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[1]Muscle Health Research Centre, School of Kinesiology and Health Science, Faculty of Health, York University, Toronto, Ontario, CANADA; [2]Department of Human Health and Nutritional Sciences, College of Biological Sciences, University of Guelph, Guelph, Ontario, CANADA

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[1]Muscle Health Research Centre, School of Kinesiology and Health Science, Faculty of Health, York University, Toronto, Ontario, CANADA; [2]Department of Human Health and Nutritional Sciences, College of Biological Sciences, University of Guelph, Guelph, Ontario, CANADA

Address for correspondence: Dr. Arthur J. Cheng, 351 Farquharson Life Sciences Building, York University, Toronto, Ontario, Canada M3J 1P3; E-mail: ajcheng@yorku.ca; Phone: 1-416-736-2100 ext. 30030

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ABSTRACT

Introduction: We aimed to investigate the neuromuscular contributions to enhanced fatigue resistance with carbohydrate ingestion, and to identify whether fatigue is associated with changes in interstitial glucose levels assessed using a continuous glucose monitor (CGM). **Methods:** Twelve healthy participants (6 males, 6 females) performed isokinetic single-leg knee extensions (90°/s) at 20% of the maximal voluntary contraction (MVC) torque until MVC torque reached 60% of its initial value (i.e, task failure). Central and peripheral fatigue were evaluated every 15 min during the fatigue task using the interpolated twitch technique (ITT), and electrically evoked torque. Using a single-blinded cross-over design, participants ingested carbohydrates (CHO) (85g sucrose/h), or a placebo (PLA), at regular intervals during the fatigue task. Minute-by-minute interstitial glucose levels measured via CGM, and whole blood glucose readings were obtained intermittently during the fatiguing task. **Results:** CHO ingestion increased time to task failure over PLA (113 ± 69 vs. 81 ± 49 min; mean ± SD; $p < 0.001$) and was associated with higher glycemia as measured by CGM (106 ± 18 vs 88 ± 10 mg/dL, $p < 0.001$) and whole blood glucose sampling (104 ± 17 vs 89 ± 10 mg/dL, $p < 0.001$). When assessing the values in the CHO condition at a similar timepoint to those at task failure in the PLA condition (i.e., ~81 min), MVC torque, % voluntary activation, and 10 Hz torque were all better preserved in the CHO vs. PLA condition ($p < 0.05$). **Conclusions:** Exogenous CHO intake mitigates neuromuscular fatigue at both the central and peripheral levels by raising glucose concentrations rather than by preventing hypoglycemia.

Key Words: GLYCEMIA, BLOOD SUGAR, ENDURANCE EXERCISE, CONTINUOUS GLUCOSE MONITOR, CENTRAL FATIGUE, MUSCLE FATIGUE

Introduction

Carbohydrates (CHO) are one of the most widely used nutrition supplements in endurance sports (1-5), with the common belief that acute CHO intake can extend fatigue resistance, and this may occur by rapidly providing energy substrate to the body to maintain skeletal muscle force and power generation during voluntary exercise. Fatigue here is defined as any exercise-related reduction in muscle force (or power) generation during a given exercise task, regardless of whether the task can be sustained (6). The neuromuscular system is responsible for skeletal muscle force production beginning with motor unit activation and the propagation of action potentials descending the motor neurons, leading to activation and contraction of skeletal muscle. Yet, the ability of exogenous CHO, typically by oral ingestion, to preserve neuromuscular function during fatiguing exercise remains incompletely understood.

Carbohydrate ingestion has been identified as a method to slow the onset of central fatigue during exercise (7-12). Nybo et al. showed at task failure following 3 h of cycling at a workload of 60% of VO_{2max} that central fatigue occurred to a lesser degree upon the frequent ingestion of glucose (6% glucose polymer every 15 min; ~200 g in total) during 3 h exercise compared with the placebo condition through a significant preservation in voluntary activation capacity (10). In the same study, the preservation of voluntary activation with CHO feeding was associated with the maintenance of blood glucose concentration, as measured with antecubital venous sampling every hour. In contrast blood glucose concentration markedly decreased in the placebo condition and reached the clinical definition of level 2 hypoglycemia (≤54 mg/dL) (13) by the end of exercise wherein voluntary activation failure occurred, thereby implicating a possible role for circulating glucose concentration (or glucose delivery to the central nervous

system) in mitigating central fatigue. It has been commonly proposed that, at the intramuscular level, glycogen depletion is a major cause of muscular fatigue during endurance exercise because glycogen serves as a readily available intramuscular storage of glucose that can be broken down rapidly to supply ATP to the contracting muscles (7, 14-17). Nonetheless, a similar decline in muscle glycogen content exists during prolonged exercise when CHO or a placebo (i.e., CHO-free solution) are consumed, yet the endurance time is extended with the CHO feeding (7). These findings (7), in addition to other supporting literature (7, 18-20), indicate that by increasing total energy substrate availability, CHO ingestion can independently augment fatigue resistance irrespective of any effects on muscle glycogen sparing.

Continuous glucose monitors (CGM) have long been used by individuals with type 1 diabetes to reduce the risk of hypo- or hyperglycemia during exercise, by helping to inform when to consume or avoid CHO ingestion (21, 22). Current systems are deemed reasonably accurate for use during exercise for glucose concentration ranging from ~35-400 mg/dL (~13% mean absolute relative difference from clinical measures of glucose in the bloodstream), which can be observed during exercise in those living with type 1 diabetes (21). An emerging application of CGMs, however, has been its use by non-diabetic athletes to optimize CHO intake prior to and during exercise based on a continuous tracking of interstitial glucose (22-25). The use of CGM might help ensure that sufficient CHO is being ingested to help maintain circulating glucose levels to act as a fuel for contracting muscle, and the central nervous system, amidst fatigue onset. Furthermore, CGMs potentially mitigate previous research limitations caused by sparse blood glucose readings, improving our insight into the effects of real-time glucose fluctuations on exercise performance (25, 26).

This study aimed to investigate the role of CHO ingestion on time-course changes in fatigue developed at the level of the neuromuscular system. This approach seeks to elucidate the role of CHO intake and glycemia in fatigue at both central and peripheral levels. Additionally, it offers deeper understanding of how monitoring interstitial glucose levels in real-time aids in comprehending the impact of endurance exercise, with or without CHO consumption, on neuromuscular function. We utilized a randomized crossover study design in which healthy, young participants consumed either CHO or a placebo during a low-intensity endurance exercise task involving repeated isokinetic 90°/s single-legged knee extensions at a submaximal load (20% of MVC torque) until task failure. We hypothesized that 1) time to task failure (TTF) would be increased by the ingestion of CHO, 2) both central and peripheral fatigue would be lessened by the ingestion of CHO, and 3) the onset of neuromuscular fatigue during the placebo condition would coincide with a critical interstitial glucose concentration, thought to be at the threshold for biochemical hypoglycemia (~3 mmol/L).

