Inadequate Carbohydrate Intake Following Prolonged Exercise Does Not Increase Muscle Soreness After 15 Minutes of Downhill Running

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In Delayed Onset Muscle Soreness (DOMS), muscles become sore 24 to 48 hours after eccentric and unaccustomed activity. Fiber stiffness, due to decreased muscle glycogen, may predispose muscle to greater damage during eccentric exercise. This study sought to determine if inadequate carbohydrate intake following a protocol to decrease muscle glycogen would increase DOMS after 15 min of downhill running. Thirty-three male subjects (age, 18–35 years) were randomized into 3 groups for testing over a 7-day period. The depletion (DEP) group ($n = 12$) underwent a glycogen depletion protocol prior to a 15-min downhill run designed to induce DOMS. The repletion (FED) group ($n = 10$) underwent a glycogen depletion protocol followed by a carbohydrate repletion protocol (>80% CHO) prior to downhill running. The third (ECC) group ($n = 11$) performed only the downhill running protocol. Subjective muscle soreness, isometric force production, relaxed knee angle, and thigh circumference were measured pretreatment and on days 1, 2, 3, 4, and 6 post treatment. Subjective muscle soreness for all groups increased from 0 cm pretreatment to $3.05 \pm 0.72$ cm (on a 10-cm scale) on day 1 post treatment ($p < .05$). All groups were significantly different from baseline measurements until day 4 post treatment. Each group experienced a decline in isometric force from $281 \pm 45$ N pre- to $253 \pm 13$ N on day 1 post treatment ($p < .05$). The decrease in isometric force persisted in all groups for 4 days post treatment. Increases in thigh circumference and relaxed knee angle elevations in all 3 groups were statistically different ($p < .05$) from pretreatment until day 4. No differences were noted between groups for any of the parameters examined. In the current study, 15 min of downhill running is sufficient to cause DOMS with the associated functional and morphological changes; however, inadequate carbohydrate intake after a glycogen depleting exercise does not appear to exacerbate DOMS and the associated symptoms.

*Key Words:* muscle damage, carbohydrate, DOMS

**Introduction**

Delayed onset muscle soreness (DOMS) is the muscular pain that occurs 24 to 48 hours following eccentric and unaccustomed activity of moderate intensity and...
duration (24). Muscles exhibit warmth, become painful to the touch, and swell, and joints undergo a decreased range of motion (7, 18). Histological preparations from muscle biopsy samples show mechanical damage as revealed by Z-band streaming and disruption of the A-band in muscle fibers (15, 16, 27). Myoproteins increase in the plasma (3), and an inflammatory response occurs in the damaged muscle fibers (18, 35).

As common as DOMS is, little is known regarding the specific cellular mechanisms that induce muscle soreness (1, 2). Lieber and Friden have postulated that fiber oxidative capacity may be a key to understanding the mechanism responsible for DOMS (27, 29). Using a rabbit model, they observed that muscle fibers, after eccentric treatments, displayed structural abnormalities primarily in fast-twitch muscle and stained negative for glycogen. Human models have also shown selective damage to fast-twitch muscle fibers after eccentric exercise protocols (17, 25). Metabolic studies of human muscle further indicates that eccentric activity rapidly depletes fast-twitch fibers of glycogen when performed above VO₂max (15, 23), uses significantly more muscle glycogen than concentric activity at similar intensities (38), and impairs glycogen uptake post-exercise compared to muscle depleted by concentric activity (9, 10, 38, 44).

Lieber and Friden proposed that fast-twitch fibers, which lack the higher oxidative capacities of slow-twitch fibers, fatigue early in an exercise period and become unable to generate enough adenosine triphosphate (ATP) to support the contractile mechanism. According to the current theories of muscle contraction, the release of the myosin head from the actin filament requires the addition of a new ATP. Insufficient ATP causes this release mechanism to fail, and the fiber enters a rigid or highly stiff state (11). Lieber and Friden theorized that subsequent eccentric stretch of stiff fibers would mechanically disrupt the sarcolemma, sarcoplasmic reticulum, and myofilaments, resulting in the observable cytoskeletal and myofibrillar damage leading to an inflammatory response and the concomitant delayed muscle soreness.

