Original Research

Exercise-Induced Glycogen Reduction Increases Muscle Activity

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ABSTRACT

International Journal of Exercise Science 9(3): 336-346, 2016. Intramuscular glycogen stores are an important energy source during extended bouts of strenuous exercise. A substantial reduction in glycogen could influence neural muscular drive and result in a decreasing quality of exercise performance and potentially increased injury rates. The aim of this study was to examine the effect of glycogen reduction on motor drive as determined by the surface electromyogram (EMG) amplitude and median frequency during a cycling graded exercise test. Eight trained cyclists performed a discontinuous cycling graded exercise test to exhaustion under both normal and glycogen reduced conditions. EMG was collected from the vastus lateralis. Repeated measures regression models indicated that EMG amplitudes were elevated at cycling workloads higher than 196 Watts and metabolic workloads higher than 40.8 ml/kg/min, corresponding to 77% VO_{2max}. There was no effect of increases in workload or glycogen reduction on EMG median frequency. Changes in mechanical and metabolic workload had a substantial effect on EMG amplitude (Cohen's $f^2 = 0.227$ and 0.247, respectively), but not median frequency (Cohen's $f^2 = 0.026$ and 0.033, respectively). Thus, EMG amplitude is a more effective and reliable measure to examine changes in motor drive during variable workload conditions and metabolic perturbations. The results suggest that healthy glycogen reduced humans require higher levels of muscle activity in order to attain a given mechanical and metabolic workload. This may affect the long term performance of professional and military athletes who need to be able to perform at a high level for extended periods of activity.

KEY WORDS: Electromyography, surface EMG, median frequency, cyclists, sample entropy

INTRODUCTION

The ability to perform at a high level in a state of muscular fatigue is important for endurance athletes and military personnel, especially considering liver and muscle glycogen is reduced during recent or prolonged activity. Gollnick et al. (10) have shown that a substantial amount of muscle and liver glycogen is reduced after only 120 minutes of continuous exercise at ~64% of maximal aerobic capacity. Soldiers, for example, routinely march for periods greater than 120 minutes on level and

inclined surfaces with loads meeting and exceeding the 64% VO_{2max} threshold for extended periods (33). This fatigued and lower energetic state leads to an increased likelihood of muscular (20), ligamentous (2) and/or bony injury (6). It is unclear whether the decreases in muscle glycogen result in changes in motor drive, which could contribute to the increased injury. Understanding changes in motor drive due to decreases in muscle glycogen are vital to discerning physiological status of the soldier or endurance athlete and their ability to perform under duress.

In healthy humans, surface muscle electromyography (EMG) after glycogen reduction has been previously examined in isometric exercise (11), cycling time-trials (23) and incremental cycling (9). Grisdale et al. (11) demonstrated that vastus lateralis EMG amplitude increased and frequency power spectrum decreased after glycogen reduction in a series of submaximal isometric knee extensions. On the contrary, Osborne and Schneider (23) reported that median EMG frequency increased during constant-load cycling above the ventilatory threshold in a glycogen reduced state. Glass et al. (9) used glycogen reduction in incremental cycling to examine the EMG amplitude threshold, a controversial phenomenon not always observed (1, 13, 14, 18, 30, 31). Nonetheless, a later onset of EMG amplitude increase and a non-significant decreased EMG amplitude at maximal workload may indicate that motor drive is lower in glycogen depletion (9). Our research group has previously examined how glycogen reduction alters surface EMG during maximal contractions interspersed within a graded cycling exercise test, which suggested that EMG amplitude may increase in a glycogen reduced state (32); however, interspersed maximal isometric contractions may not represent the submaximal dynamic contractions performed during cycling. The conflicting results and differing methodologies from previous cycling and isometric data with respect to EMG amplitude and median frequency provide an unclear picture regarding the changes in EMG parameters during exercise in a glycogen reduced state.

