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Exercising with low muscle glycogen content increases fat oxidation and decreases endogenous, but not exogenous carbohydrate oxidation

Lee M. Margolis [a,b,*], Marques A. Wilson [a], Claire C. Whitney [a], Christopher T. Carrigan [a], Nancy E. Murphy [a], Adrienne M. Hatch [a], Scott J. Montain [a], Stefan M. Pasiakos [a]

[a] U.S. Army Research Institute of Environmental Medicine, Natick, MA, United States of America
[b] Oak Ridge Institute of Science and Education, Oak Ridge, TN, United States of America

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ABSTRACT

Background: Initiating aerobic exercise with low muscle glycogen content promotes greater fat and less endogenous carbohydrate oxidation during exercise. However, the extent exogenous carbohydrate oxidation increases when exercise is initiated with low muscle glycogen is unclear.
Purpose: Determine the effects of muscle glycogen content at the onset of exercise on whole-body and muscle substrate metabolism.
Methods: Using a randomized, crossover design, 12 men (mean ± SD, age: 21 ± 4 y; body mass: 83 ± 11 kg; VO_{2peak}: 44 ± 3 mL/kg/min) completed 2 cycle ergometry glycogen depletion trials separated by 7-d, followed by a 24-h refeeding to elicit low (LOW; 1.5 g/kg carbohydrate, 3.0 g/kg fat) or adequate (AD; 6.0 g/kg carbohydrate, 1.0 g/kg fat) glycogen stores. Participants then performed 80 min of steady-state cycle ergometry (64 ± 3% VO_{2peak}) while consuming a carbohydrate drink (95 g glucose +51 g fructose; 1.8 g/min). Substrate oxidation (g/min) was determined by indirect calorimetry and ^{13}C. Muscle glycogen (mmol/kg dry weight), pyruvate dehydrogenase (PDH) activity, and gene expression were assessed in muscle.
Results: Initiating steady-state exercise with LOW (217 ± 103) or AD (396 ± 70; $P < 0.05$) muscle glycogen did not alter exogenous carbohydrate oxidation (LOW: 0.84 ± 0.14, AD: 0.87 ± 0.16; $P > 0.05$) during exercise. Endogenous carbohydrate oxidation was lower and fat oxidation was higher in LOW (0.75 ± 0.29 and 0.55 ± 0.10) than AD (1.17 ± 0.29 and 0.38 ± 0.13; all $P < 0.05$). Before and after exercise PDH activity was lower ($P < 0.05$) and transcriptional regulation of fat metabolism (FAT, FABP, CPT1a, HADHA) was higher ($P < 0.05$) in LOW than AD.
Conclusion: Initiating exercise with low muscle glycogen does not impair exogenous carbohydrate oxidative capacity, rather, to compensate for lower endogenous carbohydrate oxidation acute adaptations lead to increased whole-body and skeletal muscle fat oxidation.

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1. Introduction

The practice of initiating aerobic exercise with low muscle glycogen content has grown in popularity over recent years [1–5]. Lowering muscle glycogen through prolonged aerobic exercise and high fat, low carbohydrate feeding increases reliance on fat as a fuel source, while sparing endogenous carbohydrate during subsequent exercise bouts [6–11]. While this strategy optimizes fat oxidation, common nutritional guidance is to consume carbohydrate during prolonged exercise, particularly when muscle glycogen content is low, to sustain submaximal performance [12]. The limited number of studies [13–15] assessing the impact of pre-exercise muscle glycogen on exogenous carbohydrate oxidation have yielded conflicting results, reporting an increase [13], decrease [14], or no change [15] in exogenous carbohydrate oxidation. Given the inconsistencies in the current literature, as well as limited sample size ($n \leq 7$) and lack of direct muscle glycogen measurements in past studies [13–15], it is unclear if initiating exercise with low muscle glycogen impairs exogenous carbohydrate oxidative capacity. As carbohydrate intake is an important factor sustaining performance during exercise, understanding how pre-exercise muscle glycogen affects

Abbreviations: FAT, fatty acid translocase; HADHA, hydroxyacyl-CoA dehydrogenase; PDK4, pyruvate dehydrogenase kinase 4; PDH, pyruvate dehydrogenase; PGC-1α, Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1α; DEXA, dual energy x-ray absorptiometry; BL, baseline; GD, glycogen depletion; LOW, low glycogen; AD, adequate glycogen; PRE, pre steady-state exercise; POST, post steady-state exercise; VO_{2peak}, peak oxygen consumption; MRE, meal, ready-to-eat; VPDB, Vienna Pee Dee Belemnite; B2M, beta-2-microglobulin; FABP, fatty acid binding protein; CPT1a, Carnitine Palmitoyl transferase 1a; ACOX1, Acyl-CoA Oxidase 1; PPARδ, Peroxisome Proliferator-Activated Receptor-δ; PPARγ, Peroxisome Proliferator-Activated Receptor-γ; GSK3α, Glycogen Synthase Kinase-3α; GLUT4, Glucose Transporter type 4; HK2, Hexokinase 2.

* Corresponding author at: 10 General Greene Ave, Building 42, Natick, MA 01760, United States of America.
E-mail address: lee.m.margolis.civ@mail.mil (L.M. Margolis).

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exogenous carbohydrate oxidation is integral to make appropriate fueling recommendations.