MATERIALS AND METHODS

Subjects

Twelve healthy participants (6 M, 6 F) were recruited for this study. The participants in this study were 24.1 ± 5.8 years of age with an average height of 171.6 ± 6.3 cm and mass of 71.9 ± 11.0 kg. They were recreationally active and not participating in organized sport. Participants were free of acute or chronic disease affecting their neuromuscular performance or glycemic regulation. All participants completed the 2021 Physical Activity Readiness Questionnaire *(*PAR-Q) form, which identifies the existence of significant health problems (27), and they underwent an overnight CHO restriction for ≥8 hours, as per the recommendations of

the Diabetes Canada Clinical Practice Guidelines Expert Committee (28). Participants were instructed to avoid caffeine consumption the day of and to refrain from physical activity for at least 24 hours prior to testing. All participants gave informed, written consent and the study was approved by the local ethics committee on human research (York University Human Participants Ethics Review Committee). Two familiarization sessions were conducted to ensure participants were fully habituated to the experimental protocol. During each familiarization session, participants practiced maximal voluntary contractions, ensuring they produced > 90% voluntary activation assessed via a modified interpolated twitch technique (described below) as well as becoming familiarized with the electrical stimulation to ensure they were relaxed during these procedures. The participants were also familiarized with the fatigue protocol, ensuring that they could accurately achieve the target load for each contraction and that they could follow the metronome pacing (described below).

Apparatus and Recording

A HUMAC NORM ™ dynamometer (Computer Sports Medicine Inc., Stoughton, MA, USA) system was used to assess knee extension torque, velocity, and range of motion (ROM). Subjects were seated in the adjustable HUMAC NORM with a knee joint angle set at 90□ using a goniometer and a hip angle of 95□.□Two straps applied over the chest and one strap across the hip minimized extraneous movements during contractions. Proper alignment of the left knee was visually maintained by modifying the chair position, such that the knee's axis of rotation (tibio-femoral joint) was aligned with the axis of rotation of the dynamometer's attachment arm. The leg was secured to the attachment arm using wide Velcro straps on the anterior side of the quadriceps femoris and another strap around and directly above the ankle. ROM for all subjects

was maintained from 0–90° knee flexion (90° from start to end of knee extension). Complete knee extension is in anatomical reference to 0° of knee flexion.

Two custom-made electrodes were used to transcutaneously stimulate the quadriceps femoris. The electrodes were made by wrapping conducting gel-coated aluminum foil sheets with a paper towel soaked on the side that is in contact with the skin (29). The electrodes were sized ~ 7 cm in length, with the width adjusted to ensure that the largest possible surface area would be covered at the proximal and distal portions of the quadriceps without interference from antagonist muscles. Palpation and visual inspection of the quadriceps muscles ensured that only the knee extensors were activated during stimulation. Electrodes were connected to a DS7R high voltage constant current stimulator (Digitimer North America LLC, Fort Lauderdale, FL). The current that elicited maximal 100 Hz doublet torque was found for each participant with the DS7R stimulator (200 μs pulse width, 10 ms inter-stimulus gap) by stimulating the participants until the doublet torque no longer increases (30). This stimulation current was increased by 10% to ensure supramaximal activation of the muscle, and this doublet was used to estimate voluntary activation (described below). Also, the current eliciting 10 Hz and 50 Hz torque (200 μs pulse width, 1.5 s duration) was found for each participant by stimulating the participants until 50 Hz torque was 50% of maximum voluntary isometric contraction (MVC) torque (30, 31). MVC torque was determined by providing strong verbal encouragement and visual feedback of torque production on a computer monitor positioned approximately 2 m from the participant. Guidelines were displayed to provide motivation to attain a higher maximum force with each attempt. The participants were given a minimum of three attempts separated by 5 min of rest. Criteria to deem an MVC as maximal effort was that there was no further increase in force between attempts and

that voluntary activation was ≥ 90%. The MVC torque is reported as the peak torque measured prior to the superimposed doublet for the assessment of voluntary activation (Fig. 1).

The dynamometer's isokinetic mode was used to control the knee extension velocity, which was set at 90°/s and during the periodic isometric MVC torque measurements, the interpolated twitch measurements, and the 10 Hz and 50 Hz stimulations, the dynamometer was set to isometric mode. Visual feedback on leg position and torque was provided to participants using Spike2 ™ V. 10 software (Cambridge Electronic Design Ltd., Cambridge, UK) with target lines set on the computer monitor to ensure a full range of motion and attainment of 20% of the participant's initial MVC torque during each knee extension. A metronome was used to pace participants at 23 beats per minute such that they completed a full cycle of knee extension between each tick of the metronome. This pace of knee extension was determined through pilot testing to be the most suitable in allowing a low intensity of exercise and a sufficient period for participants to reach a full range of motion using the predetermined velocity of knee extension at 90°/s, while ensuring sufficient rest was provided between contractions to mimic prolonged endurance exercise.

Experimental Overview

Following the completion of the two familiarization sessions, participants underwent two trials through a randomized crossover design with one trial involving CHO ingestion and the other involving a placebo. A powder-based fruit-flavoured dessert (Jell-O ™, Kraft Foods, Chicago, IL, USA) was used for both the CHO ingestion trial and the placebo trial. In the CHO ingestion trial, a cup of Jell-O ™ consisting of 17 g of sucrose was given to participants prior to

the start of their endurance exercise protocol and every 15 minutes of performance, which amounted to 85 g of sucrose per hour of endurance exercise, with the level of CHO ingestion in line with previous literature denoting optimal level of CHO consumption during endurance exercise (2). The continuous ingestion of CHO throughout the exercise session was performed based on previous evidence showing an improved endurance exercise performance upon continuous intake of CHO in comparison with a single bolus of equivalent total CHO ingestion prior to exercise (32). The placebo control trial involved the use of sugar-free Jell-O ™ which was similar in colour, flavour, and texture to the CHO ingestion trial. No participants experienced any gastrointestinal symptoms related to the CHO or placebo ingestion. CHO and placebo ingestion occurred every 15 min as this was the most convenient moment when neuromuscular function assessments were being made and it also provided a sufficient time period to fully ingest the Jell-O ™. Neuromuscular fatigue measurements were taken prior to, and every 15 minutes during the exercise (Fig. 1). Task failure was determined when MVC torque during these neuromuscular fatigue measurements reached 60% of its pre-exercise value. The neuromuscular measurements involved assessments of isometric MVC torque, a modified ITT using a 100 Hz doublet (31, 33), and a 10 Hz as well as 50 Hz tetanus (1.5 s duration) to determine the 10:50Hz torque ratio (Fig. 1, Supplemental Fig. 3, Supplemental Digital Content, http://links.lww.com/MSS/C995 , Original records from one participant showing fatigue-induced changes in voluntary and electrically stimulated torque during the PLA and CHO trials). The 10:50 Hz torque ratio calculations were used to assess peripheral fatigue development, whereby fatigue-related reductions in sarcoplasmic reticulum calcium release and impaired myofibrillar calcium sensitivity within skeletal muscle cause greater impairments in low- vs high-frequency

force (14, 34). Furthermore, the 10 and 50 Hz tetani were 1.5 s in duration since this is a sufficient duration to reach peak force production during tetanic stimulations (35).

