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Accelerating endogenous carbohydrate oxidation impairs durability of the moderate-to-heavy intensity transition

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Title: Accelerating endogenous carbohydrate oxidation impairs durability of the moderate-to-heavy intensity transition

Running title: Accelerated endogenous carbohydrate oxidation reduces durability

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Abstract

We examined the hypothesis that accelerating endogenous carbohydrate oxidation exacerbates prolonged exercise-induced reductions in power output at the intensity domain transitions. Eleven trained cyclists undertook assessments of power output at the moderate-to-heavy (first ventilatory threshold [VT_1]) and heavy-to-severe transition (three-minute all-out test end-test power [CP_{3MAOT}]) on four occasions: without prior cycling (CON); without prior cycling, but two hours after 500 mg niacin ingestion and with mild heat stress applied locally to the upper legs for two hours prior to assessments (CON+NH); with two hours of prior cycling (EX); and with two hours of prior cycling, 500 mg of pre-exercise niacin ingestion, and mild heat stress applied locally to the upper legs during two hours of prior cycling (EX+NH). Endogenous carbohydrate oxidation was ~19% higher in EX+NH than EX. Power output at the intensity domain transitions were not different between CON and CON+NH. Power output at VT_1 was reduced by ~7% in EX vs. CON, and a further ~7% in EX+NH. The lower power output at VT_1 in EX and EX+NH was largely attributable to reduced metabolic power, but the larger reduction in power output at VT1 in EX+NH was attributable to effects on metabolic efficiency. CP_{3MAOT} was not different between-trials, and W' was ~25% lower in EX and EX+NH than CON and CON+NH, with no difference between exercise conditions. These data support a role for endogenous carbohydrate depletion in durability of the moderate-to-heavy but not heavy-to-severe transition during prolonged exercise.

New and noteworthy

Accelerating endogenous carbohydrate oxidation during prolonged cycling exacerbated the reduction in power output at the moderate-to-heavy intensity transition. However, this did not have a significant effect on power output at the heavy-to-severe transition or work capacity above the heavy-to-severe intensity transition. Endogenous carbohydrate depletion may be an important mechanistic factor in durability of the moderate-to-heavy transition.

Keywords

Metabolism, cycling, endurance, glycogen

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Introduction

Exercise intensity can be demarcated into three distinct domains (1). The moderate-, heavy-, and severe-intensity domains feature attainment of a rapid steady state, delayed steady state, or failure to achieve a steady state in physiological responses to exercise such as pulmonary oxygen uptake ($\dot{V}O_2$), blood lactate concentrations, and muscle metabolic and ionic variables, respectively (2–4). Accordingly, power output at the intensity domain transitions is often used for training stress management, i.e. programming training sessions and monitoring training load (5). However, recent studies have shown that power output at the intensity domain transitions deteriorates during prolonged exercise (6–11). An individual's resilience to the effects of prolonged exercise on the intensity domain transitions has been termed 'durability' (12), and durability has been proposed as an important determinant of endurance performance (13). Durability has clear implications for the application of intensity domain transition data to training stress management, as an initially moderate-intensity power output could eventually lead to a heavy- or even severe-intensity response, and hence yield a markedly different physiological response (14, 15).

It has been suggested that depletion of endogenous carbohydrate stores may explain the reduction in power output at the intensity domain transitions during prolonged exercise. Skeletal muscle glycogen is reduced during prolonged exercise (16), and this has been linked to impaired muscle contractile function via effects on sarcoplasmic reticulum Ca^{2+} handling and action of specific muscle ATPases (17–21). Preferential glycogen depletion in type I fibres during prolonged exercise may result in a progressively less-oxidative phenotype as exercise progresses due to glycogen depletion-induced inactivation (22), and this may reduce the power output achieved at the intensity domain transitions given strong relationships between muscle fibre type profile and power output at the heavy-to-severe intensity transition (23). Similarly, liver glycogen depletion occurs during prolonged exercise (24–26). Liver glycogen depletion may contribute to the decline in blood glucose concentrations observed as exercise progresses (27–29), which may in turn impair neuromuscular function (30) and contribute to reduced power output at intensity domain transitions. Supporting a role of carbohydrate availability in durability, recent studies have shown improved maintenance of power output at the intensity domain transitions during prolonged exercise when carbohydrates are ingested (8, 31, 32). However, whilst carbohydrate ingestion during prolonged exercise spares liver glycogen (25) and may have a small sparing effect on muscle glycogen (33), it exerts a range of other effects, including the activation of reward-related brain regions (34). We have also shown that reduced carbohydrate availability via a 24-h exercise and diet protocol to reduce muscle glycogen stores increased electromyographic median power frequency at submaximal work rates, worsened gross cycling efficiency, and reduced power output at the moderate-to-heavy, but not heavy-to-severe, transition (35). Whilst these data support a

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role for glycogen availability in power output at the moderate-to-heavy intensity transition, they cannot confirm an effect of glycogen depletion on durability as intensity domain transitions were assessed when fresh, and acute prolonged exercise exerts effects beyond glycogen depletion. Therefore, no studies to our knowledge have investigated the hypothesis that maintenance of endogenous carbohydrate substrate availability contributes to durability directly.