Skeletal muscle molecular adaptations are the primary mechanism regulating shifts in whole-body substrate oxidation when aerobic exercise is performed with low muscle glycogen [16–18]. To reduce carbohydrate oxidation when muscle glycogen is low, upregulation of pyruvate dehydrogenase kinase 4 (PDK4) [19] reduces glycolytic flux into the tricarboxylic acid cycle by suppressing pyruvate dehydrogenase (PDH) activity [20,21]. To compensate for lower carbohydrate oxidation there is an upregulation in transcription factors peroxisome proliferator activated receptors (PPARs) and PPAR gamma coactivator 1α (PGC-1α), which are central regulators of mitochondrial function and oxidative capacity [22,23]. These factors target and upregulate the transcription of fatty acid translocase (FAT), carnitine palmitoyl transferase 1a (CPT1a) and hydroxyacyl-CoA dehydrogenase (HADHA) when muscle glycogen content is low [19,24–26]. Alterations in transcriptional regulation of fatty acid uptake, transport, and oxidation contribute to greater whole-body fat oxidation [24]. However, when carbohydrate is consumed after aerobic exercise that produces low muscle glycogen content, the acute molecular adaptations governing substrate oxidation are suppressed compared to no or minimal carbohydrate intake [25,26]. Whether acute molecular adaptations that spare endogenous carbohydrate and enhance fat oxidation are maintained when carbohydrate is consumed during exercise initiated with low muscle glycogen content is undetermined.

Discordant results on exogenous carbohydrate oxidation from past studies [13–15], make it difficult to provide recommendations on carbohydrate fueling to sustain physical performance when muscle glycogen is low at the onset of exercise. Additionally, lack of direct measurement of muscle glycogen and acute molecular adaptations limit the interpretation of results from these previous studies. As such, the purpose of this study was to determine if there is a difference in exogenous carbohydrate oxidation when steady-state aerobic exercise is initiated with low or adequate muscle glycogen concentrations. We hypothesized that exogenous carbohydrate oxidation would not be different, and exogenous carbohydrate oxidation would be lower when exercise is performed with low versus adequate glycogen content. Furthermore, the current study sought to examine molecular adaptations to link mechanistic alterations within skeletal muscle to changes in whole-body substrate oxidation.

2. Methods

2.1. Participants

Participants in this study were part of a larger investigation assessing the impact of the influence of muscle glycogen content on microRNA expression. However, analysis of exogenous carbohydrate oxidation was set as a separate objective from microRNA analysis prior to data collection (www.clinicaltrials.gov; NCT03250234). Twelve healthy, nonobese, recreationally active men between the ages of 18–39 years were recruited to participate in this randomized, crossover study. Individuals were excluded from analysis if they were not in good health, refused to abstain from use of alcohol, nicotine, and dietary supplement use during the study, had musculoskeletal injury that compromised ability to exercise, or donated blood with 8 weeks of beginning the study. One participant identified as using tobacco products prior to the start of the study. Information was not captured on the type, frequency, or amount of tobacco product use. While cessation of smoking can impact substrate metabolism [27], data from this participant did not appear as an outlier and directional changes in their data were consistent with change in overall group mean differences between LOW and AD treatments. After providing written informed consent, height was measured to the nearest 0.1 cm using a stadiometer (Seritex, Inc., Carlstadt, NJ, USA), and body mass was measured after a 10-hr overnight fast, using a calibrated digital scale (WB-110A, Tanita, Tokyo, Japan) to the nearest 0.1 kg. Body composition was determined using dual energy x-ray absorptiometry (DEXA, DPX-IQ, GE Lunar Corporation, Madison, WI, USA). These data were used for participant descriptive characteristics (age: 21 ± 4 y; height: 1.8 ± 0.1 m; body mass: 83 ± 11 kg; body mass index: 26 ± 2 kg/m^2; fat mass: 19 ± 10 kg; fat-free mass: 63 ± 9 kg; VO_{2peak}: 44 ± 3 mL/kg/min). All data collection took place at the US Army Research Institute of Environmental Medicine (USARIEM, Natick, MA), between August 2017 to May 2018. This study was approved by the Institutional Review Board at the US Army Medical Research and Materiel Command (MRMC, Fort Detrick, MD).

2.2. Study Design

To normalize glycogen content, 48 h prior to testing, participants completed a glycogen depletion protocol by cycling at various intensities until failure [28], followed by ingestion of a controlled diet to replete glycogen content (Fig. 1). At the conclusion of the repletion phase, a baseline (BL) muscle biopsy was obtained from the vastus lateralis under local anesthesia (lidocaine) after a 10-h overnight fast. Participants then completed the same glycogen depletion (GD) protocol, immediately followed by a muscle biopsy. Participants then consumed a controlled diet designed to elicit low (LOW) or adequate (AD) glycogen stores for 24 h. Participants returned to the laboratory the following morning after a 10-h overnight fast, and performed 80 min of steady-state cycle ergometry (64 ± 3% VO_{2peak}) while ingesting 146 g of carbohydrate (95 g glucose +51 g fructose; 1.8 g/min). Substrate oxidation during exercise was determined by indirect calorimetry and tracer techniques (^{13}C-glucose and ^{13}C-fructose). Muscle biopsies were obtained before (PRE) and after (POST) steady-state exercise. Following a minimum 7-d washout period, participants returned to the laboratory to complete the second arm of the investigation. Treatment (LOW vs. AD) order was randomized using a random numbers generator to avoid order bias.