McArdle's disease, where patients are unable to metabolize muscle glycogen (21), provides an interesting pathologic model for understanding the effect of glycogen reduction on motor drive. A low-force sustained isometric contraction of the plantar flexors results in decreases in EMG median frequency for both McArdle's patients and controls, but only McArdle's patients show substantial increases in EMG amplitude (34). During constant-load cycling at ventilatory threshold, McArdle's patients demonstrated greater vastus lateralis EMG activity compared to controls (25). Furthermore, at graded cycling exercise test workloads as low as 40 watts, elevated vastus lateralis activity is evident in McArdle's patients (25). Therefore, increased motor drive is necessary to complete constant load tasks when glycogen is reduced or inaccessible (11, 23, 25, 34). Graded exercise may also result in increased motor drive when glycogen availability is reduced (25), though this has not been studied in healthy populations. The EMG frequency spectrum may shift lower (11) or higher (23) during constant-load exercise and there is no EMG frequency data available for a glycogen reduced state during graded exercise. The lack of consensus in the field regarding EMG

frequency metrics in glycogen reduction may be a result of the low number of studies overall.

The goal of the present study was to examine the effect of glycogen reduction on EMG amplitude and median frequency during a graded cycling exercise test in a healthy population. We hypothesized that glycogen reduction results in increased muscle activity (EMG amplitude) and decreased median frequency, due to the onset of fatigue. Furthermore, we quantified the absolute workload and relative metabolic workload necessary to elicit the changes in EMG amplitude and median frequency.

METHODS

Participants

The participant characteristics and exercise methodologies have been previously discussed (22, 32). Briefly, eight (4 males and 4 females) cyclists and triathletes participated in this study (ages 21-38). The trained participants (VO_{2max}: 53.2±8.6 ml/kg/min; mean body mass: 68.4±8.1 kg; mean height: 173±10 cm) had been training for the sport a minimum of 90 days. All participants gave informed consent and all study protocols were approved by the university's Institutional Review Board.

Protocol

Exercise testing and glycogen reduction were performed on Monark 828E cycle ergometer (Varberg, Sweden). The ergometer was modified to accommodate the participant's own clipless pedals, shoes and saddle (seat) in an attempt to approximate the participant's normal motor patterns when cycling on his/her road bike. Oxygen uptake (VO_2) was measured by a ParvoMedics TrueMax 2400 Metabolic System (ParvoMedics, Ogden, Utah) that was gas and volume calibrated prior to every testing session. Surface EMG was collected via a Delsys Bagnoli system (Delsys Inc., Boston, Massachusetts). This system configuration is: interelectrode distance = 10 mm; amplification factor = 10,000 (20–450 Hz); CMMR @60 Hz > 80 dB. EMG was collected at 4,096 Hz.

Each subject completed two graded exercise tests separated by at least a week to allow for glycogen replenishment (19) and minimize any muscle soreness. One test was the control and was performed under normal conditions. The experimental test was preceded by a cycling time trial, performed 8 hours prior, designed to reduce glycogen stores. The order of testing was counterbalanced.

The glycogen reduction time trial was developed and validated by invasive muscle biopsies (10) and the protocol was previously reported effective by decreased blood lactate production in this cohort (22). The session consisted of the participant cycling at 65-70% of his/her predicted VO_2max for 120 minutes. VO_2 and heart rate were monitored every 20 minutes in order to maintain proper intensity. The participants were instructed to not consume anything, except water, between the glycogen reduction time trial and the experimental exercise trial the following morning. Prior to testing in the experimental trial, verbal confirmation of adherence to the study protocol was obtained.

All participants were asked to refrain from vigorous exercise 24 hours prior to exercise testing (in the case of the control trial) and to

avoid caffeinated or alcoholic beverages 8 hours prior to the exercise test.

Prior to exercise testing, preamplified EMG electrodes were secured over the vastus lateralis of the dominant leg at the midpoint between the greater trochanter and patella approximating the muscle fiber angle. The electrode was outlined in black permanent marker to facilitate similar placement between control and glycogen reduced trials. The skin of the participant was shaved, abraded and cleaned prior to electrode placement. Manual muscle tests were performed to confirm proper electrode placement and limit cross-talk from adjacent muscles. Maximal voluntary isometric contractions were performed to verify appropriate signal-to-noise ratio.