One means of exploring the role of endogenous carbohydrate availability on durability is to experimentally manipulate endogenous carbohydrate oxidation during prolonged exercise. Niacin inhibits adipose tissue lipolysis (36–39), and therefore can accelerate carbohydrate oxidation and muscle glycogenolysis in compensation for reduced fatty acid availability (40, 41). Similarly, increasing skeletal muscle temperature during exercise accelerates muscle glycogenolysis (42, 43). Accordingly, we used a combined strategy of pre-exercise niacin ingestion and application of mild heat stress locally to the upper leg muscles during exercise to accelerate endogenous carbohydrate oxidation during prolonged cycling and examine its effects on power output at the intensity domain transitions. We hypothesised that prolonged cycling would reduce power output at the intensity domain transitions, and this effect would be exacerbated by accelerated endogenous carbohydrate oxidation.

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Methods

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Auckland University of Technology Ethics Committee (25/199).

Experimental design

A randomised crossover design was used for this study. Participants completed an initial characterisation trial to confirm eligibility and set the parameters for the subsequent four experimental trials, which were conducted in random order, 9 ± 7 days apart. Visits were separated by 3-15 days, other than one instance of a 21-day gap and one instance of a 40-day gap between trials due to a technical issue. As habitual training was held constant over this time, these data were included. Participants arrived at the laboratory for the experimental trials having consumed a meal containing ~1 $g \cdot kg^{-1}$ carbohydrate ~1 h prior to arriving, refrained from vigorous exercise and caffeine for 24 hours, and having recorded and subsequently replicated their three-day diet and training record. Experimental trials consisted of submaximal incremental cycling tests for measurement of power output at moderate-to-heavy intensity transition via the first ventilatory threshold (VT_1), and power output at the heavy-to-severe intensity transition via the three-minute all-out test end-test power (CP_{3MAOT}). These assessments were performed without prior cycling (CON), without prior cycling, but following 500 mg niacin ingestion two hours beforehand, and with mild heat stress applied locally to the upper legs for two hours prior to assessments (CON+NH), with two hours of prior cycling (EX), and with two hours of prior cycling, 500 mg of pre-exercise niacin ingestion, and mild heat stress applied locally to the upper legs during the two hours of prior cycling (EX+NH).

Participants

Eleven endurance-trained cyclists participated in this study (eight male, three female). To be eligible, participants self-reported training >8 hours per week and a best-effort 20-min power >3 $W \cdot kg^{-1}$ for males or >2·5 $W.kg^{-1}$ for females. In addition to this they were free of injury and infection, had no metabolic disorder, gut condition, gout and cardiovascular disease, and were not taking medication or niacin. A priori sample size calculation determined that nine participants are required to detect an effect size of dz = 1.07 with 80% statistical power and an alpha value of 0.05 (G*Power, Aichach, Germany). This effect size was used based on the effect of carbohydrate ingestion during prolonged exercise on power output at the moderate-to-heavy intensity transition (31). One participant completed three of the four experimental trials before dropping out due to an injury unrelated to the study.

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Characterisation trial

Participants reported to the laboratory having fasted overnight for ~10 h and having refrained from alcohol and caffeine consumption for ~18 h. After providing written, informed consent, height and body mass were measured. Cycling commenced at 60 W (females) or 95 W (males) on an electromagnetically-braked ergometer (Excalibur Sport, Lode, Groningen, The Netherlands). Power output increased by 35 W every three minutes until a clear rise in the ventilatory equivalent for oxygen ($\dot{V}_E \cdot \dot{V}O_2^{-1}$) emerged, after which the power output was increased by 35 W every minute until volitional exhaustion. Expired gases were collected continuously (Quark CPET, COSMED, Rome, Italy). The peak rate of oxygen consumption ($\dot{V}O_2$peak) was accepted as the highest 15-s rolling average $\dot{V}O_2$. The $\dot{V}O_2$ at VT_1 was determined as the breakpoint in the $\dot{V}_E \cdot \dot{V}O_2^{-1}$ vs. $\dot{V}O_2$ relationship by average of visual inspection by two researchers. The $\dot{V}O_2$ at VT_1 was converted to power output by linear regression of the power output vs. $\dot{V}O_2$ relationship, using the last minute of expired gas data in each of the three-minute stages.

Following the incremental test, participants rested for ~15 min before completing a full familiarisation of the three-minute all-out test. The three-minute all-out test began with participants performing a warm-up at 100 W for 5 min. Participants then rested for 30 s and, during the last 5 s, increased cadence to approximately 120 rev·min^{-1}. Participants then cycled all-out for 3 min. Participants were instructed to reach peak power output as quickly as possible and maintain an all-out effort throughout. Loud verbal encouragement was given, but the subjects were not informed of the elapsed time. The resistance to pedalling during the test was set so that participants attained a power output of 50% of the difference between VT_1 and $\dot{V}O_2$peak at their preferred cadence, using the linear factor (linear factor = power/cadence^2) (44, 45). Expired gases and heart rate were collected throughout. End-test power (analogous to critical power) was calculated as the mean power output in the last 30-s of the 3-min all-out test (CP_{3MAOT}). The CP_{3MAOT} is a valid measure of the heavy-to-severe intensity transition (45). Work completed above CP_{3MAOT} (W') was also calculated.