2.3. Glycogen Depletion Protocol

Exercise intensities were based on peak oxygen uptake (VO_{2peak}) determined using a progressive-intensity cycle ergometer (Lode, BV, Netherlands) test and an indirect, open circuit respiratory system (True Max 2400, Parvomedics, Sandy, Utah, USA). To deplete muscle glycogen, participants completed 2 min of high-intensity cycling (work period) at 90% VO_{2peak}, followed by a 2-min recovery period cycling at 50% VO_{2peak} [28]. This work-to-recovery ratio was maintained until the participant was no longer able to complete 2 min of cycling at 90% VO_{2peak}. Cycling intensity during the work period was progressively lowered to 80%, 70%, and 60% VO_{2peak} when the participant was unable to complete 2 min of cycling at the given workload. Once the participant could not complete 2 min of cycling at 60% VO_{2peak}, exercise was stopped. The recovery period was maintained at 50% VO_{2peak}. Participants performed two practice sessions to ensure they were familiar with the protocol before testing.

2.4. Study Diets

Dietary intake was controlled for 48 h before testing began, to ensure participants presented for both trials with similar glycogen stores. Participants were provided all food and beverages (except water), consuming on average 5.7 ± 0.6 g/kg/d carbohydrate, 1.2 ± 0.1 g/kg/d protein, and 1.0 ± 0.1 g/kg/d fat in both study arms during the normalization phase. On glycogen depletion protocol days, participants consumed a high fat or high carbohydrate diet for 24 h to elicit LOW or AD glycogen stores at the start of steady-state exercise (Table 1). Study dietitians prepared and administered all meals, derived from Meal, Ready-to-Eat (MRE; Ameriqual, Evansville, IN, USA) combat rations and commercially available food items. Participants returned all

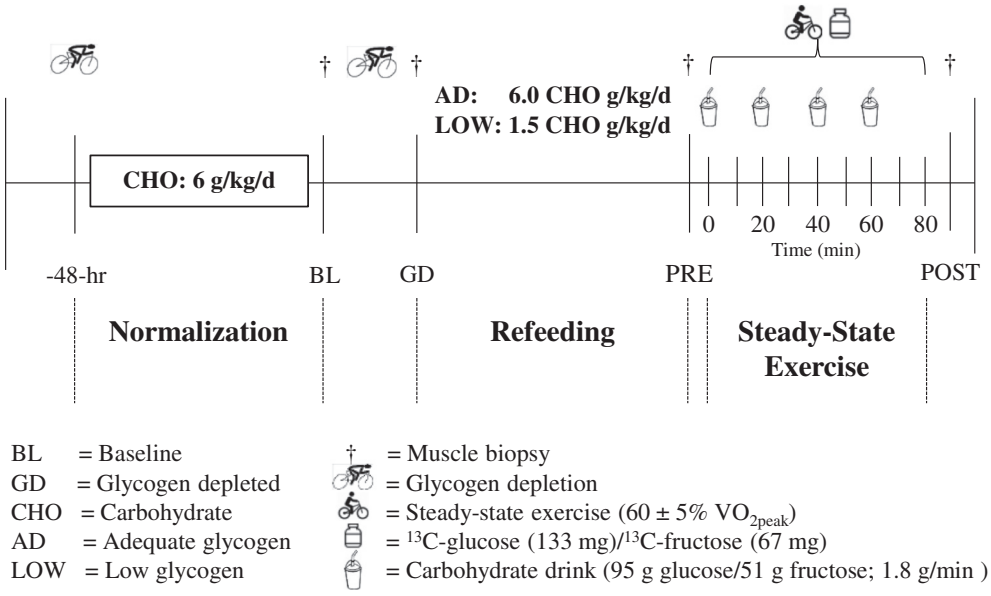

Fig. 1. Study design.

wrappers to dietitians to confirm intake. Participants abstained from nicotine and alcohol use during the study.

2.5. Steady-State Exercise Protocol

Participants ingested 146 g total carbohydrate (95 g glucose +51 g fructose), dissolved in 1450 mL of water, at an average ingestion rate of 1.8 g/min. The natural enrichment of the glucose-fructose drink was −11.10‰ δ ^{13}C versus internationally accepted standard for carbon isotope measures, Vienna Pee Dee Belemnite (VPDB). The carbohydrate drink was enriched with 200 mg of ^{13}C-glucose (133 mg) and ^{13}C-fructose (67 mg; Cambridge Isotope Laboratory, Andover, MA, USA) to match the ratio of glucose and fructose in the drink to achieve a final enrichment of 104.92‰ δ ^{13}C versus VPDB [29]. Isotope enrichment was used to ensure a significant increase in isotopic enrichment above natural abundance levels to allow for optimal measurement of exogenous carbohydrate oxidation. Immediately before starting the exercise bout, participants ingested 550 mL of the carbohydrate drink. Participants then began cycling for 80 min at their target VO_2 (64 ± 3% VO_{2peak}). Participants consumed 300 mL of the carbohydrate drink at 20, 40, and 60 min during exercise. The carbohydrate drink was prepared by the Combat Feeding Directorate (Natick, MA, USA) contained corn-derived crystalline fructose (KRYSTAR® 300, Tate and Lyle Sugars, London, UK), maltodextrin (MALTRIN QD® M500, Grain Processing Corporation, Muscatine, IA, USA) and dextrose (CERELOSE®, Ingredion, Westchester, IL, USA). Nutrient content was confirmed before use using gas chromatography (Covance Laboratories, Inc., Madison, WI, USA).