Because Lieber and Friden noted that injured fast glycolytic muscle fibers were depleted of glycogen, we hypothesized that fasted subjects, following a protocol to decrease muscle glycogen, might be more predisposed to DOMS than subjects with decreased muscle glycogen who had been fed a diet high in carbohydrates. Theoretically, drastically decreasing muscle glycogen would lead to a decrease in the availability of ATP, which would compromise the release of myosin cross-bridges from the actin filaments, resulting in stiffness, injury, soreness, and dysfunction (22, 33, 34). The present study was designed to test the hypothesis that there would be greater muscle soreness, stiffness, injury, and dysfunction in glycogen-depleted subjects compared to subjects with normal muscle glycogen content.

Methods

Subjects

Thirty-three male subjects between the ages of 18 to 35 were recruited from the local population. Subjects were excluded on the basis of a questionnaire if they responded yes to any of the following: lower extremity pain, history of knee surgery, used medications that would prevent an inflammatory response, involved in a lower
extremity strength training program in the past 6 months, eccentric damage of their lower extremities in the past 90 days, had Type I or II diabetes, or were avid cyclists or runners. An informed consent document consistent with the university policy for the protection of human subjects was given to the subjects, and written consent was obtained.

**Experimental Design**

Subjects were randomized into three groups (Figure 1). The depletion group (DEP) underwent a glycogen depletion protocol (21), fasted for 12 hours, and then performed an eccentric exercise protocol (5). The repletion group (FED) underwent a glycogen depletion protocol, followed immediately by the consumption of a meal, designed to replenish glycogen, before undergoing the eccentric treatment 12 hours later. The third group (ECC) was a control and only participated in the eccentric treatment.

All subjects were tested for maximal oxygen consumption using a cycle ergometer to determine cycling resistance levels for the glycogen depletion protocol that followed 1 week later. The DEP and FED groups performed the glycogen depletion protocol before the eccentric exercise protocol. The DEP group consumed only water during the 12 hours between the depletion and eccentric protocols. Following the depletion protocol, the FED group consumed a meal consisting of 80% carbohydrates, 10% fat, and 10% protein. Food consisted of pasta, sauce, breads, and Gatorade®. Portions were determined by calculating 2 g of carbohydrates per kilogram of bodyweight (6, 23) from the Recommended Dietary Allowances packaging label on each food item. Portions were fed to the subjects within an hour of the depletion protocol. The ECC group performed the maximal test of oxygen consumption and, 1 week later, the downhill running protocol.

All groups underwent muscle damage marker assessment 1 day prior to, and on days 1, 2, 3, 4, and 6 after, the eccentric exercise bout. Subjects refrained from any activity that would induce muscle soreness during the experiment.

![Figure 1 — VO\textsubscript{2max} tests were performed by all subjects 1 week prior to a glycogen depletion protocol. The study groups DEP and FED both performed the depletion protocol. The DEP group fasted between the depletion protocol and the downhill run, while the FED group consumed 2 g CHO/kg body weight before their downhill run. The ECC group performed only the VO\textsubscript{2max} test and the eccentric protocol. Each group received DOMS assessment on days 1, 2, 3, 4, and 6 post eccentric activity.](image-url)
Test of Maximal Oxygen Consumption

All groups performed a graded exercise test to exhaustion to determine maximal oxygen consumption. The VO$_{2\text{max}}$ test was performed on an electromagnetically braked cycle ergometer (Lode Excalibur, Lode, Groningen, Netherlands). The cycling protocol was a concentric activity that would not induce DOMS.