Experimental trials

Participants reported to the laboratory for experimental trials having consumed a meal containing ~1 g·kg^{-1} carbohydrate ~1 h prior to arriving, having refrained from alcohol and caffeine consumption for ~18 h, and having recorded and subsequently replicated their diet and training for three days. All experimental trials took place at the same time of day in a climate-controlled laboratory (~18°C, ~60% relative humidity).

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Assessment of the moderate-to-heavy intensity transition. In all trials, the moderate-to-heavy intensity transition was assessed using a five-step submaximal incremental cycling test, as previously described (9). Cycling commenced at 100 W for 5 min prior to the first step, which occurred at 50 W below the estimated VT_1 power output from the characterisation trial. Power output then increased by 25 W every 4 min, such that the fifth step was 50 W above the estimated VT_1 power output from the characterisation trial. Expired gases were collected throughout and used to measure power output at VT_1 using the same methods as described for the characterisation trial. Assessments were made by two researchers with a within-subject coefficient of variation of ~3.6%.

The mechanical power output achieved at the moderate-to-heavy intensity transition is, mathematically, determined by the rate of metabolic energy expenditure at the transition (termed 'metabolic power') and the conversion of that metabolic energy expenditure into mechanical power output (termed 'metabolic efficiency'). We quantified the proportion of changes to power output at the moderate-to-heavy intensity transition in EX and EX+NH vs. CON. We first estimated metabolic power at VT_1 for each trial by linear regression of the power output vs. metabolic energy expenditure ($kcal \cdot min^{-1}$) relationship in the five-step submaximal test (Eq. 1) (46). We then used metabolic power at VT_1 in EX and EX+NH to estimate the power output at VT_1 that would be achieved with the metabolic efficiency in CON (termed '$EX_{Power}CON_{Eff}$' and '$EX+NH_{Power}CON_{Eff}$', respectively). To estimate $EX_{Power}CON_{Eff}$ and $EX+NH_{Power}CON_{Eff}$, we converted metabolic power in EX and EX+NH ($kcal \cdot min^{-1}$) to mechanical power output using linear regression of the mechanical power output (W) vs. metabolic energy expenditure ($kcal \cdot min^{-1}$) relationship in CON. Accordingly, the proportion of EX/EX+NH-induced changes in power output at the moderate-to-heavy intensity transition attributable to changes in metabolic efficiency and metabolic power was calculated using the below equations (Eq. 2).

$$\text{Energy expenditure } (kcal \cdot min^{-1}) = (4.471 \times \dot{V}O_2) + (0.55 \times \dot{V}CO_2)$$

Eq. 1 where $\dot{V}O_2$ and $\dot{V}CO_2$ are in $L \cdot min^{-1}$.

Contribution of metabolic efficiency to between-trial difference in power output at the intensity domain transition in EX = $EX - EX_{Power}CON_{Eff}$

Contribution of metabolic power to between-trial difference in power output at the intensity domain transition in EX = $EX_{Power}CON_{Eff} - CON$

Contribution of metabolic efficiency to between-trial difference in power output at the intensity domain transition in EX+NH = $EX+NH - EX+NH_{Power}CON_{Eff}$

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206 Contribution of metabolic power to between-trial difference in power output at the intensity
207 domain transition in EX+NH = $EX+NH_{Power}CON_{Eff}$ – CON

208 Eq. 2 where CON = power output at the moderate-to-heavy intensity transition in CON, EX =
209 power output at the moderate-to-heavy intensity transition in EX, and $EX_{Power}CON_{Eff}$ = power
210 output that would be produced in CON using the metabolic power at the moderate-to-heavy
211 intensity transition in EX, EX+NH = power output at the moderate-to-heavy intensity transition
212 in EX+NH, and $EX+NH_{Power}CON_{Eff}$ = power output that would be produced in CON using the
213 metabolic power at the moderate-to-heavy intensity transition in EX+NH.

214

215 Assessment of the heavy-to-severe intensity transition. After ~15 min, participants completed the three-
216 minute all-out test according to the procedures described in the characterisation trial.

217 Experimental interventions. Participants arrived in the laboratory ~150 min prior to the exercise tests.
218 In CON, participants sat at rest prior to the assessment of the moderate-to-heavy intensity transition. In
219 CON+NH, participants ingested 500 mg of niacin (Bulk Supplements, Henderson, NV, USA) in ~50
220 mL water on arrival and then rested. Mild heat stress was applied locally to the upper legs for the 120-
221 min prior to the assessment of the moderate-to-heavy intensity transition using wired heat pads with
222 integrated heating elements (VXHP-01, Vidawell, Auckland, New Zealand). In EX, an antecubital
223 venous cannula was inserted upon arrival and an ~10 mL sample was drawn at baseline. The cannula
224 was flushed with saline every 15 min. Subsequently, participants cycled at 90% of the power output at
225 VT_1 estimated in the characterisation trial. Expired gas was collected for 4 min every 15 min and used
226 to estimate whole-body rates of energy expenditure (Eq. 1), carbohydrate oxidation and fat oxidation
227 (Eq. 3) (46) for each 15-min period, and ~10 mL venous blood was sampled after 40, 80, and 120 min.
228 In EX+NH, the same procedures as EX were followed, but with 500 mg of niacin ingestion ~15 min
229 prior to cycling and application of mild heat stress to the upper legs during the prolonged cycling phase.
230 Previously, we saw that unilateral mild local heat stress application using this method during cycling
231 increased estimated muscle temperature by ~2.5°C vs. an unheated, exercising limb (47). A niacin
232 'flush' occurred in all participants, but this was largely resolved prior to the prolonged phase in EX+NH,
233 and in all cases resolved before assessments of the intensity domain transitions. The cannula and mild
234 local heat stress was withdrawn 5 min prior to the assessment of the moderate-to-heavy intensity
235 transition (following prolonged exercise in EX+NH). Participants had ad libitum access to plain water
236 from arrival in the laboratory to the assessment of the moderate-to-heavy intensity transition.