The graded exercise test consisted of discontinuous, 5-minute stages of cycling at progressively higher workloads using a branching protocol (McMurray & Tenan, 2010). Each stage was performed at 80 rpm and the exercise was ceased once the participant was unable to maintain at least 70 rpm for more than 30 seconds. $VO_2$was collected continuously throughout each stage. A five-minute rest occurred between stages. The final minute of VO_2 from each stage was utilized for analysis, while EMG was collected during the final 30 seconds of each stage. The participant was required to remain in the saddle throughout each stage to maintain consistent motor patterns and pedal cadence.

All EMG data was processed in Matlab R2011b (Mathwork, Natick, Massachusetts). The onset and offset of muscle activity was detected using the sample entropy methodology proposed and validated by Zhang and Zhou (35). Briefly, sample entropy is a metric of signal complexity which assesses the probability $Bm(r)$ that two sequences match m points by counting the average number of vector pairs where the distance is lower than the tolerance r. $Am(r)$ is defined as the embedded dimension of $m+1$; therefore Sample entropy is calculated as: $-ln(Am(r)/Bm(r))$. In accordance with the conventions established by Zhang and Zhou (35), we set $m = 2$ and $r = 0.25 \times SD$ of the original time series. The onset detection threshold for the sample entropy algorithm was set at 0.65 because this was previously reported as an ideal threshold (35) and pilot testing in our data confirmed its use. Additionally, any detected bursts shorter than 100 milliseconds were removed from analysis because the burst detection was either aberrant or not representative of vastus lateralis muscle activity during a seated cycling pedal stroke. The active muscle EMG was assessed for amplitude and median frequency. For amplitude, each burst was full-wave rectified and the mean value was obtained and averaged across all bursts for a given exercise stage. The amplitude for each averaged burst was normalized to the highest singular data point collected during the exercise trial. For median frequency, only bursts which were greater than 250 milliseconds (1024 samples) were analyzed to obtain reliable frequency estimates. The power spectral density estimate were obtained using Welch's averaged periodogram with a linear detrending on 512 sample windows with 50% overlap, up to 2048 samples. This method results in a 1.17 Hz resolution. The median frequency is calculated as the frequency which divides the power spectrum in half and averaged across all applicable bursts within each stage. The

average median frequency of all the bursts within a stage is the median frequency for that stage. Median frequency is typically utilized as an indicator of neuromuscular fatigue; therefore, all median frequencies are reported as a percent change from the first 40 watt stage of the exercise test.

Statistical Analyses

All primary statistical analyses were performed using SAS 9.3 (SAS, Cary, North Carolina). Secondary interaction analyses were performed in an online R-based calculator (24). Four repeated-measures regression models were constructed. Each model used the maximal likelihood estimation technique and a first-order autoregressive covariance structure to account for the repeated-measures nature of the study. The first-order autoregressive structure assumes that the further apart measurements are in workload (absolute or metabolic) the less correlated the measures will be. This structure is theoretically superior to the typical ANOVA covariance structure, compound symmetry, which assumes that there is a constant variance between measures. The assumption of compound symmetry is particularly violated when workloads are not evenly spaced, as with the VO_2 within each testing session.

Each model included either EMG amplitude or percent change in median frequency as the dependent variable. When percent change in median frequency was assessed, the first 40 Watt stage was removed from analysis because inclusion of this data point (100% for each observation) would bias the regression. The impact of glycogen depletion and workload as well as the interaction of these effects were the independent variables. Separate models were run to assess different effects of absolute workload (Watts) and metabolic workload (VO_2, ml/kg/min).

When a significant interaction effect was obtained in one of the models, a secondary analysis of that interaction was performed to ascertain the "region of significance" (24), or where along the continuous workload variable there were differences between control and glycogen reduced trials. In order to perform this analysis, the continuous workload variable must be mean-centered at zero. This transformation does not change the fit of the regression model, impact statistical power or affect the reliability of the product terms (5). To facilitate interpretation, the mean-centered variables are converted back to the original units in the results section. The interaction analysis technique utilizes the 95% confidence intervals to determine the region of significance. Therefore, for both the primary model and secondary interaction analysis, alpha is maintained at 0.05.