Each subject began cycling at a resistance of 125 W. The resistance was increased by 50 W every 3 min until volitional exhaustion. Each subject met three criteria to determine VO$_{2\text{max}}$. First, oxygen consumption plateaued during the last minutes of the test, rising no more than 2 ml · kg$^{-1}$ · min$^{-1}$ in between the final test stages. Second, the respiratory exchange ratio increased to 1.15 or higher. Last, each subject’s heart rate increased to within 10 beats of the age-predicted maximum. The last three VO$_{2}$ readings were averaged to determine a maximal oxygen uptake value. Expired volume was determined by a Fleish pneumotach, and exhaled O$_{2}$ and CO$_{2}$ were determined by a mass spectrometer (Marquette Inc.). Oxygen uptake and carbon dioxide production were analyzed every 30 s by an online computer program (Consentius Inc.). The mass spectrometer was calibrated prior to testing using certified medical gases of known concentration. Heart rates were monitored by radiotelemetry (Polar Electro Inc., Port Washington, NY, USA), and a rating of perceived exertion (RPE) was recorded at 2-min intervals.

Depletion Protocol

Muscle glycogen depletion was induced with the protocol of Gollnick et al. (20). This protocol depleted both slow- and fast-twitch fibers of glycogen in the anterior thigh muscles (vastus lateralis, vastus medialis, and rectus femoris). Subjects rode an electromagnetically braked cycle ergometer (Lode Excalibur, Lode, Groningen, Netherlands) for 1.5 hours at a workload corresponding to 60% of their maximal oxygen uptake. Subjects then performed six 1-min sprints at 150% of their VO$_{2\text{max}}$. Ten-minute rests were interposed between each sprint. This depletion protocol and others comparable to it have shown to result in glycogen depletion in similar subjects (6, 23).

Eccentric Exercise Protocol to Induce DOMS

To induce muscle soreness, all subjects performed a downhill running protocol (5) on a treadmill (Quinton Instruments, Seattle, WA, USA). Downhill running activates the anterior thigh muscles measured for glycogen depletion in the Gollnick cycling protocol (4, 12, 13, 40). Subjects in the DEP and FED groups reported to the lab 12 hours after the glycogen depletion cycle protocol. Subjects in the ECC group performed the protocol at the same time of day as the other two groups. The subjects familiarized themselves with the treadmill by running for 5 min at a 0% grade at a target pace that elicited a heart rate of 170 bpm. After 5 min at 170 bpnms, the slope of the treadmill was changed to –10%, while the speed remained at the target pace, and subjects ran continuously for 15 min. Subjects’ heart rate was recorded every 5 min. Since muscle damage occurs early in the eccentric exercise period and to prevent severe DOMS, the protocol in this study was modified by having subjects exercise for 15 min. This modification was verified during our pilot study in which 5 subjects, who exercised for 15 min, demonstrated an average muscle soreness of 5.3 on a 10-cm subjective pain scale.
Dependent Variables

Perceived muscle soreness, maximal isometric force, relaxed limb angles, and circumference of the lower anterior thigh were used as indirect muscle damage indicators (43). Each variable was collected on days 0, 1, 2, 3, 4, and 6.

Perceived Muscle Soreness

Each subject assessed pain of the contracted muscles of the anterior thigh (vastus medialis, vastus lateralis, and rectus femoris) with the knee joint at 90° using a horizontal visual analogue scale with a continuous, unmarked 10-cm line. The 0-cm end represented no soreness, and the 10-cm end represented very, very sore (41). Each subject was seated on a table with his or her knees at 90° angles and legs unable to touch the floor. Each subject performed a maximal isometric knee extension at 90° against an immovable fixture, after which subjects were asked to rate their perceived muscle pain by marking the 10-cm line.

Maximal Isometric Force

Isometric force measurements were obtained using a BIODEX System 3 Isokinetic Dynanometer (Biodex Medical Systems, Shirley, NY, USA). Subjects were secured on the BIODEX with leg, thigh, waist, and shoulder straps. Seat and limb positions were recorded to maintain the same position in all trials. Knee angle was set at 60°. Subjects were given three periods of 10 s in which to develop maximal isometric torque. Peak torque generated during each period was taken, and all three maximum isometric peak torque measurements were averaged. The average derived from the three contractions was used as peak torque. Subjects rested for 2 min between maximal isometric contraction periods.

