Effects of dietary carbohydrate on delayed onset muscle soreness and reactive oxygen species after contraction induced muscle damage

G L Close, T Ashton, T Cable, D Doran, C Noyes, F McArdle, D P M MacLaren

Background: Delayed onset muscle soreness (DOMS) occurs after unaccustomed exercise and has been suggested to be attributable to reactive oxygen species (ROS). Previous studies have shown increased ROS after lengthening contractions, attributable to invading phagocytes. Plasma glucose is a vital fuel for phagocytes, therefore carbohydrate (CHO) status before exercise may influence ROS production and DOMS.

Objective: To examine the effect of pre-exercise CHO status on DOMS, ROS production, and muscle function after contraction induced muscle damage.

Method: Twelve subjects performed two downhill runs, one after a high CHO diet and one after a low CHO diet. Blood samples were drawn for analysis of malondialdehyde, total glutathione, creatine kinase, non-esterified fatty acids, lactate, glucose, and leucocytes. DOMS and muscle function were assessed daily.

Results: The high CHO diet resulted in higher respiratory exchange ratio and lactate concentrations than the low CHO diet before exercise. The low CHO diet resulted in higher non-esterified fatty acid concentrations before exercise. DOMS developed after exercise and remained for up to 96 hours, after both diets. A biphasic response in creatine kinase occurred after both diets at 24 and 96 hours after exercise. Malondialdehyde had increased 72 hours after exercise after both diets, and muscle function was attenuated up to this time.

Conclusions: Downhill running resulted in increased ROS production and ratings of DOMS and secondary increases in muscle damage. CHO status before exercise had no effect.

Methods

Subjects

Twelve physically active male subjects of mean (SEM) age 23.3 (0.98) years, height 175 (1.56) cm, body mass 76.7 (1.73) kg, maximal oxygen uptake ($\dot{V}O_2\text{MAX}$) 4.2 (0.14) litres/min, and body fat 14.6 (1.06)% were naïve to downhill running, volunteered for the study. All were informed verbally and in writing about the nature of the study, including all potential risks. Written consent was obtained before participation, and ethical approval was granted by the ethics committee of Liverpool John Moores University.

Abbreviations: CHO, carbohydrate; CK, creatine kinase; DOMS, delayed onset muscle soreness; MDA, malondialdehyde; NEFA, non-esterified fatty acids; RER, respiratory exchange ratio; ROS, reactive oxygen species; $\dot{V}O_2\text{MAX}$, maximal oxygen uptake.

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Delivered onset muscle soreness (DOMS) is a symptom of a type 1 muscle strain injury that presents as tender or aching muscles, usually felt during palpation or movement and can affect any skeletal muscle. It is associated with unaccustomed exercise, particularly if the exercise involves lengthening contractions. Although there have been many proposed mechanisms to account for DOMS, its exact aetiology remains unresolved. The most recent suggestion is that reactive oxygen species (ROS) may be responsible, although this remains questionable. We have recently shown, using electron spin resonance spectroscopy, that 48–72 hours after downhill running there is a significant increase in ROS production, which is likely to be from invading phagocytes. This finding is similar to those of a previous study which reported increased ROS using indirect measures 96 hours after downhill running. The 48–96 hour delay in the production of ROS in these studies suggests that the likely source is invading phagocytes. However, the exact role of this increased ROS production in the aetiology of DOMS is not fully established.

It is known that plasma glucose is a vital fuel for several cells of the immune system including phagocytes. It has been shown that carbohydrate (CHO) status before exercise and CHO ingestion during exercise is associated with smaller shifts in the number of circulating leucocytes, as well as attenuation of many immune cell functional responses. Increasing CHO status before exercise through a high CHO diet is designed to maximise liver and muscle glycogen stores, thereby maintaining blood glucose and ultimately minimising any immunosuppression. Conversely, depletion of CHO stores through a low CHO diet should result in decreased liver glycogen and thus reduced availability of blood glucose during exercise, resulting in a greater stress response and an associated immunosuppression.

