Protection of muscle membrane excitability during prolonged cycle exercise with glucose supplementation


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Stewart RD, Duhamel TA, Foley KP, Ouyang J, Smith IC, Green HJ. Protection of muscle membrane excitability during prolonged cycle exercise with glucose supplementation. J Appl Physiol 103: 331–339, 2007. First published April 5, 2007; doi:10.1152/japplphysiol.01170.2006.—To determine if exercise-induced depressions in neuromuscular function are altered with oral glucose supplementation, 15 untrained participants (VO₂peak = 45 ± 2 ml·kg⁻¹·min⁻¹, mean ± SE) performed prolonged cycle exercise at ~60% VO₂peak on two occasions: without glucose supplementation (NG) and with oral glucose supplementation (G). The oral G began at 30 min of exercise and was administered every 15 min (total ingested = 1.23 ± 0.11 g carbohydrate/kg body mass). Quadriceps isometric properties and membrane excitability were assessed prior to exercise, after 90 min of exercise, and at fatigue. Cycle time to fatigue was greater (P < 0.05) in G compared with NG (137 ± 7 vs. 115 ± 6 min). Progressive reductions (P < 0.05) in maximal voluntary contraction (MVC, N) were observed for NG at 90 min (441 ± 29) and at fatigue (344 ± 33) compared with pre-exercise (666 ± 30). At fatigue in G, the reduction in MVC was not as pronounced (P < 0.05) as in NG. Motor unit activation assessed with the interpolated twitch technique during an MVC following exercise was not different between conditions. During cycling, the G condition also resulted in a higher (P < 0.05) muscle compound potential (M-wave) amplitude (mV) at both 90 min (+50%) and at fatigue (+87%) compared with NG. Similar effects were also found M-wave area (mV·ms). These results suggest that the ergogenic effect of glucose supplementation occurs not as a result of decreased neural activation but to improved muscle function, possibly as a consequence of protection of muscle membrane excitability.

quadriceps; compound muscle action potential; blood glucose; fatigue; central and peripheral fatigue

PROLONGED SUBMAXIMAL CYCLING exercise ultimately results in neuromuscular fatigue as indicated by an inability to maintain power output. The inability to maintain power output has been associated with reductions in both voluntary and stimulated force production as measured soon after the cycling activity both isometrically by reductions in maximal voluntary contraction (MVC) (13, 43) and reductions in involuntary force, particularly at low frequencies of electrical stimulation (13, 43) and dynamically (32). Conceptually, the impairment in mechanical function observed following prolonged exercise could be due to failure in one or more processes involved in either neural signaling to the muscle (i.e., central) to the translation of the neural signal into an appropriate force response by the muscle (i.e., peripheral) or to a combination of both (18).

To isolate the role of central vs. peripheral failure in the reduction in MVC force that occurs, measurements of surface electromyography (EMG) and/or the interpolated twitch have been performed (4). Reductions in the EMG, a measure of the compound action potentials, elicited during an MVC, have been interpreted as a failure in central drive resulting in an inability to fully exploit the muscle force-generating power (41, 47). With the interpolated twitch, however, a technique that artificially increases central drive (5), the reduction in force observed during an MVC appears to occur not as a result of a compromised central drive but as a result of problems in the muscle (4). The limitation of both techniques as a measure of central drive is that it is assumed that transmission of the neural signal across the neuromuscular junction and the muscle fiber membrane is not impaired (34).

The manifestation and persistence of decrements in force at low frequencies of stimulation, commonly referred to low frequency fatigue (LLF) (16), are consistent with the notion that the primary site of the failure occurs within the muscle itself, namely to one or more of the excitation and contraction processes (18). Excitation-contraction coupling (E-C coupling) failure resulting in a decrease in cytosolic free calcium concentration ([Ca²⁺]), which occurs as a result of reductions in sarcoplasmic reticulum (SR) Ca²⁺ release, has been shown to be the major factor in LFF (1). The reductions in [Ca²⁺] could be due to a failure of plasma membrane to appropriately translate the neural command into repetitive action potentials, an uncoupling of the signaling between T-tubule depolarization and the calcium release channels (CRC), or to a direct disturbance in the SR itself (2). Evidence exists from different experimental protocols to support the importance of all three E-C coupling processes depending on the requirements of the task (1, 9, 48).