Relaxed Limb Angle

The anatomic reference points for goniometer placement on the leg were taken from Norkin and White (36). They included the lateral malleolus, the lateral head of the tibia, and the anterior inferior iliac spine. Anatomical reference points of the left leg were marked with a permanent marker and were remarked if fading occurred between tests. Subjects sat on a table with their left knee joint relaxed and both feet even above the ground. Initially, a mark was made 2 in. from the knee joint, where the posterior thigh and the edge of the table met. Subjects were told to relax their quadriceps. Knee angle measurements were performed in duplicate.

Thigh Circumference

Thigh circumference was determined with a Gulick tape measure at one fourth and one half the distance up the thigh from the top of the patella to the inguinal line while the subject was in a standing position. Corresponding marks were made on the anterior and posterior thigh at both distances and were remarked between tests as needed. Subjects were asked to stand with their weight evenly distributed on both feet and to relax as a Gulick tape measure (accuracy to 1/16 in.) was placed around their thighs, lining up at the anterior and posterior marks. The tape was then removed and the measurement repeated.
**Statistical Analysis**

Data were analyzed with repeated measures ANOVA. The dependent variables were subjective muscle soreness, maximal isometric force, relaxed knee angle, and thigh circumference. Independent variables were Time and Group. Between group differences were checked by the Wilks’ Lambda test. Group and Time significance were accepted at a $p < .05$. Data were analyzed using SAS 8.0 (SAS Institute Inc., North Carolina, USA).

**Results**

No significant differences existed between groups in any of the measured variables at any time point. Muscle soreness, thigh circumference, and relaxed knee angle were significantly different from baseline on days 1, 2, and 3 post exercise. Maximal isometric force was statistically different from baseline measurements for 4 days post treatment.

**Perceived Muscle Soreness**

Baseline muscle soreness for all groups was zero (Figure 2). DEP soreness peaked at 24 hours at 3.68 ± 0.67 cm ($p < .05$). FED and ECC peaked the 1st day as well, with means of 2.27 ± 0.76 and 3.21 ± 0.75, respectively ($p < .05$). At 48 hours post eccentric activity, all groups were still significantly different ($p < .05$) from baseline, with DEP at 2.84 ± 0.54, FED at 2.23 ± 0.34, and ECC at 2.68 ± 0.63. Each subject returned to baseline by the end of the measuring period. Interaction effect was non-significant ($F_{10, 145} = 0.92, p = .51$), indicating no differences between groups. Group effects were non-significant ($F_{2, 29} = 0.55, p = .58$), while Time effects were significant ($F_{5, 145} = 39.89, p < .05$).

**Maximal Isometric Force**

Baseline isometric force values were 275 ± 35, 283 ± 56, and 285 ± 45 N for DEP, FED, and ECC, respectively ($p > .05$; Figure 3). Twenty-four hours following the eccentric exercise, each group showed maximal force decreases ranging from 22–31 N ($p < .05$). On the 2nd day post treatment, FED decreased 5 N, and ECC remained the same ($p < .05$). All groups after the day 2 measurement increased in force from their maximal force deficit and returned to baseline values by the end of the 6th day. Interaction effect was non-significant ($F_{10, 145} = 0.83, p = .60$). Group effect was non-significant ($F_{2, 29} = 0.16, p = .85$). Time effects were significant ($F_{5, 145} = 57.22, p < .05$).

**Thigh Circumference**

Thigh circumference baseline values were 17.25 ± 1.54, 17.25 ± 1.48, and 18.45 ± 2.66 in. for DEP, FED, and ECC, respectively ($p > .05$; Figure 4). Circumference increased significantly in all groups by day 2 but returned to baseline values by day 6. Interaction effect was significant ($F_{10, 145} = 2.27, p < .05$), indicating differences between Groups over Time. Group effect was non-significant ($F_{2, 29} = 1.42, p = .25$). Time effect was significant ($F_{5, 145} = 37.46, p < .05$).
Baseline knee angle values were 110.3 ± 1.06°, 111.1 ± 0.91°, and 108.0 ± 0.92° for DEP, FED, and ECC, respectively, with no significant differences between baseline values (Figure 5). DEP and ECC had maximum angle values at day 2 post treatment, while FED had its maximum value on day 1 post treatment. All groups decreased to baseline levels by the end of the measurement period; however, changes over time were not different between groups. Interaction effect was non-significant ($F_{10, 145} = 0.58, p = .82$), indicating no differences between groups. Group effect was non-significant ($F_{2, 29} = 2.31, p = .11$). Time effect was significant ($F_{5, 145} = 46.06, p < .05$).