In addition to assessing the effect of exercise workload on changes in median frequency and EMG amplitude, the effect size of workload on median frequency and amplitude was also assessed. Cohen's f^2 can be used to assess the effect size of individual variables was calculated for changes in median frequency and EMG amplitude within each model according to the methodology prescribed by Selya et al. (28).

RESULTS

There was a main effect of cycling workload on increased EMG amplitude ($p < 0.001$), but glycogen reduction had no significant effect

(p=0.122; see Figure 1). However, when comparing the control and experimental trials there was a significant interaction effect between glycogen reduction and cycling workload (p = 0.013), with the glycogen reduction trial resulting in increased EMG amplitude at workloads greater than 196 W (shaded region in Figure 1). Neither glycogen reduction (p = 0.513) nor cycling workload (p = 0.191) significantly influenced EMG median frequency, nor did these variables interact to influence EMG median frequency (p = 0.279; see Figure 2).

There was a main effect for both glycogen reduction (p = 0.037) and changes in VO_2 during the exercise test (p < 0.001) on EMG amplitude (see Figure 3). There was a notable interaction effect (p = 0.007), with significantly lower EMG amplitudes below 7.1 ml/kg/min and higher amplitudes above 40.8 ml/kg/min (shaded region Figure 3) in a glycogen reduced state. The EMG amplitudes below 7.1 ml/kg/min are a regression projection because this value is lower than any VO_2 recorded in this study. However, median frequency was not influenced by glycogen reduction (p = 0.205), VO_2 changes (p = 0.206) or the interaction of these factors (p = 0.207) (see Figure 4).

Both mechanical and metabolic workloads resulted in large effect sizes on EMG amplitude (Table 1). In contrast, mechanical and metabolic workloads resulted in small effect sizes on changes in EMG median frequency (Table 1).

Table 1. Effect size (Cohen's f^2) of mechanical and metabolic workload on EMG amplitude and EMG median frequency during the discontinuous graded exercise test.

Dependent Variable	Independent Variable	Effect Size (Cohen's f^2)
EMG Amplitude	Mechanical workload (W)	0.227
EMG Median Frequency	Mechanical workload (W)	0.026
EMG Amplitude	Metabolic Workload (ml/kg/min)	0.249
EMG Median Frequency	Metabolic Workload (ml/kg/min)	0.033

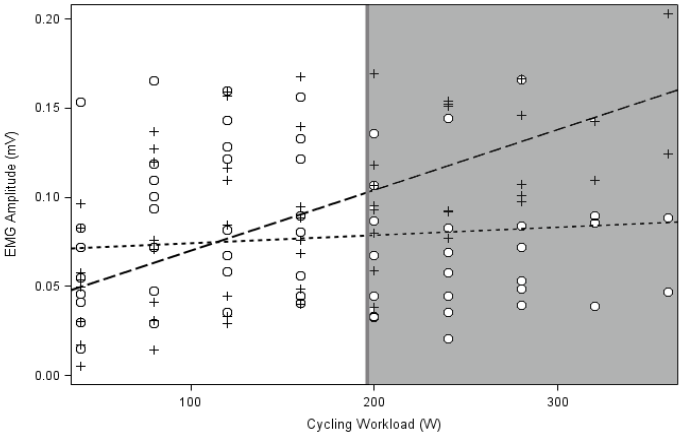

Figure 1. Average EMG amplitude with changes in cycling workload. Control data points (circles) are associated with the (--) model regression line. Glycogen reduced data points (crosses) are associated with the (— —) model regression line. The shaded area indicates the region of significance where the control and glycogen reduced data is statistically different (p < 0.05).