Oral glucose supplementation during prolonged exercise has been shown to have an ergogenic effect (10, 11, 22), possibly by altering the E-C coupling processes (37, 49, 54). Recently, it has been demonstrated that artificially induced elevations in arterial blood glucose in the anaesthetized rat resulted in increased fatigue in the repetitively stimulated plantaris muscle (27). The attenuation in fatigue was also accompanied by a decreased disturbance in membrane excitability as measured by the properties of the muscle compound action potential (M-wave) (28) and not to changes at the neuromuscular junction as assessed by maximal direct stimulation (27). The M-wave is a measure of the total current induced by a single supramaximal twitch and is used as a measure of membrane excitability (23). Since previous studies have demonstrated impaired membrane excitability using M-wave properties during prolonged cycling in the untrained (31, 32), it is conceiv-
able that oral glucose ingestion serves to protect membrane function. If such is the case, better protection of both MVC and M-wave properties would be expected in active muscle during prolonged cycling with glucose supplementation.

The purpose of this study was to investigate the role of oral glucose supplementation on fatigue resulting from prolonged cycle exercise. We have hypothesized that with comparable work, there would be less of a reduction in membrane excitability during cycling with glucose feeding, which would also be accompanied by less of a decline in MVC and the force generated at different frequencies of stimulation. Moreover, we have also hypothesized that central activation of the muscle, although reduced with exercise, would not be affected by the glucose intervention.

The issues addressed in this paper are part of a much larger study in which we have investigated muscle substrate utilization, metabolism and cation pump regulation, and blood hormonal responses.

METHODS

Participants

Fifteen healthy untrained volunteers participated in the study (14 males, 1 female). Peak aerobic power [peak oxygen consumption ($V_{O_2\text{peak}}$)], as assessed during progressive cycle exercise to fatigue, was $45.0 \pm 2.0 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. The study had the official approval of the Office of Research Ethics at the University of Waterloo. All participants were informed of all the experimental procedures and risks before providing written consent.

Experimental design. To investigate the relationship between submaximal cycling exercise to fatigue and glucose supplementation, participants cycled at $\sim 60\% V_{O_2\text{peak}}$ for 90 min, received a 5-min rest, and then continued to cycle until fatigue on two separate occasions, namely with no glucose (NG) and with glucose (G) supplementation. For both conditions, the same absolute power output was employed. The number of pedal revolutions per min was standardized at 60. The conditions were separated by $\sim 4$ wk. Participants were randomly assigned and blinded to either G or NG as their first condition. As expected, there was no order effect for any of the properties examined. Muscle mechanical properties were measured 15 min prior to exercise, during rest immediately after 90 min of exercise, and at fatigue in both NG and G. In addition, 10 supramaximal twitches were delivered to the quadriceps muscle of each volunteer at a rate of 1 twitch/s while seated on the cycle at rest prior to exercise, at 30 min, 60 min, and 90 min of exercise, and at fatigue. Participants were required to visit the laboratory 2 wk before the first experimental condition to have their $V_{O_2\text{peak}}$ measured and to become familiarized with the protocols used for assessment of muscle function. Prior to each condition, which was conducted in the morning, participants were also asked to abstain from any caffeine or alcohol use and to avoid vigorous activity for 48 h. Participants were also instructed to fast for 12 h prior to each testing session. All the testing was performed in a neutral environment ($\sim 20°C$; $\sim 50\%$ relative humidity). The cycle task was continued until volitional fatigue.

Diet Analysis and Glucose Supplementation

During NG, every 15 min beginning at 30 min of exercise, participants ingested a placebo drink of $\sim 200$ ml that contained a noncarbohydrate sweetener (Sugar Twin; Alberto-Culver Canada, Toronto, ON, Canada) consisting of a 7.5% solution of water, sodium cyclamate, benzoic acid, and methylparaben. During G, participants were given a $\sim 6\%$ glucose drink at matched time points. Total carbohydrate ingested during G was $\sim 1.23 \pm 0.11$ g/kg body mass. No electrolytes were included in either the sweetener or the glucose drink. For each subject, the volume of fluid ingested at each time point was matched between conditions. Beverages were served at room temperature ($\sim 20°C$). No other supplements, either beverage or solid foods, were provided during the exercise in either NG or G. All participants were also required to submit a detailed 7-day diet journal for the period prior to each experimental condition to ensure that there was minimal intersubject variability of macronutrient ingestion.

