contact sports a small weight gain can many times be beneficial, especially when the individual is one who habitually initiates the contact. Thus, for these athletes, creatine loading would not only provide them an ergogenic benefit for the high-intensity work but also could enable them to replenish needed glycogen stores more rapidly between games.

In summary, this study shows that a muscle which has enhanced creatine stores is able to store more glycogen during a standard 3-d glycogen depletion/loading protocol. This increase in the 3-d accumulation of muscle glycogen is presumed to be due to a creatine induced cell volume increase stimulating glycogen synthesis.

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