The participants wore a minute-by-minute continuous glucose monitor (CGM, Abbott Libre Sensor Glucose Sports Biosensor, Abbott Diabetes Care, USA) sensor for 14 days (sensor wear duration) under free-living conditions. The CGM measures the interstitial fluid glucose concentration (55-200 mg/dL range) by means of a subcutaneous sensor and logs interstitial fluid glucose values via Bluetooth and a smartphone App (Supersapiens Inc., USA). The CGM sensor was placed on the upper left arm of all participants. A period of 24 hours between the installation of the CGM and the collection of data was employed to ensure that there was a sufficient equilibrium (stabilization) period, as suggested by previous literature (36, 37). Minute-by-minute interstitial glucose levels were collapsed over 5-minute averages during the exercise tasks for visualization and analyses purposes (Fig. 1). Additionally, whole blood glucose readings were obtained at baseline (before task initiation) and intermittently during the exercise task using a commercially available blood glucose meter (Contour ® Next, Bayer AG Corp., Basel, Switzerland) from capillary blood samples (38). To minimize participant discomfort with excessive capillary samples while ensuring sufficient time course measurements in blood glucose during fatigue induction, blood glucose measurements during the fatiguing task were gathered at every 10% decline in isometric MVC torque with respect to the pre-exercise value, for a maximum of five blood glucose measurements per exercise session.

Statistical Analyses

Two-way repeated measures ANOVA and paired t-tests were performed as appropriate using GraphPad software *(Prism* 10.0.3, San Diego, CA, USA). Due to the collection of fatigue-specific data occurring at intervals of 15 minutes, subjects with a total time to task failure (TTF) of <60 min in either condition could not have TTF evenly divided over intervals of PRE, 25%, 50%, 75%, and POST. Therefore, a mixed-effects model for repeated measures was used instead in these specific cases. Post hoc analysis was performed using Tukey's test. A Mann-Whitney U-test was performed to assess possible difference in baseline glucose concentrations between the PLA and CHO conditions. To evaluate the disparities between the CGM and blood glucose measurements, we performed a Bland-Altman bias and agreement analyses. Data within the text and in figures are represented as means ± SD. Statistically significant differences were determined at $p < 0.05$.

RESULTS

Baseline measurements

There were no significant differences in the baseline variables between the two conditions (see Table).

Interstitial glucose and blood glucose concentration changes during exercise

We assessed the potential accuracy of the interstitial glucose recordings relative to the blood glucose measurements. The Bland-Altman analyses depicted a mean bias (SD) of 0.10 (10) mg/dL, with a limit of agreement spanning from -19 to 20 mg/dL. The mean difference is not a significant deviation from zero, and the limit of agreement appears relatively narrow. We

elected to plot a modified Bland-Altman plot (Supplemental Fig. 1, Supplemental Digital Content, Modified Bland-Altman plot with acceptable limits of sensor bias, http://links.lww.com/MSS/C995) to examine sensor bias against accepted thresholds. These criteria stipulate a deviation within ±15mg/dL of reference readings (fingerstick in this study) for glucose levels <100 mg/dL, and within ±15% for glucose levels ≥100 mg/dL. Among readings <100 mg/dL, 4/51 data points exceeded the limits, while for readings ≥100 mg/dL, 3/35 data points fell outside the bounds. These findings collectively suggest a good accuracy in the CGM readings. As presented in Supplemental Fig. 2 (Supplemental Digital Content, http://links.lww.com/MSS/C995)**,** among the 87 data points encompassing CGM and corresponding fingerstick readings, a modified Clarke-Error grid revealed just 3 CGM readings (3%) in Zone B (indicating values which could lead to inappropriate decision making.) and 2 (2%) in Zone C (suggesting values which would lead to inappropriate decision making.). These findings again suggest reasonable accuracy for this CGM device for the cohort of subjects in this study.

Just prior to the start of the fatigue tasks, baseline interstitial glucose concentration was 89.8 ± 11.2 and 90.6 ± 10.2 mg/dL in PLA and CHO groups, respectively. During the CHO trial, interstitial glucose levels rose significantly ($p < 0.01$) from baseline by 19.2 mg/dL reaching a maximum level of 109.8 ± 21.7 mg/dL during the task, without any notable decline during the task, resulting in a final value of 107.6 ± 7.5 mg/dL (Fig. 2). During the PLA condition, interstitial glucose levels remained relatively stable from baseline with a final value at task failure of 87.8 ± 9.7 mg/dL ($p = 0.40$) (Fig. 2).

Time to task failure (TTF) and the contributions of central and peripheral fatigue.

When comparing TTF between both conditions, the TTF during PLA was 81.3 ± 48.5 min, whereas with CHO feeding, TTF was ~38% longer at 112.5 ± 73.4 min (Fig. 3). Thus, CHO feeding significantly extended endurance performance when compared to the PLA condition (p = 0.023). To determine the fatigue-induced changes in gross neuromuscular function during the CHO and PLA conditions, we assessed MVC torque every 15 min during the exercise task until task failure. In both the CHO and PLA conditions, fatigue-induced changes in MVC torque showed a consistent pattern with three phases: an early decline between 0-50% TTF (p < 0.01), thereafter it exhibited a plateau from 50-75% TTF (p = 0.9), and finally there was a steep reduction between 75 to 100% TTF (p < 0.01) (Fig. 4A). In both the CHO and PLA conditions, percent voluntary activation (%VA) showed a similar pattern as MVC torque with three phases: an early decline in VA from 0-50% TTF (p < 0.01) followed by a plateau phase from 50-75% TTF (p = 0.94) and thereafter a steep decrease between 75 to 100% TTF (p < 0.01) (Fig. 4B). Thus, central fatigue appears to contribute to both the early and late phases of the fatigue-induced decline in MVC torque. Regarding peripheral fatigue assessed via evoked contractions, 10 Hz torque underwent a large decline from 0-50% TTF (p < 0.01) and a non-significant change from 50-100% TTF (p = 0.40) (Supplemental Fig. 4, Supplemental Digital Content, Time course changes in 10 and 50 Hz torque, http://links.lww.com/MSS/C995). The 50 Hz torque showed a significant decline at 0-25% TTF (p < 0.01) and then it did not significantly decrease further from 25-100% TTF (p = 0.72) (Supplemental Fig. 4, Supplemental Digital Content, http://links.lww.com/MSS/C995). The 10:50 Hz torque ratio displayed a large decrease from 0-50% TTF (p < 0.01) and thereafter a non-significant change from 50-100% TTF (p = 0.11) (Fig. 4C).

Neuromuscular function assessment at a matched time to task failure

When normalized to the total time to task failure, we observed no significant differences between CHO vs. PLA conditions in the fatigue-induced changes in MVC torque at task failure (133.5 ± 39.4 N·m vs. 136.0 ± 48.7 N·m respectively, p = 0.77), VA (75.9 ± 12.9% vs. 74.5 ± 14.3% respectively, p = 0.80), 10 Hz torque (29.5 ± 20 N·m vs. 23.1 ± 14.6 N·m respectively, p = 0.06), and 10:50 Hz ratio *(0.33 ± 0.09 vs. 0.31 ± 0.09 respectively, p* = 0.48), although there was a significant difference for higher 50 Hz torque in the CHO vs. PLA condition (89.7 ± 47.9 N·m vs. 72.6 ± 35.5 N·m respectively, p = 0.03). Taking into consideration that CHO extended TTF, we took the time to task failure in the PLA condition for each participant and then matched for the neuromuscular fatigue assessment that was performed at the equivalent time in the CHO condition to determine whether CHO ingestion led to better preservation of neuromuscular function at equivalent time (Fig. 4 D-F). When the values in the CHO condition are obtained at the equivalent time point as when time to task failure occurred in the PLA condition (i.e., which was ~ 81 min), a significant difference in the MVC torque *(p* = 0.023) (Fig. 4D), %VA *(p* < 0.01) (Fig. 4E), and 10 Hz torque *(p* = 0.026) were observed, whereby MVC torque, %VA, and 10 Hz torque were all better preserved in the CHO vs. PLA condition. The 50 Hz torque was not significantly different between PLA and CHO at the matched timepoint *(p* = 0.5). The 10:50 Hz torque ratio was not significantly different between the CHO vs. PLA condition at the matched timepoint although it was close to achieving a statistically significant difference with a p-value of 0.062 (Fig. 4F), indicating potentially greater depression of low-frequency compared to high-frequency torque in the PLA vs. CHO condition.