237

238 Carbohydrate oxidation ($g \cdot min^{-1}$) = (4.21 x $\dot{V}CO_2$) – (2.962 x $\dot{V}O_2$)

239 Fat oxidation ($g \cdot min^{-1}$) = (1.695 x $\dot{V}O_2$) – (1.701 x $\dot{V}CO_2$)

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Eq. 3 where $\dot{V}O_2$ and $\dot{V}CO_2$ are in $L \cdot min^{-1}$.

Plasma analysis. Venous blood samples were collected into ethylenediaminetetraacetic acid tubes and kept on ice until trial cessation. A small sample of whole blood was aliquoted into capillary tubes for manual, triplicate measurement of haematocrit following micro-centrifugation (Haematospin 1400, Hawksley & Sons, Ltd., England). Remaining whole-blood was centrifuged for 10 min at 4°C and 3500 rpm, and plasma was stored at -80°C prior to analyses. Plasma glycerol and non-esterified fatty acids concentrations were measured in duplicate using colourimetric assays (ab65337 and ab65341, Abcam). Plasma glucose and lactate concentrations were measured were measured via enzymatic colorimetric diagnostic assays (cobas Modular P800, Roche Diagnostics New Zealand Ltd, Auckland, NZ). Plasma variables were corrected for within-trial changes in plasma volume vs. baseline using haematocrit data (48).

Statistical analysis. Data are reported as mean ± standard deviation, unless otherwise stated. Substrate oxidation responses during experimental trials are only reported for N = 7 due to a technical fault with $\dot{V}CO_2$ measurement impacting four participants. All statistical analyses were undertaken in GraphPad Prism (version 10.4.2, 2025, GraphPad Software, San Diego, CA, USA). Shapiro-Wilk tests were used to assess normality of data distributions. Power output at the intensity domain transitions were compared between-trials using mixed-effects analyses due to one missing data point. Tukey tests were used to locate variance post-hoc. Two-way repeated measures analyses of variance, with trial and timepoint as factors, were used to compare substrate oxidation rates and plasma variables between EX and EX+NH. Šídák's multiple comparisons tests were used to locate variance post-hoc. Total carbohydrate and fat oxidised during the preload phase of EX and EX+NH were compared using paired t-tests. Contributions made by metabolic efficiency and metabolic to power to loss of power output at the intensity domain transitions were compared between EX and EX+NH using paired t-tests. Substrate oxidation responses during experimental trials are only reported for N = 7 due to a technical fault with $\dot{V}CO_2$ measurement impacting four participants. For these variables, effect sizes (d_z) and 95% confidence intervals are reported, along with observed statistical power. Statistical significance was accepted when $P \leq 0.05$).

***Figure 1

Figure 1. Schematic illustration of the study design. On four occasions, participants undertook assessments of the moderate-to-heavy and heavy-to-severe intensity transitions following two hours of seated rest (CON) or moderate-intensity cycling (EX), with (+NH) and without 500 mg of niacin ingestion and mild, local heat stress application.

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Results

Metabolic responses during prolonged cycling. A main effect of trial (P = 0.044) was observed for carbohydrate oxidation during the prolonged cycling phase (Figure 2A). Overall carbohydrate oxidation during the prolonged cycling phase was ~19% greater in EX+NH than EX (250 ± 77 vs. 214 ± 65 g, d_z = 1.00 [0.07, 1.92], achieved power, ~57%, P = 0.038, Figure 2B). A main effect of trial (P < 0.001) and time x trial interaction (P = 0.004) was observed for fat oxidation (Figure 2C). Overall fat oxidation during the prolonged cycling phase was ~12% lower in EX+NH than EX, but this did not reach the threshold for statistical significance (68 ± 24 vs. 76 ± 18 g, d_z = -0.79 [-1.71, 0.14], achieved power, ~40%, P = 0.082, Figure 2D). No main effect of trial (P = 0.719) or time x trial interaction (P = 0.656) was observed for rate of energy expenditure during the prolonged cycling phase (Figure 2E). Overall energy expenditure during the prolonged phase was not different between-trials (P = 0.448, Figure 2F). A main effect of time (P < 0.001) was observed for plasma glycerol concentration during the prolonged cycling phase (Figure 3A), but no effect of trial (P = 0.118) or trial x time interaction (P = 0.159). A main effect of time (P < 0.001) and time x trial interaction (P = 0.022) was observed for plasma NEFA concentration during the prolonged cycling phase (Figure 3B). Specifically, plasma NEFA concentration was lower in EX+NH than EX at the 80-min timepoint. A main effect of time (P = 0.01) was observed for plasma glucose concentration during the prolonged cycling phase (Figure 3C), but no effect of trial (P = 0.558) or trial x time interaction (P = 0.844). A main effect of trial (P = 0.036) was observed for plasma lactate concentration during the prolonged cycling phase (Figure 3D), but there was no effect of time (P = 0.085) or time x trial interaction (P = 0.408). Specifically, plasma lactate concentration was higher in EX+NH than EX at the 40-min timepoint.