Respiratory gas exchange was measured during steady-state exercise using indirect calorimetry (Parvo Medics). Measurements were collected at 0, 15, 30, 45, 60, and 75 min during exercise. Breath samples were collected at 0, 20, 40, 50, 60, 65, 70, 75, and 80 min during exercise to assess expired ^{13}C (Quin-Tron Instrument Company, Milwaukee, WI, USA). Heart rate was monitored throughout the exercise bout (Polar Electro, Inc., Oulu, Finland).

2.6. Substrate Metabolism

Total carbohydrate and fat oxidation were calculated as [30]:

Fat oxidation (g/min) = $1.695 \times VO_2$ (L/min) − $1.701 \times VCO_2$ (L/min).

Total carbohydrate oxidation (g/min) = $4.585 \times VCO_2$ (L/min) − $3.226 \times VO_2$ (L/min).

Measurement of $^{13}C/^{12}C$ in expired CO_2 were measured using isotope-ratio mass spectroscopy (Metabolic Solutions, Inc., Nashua,

Table 1
Diet intervention to induce low (LOW) or adequate (AD) muscle glycogen.

	LOW	AD	*P* value
Absolute			
Energy (kcal/d)	3081 ± 374	3086 ± 347	0.77
Carbohydrate (g/d)	125 ± 18	498 ± 56	< 0.01
Protein (g/d)	106 ± 13	98 ± 11	< 0.01
Fat (g/d)	256 ± 30	86 ± 11	< 0.01
Relative			
Energy (kcal/kg/d)	37.1 ± 0.6	37.1 ± 0.7	0.87
Carbohydrate (g/kg/d)	1.5 ± 0.1	6.0 ± 0.2	< 0.01
Protein (g/kg/d)	1.3 ± 0.5	1.2 ± 0.5	< 0.01
Fat (g/kg/d)	3.0 ± 0.5	1.0 ± 0.5	< 0.01
Percent Total Energy			
Carbohydrate (%)	16 ± 1	65 ± 1	< 0.01
Protein (%)	14 ± 1	13 ± 1	< 0.01
Fat (%)	72 ± 1	25 ± 1	< 0.01

Values mean ± SD, n = 12.

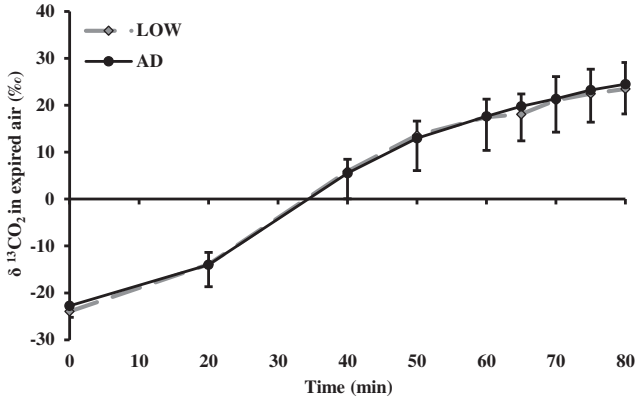

Fig. 2. Expired δ ^{13}C versus VPDB (Vienna Pee Dee Belemnite) during steady-state cycling, while consuming 1.8 g/min carbohydrate (95 g glucose +51 g fructose) enriched with ^{13}C-glucose (133 mg) and ^{13}C-fructose (67 mg) with low (LOW) or adequate (AD) glycogen stores. Values mean ± SD, *n* = 12.

NH, USA). The isotopic enrichments were expressed as ‰ δ versus VPDB (Fig. 2). Exogenous carbohydrate oxidation was calculated as:

$$\text{Exogenous carbohydrate (g/ min)} = VCO_2\,[(Rexp - Rref)/(Rexo - Rref)]/k$$

where VCO_2 is in L/min, Rexp is the observed isotopic composition of expired CO_2, Rref is the isotopic composition of expired CO_2 at rest before ingestion of the first dose of ^{13}C-glucose and ^{13}C-fructose, Rexo is the isotopic composition of the exogenous glucose ingested, and k (0.747 L/g) is the volume of CO_2 provided by the complete oxidation of glucose. Endogenous carbohydrate oxidation was calculated by subtracting exogenous carbohydrate oxidation from total carbohydrate oxidation. The first 40 min of steady-state exercise were used to allow for equilibration between the $^{13}C/^{12}C$ in expired CO_2 and the $^{13}C/^{12}C$ in CO_2 produced in tissues [31].

2.7. Muscle Analysis

Glycogen concentration was determined in ~3 mg (dry weight) freeze dried muscle homogenized in water using a TissueLyser II with a 5-mm steel bead (Qiagen, Valencia, CA, USA). Homogenates were boiled at 100 °C for 5 min and centrifuged at 13,000×*g* for 5 min at room temperature. Supernatant was removed and muscle glycogen concentrations were assessed using an endpoint colorimetric assay (Cat# MAK016; Sigma-Aldrich, St. Louis, MO, USA).

Pyruvate dehydrogenase (PDH) activity was determined in ~20 mg muscle samples homogenized in ice-cold homogenization buffer (1:10 *w*/*v*) [32]. Homogenates were centrifuged at 10,000×*g* for 15 min at 4 °C. Supernatant (lysate) was collected and protein concentrations were determined using the 660 nm Protein Assay (Thermo Fisher Scientific, Waltham, MA, USA). PDH activity was assessed in lysates using a kinetic colorimetric assay (Cat# MAK183; Sigma-Aldrich). Plates were run at 37 °C with reads every 5 min for 60 min or until activity plateaued. PDH activity was normalized to time of run and sample protein concentrations for final analysis.