DISCUSSION

By tracking real-time changes in the central and peripheral contributions to fatigue with and without carbohydrate ingestion, in relationship to interstitial glucose levels during endurance exercise, we showed for the first time that in the early stages of fatiguing exercise, CHO ingestion is important in mitigating both central and peripheral fatigue. However, the importance of CHO ingestion goes beyond the prevention of hypoglycemia. Indeed, in the late stages of neuromuscular fatigue, CHO feeding that increases glucose levels well above baseline appears to play a more important role in minimizing central fatigue than peripheral fatigue.

Use of CGMs in sports performance for non-diabetics

First generation retrospective (blinded) CGM devices were initially used as a glucose assessment tool for individuals living with diabetes on insulin therapy (39). Succeeding real time and/or intermittently scanned CGM devices were adopted more widely by individuals with both insulin-requiring and non-insulin-requiring diabetes, and pre-diabetes (40). However, in recent years, the use of CGM for sports performance in non-diabetic athletes has emerged (41). While the use of CGM in non-clinical conditions has been criticized, primarily due to a lack of data demonstrating their utility in the “healthy” population (42), other studies have demonstrated that, during exercise, apparently healthy and physically active individuals without diabetes often exhibit interstitial glucose levels outside of the tight glycemic range (70-120 mg/dL) (43, 44). Additionally, they can experience reactive hypoglycemia during exercise resulting from poorly timed carbohydrate intake prior to the activity (45). This study aimed to determine if glycemia, as observed using CGM technology, could predict the onset of exercise-induced fatigue. First, we showed that the changes in interstitial glucose concentrations recorded by the CGM showed a

good accuracy compared to blood glucose readings. While hypoglycemia was not observed at the point of fatigue in our study, higher CGM values tended to be associated with delayed fatigue, both at the central and peripheral levels. We acknowledge that the use of CGM to enhance endurance performance in healthy individuals and elite-level athletes, is not yet proven and several limitations exist with this technology (*see Methodological Considerations* below). Nonetheless, our findings support the notion that CGM-informed carbohydrate feeding, with the goal of preventing a drop in glucose concentration during prolonged exercise, might be valuable in enhancing endurance performance, as previously suggested (41).

Interstitial Glucose Concentration Changes in PLA and CHO During Fatigue Induction

The significant increase in interstitial glucose upon the ingestion of CHO is to be expected following absorption through the gut and uptake into the bloodstream for systemic transport, and then measured in the interstitium via CGM. Indeed, the consumption of CHO every 15 minutes resulted in sustained and elevated interstitial glucose levels by more than 20 mg/dL over the placebo ingestion trial throughout the endurance exercise task, and this was associated with improved endurance compared with the PLA trial. Nonetheless, irrespective of the elevated interstitial glucose levels in the CHO trial, task failure occurred, albeit at a later time point, even though glucose levels failed to drop significantly. These findings are in contrast to previous research which showed that voluntary activation failure is associated with the onset of hypoglycemia following prolonged endurance cycling with placebo intake (10). One potential explanation for the differences observed is the single leg exercise employed in our study that activates a limited muscle mass and which may not fully deplete liver glycogen stores compared to cycling exercise. However, the findings of this current study remain in agreement with several

previous studies which indicated that glucose levels do not necessarily reach hypoglycemia during endurance tasks lasting up to 3h, often with task failure still developing in healthy non-diabetic individuals (23, 46, 47). It is unclear what explains the discrepancy as to whether hypoglycemia is observed following exercise without CHO ingestion but there could be a variety of factors including the type and duration of exercise, the extent of fatigue induced during the exercise, as well as training status which affects both glucose and glycogen utilization. In the current study, exercise of this nature (i.e., single leg exercise involving the activation of a limited muscle mass) does not appear to pose a threat to interstitial glucose levels in healthy individuals, perhaps because glucose regulation is tightly regulated by a multitude of mechanisms to minimize the risk of developing hypoglycemia (48, 49). Nonetheless, based on our results, we conclude that improved endurance performance is possibly associated with elevations in interstitial glucose levels, rather than the prevention of hypoglycemia per se and that the "excess" CHO might serve as a valuable fuel for the neuromuscular system. Based on our findings, we propose that endurance performance may be enhanced if glycemia is stabilized between ~100-140 mg/dL (5.6-7.8 mmol/L). Future studies are needed to confirm this range and further help establish the utility of CGM technology for recreationally active and/or competitive athletes who are not living with diabetes.

MVC Torque, Central Fatigue, and Peripheral Fatigue at Matched Timepoint

We observed three phases to the fatigue-induced decline in maximal voluntary strength (i.e., MVC torque), with a pronounced reduction in the early phase of the fatigue task, followed by a plateau phase, and thereafter a final steep decline in the late phase leading to task failure. It is unclear what central or peripheral mechanism underlies the initial decline in MVC torque, but

it appears that the contributions of CHO ingestion have a noticeable effect on preserving voluntary activation, at least as compared to 10:50 Hz torque in the early stages of the exercise (Fig. 4). Ingestion of CHO may reduce central fatigue by providing additional energy substrate to the brain, or it may be energy independent, and simply related to taste sensations, as previous research has shown that simply rinsing the mouth with CHO is sufficient to improve exercise performance, perhaps via neural circuits that connect the mouth and the brain (50). In the late phases of the fatigue task, our findings emphasize a key role of CHO ingestion in mitigating central fatigue, which is consistent with previous literature (8, 10). At the intramuscular level, a likely explanation for rapid decline in MVC torque in the early stages of exercise is muscle glycogen depletion, which has been the major proposed mechanism causing skeletal muscle fatigue during prolonged endurance exercise (15, 17, 51). Furthermore, at the matched timepoint between the CHO and PLA condition, we observed that 10 Hz torque, was better preserved with CHO ingestion and mild hyperglycemia (~110-120 mg/dL) as compared with placebo and euglycemia (~80-90 mg/dL), and this is in line with our previous data in mouse intact single muscle fibers showing that low-frequency force is more greatly impaired than high-frequency force with glycogen depletion due to decreased sarcoplasmic reticulum Ca^{2+} release (14). In summary, CHO ingestion plays a vital role in increasing fatigue resistance, but ultimately glycogen depletion, or some other factor, within the neuromuscular system is still likely responsible for task failure despite elevated glucose availability.