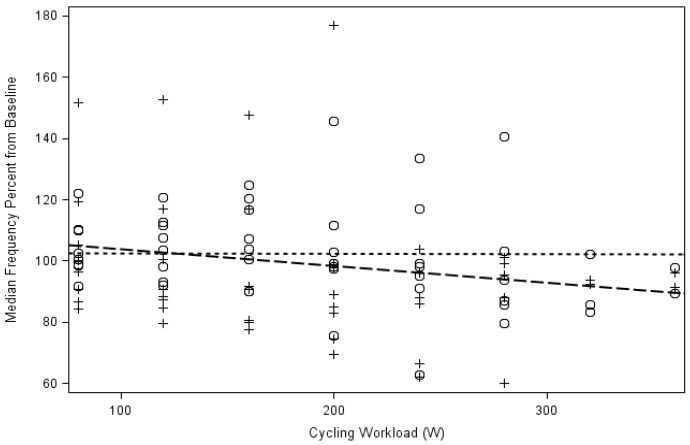

Figure 2. EMG median frequency percent change from baseline with changes in cycling workload. Control data points (circles) are associated with the (--) model regression line. Glycogen reduced data points (crosses) are associated with the (— —) model regression line.

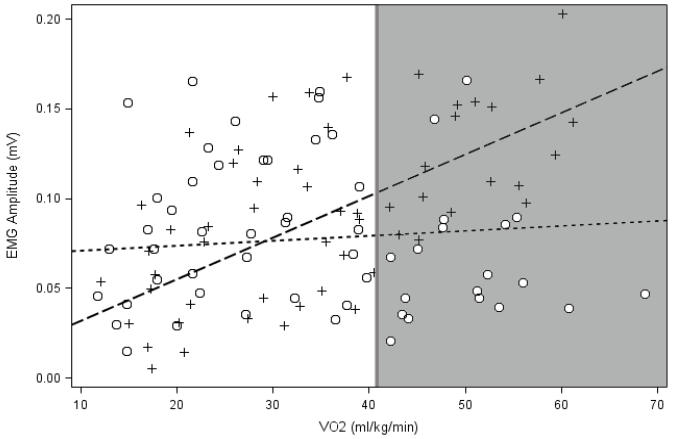

Figure 3. Average EMG amplitude with changes in metabolic workload. Control data points (circles) are associated with the (--) model regression line. Glycogen reduced data points (crosses) are associated with the (— —) model regression line. The shaded area indicates the region of significance where the control and glycogen reduced data is statistically different ($p < 0.05$).

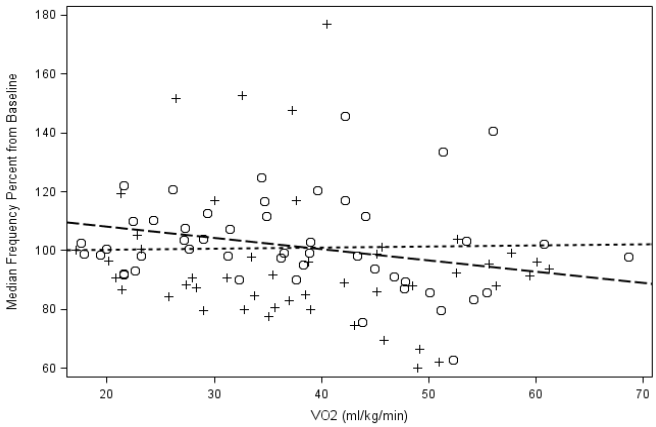

Figure 4. EMG median frequency percent change from baseline with changes in metabolic workload. Control data points (circles) are associated with the (--) model regression line. Glycogen reduced data points (crosses) are associated with the (— —) model regression line

DISCUSSION

The present analysis confirms the hypothesis that in a healthy population muscle activity is increased when glycogen stores are reduced. Further, the increase in muscle activity was found to be elevated only after a specific absolute (196 Watts) and metabolic (40.8 ml/kg/min) workload was reached. In our cohort, the metabolic workload eliciting a difference in muscle activation was 77% of VO_2 max on average. On the contrary, neither glycogen status nor workload had an effect on changes in EMG median frequency.