Figure 2 — Perceived muscle soreness prior to (0) and 6 days following the downhill running protocol. This graph compares the muscle soreness scores (cm) among the depleted (DEP), repleted (FED), and control (ECC) groups. *Significant differences of all three groups from baseline measurements ($p < .05$). There were no significant differences between groups.

Relaxed Knee Angle

Baseline knee angle values were 110.3 ± 1.06°, 111.1 ± 0.91°, and 108.0 ± 0.92° for DEP, FED, and ECC, respectively, with no significant differences between baseline values (Figure 5). DEP and ECC had maximum angle values at day 2 post treatment, while FED had its maximum value on day 1 post treatment. All groups decreased to baseline levels by the end of the measurement period; however, changes over time were not different between groups. Interaction effect was non-significant ($F_{10, 145} = 0.58, p = .82$), indicating no differences between groups. Group effect was non-significant ($F_{2, 29} = 2.31, p = .11$). Time effect was significant ($F_{5, 145} = 46.06, p < .05$).
Discussion

The present study sought to determine if inadequate carbohydrate intake after exercise to decrease muscle glycogen would increase the degree of DOMS in subjects after a downhill run. The results of the indirect measures of muscle damage indicate that DOMS did occur in all subjects. The loss of maximal isometric force, perceived muscle soreness, decrease in relaxed knee angle, and increase in thigh circumference is consistent with DOMS as seen in other studies (7, 8, 18, 34). The etiology of each of the above responses to muscle damage has been discussed further in other studies (26, 34, 37, 42).

Lieber and Friden suggested that fast-twitch fibers fatigue during the early phases of intense exercise (5–15 min) and, based upon their inability to regenerate

Figure 3 — Maximal isometric strength prior to (0) and 6 days following the downhill running protocol. This graph compares DEP, FED, and ECC group strength loss over the measuring period. *Significant differences of all three groups from baseline measurements (p < .05). There is no significant difference between any of the groups.
ATP, enter a rigid and highly stiff state (28). Further stretching of these stiff fibers during eccentric contraction disrupts the fiber membranes, allowing an influx of destructive metabolites into the cell, resulting in the observed cytoskeletal and myofibrillar damage of DOMS (27, 28). Since glycogen depletion is a well-known cause of fatigue, and since Lieber and Friden showed that fatigued and damaged muscles were also depleted of glycogen, we hypothesized that a diet deficient in carbohydrate would predispose whole muscle with decreased muscle glycogen to greater damage from eccentric exercise than muscle allowed to increase glycogen stores through ample carbohydrate intake. We also hypothesized that these subjects would also experience a greater degree of DOMS and its associated symptoms.

Figure 4 — Measurement of thigh circumference prior to (0) and 6 days following the downhill running protocol. Average DEP, FED, and ECC group thigh circumferences are shown over the measuring period. *Significant difference of all three groups from baseline measurements ($p < .05$). Statistical analysis failed to show any differences between groups.
Our results indicate that 15 min of downhill running in an untrained population is sufficient to induce DOMS; however, decreased muscle glycogen of whole muscles followed by a state of fasting did not increase muscle soreness. Thigh circumference measures revealed a significant difference at one time point between the DEP group and the other two groups. The other three measures indicated no

Figure 5 — Relaxed knee angle measurements prior to (0) and 6 days following the downhill running protocol. The graph shows the DEP, FED, and ECC group angle measurements over the measuring period. *Significant difference of the three group measurements over baseline values ($p < .05$). There are no significant differences between groups.
differences between groups at this time point; therefore, we question the physiological significance of circumference differences at a single time point. Interestingly, Lieber and Friden postulated that eccentric damage to muscles occurs in the first 5–15 min of eccentric exercise. Therefore, considering the state of the decreased muscle glycogen of the subjects, the downhill running time of 15 min should have been sufficient to invoke significant differences of muscle soreness.