Muscle Stimulation and Force Measurements

Muscle measurements were initiated $\sim 2$ min following the cycling, the time needed to position the participant for the mechanical assessment. The time was standardized for each subject for each condition. The muscle properties assessed included MVC force, the forces elicited at different frequencies of electrical stimulation (ES), the force generated by a single supramaximal twitch superimposed on an MVC [the interpolated twitch (IT) technique], and the surface EMG recorded during an MVC with a supramaximal twitch. The experimental setup for the quadriceps muscle has been previously reported (43, 52).

In brief, muscle measurements were based on isometric knee extension performed on the quadriceps muscle with the participant sitting in a straight-backed chair with the ankle secured at $90°$ with a plastic cuff attached to a linear variable differential force transducer (LVDT). The LVDT was amplified by a Daytronic carrier amplifier at $11$ Hz, converted to a digital signal, and fed into an IBM computer for analyses. Each participant was secured in the chair by a waist belt and was instructed to keep their arms crossed over his/her chest during the measurement protocol. Electrical impulses were delivered to the quadriceps muscle through two aluminum chloride electrodes ($8 \times 13$ cm) by a Grass model S48 stimulator with an isolation unit. Electrodes were coated with warm conduction gel and placed on the proximal belly of the vastus lateralis near the hip (active electrode) and 5 cm above the patella (ground electrode). The electrodes were secured to the quadriceps by wrapping a stretchable athletic tape around the electrodes and quadriceps. All measurement equipment was calibrated with weights of known amount prior to each testing session. Twitch measurements were performed by delivering a single supramaximal impulse ($150$ V) for $50\, \mu$s. The supramaximal impulse was determined by progressively increasing voltage until a plateau in force was observed and then increasing voltage a further $25\%$. A force-frequency relationship was determined by delivering impulses of $50\, \mu$s at a train duration of $1$ s at frequencies of $10$, $20$, $30$, $50$, and $100$ Hz. The voltage used to measure the force-frequency relationship was determined by adjusting the voltage of a $100$-Hz stimulation until $50\%$ of the participant’s MVC was attained. This voltage was reassessed prior to both conditions to avoid possible changes in conductance over the 4-wk period between conditions. In a previous study (26), we have found that the reliabilities for different frequencies of stimulation, as assessed by the correlation coefficient, ranged between 0.91 and 0.96 for 10 and 100 Hz, respectively. For all stimulation frequencies, the maximal force ($P_m$, $P_s$), the maximal rate of force development ($+dP/dt_{\text{max}}$) and the maximal rate of force decline ($-dP/dt_{\text{max}}$) were assessed. In addition, contraction time (CT) and one-half relaxation time (1/2 RT) were measured for the twitch only. The percent motor unit activation (MUA) was assessed by the IT technique (5), which was performed by delivering a supramaximal stimulus at the peak of the participant’s MVC.

EMG

EMG activity (20- to $500$-Hz bandwidth) was measured by placing two Ag-AgCl Medtracite recording electrodes (10 mm diameter) on the vastus medialis muscle. Signals were collected at a sampling rate of $2,048$ Hz, amplified, and converted from analog to digital signals.
by a National Instruments AT-MIO-16H multifunction board. EMG signals for MVCs were full-wave rectified and averaged by 1-s blocks over the complete voluntary activation to yield an average EMG (aEMG) activation. M-waves were collected during the supramaximal twitches and were assessed for amplitude, duration, and area. M-wave amplitude is measured as the difference in mVs between the two peaks of the biphasic signal, M-wave duration is measured from the beginning to the end of the biphasic signal, and area is the integral of the absolute value of the biphasic wave form (19). It should be noted that since stimulation was induced in the vastus lateralis and M-wave properties measured in the vastus medialis, the possibility exists that different response patterns exist between the two muscles. This protocol was necessary because the area required for the stimulation electrodes and for tissue sampling precluded the use of the vastus lateralis for EMG recordings. In earlier work, we have found that the changes in the vastus lateralis EMG correlated with the changes in vastus lateralis EMG at the specific joint angles employed in the experiment (19).