Muscle activation is increased when glycogen stores are low. The present study demonstrated that the findings using McArdle's patients (25) can be reproduced, to a some degree, in healthy subjects. While differences between McArdle's and control subjects were evident when cycling at 40 W of power output, our glycogen reduction

protocol elicited increased neural drive at power output exceeding 196 Watts. The metabolic workload, 40.8 ml/kg/min, needed to elicit increased neural drive in a glycogen reduced state can be attained by athletes engaging in activities lasting 2-h or longer.

Elevated vastus lateralis muscle activity in a glycogen reduced state implies that even maximal cycling tests do not require either maximal motor unit recruitment or maximal motor unit discharge under normal conditions. Glycogen reduction makes it necessary to recruit higher threshold motor units or increase the rate coding of active motor units in order to maintain the same absolute and metabolic workload. Previous research on McArdle's patients has demonstrated that intracellular pH increases during sustained contractions, as opposed to decreasing pH in control subjects (34). The increased pH is caused by a lack of lactate formation with concomitant hyperventilation and subsequent alkalization by the release of negatively charged phosphate groups from ATP breakdown. To a lesser extent, this process may occur in a healthy glycogen reduced population as this cohort has been previously shown to have substantially lower blood lactate production when glycogen reduced (22). The pH difference may be sensed by the Group III/IV afferents (26) and result in increased descending motor drive to complete the given workload.

The localized effect size measurements utilized in this study affirm the use of EMG amplitude for the assessment for muscle activity changes during graded cycling exercise; however, the effect size of workload on changes in EMG median frequency was very low. Given median frequency has been used as a measure of type 2 muscle fiber recruitment (7, 23) and fatigue (3, 8), the low effect size in a graded exercise test to exhaustion is surprising. It is likely that true neuromuscular fatigue does not occur in a graded exercise cycling test of relative short duration. Additionally, the recruitment of larger motor units, which contribute to high frequency content in the surface electromyogram, may obscure any fatigue-related frequency decreases. The EMG amplitude data in our study does not indicate that maximal motor unit recruitment necessarily occurred, even after glycogen reduction. The slope of median frequency decrease does appear to be greater after glycogen reduction, though the effect is low and changes are not statistically significant. The exercise test was terminated when the participant was unable to maintain power output even though no measurable changes occurred in EMG median frequency; therefore, human factors and exercise performance research may be better served using EMG amplitude measures over standard frequency metrics when the task is dynamic and not constant-load. Research has been ongoing in the use of wavelets and time-frequency distributions to assess neuromuscular performance (12, 16, 29), though the field has not yet adopted a standard processing technique for dynamic exercise.

For both the endurance athlete and military athlete, it is not always feasible to suggest mitigating fatigue and injury by decreasing exercise length or workload. Cycling "Tours", ultra-endurance runs, and a military march have fixed lengths and must be completed at a prescribed intensity, sometimes for days on end. Therefore, it is

necessary to find creative methods which decrease workload and decrease net glycogen turnover. Appropriate nutritional practices should also mitigate glycogen turnover during extended bouts of exercise. Ingesting carbohydrate during strenuous exercise does not alter the pattern of endogenous glycogen utilization (4); thus, it may not blunt the observed increases in muscle activity required to complete the task. When 50% carbohydrate meal is consumed prior to exercise, however, the vast majority of oxidized carbohydrate is derived from the meal instead of endogenous glycogen sources (27). The timing of carbohydrate ingestion may provide a simple solution to alleviate the observed increases in muscle activity with glycogen depletion.

EMG amplitude is a more reliable and useful measure of motor activity than EMG median frequency during dynamic graded exercise. This study demonstrates that glycogen reduction in healthy humans results in greater muscle activation, similar to McArdle's patients who are unable to metabolize glycogen. The increases in muscle activity necessary to complete a given workload may affect the long-term performance of the endurance athlete and military personnel unless steps are taken to alter the pattern of muscle glycogen reduction during prolonged, strenuous exercise.

ACKNOWLEDGEMENTS

Research was performed at the University of North Carolina at Chapel Hill

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