Methodological Considerations

Although the methodology employed in our current study involving isokinetic single-legged knee extensions offers the advantage of being able to simultaneously track changes in

neuromuscular fatigue along with interstitial glucose levels, it differs from previous studies examining blood and interstitial glucose level changes during whole-body exercise. Thus, the interpretations made in the current study based on single-legged endurance exercise may not fully reflect what might occur during whole-body exercises where the involvement of a greater muscle mass would result in larger fluxes in glucose delivery and utilization. Another potential limitation of this study is the single-blinded crossover design, as the experimenters were required to take live readings of the interstitial and blood glucose levels during the exercise, but which could have potentially biased the outcomes of the current study. Furthermore, although manufacturer recommendations stipulate installing the CGM on the upper arm, CGM sensor location can affect the accuracy of the glucose delivery to the muscle (52). However, based on the results of our study where we show that both central and peripheral factors contribute to exercise-induced fatigue, the arm placement of the CGM may better reflect the glucose levels available systemically to the whole neuromuscular system. Also, it is important to mention that CGM technology can sometimes inaccurately reflect circulating glucose, with error rates exceeding 15-20% (see Supplemental Fig. 1, Supplemental Digital Content, http://links.lww.com/MSS/C995). Additionally, a major limitation of CGMs is the time lag in the interstitial glucose recording which has a delay of ~12 min compared with a blood glucose measurement obtained by finger stick, which can be problematic when assessing sensor accuracy against whole blood glucose measurement, as in this study, and when trying to use the device as a trigger to consume CHO for exercise performance and/or glucose stability (53). However, it is worth noting that we did not detect a time course alteration in interstitial glucose concentration that was associated with a fatigue-induced decline in neuromuscular function in either the

placebo or CHO-fed group, suggesting that the time lag did not affect the interpretation of our findings.

CONCLUSIONS

We show that exogenous CHO intake has a clear impact on mitigating neuromuscular fatigue at both the central and peripheral levels, perhaps by raising glucose concentrations rather than preventing hypoglycemia per se.

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Conflict of Interest

Supersapiens Inc. provided the CGMs as well as technical assistance in this study. However, final interpretations of the data in this study were made at the discretion of the authors of this manuscript. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. M.C.R. serves on the scientific advisory committee for two CGM-based companies (Dexcom Inc., and Supersapiens Inc.). The other authors report no conflict of interest.

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FIGURE LEGENDS

Figure 1. Schematic of the experimental protocol. Placebo (PLA) and carbohydrate fed (CHO) condition; interpolated twitch (ITT), maximum voluntary contraction (MVC). Illustration created at Biorender.com.

Figure 2. Interstitial glucose levels in the placebo (PLA) and carbohydrate fed (CHO) conditions over time. Data are shown as mean (± SD) (n = 12). Significant difference between PLA and CHO interstitial glucose from 20% to POST TTF († $P < 0.05$). A significant time course change in interstitial glucose in CHO over time compared to PRE (** $P < 0.05$). The dotted lines depict interstitial glucose concentrations representing level 1 and 2 hypoglycemia.

Figure 3. Increased time to task failure in carbohydrate fed (CHO) compared to placebo (PLA) condition. Individual time to task failure are shown for all participants, as well as mean (± SD) data (n = 12). CHO is significantly different from PLA (* $P < 0.05$).

Figure 4. Time course changes in neuromuscular function. MVC torque (A), % voluntary activation (B), and 10:50 Hz ratio (C) between carbohydrate fed (CHO) and placebo (PLA) conditions at 0, 25, 50, 75, 100% time to task failure. Data are shown as mean (± SD) (n = 8-12). Individual data points and box and whisker plots showing mean (± min/max) values of MVC torque (D), % voluntary activation (E), and 10:50 Hz ratio at a matched timepoint (i.e., ~81 min in both CHO and PLA conditions as denoted by the dashed line in panels A-C) (n = 12). *$P < 0.05$. **$P < 0.01$.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplementary figure_FINAL.docx