Expression of genes regulating carbohydrate and fat oxidation were determined from total RNA isolated in ~20 mg muscle samples using TRIzol reagent (Thermo Fisher). RNA quantity and quality were assessed using a Nanodrop ND-2000 spectrophotometer (Nanodrop, Wilmington, DE, USA). Equal amounts of total RNA (500 ng) were reverse-transcribed using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) conducted in a T100™ Thermal Cycler (Bio-Rad, Hercules, CA, USA). Amplifications were performed using a StepOnePlus Real-Time PCR System (Applied Biosystems). Samples were run in 10 μL reactions in duplicates using TaqMan® fast advanced master mix and commercially available TaqMan® probes (Applied Biosystems; **Supplemental Table 1**). All mRNA were normalized to B2M. Fold changes for PRE and POST steady-state exercise were calculated using the ΔΔ cycle threshold (ΔΔCT) method [33] and expressed as fold change relative to individual BL values.

2.8. Statistical Analysis

Statistical power and sample size were determined based on the primary outcome of this investigation, influence of carbohydrate availability on microRNA expression. A sample size of 9 was determined using a mean and standard deviation of 1.5 ± 1 fold change based on recent publications assessing change in microRNA expression in skeletal muscle [34–36], with an alpha of 0.05 and 80% power. A total of 12 participants were requested to account for potential inter-individual variance. Based an effect size of 1.1 ($n = 6$) for differences in exogenous carbohydrate oxidation from Pérronet et al. [13], a samples size of 12 provided ~95% power to detect differences between treatments in the current study.

Paired *t*-tests were used to assess differences in energy and macronutrient intake, exercise intensity for glycogen depletion, and steady-state exercise between treatments (LOW vs. AD). Mixed-model repeated measures ANOVA was used to assess substrate oxidation, glycogen, PDH activity and mRNA expression for effects of time, treatment, and their interactions. Bonferroni adjustments for multiple comparisons were performed if significant interactions were observed. All data are presented as mean ± SD. The α level for significance was set at $P < 0.05$. Data were analyzed using IBM SPSS Statistics for Windows Version 22.0 (IBM Corp. Armonk, NY, USA).

3. Results

3.1. Glycogen Depletion and Steady-State Exercise Intensity

There were no differences in total exercise time (LOW: 84 ± 25, AD: 88 ± 24 min), mean power (LOW: 164 ± 26, AD: 161 ± 25 watts), and heart rate (LOW: 159 ± 14, AD: 159 ± 13 BPM) between treatments for glycogen depletion cycle ergometry. There was no differences in percent VO_{2peak} (LOW: 65 ± 4, AD 64 ± 3%), power (LOW: 138 ± 19, AD: 135 ± 21 watts), and heart rate (LOW: 147 ± 8, AD: 147 ± 12 BPM) between treatments during steady-state cycle ergometry.

3.2. Muscle Glycogen

Muscle glycogen concentrations were not different at BL and declined ($P < 0.05$) similarly at GD in both LOW and AD (Fig. 3). Following 24 h refeeding, PRE steady-state glycogen was lower ($P < 0.05$) in LOW than AD. POST steady-state glycogen in AD was lower ($P < 0.05$) than PRE but remained higher ($P < 0.05$) than LOW.

3.3. Substrate Oxidation

Fat oxidation was higher ($P < 0.05$) in LOW (0.55 ± 0.10 g/min) than AD (0.38 ± 0.13 g/min; Fig. 4A), independent of time. Total carbohydrate oxidation was lower ($P < 0.05$) in LOW (1.59 ± 0.40 g/min) than AD (2.03 ± 0.36 g/min; Fig. 4B), regardless of time. Exogenous carbohydrate oxidation was not different between LOW (0.84 ± 0.14 g/min) or AD (0.87 ± 0.16 g/min; $P > 0.05$), and increased similarly in both treatments over time during steady-state exercise (Fig. 4C). Independent of time, endogenous carbohydrate oxidation was lower in LOW (0.75 ± 0.29 g/min) than AD (1.17 ± 0.29 g/min; $P < 0.05$), but decreased ($P < 0.05$) similarly in both treatments over time during steady-

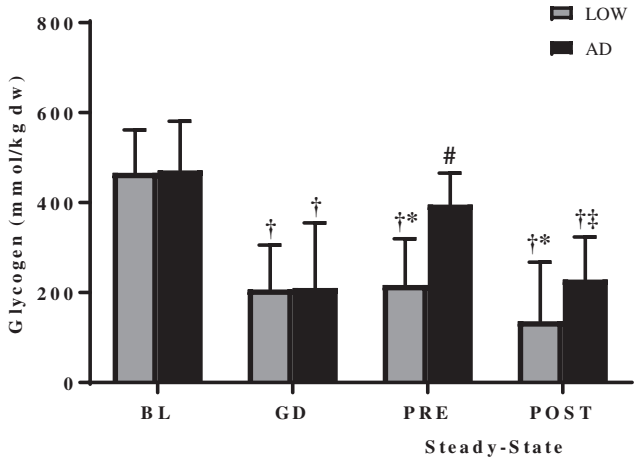

Fig. 3. Glycogen concentration at baseline (BL), after glycogen depletion (GD) and, before (PRE) and after (POST) steady-state cycling following 24-hr refeeding designed to elicit low (LOW) or adequate (AD) glycogen stores. Values mean ± SD, $n = 11$. †Different than PRE glycogen depletion, $P < 0.05$. ‡Different than PRE steady-state, $P < 0.05$. #Different than POST glycogen depletion, $P < 0.05$. *Different than AD, $P < 0.05$.