Byrnes and his colleagues (5) showed that running on a –10° slope for 30 min produced DOMS in all 22 trained college-age subjects. These subjects were maximally sore at 40 hours post treatment and, on a subjective pain questionnaire, rated their muscle soreness at specific body sites over time on a scale of 1 to 10. Our protocol was modified for untrained individuals to run for 15 min instead of 30 min (19). This change allowed the protocol to be completed by untrained subjects and to prevent potentially severe DOMS that would obscure any possible differences due to treatments. Our modified protocol produced soreness similar to the Byrnes study, but did not produce any significant differences in subjective muscle soreness between groups. Subject soreness peaked at 24 hours, with an average of 3.05 out of 10 on a subjective soreness scale.

Two possible limitations to the study were that we did not directly measure muscle glycogen levels in our subjects and that the muscle fibers depleted of glycogen via the bicycle protocol were not recruited during the treadmill protocol. In response to both limitations, we followed the same protocol as Gollnick et al. (20), in which subjects experienced nearly complete depletion of glycogen in both slow- and fast-twitch muscle fibers of the vastus lateralis muscle. Hickner et al. (23) used a similar protocol with untrained subjects and also obtained severe glycogen depletion in the quadriceps femoris muscle of both slow- and fast-twitch fibers. Not only are the same muscles utilized in the downhill run as the bicycle protocol (4, 12, 13, 40), but it is also the fast-twitch fibers that are preferentially depleted in this type of eccentric activity (16, 17, 27, 29). Concerning the depleted muscle fibers, Hickner as well as others (6, 30, 32) have shown that untrained subjects, 12 hours after depleting exercise on a high carbohydrate diet (>80%), were mostly repleted with muscle glycogen. Therefore, we believe that glycogen repletion occurred in the FED group in response to the high carbohydrate meal consumed immediately following the depleting exercise. Fasted subjects undergoing depleting protocols show extremely minimal increases in muscle glycogen levels up to 4 hours post activity but show no further increases from the 4-hour time point to the 12-hour time point (31, 39, 44). Therefore, based on the literature, we are confident that significant muscle glycogen repletion did not occur in the DEP group.

An underlying assumption of our work is that fatigued muscle fibers became stiff due to decreased glycogen, decreasing the amount of ATP that is necessary for the release of the myosin cross bridge from the actin active site (11). This assumption can be contested. There is considerable evidence that suggests that fatigued muscle still has a normal concentration of ATP (14, 22). If that is the case, then even though decreased glycogen may predispose a muscle to be more susceptible to fatigue, it may not be more susceptible to damage, because it may not be ATP depleted.

The hypothesis of our study also fails to address the issue that fiber recruitment patterns are not altered by eccentric exercises (38). In eccentric activity below VO_{2max}, it is the slow-twitch muscle fibers that show the greatest decrease in glycogen levels, yet it is the fast-twitch fibers that demonstrate the greatest damage. These
studies also indicate that muscle glycogen uptake is impaired in all fibers post-
eccentric activity (9, 10, 38, 44), and some even suggest that this lack of glycogen
repletion could be responsible for the ultrastructural changes seen in damaged
muscle (38). The current results provide no additional insight into the mechanisms
responsible for muscle soreness, but they do indicate that soreness is not related to
manipulations of glycogen levels by carbohydrate intake and glycogen depleting
activity.

Conclusion

In the current study, 15 min of downhill running was sufficient to induce DOMS.
Furthermore, muscle strength, relaxed knee angle, thigh circumference, and per-
ceived muscle soreness indirectly demonstrated muscle damage. We proposed that
subjects who underwent a glycogen depletion protocol, followed by a 12-hour fast,
would be prone to a greater degree of stretch-induced injury compared to subjects
who had increased their intake of carbohydrates after a glycogen depletion protocol.
The data, however, reveal that was not the case. We conclude therefore that an
inadequate diet of carbohydrate after glycogen-depleting exercise does not predis-
pose one to muscle dysfunction or soreness.

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