***Figure 2

***Figure 3

Metabolic responses during incremental cycling tests. Main effects of trial were observed for carbohydrate oxidation (P < 0.001), fat oxidation (P < 0.001), and energy expenditure (P = 0.005) during incremental cycling test assessments of the moderate-to-heavy intensity transition. Specifically, at the same power output, carbohydrate oxidation rates were lower in EX and EX+NH than CON and CON+NH (P < 0.050), whilst fat oxidation rates were higher in EX and EX+NH than CON and CON+NH (P < 0.050). There were no instances of significant differences in either carbohydrate or fat oxidation rates between EX and EX+NH or CON and CON+NH (Figure 4AB). The only significant

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between-trial differences in energy expenditure were higher rates in EX+NH vs. EX in stage 1, 2, 3, and 5 ($P < 0.050$, Figure 4C).

***Figure 4

Intensity domain transitions. A main effect of trial was observed for power output at VT_1 ($P = 0.001$). All trials were different from each other ($P < 0.010$), except CON and CON+NH (Figure 5A). Specifically, prolonged exercise reduced power output at VT_1 by ~7% (233 ± 63 vs. 218 ± 62 W in EX vs. CON, respectively, $P = 0.002$). In EX+NH, power output at VT_1 (195 ± 58 W, N = 10) was reduced by a further ~7% vs. EX ($P = 0.007$), and therefore ~13% vs. CON ($P = 0.002$). There was no main effect of trial for CP_{3MAOT} ($P = 0.062$), and no two trials were significantly different to each other ($P > 0.064$, Figure 5B). A main effect of trial was observed for W' ($P = 0.001$, Figure 5C). The W' in EX+NH was significantly lower than CON ($P = 0.032$) and CON+NH ($P = 0.005$), and W' in EX was significantly lower than CON+NH ($P = 0.015$). The difference between W' in EX and CON approached significance ($P = 0.063$).

***Figure 5

Contributions to loss of power output at the moderate-to-heavy intensity transition. The loss of power output at VT_1 in EX vs. CON was mediated by a reduction in metabolic power, which was similar to the reduction in metabolic power in EX+NH vs. CON (-28 ± 20 vs. -26 ± 14 W, respectively, $P = 0.855$). The change in metabolic efficiency in EX vs. CON contributed to increased power output at VT_1, whereas in EX+NH this contributed to a mean reduction (15 ± 15 vs. -6 ± 11 W, $P = 0.010$). Therefore, the larger reduction in power output at VT_1 in EX+NH than EX was explained by effects on metabolic efficiency.

***Figure 6

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Discussion

The primary aim of this study was to determine if accelerating endogenous carbohydrate oxidation during prolonged cycling impacts durability of the intensity domain transitions. Our primary observation was that accelerating endogenous carbohydrate oxidation rates during prolonged cycling impaired durability of the moderate-to-heavy, but not heavy-to-severe, intensity transition. These data therefore suggest that durability of the moderate-to-heavy intensity domain transition is sensitive to endogenous carbohydrate availability.

Our experimental intervention, which featured pre-exercise niacin ingestion to blunt adipose tissue lipolysis and application of mild heat stress locally to the upper legs during prolonged exercise to accelerate skeletal muscle glycogenolysis, successfully increased endogenous carbohydrate oxidation by ~19% during the two-hour prolonged cycling phase in EX+NH vs. EX, although the size of the effect should be interpreted with caution given the reduced sample size for this measure (Figure 2A-B). In EX+NH, the local heat stress intervention likely directly stimulated muscle glycogenolysis (42, 43). Additionally, in EX+NH the reduction in plasma NEFA concentrations (Figure 3B) likely contributed to reduced fat oxidation rates (Figure 2C-D) via reduced fatty acid availability for uptake and oxidation (49), leading to a further compensatory increase in carbohydrate oxidation. Our plasma lactate data further support accelerated carbohydrate oxidation in EX+NH (Figure 3D). It is surprising that plasma glycerol concentrations were not different between-trials (Figure 3A), as niacin-induced suppression of adipose tissue lipolysis would be expected to present with reduced plasma NEFA and glycerol concentrations. The reason for this discrepancy could relate to differences in glycerol and NEFA clearance kinetics, hepatic glycerol uptake, or glycerol appearance in the circulation from intramuscular triacylglycerol hydrolysis (50, 51). Alternatively, we may have had insufficient statistical power to detect an effect of trial on plasma glycerol concentrations, given our study was powered for a large effect on intensity domain transitions. Importantly, energy expenditure during the prolonged phase was similar in EX+NH and EX (Figure 2E-F), so between-trial differences in intensity domain transitions cannot be attributed to differences in total metabolic work during the prolonged phase. Plasma glucose concentrations were also similar during the prolonged phase (Figure 3C), so between-trial difference in intensity domain transitions also cannot be attributed to differences in glycaemia. Furthermore, administration of the combined niacin and local heat stress application intervention per se did not impact substrate oxidation responses to incremental cycling (Figure 4A-C), nor did the intervention in the absence of prolonged cycling impact power output at the intensity domain transitions (CON+NH vs. CON, Figure 5A-B). These data therefore suggest the combined niacin and local heat stress intervention acted via effects on endogenous carbohydrate oxidation during prolonged exercise. Accordingly, our