The contribution of phagocytes to ROS production after exercise and the role of ROS in the aetiology of DOMS and loss of muscle function are unclear. Likewise, despite the popular practice by athletes of altering their CHO status before exercise to maximise performance, the effect of such dietary manipulations on DOMS, muscle function, and ROS production has not been reported. Therefore the main aims of this study were to: (1) investigate the effects of alterations in CHO status before exercise on phagocyte derived ROS production; (2) clarify the effects of dietary CHO manipulation on DOMS and muscle function.
Experimental protocol
The aerobic fitness of each subject was initially assessed by determining their VO2MAX as previously. This assessment was carried out one week before the first experimental run. All subjects then performed two downhill runs, both lasting 30 minutes at a running speed corresponding to 60% VO2MAX. One run was performed after two days on a high carbohydrate diet (HC), and the other after two days on a low carbohydrate diet (LC). All subjects were randomly allocated to one of two groups: LC first or HC first. The conditions were counterbalanced, and there was a five week interval between trials.

For each trial subjects were required to visit the laboratory on five consecutive days. On day 1 they performed the experimental run, had leg torque and pain measurements taken, and gave venous blood samples before and after the run. On the following four days, they had torque and pain assessed and gave a resting venous blood sample.

Dietary intervention
All subjects followed a given diet for 48 hours before the experimental trials. The diets were HC (77% CHO, 12% protein, 11% fat) and LC (11% CHO, 12% protein, 77% fat). They were designed for each subject ensuring that HC contained ~8 g/kg body weight CHO and LC contained ~1 g/kg body weight. The diets were produced in consultation with the subjects to ensure palatability. Fluids were consumed ad libitum, although drinks containing sugar were excluded. The diets were iso-energetic, with both diets providing ~14 MJ of energy. Table 1 summarises the characteristics of the two diets.

Day 1
Subjects arrived at the laboratory after an overnight fast, and a blood sample was taken before the run. All blood samples were collected into vacutainers with a total of three vacutainers at each sample point. Tube 1 contained EDTA and was used for the analysis of leucocytes, total glutathione, and lactate. Tube 2 contained serum separation gel and was used for the analysis of malondialdehyde (MDA). Tube 3 contained lithium heparin and was used for the analysis of non-esterified fatty acids (NEFA), glucose, and creatine kinase (CK).

Glucose, CK, and NEFA were measured by enzymatic methods using a commercially available kit (Instrumentation Laboratory, Warrington, Lancashire, UK) on an IL Lab 300 chemistry analyser. Lactate was immediately deproteinised using cold perchloric acid and later analysed on an IL Lab 300 chemistry analyser using a commercially available kit (Randox Lactate PAP; Randox Laboratories, Crumlin, Co Antrim, UK). MDA was analysed by high performance liquid chromatography (Gino 50; Dionex, Macclesfield, Cheshire, UK) using the methods of Esterbaur et al. Total glutathione was measured spectrophotometrically using a 96 well microplate reader with kinetics by the methods of Anderson. Total and differential leucocytes, haemoglobin, and packed cell volume were determined on an automated Coulter counter (Coulter MAXM analyser; Coulter Corporation, Miami, Florida, USA).

After the blood sample had been taken, subjects were asked to rate the pre-exercise muscle soreness of their gastrocnemius, tibialis anterior, hamstring, quadriceps, gluteal (both left and right sides), and lower back muscles using a visual analogue scale. Briefly, they were asked to contract the selected muscle and then make a mark along a 10 cm line. The far left point of the line represented no pain at all and the far right point represented unbearable pain. The distance from no pain at all was measured, and this represented the magnitude of DOMS for that particular muscle. Scores were recorded as the mean pain for the 11 assessed sites. Muscle function was then assessed concentrically and eccentrically using an isokinetic leg dynamometer as described previously.