Figure 1

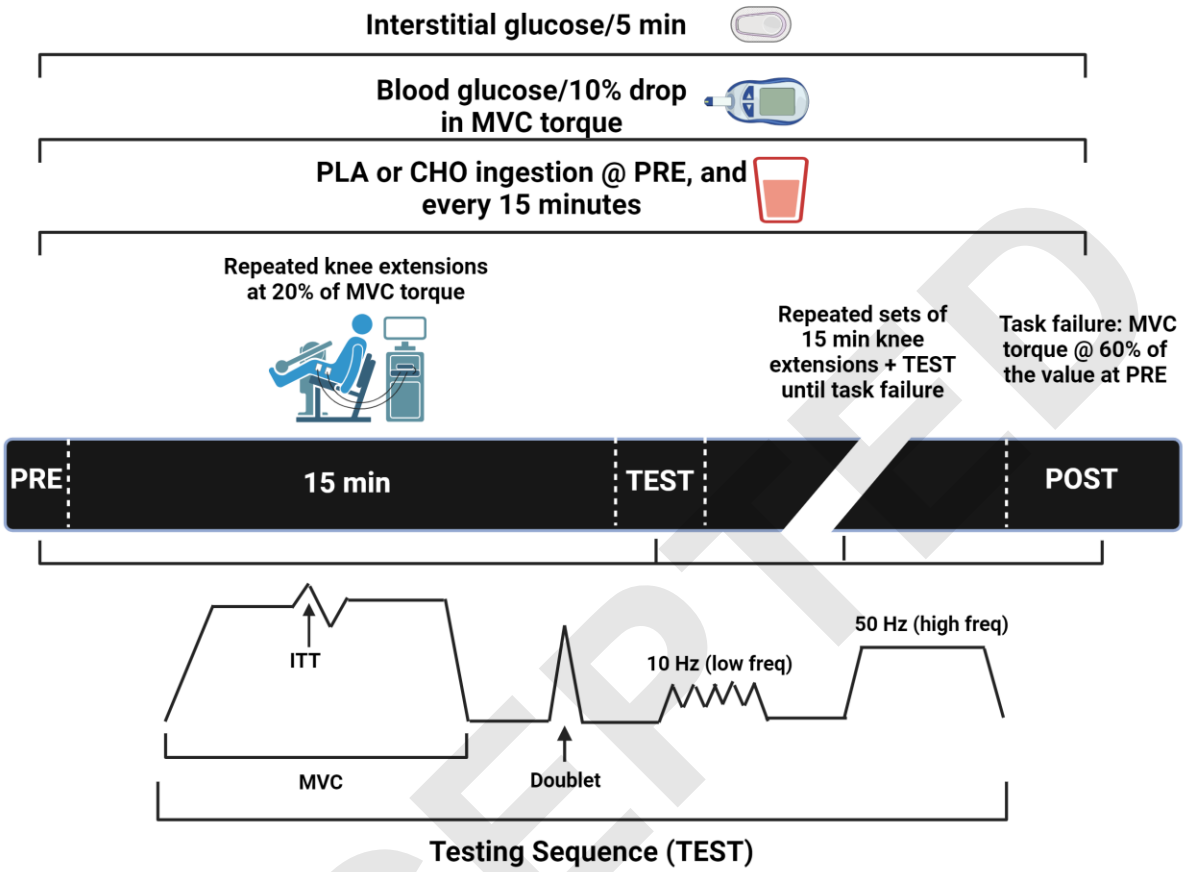


Figure 2

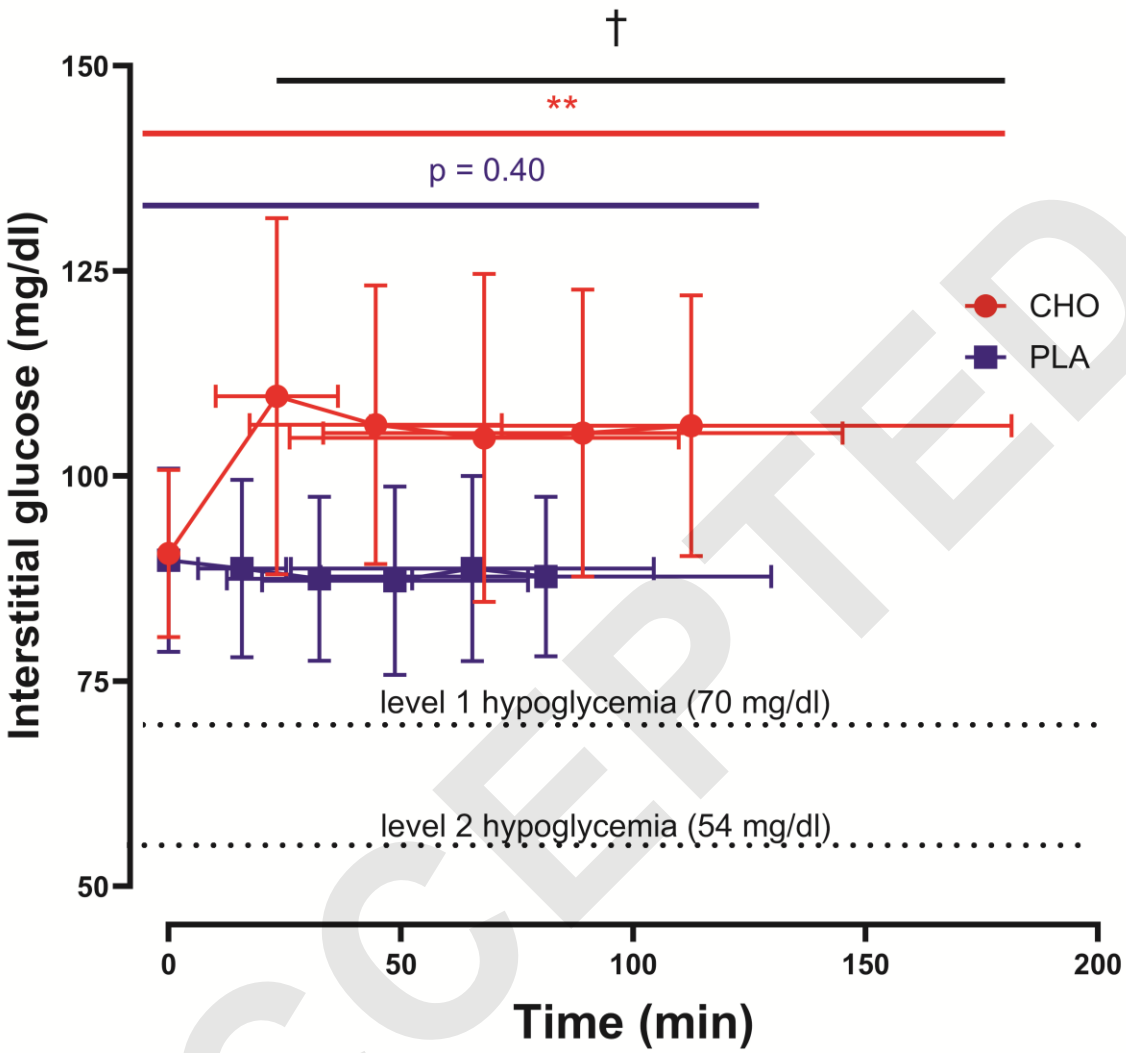


Figure 3

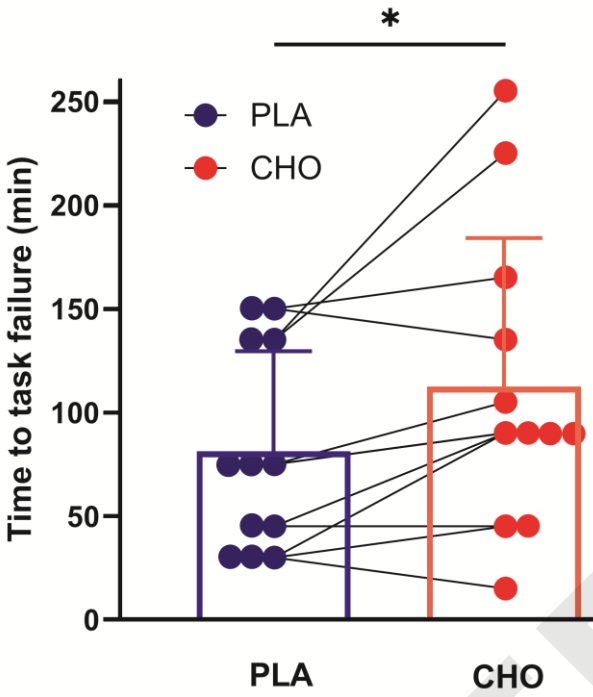


Figure 4

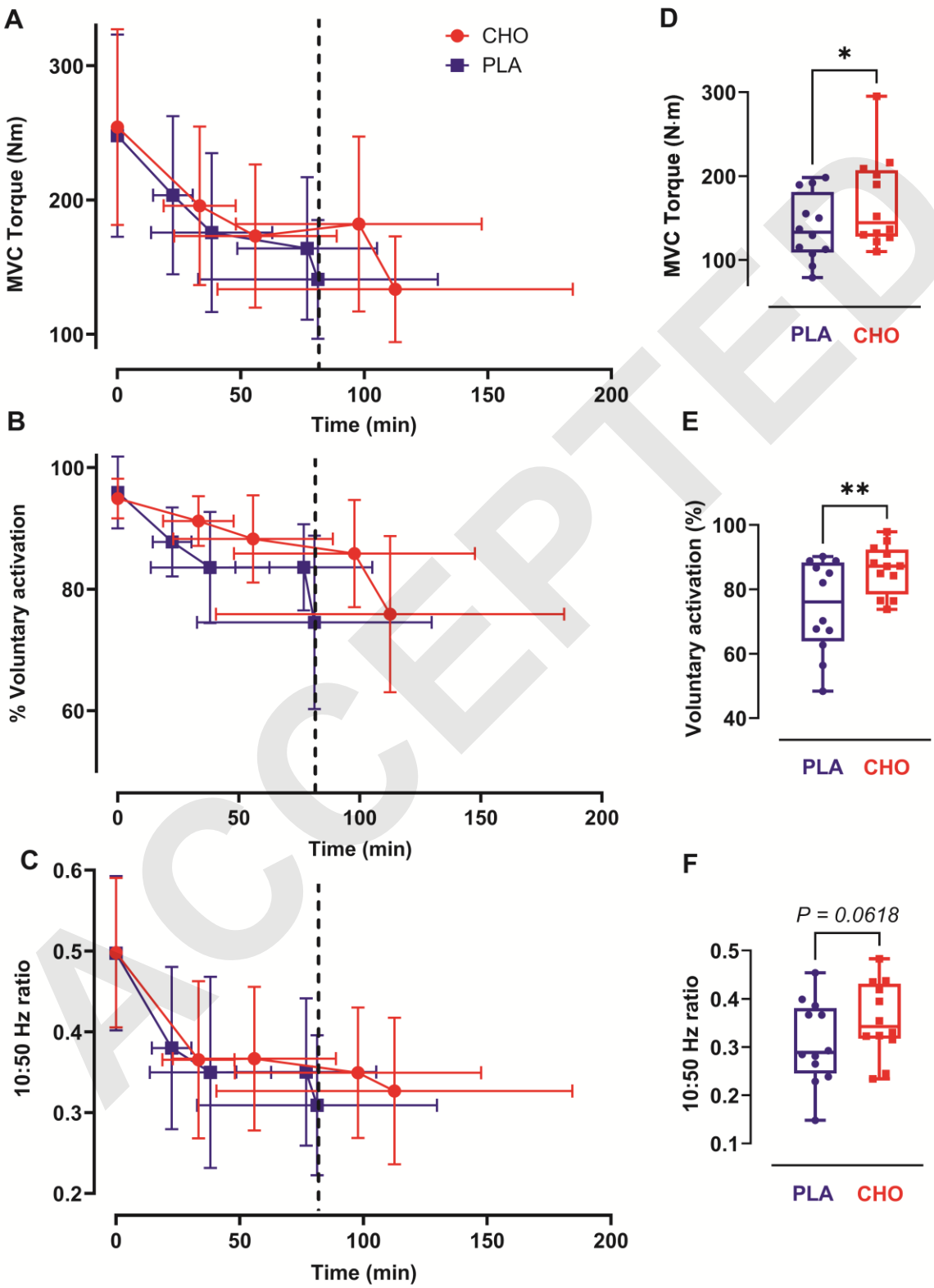
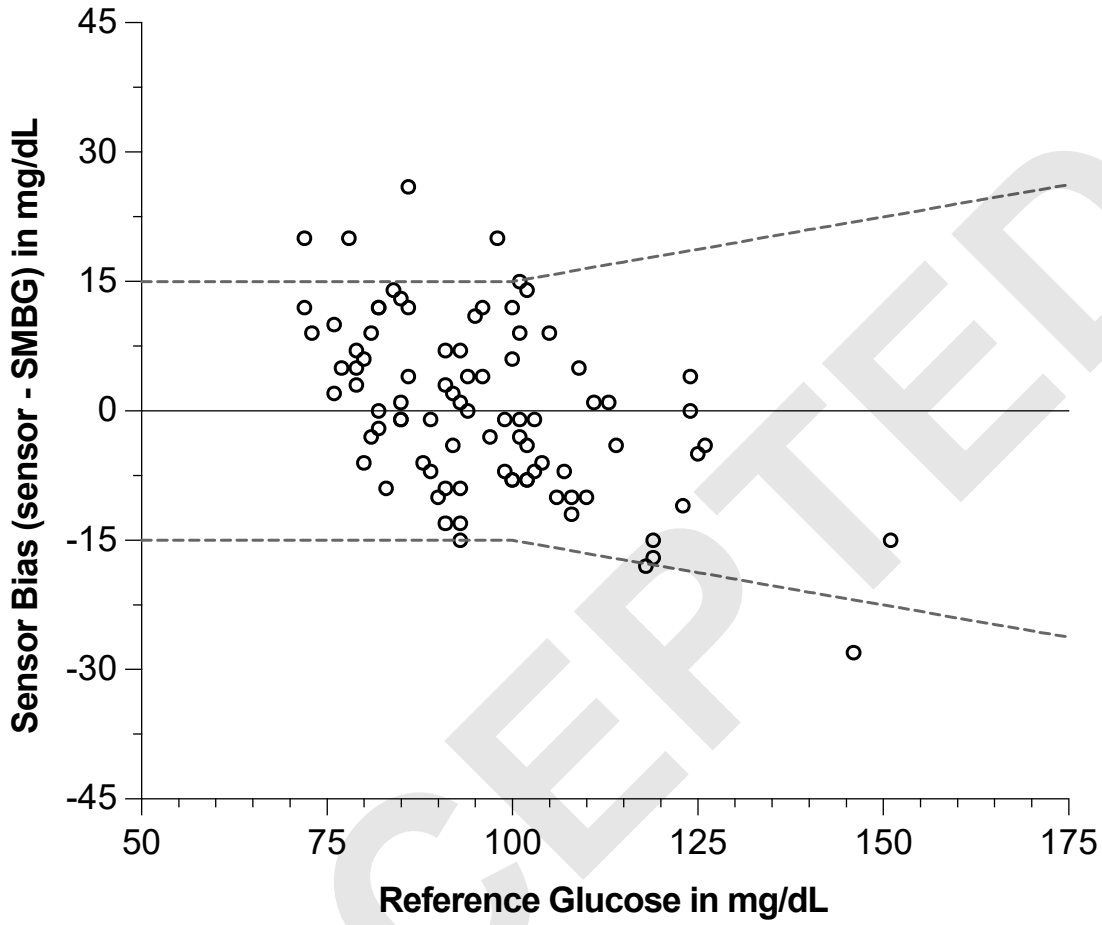


Table – Baseline measures during the placebo fed (PLA) and carbohydrate fed (CHO) trials.

	PLA trial	**CHO trial**	t-test	Mann-Whitney U-test
MVC torque (N·m)	247.9 ± 75.4	254 ± 72.8	$p = 0.34$	
Voluntary activation (%)	95.9 ± 5.9	94.9 ± 3.3	$p = 0.37$	
10 Hz torque (N·m)	58.9 ± 15.6	59.2 ± 19.9	$p = 0.91$	
50 Hz torque (N·m)	117.4 ± 33	120.8 ± 39.6	$p = 0.67$	
10:50 Hz torque ratio	0.50 ± 0.10	0.50 ± 0.09	$p = 0.72$	
Interstitial glucose level (mg/dL)	89.8 ± 11.2	90.6 ± 10.2		*p = 0.39*
Blood glucose level (mg/dL)	90.5 ± 9.7	94 ± 8.8		*p = 0.62*

Supplementary Figure 1. Modified Bland-Altman plot with acceptable limits of sensor bias. The area within the dotted lines indicates accepted limits for sensor bias. The criteria include being within ±15mg/dL of reference reading if glucose is <100 mg/dL and within ±15% if glucose is ≥100 mg/dL.



Supplementary Figure 2. Modified CGM Clarke error grid for people without diabetes.