**Cycle Ergometer M-wave and EMG Measurements**

M-wave properties and EMG were collected at 0 min (prior to exercise) at 30 min, 60 min, and 90 min of exercise and just prior to fatigue. M-wave properties during cycling were assessed by installing a trigger between the stimulator and cycle ergometer, which delivered a supramaximal twitch to the participant’s quadriceps muscle each time the participant’s knee was at a 90° angle. A rate of 1 twitch/s was attained by having the participants maintain a cadence of 60 rpm. A total of 10 twitches were delivered at each measurement point. M-wave properties were based on an average of five twitches, extracted from the middle of the set. In our experience, these provide the most stable results. Using this approach we have found that the coefficients of variation for any of the measurement points never exceeded 19, 6, and 19% for the amplitude, duration, and area of the M-wave, respectively, regardless of condition. A visual readout was present so participants could monitor their cadence. This protocol was adapted from a previously reported protocol (24). EMG collections were made while the participants exercised. EMG was full-wave rectified and averaged over 1 s intervals for each 10-s collection.

**Blood Measurements**

Blood, extracted at different time points during the exercise from a prewarmed dorsal hand vein, was placed in tubes containing 0.6 M perchloric acid and neutralized in 1.25 mM KHC03 prior to centrifugation and extraction of the supernatant. Blood glucose measurements were performed by fluorometry (33).

**Data Analysis**

Statistical analysis was performed with Statistica for Windows. Data for each individual property were analyzed by a two-way ANOVA for repeated measures to investigate the effect of time and condition. Post hoc comparisons were performed with a Newman-Keuls test. The probability level for significance was accepted at $P < 0.05$. Where differences are indicated between means in the text significance is implied.

**RESULTS**

**Pre-experimental Diet**

The participant’s average total daily caloric intake, based on a 7-day inventory completed immediately prior to the NG and G conditions, was 2,824 ± 142 calories. The percent macronutrient composition of the diet for carbohydrate, fat, and protein was 49 ± 2, 30 ± 2, and 18 ± 2%, respectively. There were no differences in total caloric intake or macronutrient content between the NG and G conditions.

**Exercise Time**

Glucose supplementation during prolonged exercise caused an increase in cycling time to fatigue (115 ± 6 vs. 137 ± 7 min).

**Mechanical Responses**

**Twitch properties.** During NG, the change in the twitch response depended on the property assessed (Table 1). For $P_t$, a reduction of ~25.3% was observed at 90 min of exercise with no further changes observed thereafter. The reduction in $P_t$ was also accompanied by 19.5% reduction in $+\text{dP}/\text{d}t_{\text{max}}$ at 90 min of exercise, and, as with $P_t$, no further changes was observed as the exercise continued until fatigue. The other measure used to characterize tension development, namely CT, was not altered with exercise regardless of duration. Of the two properties used to assess relaxation, namely 1/2 RT and $-\text{dP}/\text{d}t_{\text{max}}$, exercise only altered 1/2 RT (0 min > 90 min, fatigue). G supplementation failed to result in any differences from NG in the twitch properties that were assessed.

**Force-frequency properties.** Prolonged exercise resulted in a depression in $P_o$ at all of the stimulation frequencies examined for both NG and G conditions (Table 2). As with the twitch properties, the effect of exercise on $P_o$ was complete at 90 min regardless of the stimulation frequency employed. No additional effect of glucose was observed at any time point. When averaged over both conditions, the percent reduction in force at 90 min amounted to 50, 38.1, 18.1, 9.8, and 6.6% at 10, 20, 30, 50, and 100 Hz, respectively. $+\text{dP}/\text{d}t_{\text{max}}$ and $-\text{dP}/\text{d}t_{\text{max}}$ were altered by exercise at low stimulation frequencies (10, 20, and 30 Hz) (Fig. 1) but not at high frequencies (50, 100 Hz). At low frequency (10, 20, and 30 Hz), the reduction in $+\text{dP}/\text{d}t_{\text{max}}$ at 90 min of exercise amounted to 42, 26, and 20%, respectively. For $-\text{dP}/\text{d}t_{\text{max}}$, the change at 90 min of exercise was 41, 44, and