The downhill run was performed on the same motorised treadmill as used in the VO2MAX test. The run lasted for 30 minutes at an oxygen consumption corresponding to 60% VO2MAX. Respiratory exchange ratio (RER) and VO2 were assessed every 10 seconds throughout the run using online...
gas analysis (Metamax Cortex Biophysic GMbH, Leipzig, Germany).

Immediately after the run, subjects lay down and a second venous blood sample was taken. Their perceptions of muscle soreness were reassessed before they completed the force assessment again.

Days 2, 3, 4, and 5
Subjects visited the laboratory exactly 24, 48, 72, and 96 hours after their initial visit. They were seated for 30 minutes before a resting venous blood sample was taken from a prominent vein. The blood test took place at the same time of day as the pre-exercise blood test performed on the initial day to eliminate any circadian effects. After the blood tests, subjects were assessed for DOMS. This was followed by a five minute warm up on a cycle ergometer before muscle torque was reassessed. All subjects were then allowed five weeks of recovery before they repeated the test using the second dietary condition.

Statistical analysis
Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS Surrey, UK). All data are presented as mean (SEM). Data were analysed using a repeated measures analysis of variance to prevent familywise error. When Mauchley’s test of sphericity indicated a minimal level of violation (>0.75), the degrees of freedom was corrected using the Huynh-Feldt adjustment. When the sphericity was <0.75, the Greenhouse-Geisser correction was used. Post hoc Tukey analysis (Honestly Significant Difference (HSD)) was performed to identify where the significant differences occurred. Significance was set at the α level of 0.05 for all tests.

RESULTS
All subjects successfully completed the 30 minute run on both occasions, and were able to maintain 60% VO2max throughout. There was no significant difference in mean VO2, rating of perceived exertion, heart rate, minute ventilation, and run speed between the two dietary interventions (p>0.05, data not presented).

Metabolite data
Figure 1 shows non-protein RER during the runs under both dietary conditions. HC resulted in a significantly higher RER before exercise, and this remained elevated throughout the duration of the run. Mean RERs throughout the run were 0.91 (0.01) and 0.85 (0.01) for HC and LC respectively.

Plasma glucose, NEFA, and lactate concentrations were measured immediately before and after exercise (table 2). Before exercise, there was no significant difference in blood glucose between the dietary interventions. However, HC produced a significant (p<0.05) increase in plasma glucose

Table 2 Metabolite data obtained before and after a downhill run performed after consumption for two days of a high carbohydrate diet (HC) or a low carbohydrate diet (LC)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Before exercise</th>
<th>After exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC</td>
<td>LC</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>5.2 (0.18)</td>
<td>4.6 (0.17)</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>1.9 (0.18)†</td>
<td>1.3 (0.09)</td>
</tr>
<tr>
<td>NEFA (mM)</td>
<td>0.17 (0.02)†</td>
<td>0.38 (0.08)</td>
</tr>
</tbody>
</table>

Values are mean (SEM).
†Significant difference from before exercise (p<0.05).
*Significant difference from HC (p<0.05).
NEFA, Non-esterified fatty acids.

Figure 2 Mean (SEM) ratings of delayed onset muscle soreness (DOMS), measured using a visual analogue scale, before and after a downhill treadmill run performed after two days on a high carbohydrate diet (HC) or two days on a low carbohydrate diet (LC). *Significant difference from before exercise (p<0.05).

Figure 3 (A) Mean (SEM) serum malondialdehyde (MDA) concentrations and (B) mean (SEM) total blood glutathione concentrations before and after a downhill treadmill run performed after two days on a high carbohydrate diet (HC) or two days on a low carbohydrate diet (LC). *Significant difference from before exercise (p<0.05).
immediately after exercise whereas LC failed to do so (p>0.05). Before exercise, NEFA concentrations were significantly (p<0.05) higher after LC than after HC. Furthermore, there was a significant (p<0.05) increase in NEFA concentration after exercise for both dietary conditions, although the rise after LC was significantly (p<0.05) greater than after HC.