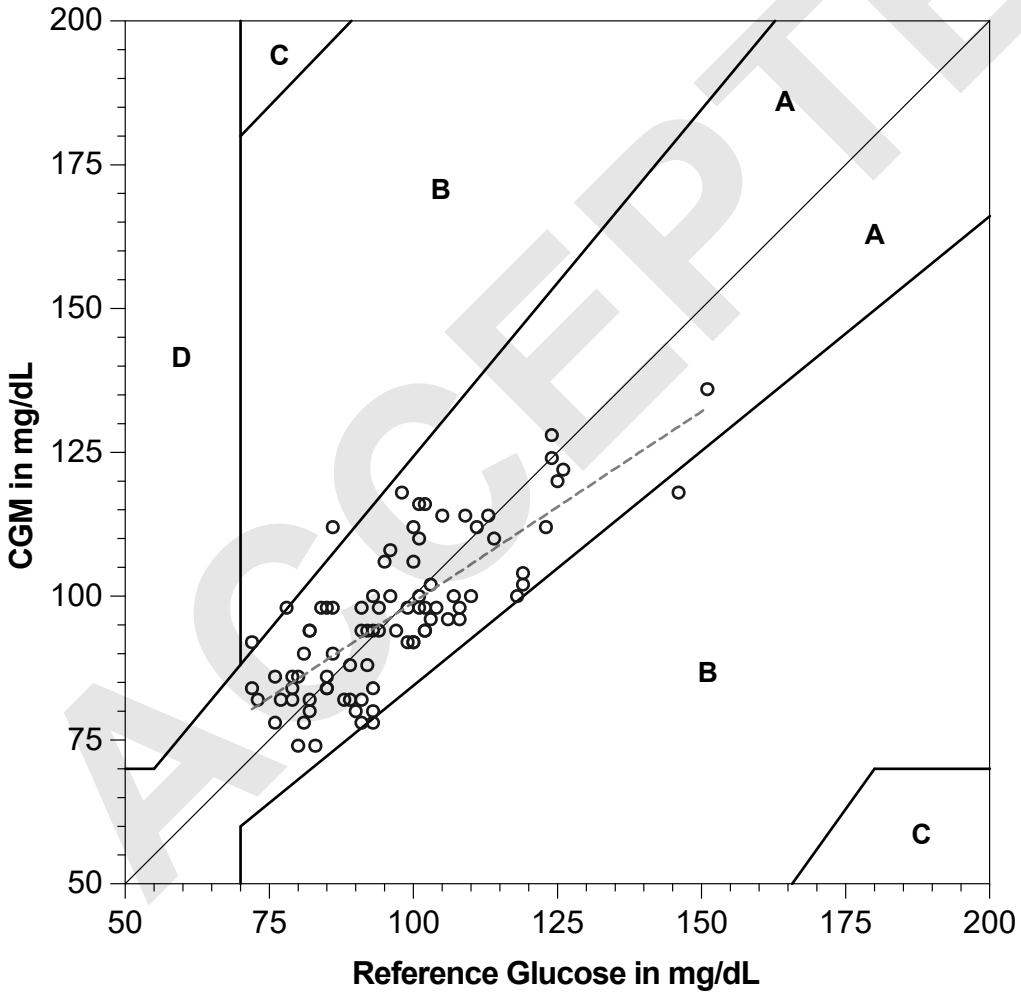
X-axis represents the whole-blood glucose concentration (or plasma glucose) as measured by a blood glucose meter or other reference tool (plasma glucose, YSI). Y-axis represents the interstitial glucose concentration as measured by a continuous glucose monitoring device (CGM). Long dashed line is a simple linear regression between reference and CGM values (y= 0.66*X + 33 mg/dL; R^2=0.60; p<0.001). Denoted regions, marked by letters A-E, represent the following error zones:

Zone A: CGM values within 20% of the reference value, appropriate decision-making zone.

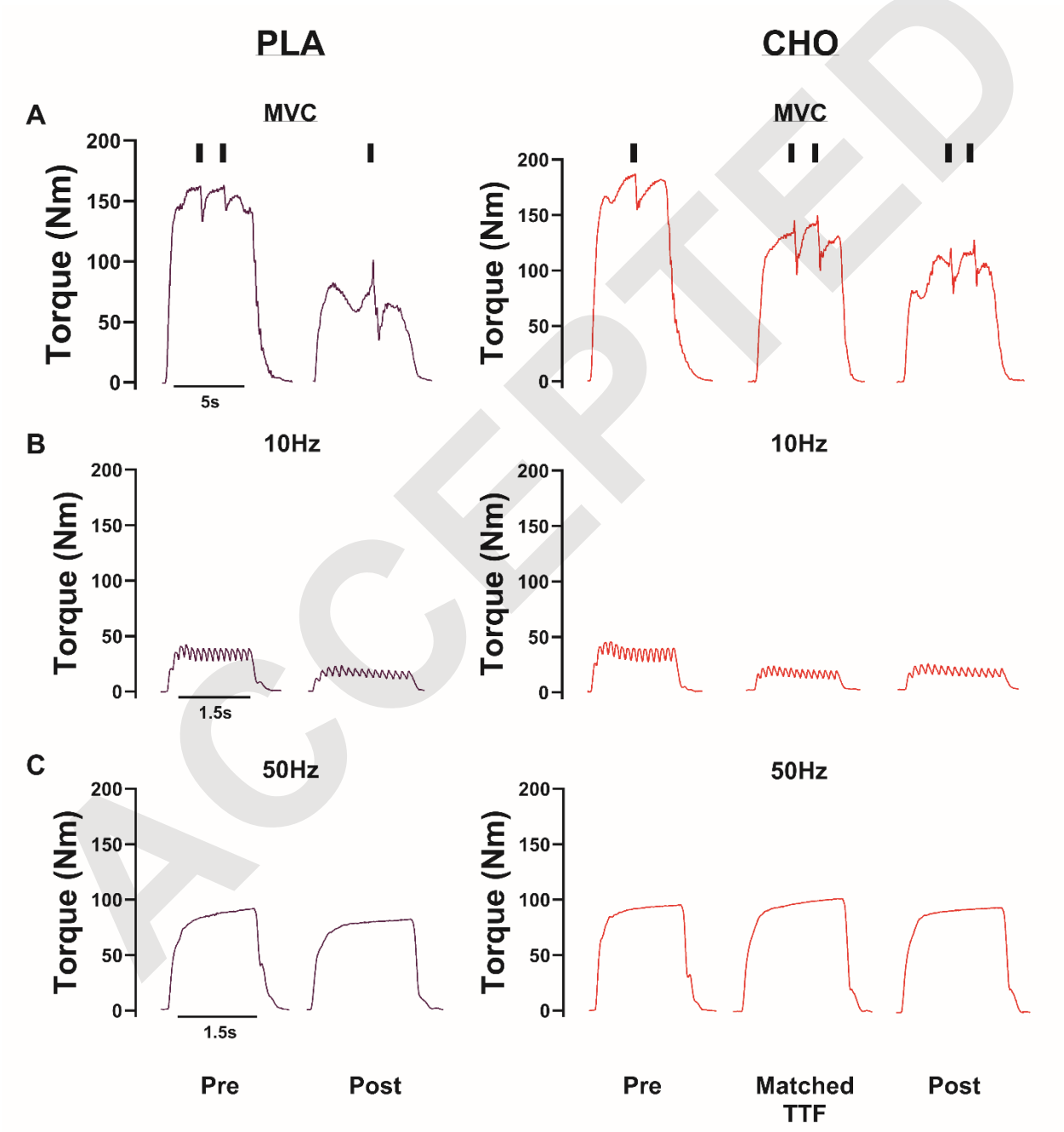
Zone B: CGM values outside 20% of the reference value, could lead to inappropriate decision making.

Zone C: CGM values reading well outside of the reference value, would lead to inappropriate decision making.

Zone D: CGM values not detecting biochemical hypoglycemia (<70 mg/dL), would lead to inappropriate decision making.



Supplementary Figure 3. Original records from one participant showing fatigue-induced changes in voluntary and electrically stimulated torque during the placebo (PLA) and carbohydrate-fed (CHO) trials. Torque traces are shown at the start (PRE) and end of exercise (POST), and in the CHO condition also at the matched time to task failure timepoint achieved in the PLA trial (Matched TTF). The black bars above the maximum voluntary contraction (MVC) torque traces indicate the timepoint in which the interpolated 100Hz doublet was superimposed during the MVC. If needed, two interpolated doublets were induced to ensure that the stimulus was applied during peak torque generation, with the highest voluntary activation value achieved recorded.



Supplementary Figure 4. Time course changes in 10 and 50 Hz torque. Shown are fatigue-induced changes between the carbohydrate fed (CHO) and placebo (PLA) conditions at 0, 25, 50, 75, 100% time to task failure. Data are shown as mean (± SD) (n = 8-12).