| Table 1. Properties of the twitch during prolonged cycle exercise with no glucose and oral glucose supplementation |
|-----------------|----------------|----------------|
|                | Time, min      | Fatigue        |
| $P_t$, N       | 186 ± 11       | 139 ± 12       | 138 ± 11       |
| $P_t$, G       | 181 ± 11       | 132 ± 10       | 128 ± 9        |
| CT, ms         | 84 ± 2         | 76 ± 3         | 77 ± 2         |
| $P_o$          | 85 ± 3         | 77 ± 4         | 84 ± 9         |
| $1/2$ RT, ms   | 53 ± 2         | 32 ± 2         | 32 ± 2         |
| $G$            | 49 ± 5         | 32 ± 3         | 32 ± 4         |
| $+\text{dP}/\text{d}t_{\text{max}}$, N/s | 3,301 ± 210 | 2,340 ± 206 | 2,339 ± 199    |
| $G$            | 3,062 ± 183    | 2,133 ± 176    | 2,114 ± 152    |
| $-\text{dP}/\text{d}t_{\text{max}}$, N/s | $-2,024 ± 118$| $-1,821 ± 138$| $-1,876 ± 138$|
| $G$            | $-1,886 ± 138$ | $-1,742 ± 161$| $-1,742 ± 126$|

Values are means ± SE (n = 15). NG, no glucose; G, glucose; 0 min, Pre-exercise. Note: time to fatigue was 115 ± 6 and 137 ± 7 min for NG and G, respectively. $P_t$, peak twitch force; CT, contraction time; $1/2$ RT, half relaxation time; $+\text{dP}/\text{d}t_{\text{max}}$, maximal rate of force development; $-\text{dP}/\text{d}t_{\text{max}}$, maximal rate of relaxation. Main effects ($P < 0.05$) of exercise were observed for $P_t$, $1/2$ RT, and $+\text{dP}/\text{d}t_{\text{max}}$. For each property, 0 min > 90 min, fatigue.
Table 2. Isometric force at different frequencies of stimulation during prolonged cycle exercise with and without oral glucose supplementation

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Time, min</th>
<th>NG</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 Hz</td>
<td>0</td>
<td>164±9</td>
<td>162±10</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>83±9</td>
<td>80±8</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td>82±9</td>
<td>78±8</td>
</tr>
<tr>
<td>20 Hz</td>
<td>0</td>
<td>287±13</td>
<td>280±14</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>175±19</td>
<td>176±19</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td>171±19</td>
<td>166±15</td>
</tr>
<tr>
<td>30 Hz</td>
<td>0</td>
<td>322±14</td>
<td>315±16</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>255±22</td>
<td>267±22</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td>248±21</td>
<td>249±20</td>
</tr>
<tr>
<td>50 Hz</td>
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<td>342±16</td>
<td>341±17</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>304±20</td>
<td>312±23</td>
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<tr>
<td></td>
<td>Fatigue</td>
<td>301±20</td>
<td>301±21</td>
</tr>
<tr>
<td>100 Hz</td>
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<td>346±15</td>
<td>336±16</td>
</tr>
<tr>
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<td>90</td>
<td>315±19</td>
<td>322±23</td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td>307±19</td>
<td>310±20</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 15). Note: time to fatigue was 115 ± 6 and 137 ± 7 min for NG and G, respectively. Main effects (P < 0.05) of exercise were found for each frequency of stimulation. For each frequency, 0 > 90 min, fatigue.

18% for 10, 20, and 30 Hz, respectively. Neither G or increased exercise time altered the response observed at 90 min for either property. The data for +dP/dt max and −dP/dt max for 20, 30, 50, and 100 Hz are not presented.

Regardless of condition, prolonged exercise also resulted in large depressions in MVC at 90 min with further reductions observed at fatigue (Fig. 2). For the NG condition, the reduction in MVC at 90 min and at fatigue amounted to 33.8 and 49.3%, respectively. At fatigue, the MVC in G was higher than in NG, indicating that force loss during maximal exertion was not as pronounced. In an attempt to determine if the loss of force at MVC was central or peripheral in origin, MUA profiles were assessed by the IT technique. Exercise, in general, resulted in small but significant progressive reductions in MUA at 90 min and at fatigue. Glucose supplements failed to modify these response patterns.

Electromyography. Measurements of aEMG recorded during cycling were reduced at fatigue compared with 30 min of exercise regardless of condition (Fig. 3). Compared with 30 min of exercise, a strong trend is also indicated at 60 min (P < 0.06) and 90 min (P < 0.08) of exercise. No differences were observed between NG and G conditions for aEMG at any time point.