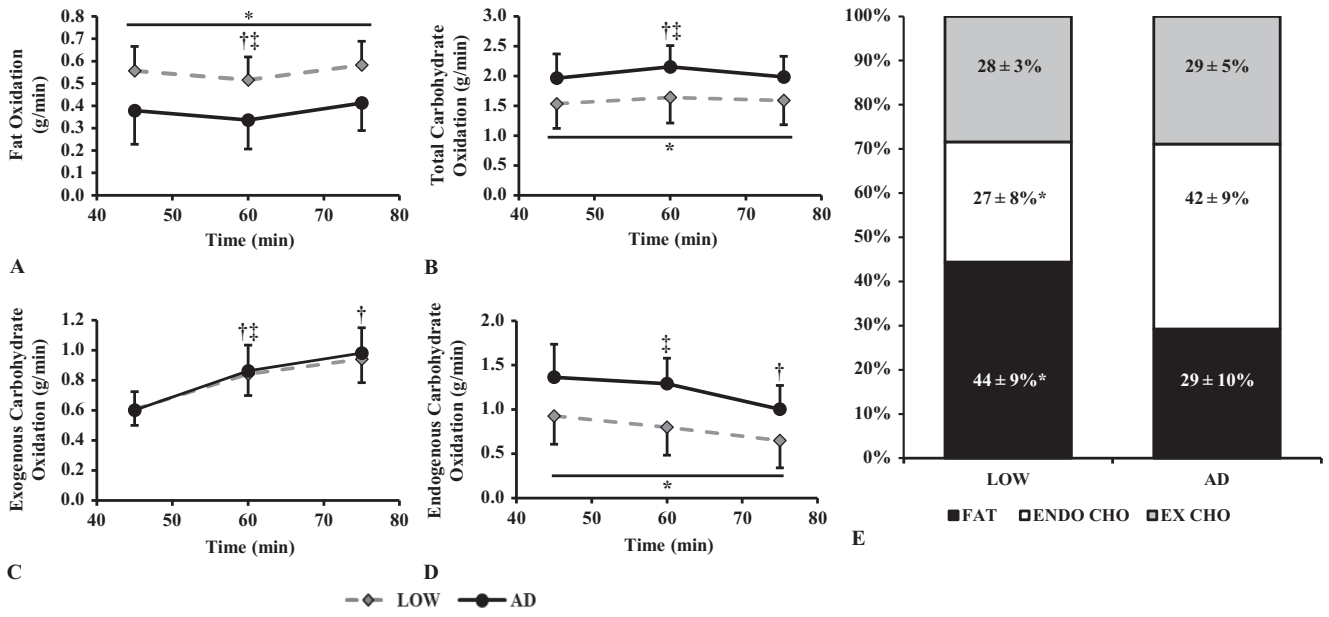

Fig. 4. Oxidation rate for fat (A), total carbohydrate (B), exogenous carbohydrate (C), and endogenous carbohydrate (D) at 45, 60, and 75 min during steady-state cycling with low (LOW) or adequate (AD) glycogen stores. Percent contribution of fat (FAT), endogenous (ENDO), and exogenous (EXO) carbohydrate (CHO) to total energy expenditure during the last 40 min of exercise. Values mean ± SD, n = 12. †Different than 45 min, $P < 0.05$. ‡Different than 75 min, $P < 0.05$.*Different than AD, $P < 0.05$.

state exercise (Fig. 4D). As a percentage of exercise-induced energy expenditure, fat constituted a higher percentage and endogenous carbohydrate a lower percentage in LOW than AD, with no difference in contribution of exogenous carbohydrate (Fig. 4E).

3.4. Acute Molecular Adaptations

Regardless of time, PDH activity was lower ($P < 0.05$) in LOW (PRE: 171 ± 102, POST 235 ± 216 nmol/μg protein/min) compared to AD (PRE: 366 ± 145, POST: 447 ± 347 nmol/μg protein/min) for steady-state exercise. PRE steady-state GSK3α was lower ($P < 0.05$) in LOW than AD (Fig. 5A). PRE and POST steady-state GSK3α were higher ($P < 0.05$) than BL in AD, while there was no difference in time in LOW. PRE and POST steady-state FAT were higher ($P < 0.05$) in LOW than AD (Fig. 5B). PRE and POST steady-state FAT were higher ($P < 0.05$) than BL in LOW, with no difference in time in AD. Independent of time, FABP, CPT1a, and HADHA were higher ($P < 0.05$) in LOW than AD (Fig. 5C-E). PRE and POST steady-state FABP, CPT1a, and HADHA were higher ($P < 0.05$) than BL, regardless of treatment. POST steady-state PPARδ was higher ($P < 0.05$) than BL and PRE in LOW, while POST steady-state PPARδ was higher ($P < 0.05$) than BL in AD, but not different than PRE (Fig. 5F). Regardless of treatment, PRE and POST HK2, PGC-1α, and ACOX1 were higher ($P < 0.05$) than BL (Table 2). There were no differences in treatment or time for GLUT4 and PPARγ.