Before exercise, lactate concentrations were significantly (p<0.05) higher after HC than after LC. Both dietary conditions resulted in a significant (p<0.05) increase in lactate after exercise, although there was no significant difference between the conditions.

Delayed onset muscle soreness
For both dietary conditions, there was a significant (p<0.05) increase in ratings of muscle soreness immediately after exercise that cannot be classed as DOMS. However, there was a further significant increase 24 hours after exercise, and this remained elevated for up to 96 hours (p<0.05) for both conditions. There were no significant differences in DOMS between the two conditions (fig 2).

Reactive oxygen species
Serum MDA and total blood glutathione were used as indicators of ROS activity (fig 3). There were no significant increases in MDA after exercise (p>0.05) although it had increased significantly (p<0.05) in both groups 72 hours after exercise and remained raised at 96 hours. There were no significant differences between the two dietary interventions. There was no significant main effect of time or group on total blood glutathione concentrations (fig 3B).

Muscle damage
Plasma CK activity was used to assess muscle damage (fig 4). There was a significant (p<0.05) increase in plasma CK activity 24 hours after exercise for both dietary conditions. This had fallen by 48 hours and returned to baseline levels at 72 hours after exercise. There was, however, a second significant (p<0.05) peak in CK 96 hours after exercise, although there was no significant difference between the two conditions.

Muscle function
Figure 5A shows the change in concentric torque at 1.04 rad/s after both dietary interventions. There was a significant reduction in muscle torque 24 hours after exercise, and this remained suppressed 72 hours after exercise, although there was no significant difference between the dietary conditions.

Concentric quadriiceps torque was also assessed at 5.20 rad/s (fig 3B). There were significant (p<0.05) torque losses immediately and 24 hours after exercise, although muscle torque had returned to baseline by 48 hours.

Muscle torque was also assessed using an eccentric protocol (fig 3C). There was a significant (p<0.05) reduction in muscle torque immediately after exercise and up to 72 hours, although there was no significant difference between the dietary conditions.

Leucocytes
Downhill running resulted in a transient leucocytosis (p<0.05) occurring immediately after exercise for both diets. This was largely due to transient neutrophilia (p<0.05) as well as a transient increase in lymphocytes (p<0.05) (fig 6). There was no change in circulating eosinophil or basophil counts after either dietary condition. Furthermore, there was no significant difference in total or differential leucocyte counts between the two diets.
tent with previous research, there was also a secondary increase in CK activity 24 hours after exercise, probably reflecting muscle damage caused by the mechanical trauma of the lengthening contractions. However, consistent with previous research, there was also a secondary increase in CK at 96 hours. As there was an increase in ROS production at 72 and 96 hours, it is possible that this secondary increase in CK represents ROS attack on lipid membranes resulting in secondary muscle damage. Previous studies in mice have also suggested that secondary muscle damage after lengthening muscle contractions is caused by ROS.

Interestingly, the study by Zerba clearly showed that treatment with “polyethylene-glycol superoxide dismutase” prevented the secondary losses of muscle force, adding further support to the theory that secondary muscle damage may be ROS mediated.

There was, however, no relation between this secondary increase in CK activity and DOMS. DOMS had fallen significantly by 96 hours after exercise and had almost returned to pre-exercise levels. This suggests that the secondary muscle damage, possibly caused by upregulation of ROS, does not prolong the sensation of DOMS. One of the aims of this study was to determine if the production of ROS played a pathological or physiological role in the aetiology ofDOMS. As the increase in ROS was not associated with further losses of muscle function or an increase in DOMS, a dissociation between ROS and DOMS is suggested. ROS were peaking at a time when muscle function and ratings of DOMS were returning to pre-exercise values, suggesting that ROS produced by phagocytic cells in the days after lengthening contractions may play a physiological role in mediating the recovery. There was also no significant difference in ratings of DOMS between the two dietary conditions, suggesting that CHO status before exercise has no affect on muscle soreness.