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data support the attribution of differences in power output at the intensity domain transitions between EX+NH and EX to effects on endogenous carbohydrate oxidation during the prolonged phase.

In line with previous studies by our group (9–11, 31, 52), and others (53), we observed a consistent, significant reduction in power output at the moderate-to-heavy intensity transition following prolonged exercise, averaging ~7% (EX vs. CON, Figure 5A). The exercise-induced reduction in power output at the moderate-to-heavy intensity transition was largely attributable to reduced metabolic power, with metabolic efficiency during the incremental test improving in EX vs. CON (Figure 4C, Figure 6). Accelerating endogenous carbohydrate oxidation during the prolonged phase by ~19% resulted in a further ~7% reduction in power output at the moderate-to-heavy intensity transition in EX+NH (Figure 5A). The ~13% reduction in power output at the moderate-to-heavy intensity transition in EX+NH vs. CON was also largely attributable to reduced metabolic power (Figure 6). However, in EX+NH, metabolic efficiency did not improve, hence the larger reduction in power output at the moderate-to-heavy intensity transition in EX+NH than EX was attributable to negative effects on metabolic efficiency. These data therefore support our hypothesis that endogenous carbohydrate availability is a key determinant of durability, and suggest that acceleration of endogenous carbohydrate oxidation during prolonged exercise exerts effects on durability via negative effects on metabolic efficiency.

It is likely that the ~19% increase in endogenous carbohydrate oxidation during the prolonged phase in EX+NH vs. EX increased muscle glycogenolysis, as niacin ingestion (40) and increased muscle temperature (42, 43) have independently been shown to accelerate muscle glycogenolysis. Consistent with increased muscle glycogenolysis, we observed higher plasma lactate concentrations in EX+NH vs. EX (Figure 3D). We previously reported that reducing muscle glycogen via a 24-h exercise and diet protocol reduced power output at the moderate-to-heavy intensity transition in the absence of acute prolonged exercise (35). In that study, the reduction in power output at the moderate-to-heavy transition occurred alongside increased recruitment of high-threshold motor units and impaired gross cycling efficiency, which suggests glycogen depletion necessitated increased recruitment of less-efficient, type II fibres to maintain power output. These data align with the present study, as accelerating endogenous carbohydrate oxidation exacerbated the negative effect of prolonged exercise on power output at the moderate-to-heavy transition via effects on metabolic efficiency (Figure 6). Given the moderate intensity of the prolonged phase, glycogen was likely preferentially depleted in type I fibres (22) and, plausibly, glycogen depletion in type I fibres may have been accelerated in EX+NH, contributing to negative effects on metabolic efficiency, and therefore power output at the moderate-to-heavy transition, vs. EX. However, it is also possible that liver glycogen depletion was accelerated during the prolonged phase in EX+NH, at least in part due to reduced NEFA availability and therefore fat oxidation (Figure

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2C-D). Similar logic follows for recent studies reporting improved durability of the moderate-to-heavy transition with carbohydrate ingestion during prolonged exercise (31), given carbohydrate ingestion lowers endogenous carbohydrate oxidation rates (54, 55) via reduced liver (25) and muscle (33) glycogenolysis. Accordingly, whilst our data are consistent with a role for endogenous carbohydrate availability in durability of the moderate-to-heavy intensity transition, we cannot through our study design isolate this effect to skeletal muscle or liver glycogen. Future studies should explore the effects of interventions that manipulate endogenous carbohydrate oxidation on durability with measurements of muscle and liver glycogen, alongside advanced electromyography to provide insight into neuromuscular and fibre type recruitment patterns via measures of amplitude and median power frequency.