M-wave properties. The properties of the M-wave were measured both while cycling and while the measurements of muscle isometric properties were being assessed. During cycling in NG, the amplitude of the M-wave was first observed to decline at 60 min with a further progressive decline observed at 90 min (Fig. 4). No further reductions in amplitude were

Fig. 1. Effects of prolonged exercise and prolonged exercise plus glucose supplementation (G) on maximal rate of force development (A) and the maximal rate of relaxation (B) at 10 Hz stimulation. Values are means ± SE (n = 15). +dP/dt max, maximal rate of force development; −dP/dt max, maximal rate of relaxation. Time to fatigue was 115 ± 6 and 137 ± 7 min for NG and G, respectively. A main effect (P < 0.05) of exercise was found for both +dP/dt max and −dP/dt max. For both properties, 0 min > 90 min, fatigue.

Fig. 2. Maximal voluntary isometric force (A) and motor unit activation (B) during prolonged exercise with and without glucose supplementation. Values are means ± SE (n = 15). MVC, maximal voluntary contraction force; 0 min, pre-exercise. Note: time to fatigue was 115 ± 6 and 137 ± 7 min for NG and G, respectively. Motor unit activation (MUA), % activation of motor unit pool. For MUA, a main effect (P < 0.05) of exercise was found. 0 min > 90 min, fatigue. *Significantly different (P < 0.05) from 0 min; †significantly different (P < 0.05) from 90 min; ‡significantly different (P < 0.05) from NG.
observed with NG at fatigue. At 90 min and at fatigue, amplitudes were higher in G compared with NG. For the other two other properties of the M-wave assessed, namely duration and area, only the area was observed to change with exercise. As with the amplitude, at 90 min and at fatigue, areas were higher with G compared with NG. This was because the exercise induced decrease in area for NG observed at these time point did not occur in G.

M-wave properties, namely the amplitude and area, assessed ~2 min after the cessation of cycling, were also found to be altered (Fig. 5). For both properties, reductions were noted at 90 min of exercise that persisted until fatigue. An effect of glucose was not observed for either amplitude or area.

**Blood glucose.** For the NG condition, blood glucose concentration did not decline until 90 min of exercise (Fig. 6). A further decline was observed in blood glucose from 90 min of exercise until fatigue. Oral glucose supplementation prevented the decline in blood glucose. At 60, 90, and 115 min of exercise, blood glucose was higher in G compared with NG.

**DISCUSSION**

**Role of Central vs. Peripheral Activation**

In this study, we showed that, as hypothesized, cycling time to fatigue was significantly longer when glucose was administered, a finding in agreement with other groups (11, 29, 36, 50). Of particular experimental interest was whether glucose acted centrally and/or peripherally to promote the increased cycle performance. To address this issue we have measured MVC and used the IT to measure central drive, expressed as %MUA. We found a reduction in MVC force during prolonged exercise that was attenuated by glucose supplementation; however, the attenuated reduction in MVC was not accompanied by attenuations in central drive, as measured by the IT technique. This is in contrast to previous work that found that oral glucose ingestion helped protect MVC following exercise that could be explained by a less of a reduction in central drive (36). However, we did find that prolonged exercise caused small but significant reductions in central drive as indicated by the %MUA in both the NG and G conditions. Interestingly, compared with the study documenting the beneficial effects of glucose supplementation on central drive (36), our blood glucose concentration during prolonged cycling without glucose was much better preserved.

Reductions in central drive could be attributed to reduced activation of the α-motor neurons, resulting in decreases in action potential frequency in efferent motor nerves or to reductions in the transmission of action potentials across the
neuromuscular junction (34). Since we found no differences in the reductions in %MUA with glucose, both of which depend on continued integrity of neuromuscular transmission, the ergogenic effect of glucose is likely not a direct result of reductions in the central drive. Rather, it would appear that peripheral factors are involved in mediating the effect of glucose.

Peripheral Fatigue

By stimulating the quadriceps muscle at different frequencies, we were able to determine if the loss of force with exercise was specific to high or low action potential frequency. As expected, we observed that the major decreases occurred at the low stimulation frequencies, namely 10 and 20 Hz, with only small reductions at the high stimulation frequencies, namely 50 and 100 Hz. The LFF that we observed has been documented on many previous occasions (2, 8, 53) and, based on single fiber experiments, has been attributed primarily to a reduction in $[\text{Ca}^{2+}]_i$, secondary to reductions in $\text{Ca}^{2+}$ release by the SR (1). The more pronounced fatigue observed at low frequencies of stimulation has been attributed to the sigmoidal shape of the force-$[\text{Ca}^{2+}]_i$ activation relation (1). At low stimulation frequency, a given change in $[\text{Ca}^{2+}]_i$ has a much greater effect on force than at high stimulation frequency.