This study also sought to determine the effects of dietary CHO manipulation on muscle function after muscle damaging exercise. There were significant losses of muscle function after both diets. However, there were no significant differences between the two dietary conditions, suggesting that pre-exercise CHO status has no effect on muscle function after contraction induced muscle damage.

It has been suggested that high fat diets, especially those high in n–3 polyunsaturated fatty acids, may inhibit neutrophil chemotaxis and the subsequent generation of superoxide by upregulation of superoxide dismutase. Although the amount of polyunsaturated fatty acids was not determined in this study, it can be concluded that two days on a 77% fat diet does not significantly inhibit the production of superoxide. Furthermore, we can also conclude from this study that high fat diets offer no protection against DOMS, as suggested by Lenn et al., or affect the recovery of muscle function.

Circulating leucocytes showed a similar response to that reported by Bishop et al. after three days on a CHO manipulated diet. There was a trend (albeit not significant) for attenuation of leucocytosis after exercise performed after the high CHO diet compared with the low CHO diet, which was largely due to attenuated neutrophilia. Interestingly, despite this attenuation of neutrophilia, there was no significant difference in ROS responses between the two conditions. The serum MDA and total blood glutathione concentrations suggested that oxidative stress and lipid peroxidation occurred 72 and 96 hours after exercise. One interpretation of these findings is that ROS produced in the days after downhill running are not related to post-exercise neutrophilia; however, as reported by Bishop et al., this type of conclusion can only be made if the functional capacity of the blood compartment is assumed to be directly related to the tissue in question, in this case the damaged muscle. One concern of this conclusion is that, in response to physical trauma, neutrophils migrate to the site of injury where they release chemotactic factors, which subsequently recruit other neutrophils to the damaged tissue. Moreover, it is known that, after lengthening muscle contractions, there is compartmentalisation of muscle damage, and therefore the site of injury will have a finite size. Therefore not all of the neutrophils in the circulation will need to infiltrate the damaged tissue and hence the circulating neutrophil count may not be a good indicator of neutrophil functional activity.
Bishop et al.\(^21\) reported that despite the attenuated neutrophilia associated with a high CHO diet, when changes in neutrophil were expressed as lipopolysaccharide stimulated elastase release per neutrophil, there was no difference between a high CHO and low CHO diet. Therefore, in relation to the present study, it would appear that, despite the trend towards attenuation of circulating neutrophils, there were still sufficient neutrophils in the circulation to migrate to the site of injury and exhibit a respiratory burst, as shown by the increased MDA production.

The subjects chose their own foods from a comprehensive list. Food was purchased and prepacked for the subjects, and the metabolite data suggest good adherence to the diets. The diets were iso-energetic and therefore any differences observed were due to the macronutrient differences and not total energy intake. Two days on a high CHO diet significantly increased resting RER and lactate concentrations and decreased NEFA concentrations before exercise, suggesting that the diets were successful in altering pre-exercise CHO status. As the diets were not combined with a bout of strenuous exercise, it is unlikely that muscle glycogen stores were significantly affected, although there was likely to have been a significant reduction in liver glycogen stores. However, despite these presumed changes in pre-exercise liver glycogen status, there were no observed differences between the two dietary interventions with regard to DOMS, ROS, or changes in muscle function. It must be noted, however, that the study did not measure liver or muscle glycogen and therefore any changes in glycogen status are deduced from the metabolic measurements. Furthermore, the dietary manipulation was only carried out for two days, and therefore conclusions on long-term dietary CHO manipulation cannot be made.

In conclusion, 30 minutes of downhill running at 60% \(\text{VO}_{2}\text{MAX}\) results in a delayed increase in ROS production and muscle damage. However, despite the vital role of glucose as a metabolic fuel, the two day alteration in CHO status before the exercise had no effect on DOMS, muscle function, or ROS production.
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