It is noteworthy that the EX+NH did not result in statistically greater deterioration of the heavy-to-severe intensity transition (Figure 5B). This raises the possibility that power output at the heavy-to-severe intensity transition is not sensitive to endogenous carbohydrate availability, at least in the context of the ~19% increase in endogenous carbohydrate oxidation observed in EX+NH. These data align with our previous study, in which we manipulated muscle glycogen via a 24-h exercise and diet intervention and observed no difference in power output at the heavy-to-severe intensity transition (35). In that study, there was an ~39% reduction in muscle glycogen in the lowered glycogen conditions, from ~406 to ~248 mmol glycosyl units·kg^{-1} dw. In contrast, another study reported improved durability of the heavy-to-severe transition following two hours of heavy-intensity cycling when 60 g·h^{-1} carbohydrate was ingested (8). In that study, muscle glycogen following prolonged exercise was higher when carbohydrate was ingested, suggesting that the improved durability of the heavy-to-severe intensity transition may have been related to muscle glycogen availability. Post-exercise muscle glycogen concentrations were lower than in our previous study, with mean values of ~200 and ~150 mmol glycosyl units·kg^{-1} dw, which may suggest that power output at the heavy-to-severe transition is sensitive to muscle glycogen availability, but only when muscle glycogen falls below threshold values. Therefore, the lack of between-trial differences in power output at the heavy-to-severe transition in the present study may be due to insufficient muscle glycogen depletion. However, in the absence of muscle glycogen data, this is speculative. Our heavy-to-severe transition data are also difficult to interpret given the lack of an effect of exercise per se, although differences approached statistical significance (~7% in EX+NH vs. CON, $P = 0.097$, Figure 5B). It is possible that the prolonged exercise protocol in this study, which was conducted at 90% of estimated VT_1, was insufficient to induce large, detectable reductions in power output at the heavy-to-severe transition. Exercise at this intensity is largely dependent on recruitment of type I fibres (56, 57). The aforementioned study in which carbohydrate ingestion improved durability of the heavy-to-severe transition featured prolonged exercise in the heavy-intensity domain, which is likely to have induced significant muscle glycogen depletion in type II fibres (8).

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However, a more recent study found that moderate-intensity cycling reduced power output at the heavy-to-severe transition, although the prolonged cycling protocol in that study was three hours, which may have been sufficient to deplete glycogen in type II fibres (32). It is therefore possible that durability of the heavy-to-severe transition is sensitive to carbohydrate availability in type II muscle fibres, and that the prolonged phase in this study did not exert consistent and/or sufficiently large effects on glycogen in type II fibres to negatively impact power output at the heavy-to-severe transition. It therefore remains possible that durability of power output at the heavy-to-severe intensity transition may have been impacted by a greater experimental acceleration of endogenous carbohydrate oxidation, or with different combinations of exercise intensity and duration. We recommend that future studies consider re-examining this effect with more-demanding prolonged exercise protocols and measurements of muscle (and ideally liver) glycogen concentrations.

We observed that whilst prolonged exercise per se reduced W' by ~25% (EX vs. CON), accelerated endogenous carbohydrate oxidation during prolonged exercise did not exacerbate this effect (Figure 5C). This aligns with our study reporting no effect of a ~39% difference in muscle glycogen on W' (35), and with a study reporting that carbohydrate ingestion during two hours of heavy-intensity cycling did not mitigate exercise-induced loss of W' (8). These data suggest that durability of W' is not sensitive to endogenous carbohydrate availability, at least in the context of the ~19% increase in endogenous carbohydrate oxidation observed in EX+NH. It remains possible that larger between-trial differences in endogenous carbohydrate oxidation could elicit effects on W', or that carbohydrate availability only affects W' below threshold glycogen values not reached in these studies.

Collectively, our data add to the growing body of literature on durability, and provide the first direct evidence that endogenous carbohydrate availability has a mechanistic role in durability of the moderate-to-heavy transition. These data therefore support that at least part of the positive effect of carbohydrate ingestion on durability of the moderate-to-heavy transition is mediated via reduced endogenous carbohydrate oxidation, and suggest that other strategies to reduce endogenous carbohydrate oxidation may have a beneficial effect on durability. Therefore, based on the present data, one might expect that increased capacity for fat oxidation during exercise, which can occur during endurance training (58–60), would translate into improved durability of the moderate-to-heavy transition, although this should be verified in future studies. In support, the capacity for fat oxidation during exercise has been linked to endurance performance outcomes (61, 62), and it is plausible that improved durability is part of this effect. Examination of this hypothesis could be achieved via investigation of the effects of New Zealand blackcurrant supplementation on durability, given this increases fat oxidation rates during exercise (63, 64), and we recommend this as a future research direction.

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In conclusion, durability of power output at the moderate-to-heavy transition, but not heavy-to-severe transition or capacity for work above the heavy-to-severe transition, is sensitive to accelerated endogenous carbohydrate oxidation. These data add to our mechanistic understanding of durability, and suggest interventions that reduce endogenous carbohydrate oxidation may lead to improved durability.

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Figure legends

Figure 1. Schematic illustration of the study design. On four occasions, participants undertook assessments of the moderate-to-heavy and heavy-to-severe intensity transitions following two hours of seated rest (CON) or moderate-intensity cycling (EX), with (+NH) and without 500 mg of niacin ingestion and mild, local heat stress application.

Figure 2. Substrate oxidation during prolonged cycling with (EX+NH) and without (EX) pre-exercise niacin ingestion and mild heat stress applied to the upper leg muscles during exercise. (a) Carbohydrate (CHO) oxidation rates, (b) total CHO oxidised, (c) fat oxidation rates, (d) total fat oxidised, (e) rate of energy expenditure, and (f) total energy expended. N = 7 due to a technical fault with $\dot{V}CO_2$ measures in four participants. * denotes $P \leq 0.05$ between-trials.