Our results indicate that the magnitude of the LFF observed was not affected by glucose supplementation, regardless of the time of exercise. These findings suggest that the critical processes responsible for LFF were not altered with glucose supplementation. This was unexpected since we had hypothesized, based on previous studies demonstrating a beneficial effect of increased carbohydrate availability on SR function (8, 30, 49, 54), that LFF fatigue would be less pronounced.

The M-wave, which is a composite record of multiple muscle fiber action potentials generated by a single supramaximal twitch stimulation (19), was used to examine membrane excitability. We have found not only that two properties of the M-wave, namely the amplitude and area, were both decreased during prolonged cycling but that the decrease was less pronounced with glucose supplementation. Since these two properties are recognized as the more robust measures of membrane excitability (3, 39), our results would indicate that the effect of glucose was to defend the ability of the sarcolemma and T-tubules to conduct repetitive action potentials during the prolonged cycling task. Although disturbances in M-wave responses have not always been a consistent finding with exercise, evidence does indicate that these properties can be...
compromised with prolonged cycling in the untrained (31). The volunteers used in this study were also untrained.

A key issue is whether or not the loss of membrane excitability can explain the pronounced LFF that we have observed following our cycling protocol. The general consensus is that LFF depends on a failure either in coupling of the T-tubular depolarization to the Ca$^{2+}$ channel (48) or to a modification in the Ca$^{2+}$ release channel itself (2). This position is also supported by studies that demonstrate that recovery of membrane excitability occurs much more rapidly than recovery of force at low frequencies of stimulation (1, 6). Given the slow recovery of LFF, it would appear that the magnitude measured after the exercise was a good approximation of what actually occurred during cycling. Therefore, based on the conditions of the experiment, we cannot implicate the reduction in membrane excitability as a cause of LFF or LFF as a factor in the ergogenic effect of glucose.

With the exception of MVC, we could find no effect of the glucose supplementation on the muscle mechanical characteristics measured following the cycling task. This was unexpected given the loss of membrane excitability that occurred during the cycling itself. It is possible that the effect of the loss of membrane excitability as measured by the properties of the M-wave would only be manifest at high frequencies of stimulation when demands for active Na" and K" transport across the plasma membrane at high action potential frequency are substantially increased (25). Unfortunately, the high-frequency stimulation employed in our measurement protocol, namely 50 and 100 Hz, were relatively brief and consequently probably insufficient to exploit the loss in membrane excitability as observed with the M-wave assessment. Future studies are needed to define the significance of graded losses in membrane excitability on the threshold effect of repeated stimulation at different frequencies on failure of the membrane to function effectively. In this regard, it should also be noted that with our stimulation protocol, only ~50% of the pre-exercise MVC force was generated. This limitation must be considered in the interpretation of our results since not all motor units in the quadriceps are activated by the stimulation. This could explain the discrepancy in the reduction in force loss as measured by an MVC and by the ES procedure. Exercise-induced increases in muscle temperature could also affect the interpretation of our results when comparisons are made with pre-exercise measurements. Force production at different frequencies of submaximal stimulation are selectively affected with increased temperature (12). Increased muscle temperature also appears to accelerate twitch force development and relaxation but not peak tension (14). The temperature effects, although influencing the time-dependent changes, would not affect the comparisons between conditions.

**Study Limitations**

An elementary issue is the mechanism underlying the ergogenic effect of glucose supplementation on prolonged submaximal cycle effort. Unfortunately, the time taken (~2 min) for transport of the participant from the cycle to the chair for preparation and administration of the isometric measurements could have resulted in some recovery sufficient to mask the disturbances as an example in the M-wave measurements that occurred during actual cycling (35, 42). Measurements after exercise clearly demonstrated reduced amplitude and duration of the M-wave; however, unlike the measurements performed during exercise the glucose effect was not significant. It should be noted that a strong trend toward higher values for both area ($P = 0.08$) and amplitude ($P = 0.07$) was observed with G.