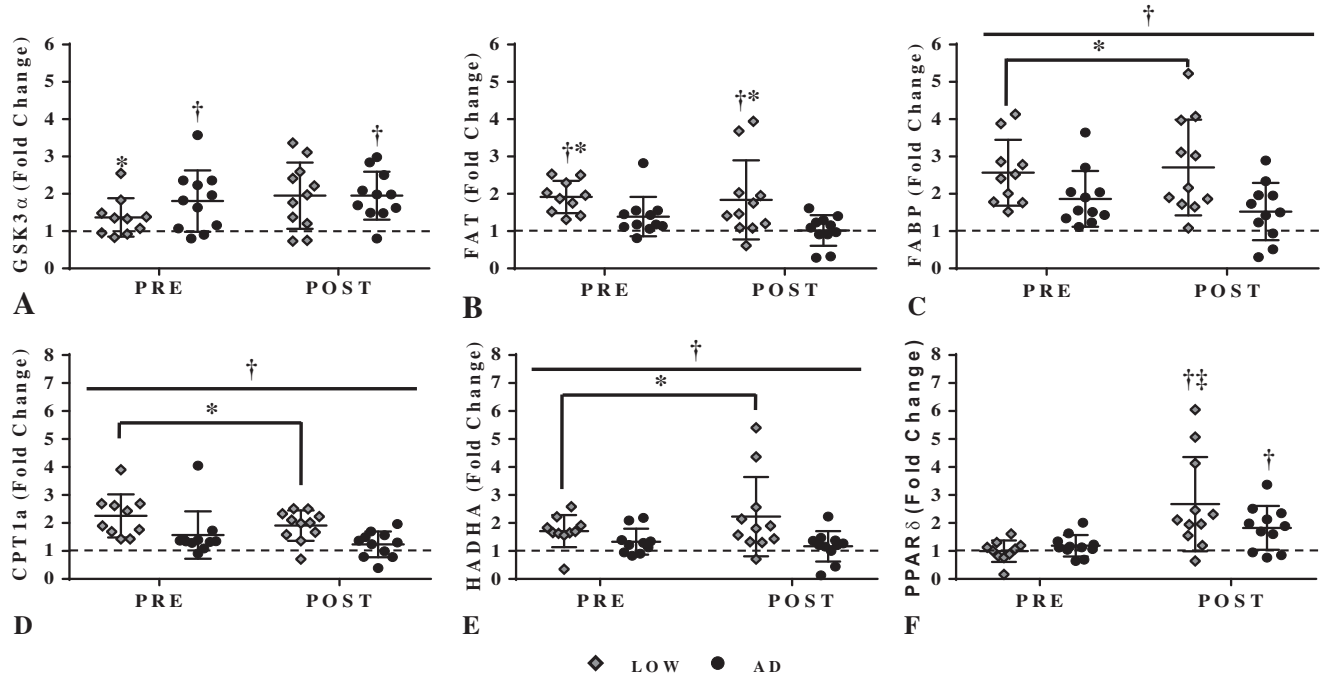

Fig. 5. Fold change before (PRE) and after (POST) steady-state for GSK3α (A) FAT (B), FABP (C), CPT1a (D), HADHA (E), and PPARδ (F) relative to baseline values (dotted line) with low (LOW) or adequate (AD) glycogen stores. Values mean ± SD, n = 11. †Different than baseline, $P < 0.05$. ‡Different than PRE, $P < 0.05$. *Different than AD, $P < 0.05$.

Table 2
Gene expression.

Gene	LOW		AD		*P* value		
(Fold Change)	PRE	POST	PRE	POST	Time	Treatment	T × T
GLUT4	1.40 ± 0.55	1.18 ± 0.56	1.27 ± 0.51	1.05 ± 0.58	0.24	0.67	0.77
HK2	2.62 ± 1.46	4.37 ± 2.99	3.01 ± 1.75	4.37 ± 2.97	< 0.01	0.94	0.59
PGC-1α	1.79 ± 0.75	2.01 ± 1.08	1.38 ± 0.51	1.71 ± 1.44	< 0.01	0.30	0.45
PPARγ	1.23 ± 0.51	1.54 ± 0.98	1.57 ± 0.64	1.47 ± 0.56	0.06	0.38	0.27
ACOX1	1.70 ± 0.33	1.37 ± 0.29	1.66 ± 0.57	1.30 ± 0.45	< 0.01	0.73	0.97

Values mean ± SD, n = 12. PRE and POST steady-state exercise data presented as fold change relative to baseline (BL). Time main effects, both PRE and POST were different than BL ($\underline{P} < 0.05$) but not different from one another. T × T, time-by-treatment interaction.

4. Discussion

The primary outcome from this study was that initiating steady-state aerobic exercise with low versus adequate muscle glycogen content did not hinder exogenous carbohydrate oxidative capacity, but did result in reduced reliance on endogenous carbohydrate, while increasing utilization of fat as a fuel source during exercise. Alterations in whole-body oxidation were driven by acute molecular adaptations resulting in reduction in PDH activity and an upregulation in skeletal muscle transcriptional control of fatty acid uptake, transport, and oxidation. These findings show that when muscle glycogen content is low, consuming carbohydrate may be used as a fueling strategy during to prolong submaximal exercise without impairing adaptations to enhance whole-body and skeletal muscle fat metabolism.