In this study, we have used the properties of the M-wave as a measure of membrane excitability. When using these properties one must acknowledge a number of limitations. Our M-wave properties were assessed using surface electrodes and transcutaneous stimulation. As such, the M-wave represents the composite of multiple action potentials obtained from activation of fibers of different types and locations in the muscle. In addition, the properties depend on not only the number of motor units activated but the dispersion of their innervation zones and conduction velocities and the shape of the action potential (17). The interpretation of the M-wave is further complicated by the degree to which the properties reflect both disturbances in sarcolemma and T-tubule excitability. It has been reported that substantial reductions can occur in the surface action potential without affecting E-C coupling and consequently twitch force (44). However, more recent studies reported a close association with M-wave disturbances and force (38, 39). Under the conditions of the current experiment, it is possible that the T-tubule may represent the primary site of failure in excitability given limited extracellular space and diffusion problems that exist (9). Several studies have implicated propagation failure in the T-tubules as a cause of fatigue (45).

Although it is tempting to conclude that the loss of membrane excitability is linked to the inability to continue cycling and coincidently to the ergogenic effects of glucose supplementation, limitations in our experimental design make this tentative. With our design, we are effectively attempting to explain the inability to continue cycling on the basis of isometric measurements performed soon after cycling. To generate the required torques for the submaximal cycling tasks, motor unit recruitment and firing rate increases occur in several locomotor muscles (40). The inability to sustain the cycling task could be due to failure either centrally or peripherally. Recent studies (41) have implicated a strong role for failure in central drive in the fatigue that occurs based on EMG (47) or on MUA (41) criteria, which assumes that neuromuscular signaling is not compromised. Since in this study the principal properties of the M-wave, namely amplitude and area, were progressively decreased with prolonged exercise, it is possible that the locus of fatigue resides not in the central drive but in the inability to conduct the excitation into the interior of the muscle cell. The fact that we have also been able to demonstrate the beneficial effect of glucose supplementation on M-wave properties during the cycling suggests that a loss of membrane excitability may be mechanistically involved in the inability to sustain cycle performance.

Experimental support for the critical role of membrane excitability in the ergogenic effect of glucose supplementation comes from animal experiments employing repetitive stimulation to the plantaris muscle. In these experiments, artificial elevations in blood glucose resulted in less fatigue and less of a disturbance in M-wave amplitude and area, both of which could not be explained by alterations in neuromuscular transmission (27). Increased protection of membrane excitability with glucose supplementation suggests improved membrane
transport of Na\(^+\) and K\(^+\). Accordingly, it would be expected that an increased catalytic activity of the Na\(^+\)-K\(^+\)-ATPase would occur (9). Increases in blood glucose levels are known to increase the maximal activity of this cation pump, either directly or via insulin-induced increases in intrinsic pump activity or transport of the Na\(^+\)-K\(^+\)-ATPase subunits from intracellular storage sites to the plasma membrane (7, 9). It is known that increased blood glucose concentration during exercise is also accompanied by increased blood insulin levels (20). Measurements of maximal Na\(^+\)-K\(^+\)-ATPase activity performed as part of this study has confirmed an increase with glucose supplementation (21), which occurred in the absence of changes in SR Ca\(^{2+}\) cycling properties (Duhamel TA, Green HJ, Stewart RD, Foley KP, Smith IC, Ouyang J, unpublished observations).

Of additional interest was whether oral glucose supplements provided during the exercise altered the substrate and metabolic responses and, as a consequence, could be implicated in the ergogenic effect that we have observed. The results of previous studies using similar protocols generally agree that the magnitude of the exercise-induced glycogen depletion (22, 46) or the degree of reduction in energy state, as assessed by ATP and PCr contents (46, 51), is not affected by glucose supplements. The results of a companion study that we have performed are consistent with these observations. We could find no influence of increased glucose availability either on glycogen loss or muscle metabolism (Duhamel TA, Green HJ, Stewart RD, Foley KP, Smith IC, Ouyang J, unpublished observations).

In summary, we have shown that as expected, oral glucose supplementation significantly increases the time to fatigue during submaximal cycling. The improved performance is also accompanied by an attenuation in the disturbances in membrane excitability as measured by M-wave properties during the cycling. Measurements performed soon after the cycling indicate that the reduction in MVC that result occurs not as a result of deficiencies central drive as measured by motor unit activation but as a result of a failure in some peripheral process.

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**REFERENCES**