Results from the current investigation show that rates of exogenous carbohydrate oxidation are not dependent on muscle glycogen content. Three past studies assessing the influence of muscle glycogen content of exogenous carbohydrate oxidation during exercise had conflicting results, reporting an increase [13], decrease [14], or no change [15] in oxidation. Discrepancies between studies could likely be attributed to differences in exercise intensity (> or <60% VO_{2peak}), timing of carbohydrate intake (before or during exercise), participants training status (recreational versus highly trained) and method to reduce muscle glycogen content (exercise plus diet manipulation or exercise alone). Of these works, the current investigation most resembles the design used by Péronnet et al. [13], which manipulated glycogen content with exercise followed by 2 days of high carbohydrate or high fat feeding. Participants in the Péronnet study then performed 120 min of steady-state exercise at 64% VO_{2max} while consuming 200 g (1.7 g/min) of carbohydrate [13]. Primary result was reported that oxidation of exogenous carbohydrate was increased by 8% when participants performed steady-state exercise with low muscle glycogen content [13]. However, alteration in exogenous carbohydrate oxidation were driven by differences observed between 40 and 80 min, with no treatment effect reported from 80 to 120 min in the exercise bout [13]. Moreover, the overall contribution of exogenous carbohydrate as a percent of exercise-induced energy expenditure was not different between the treatments, constituting ~18% and ~19% of total energy following the high carbohydrate or high fat diets, respectively [13]. Though some disparities exist, the overall results of this past work are in agreement with our study, suggesting exogenous carbohydrate oxidation is not influenced by muscle glycogen content at the onset of exercise.

Though not a direct measurement of carbohydrate oxidation, several past studies [6,7,37–40] have reported that initiating steady-state aerobic exercise with lower muscle glycogen content did not increase glucose rates of disappearance (uptake) and plasma glucose oxidation using stable isotope kinetic methodologies (6,6-$^2H_2$-glucose) in healthy participants. These past and present results indicate lower total carbohydrate oxidation in the current study are the result of lower muscle glycogen oxidation, due to lower glycogen content at the onset of exercise. To increase glucose rates of disappearance or plasma glucose oxidation during exercise, it appears that fat oxidation needs to be impaired. Zderic et al. [41] blunted fat oxidation by having participants consume a β-adrenergic receptor block [42] and complete 60 min of steady-state aerobic exercise with low or normal muscle glycogen content. Impairment of fat oxidation with the β-adrenergic receptor block resulted in an increased rate of glucose disappearance when muscle glycogen content was low compared to normal glycogen. Together, past and present findings indicate that healthy individuals possess the metabolic flexibility to primarily adapt to use fat as a fuel source when initiating steady-state aerobic exercise with low muscle glycogen, rather than increase glucose uptake into the cell to be used for fuel.

Corroborating metabolic shifts in whole-body substrate oxidation were reductions in skeletal muscle PDH activity and increased FAT, FABP, CPT1a, HADHA, and PPARδ expressions with low muscle glycogen, even when carbohydrate was consumed during exercise in the present study. Though there were marked increases in transcriptional regulators of fatty acid uptake, transport, and oxidation, upstream targets PGC-1α and PPARγ were not different between treatments. Overall, decreased PDH activity and increased transcriptional regulation of fat oxidation indicate these acute intramuscular adaptations were the primary mechanism altering whole-body substrate oxidation in our study. In agreement with findings from the current study, previous studies assessing the impact of changes in resting and post-exercise muscle glycogen on the regulation of fat oxidation demonstrated reductions in PDH activity [20] and increased FAT, CPT1a, and HADHA expression [19,24–26]. Contrary to results from the current study, past studies have shown that when carbohydrate is consumed during exercise, transcriptional regulators of fat oxidations are lower compared to a non-nutritive placebo [43–45]. The discrepancies between past and present findings are likely attributed to pre-exercise muscle glycogen content. Unlike the current study, in these previous studies [43–45], exercise was initiated with similar glycogen content between treatments. The fact that muscle glycogen content was lower at the onset of exercise, and remained lower post-exercise in LOW compared to AD in our study, likely accounted for the sustained increase in transcriptional regulation of fatty acid uptake, transport, and oxidation when carbohydrate was consumed during exercise.

While results from this investigation show agreement in treatment effects in both whole-body substrate oxidation and acute molecular adaptations in skeletal muscle, our study is not without limitations. Primarily, the lack of serial blood draws during exercise precludes analysis of changes in circulating glucose, insulin, lactate, glycerol, and free fatty acids over time and between treatments. Data on circulating substrates and hormones would have allowed for a more complete description of alterations in substrate metabolism.

In conclusion, initiating steady-state aerobic exercise with low versus adequate muscle glycogen content does not impair exogenous carbohydrate oxidative capacity. To compensate for lower endogenous carbohydrate oxidation and PDH activity, the primary adaptations to exercising with low muscle glycogen content appear to be enhanced regulation of whole-body and skeletal muscle fat oxidation. Results from this study indicate when muscle glycogen content is low, consuming carbohydrate during exercise can be used as a fueling strategy to

prolong submaximal exercise without impairing adaptations in whole-body and skeletal muscle fat metabolism.

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Statement of Competing Interest

The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70–25, and the research was conducted in adherence with the provisions of 32 CFR part 219. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations. The authors declare that they have no conflicts of interest.

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Author Contributions

L.M.M., S.J.M., and S.M.P. designed research; L.M.M., M.A.W., C.C.W., C.T.C., N.E.M., and A.M.D. performed research; L.M.M., M.A.W., C.C.W., C.T.C., C.T.C., and N.E.M. analyzed data; L.M.M., S.J.M., and S.M.P. interpreted results; L.M.M., M.A.W., C.C.W., and S.M.P. prepared tables and figures; L.M.M. drafted the manuscript; and L.M.M., M.A.W., C.C.W., C.T.C., N.E.M., A.M.D., S.J.M. and S.M.P. approved final version.

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