Figure 3. Plasma responses during prolonged cycling with (EX+NH) and without (EX) pre-exercise niacin ingestion and mild heat stress applied to the upper leg muscles during exercise. Plasma (a) glycerol, (b) non-esterified fatty acids, (c) glucose, and (d) lactate concentrations. * denotes $P \leq 0.05$ between-trials.

Figure 4. Substrate oxidation during incremental cycling with (EX) and without (CON) prior prolonged cycling, with (+NH) or without niacin ingestion prior to prolonged cycling and mild heat stress applied to the upper leg muscles prior to assessments. (a) Carbohydrate (CHO) oxidation rates, (b) fat oxidation rates, and (c) energy expenditure. N = 7 due to a technical fault with $\dot{V}CO_2$ measures in four participants. Statistical significance not shown here due to the number of comparisons (see text).

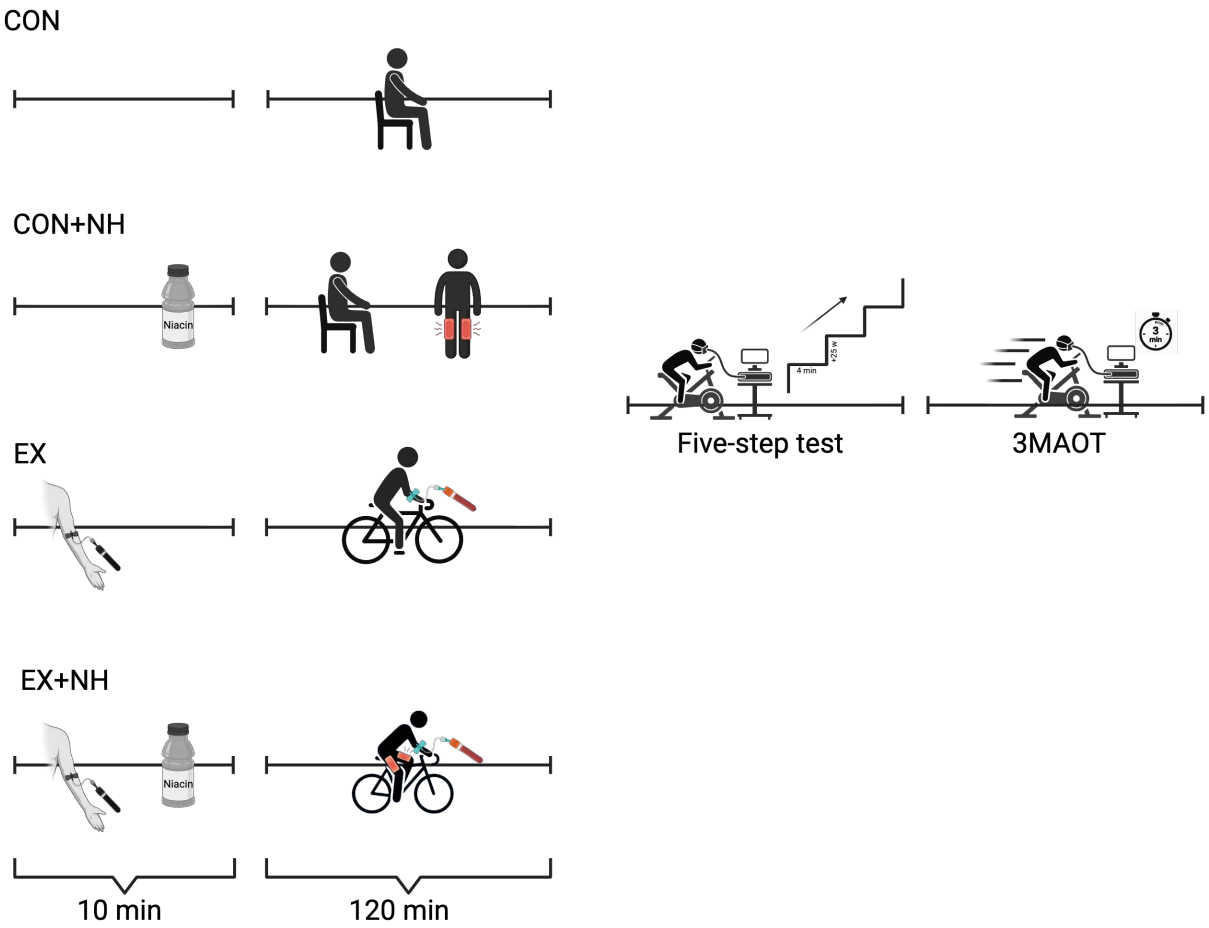
Figure 5. Intensity domain transitions measured with (EX) and without (CON) prior prolonged cycling, with (+NH) or without niacin ingestion prior to prolonged cycling and mild heat stress applied to the upper leg muscles prior to assessments. (a) Power output at the first ventilatory threshold (VT_1), (b) three-minute all-out test end-test power output (CP_{3MAOT}), and (c) work above three-minute all-out test end-test power output (W'). Data are raw means (bars) with individual responses (lines with markers). ** denotes $P \leq 0.01$.

Figure 6. Contributions to loss of power output at the moderate-to-heavy intensity transition (first ventilatory threshold) after prolonged cycling with (EX+NH) or without (EX) pre-exercise niacin

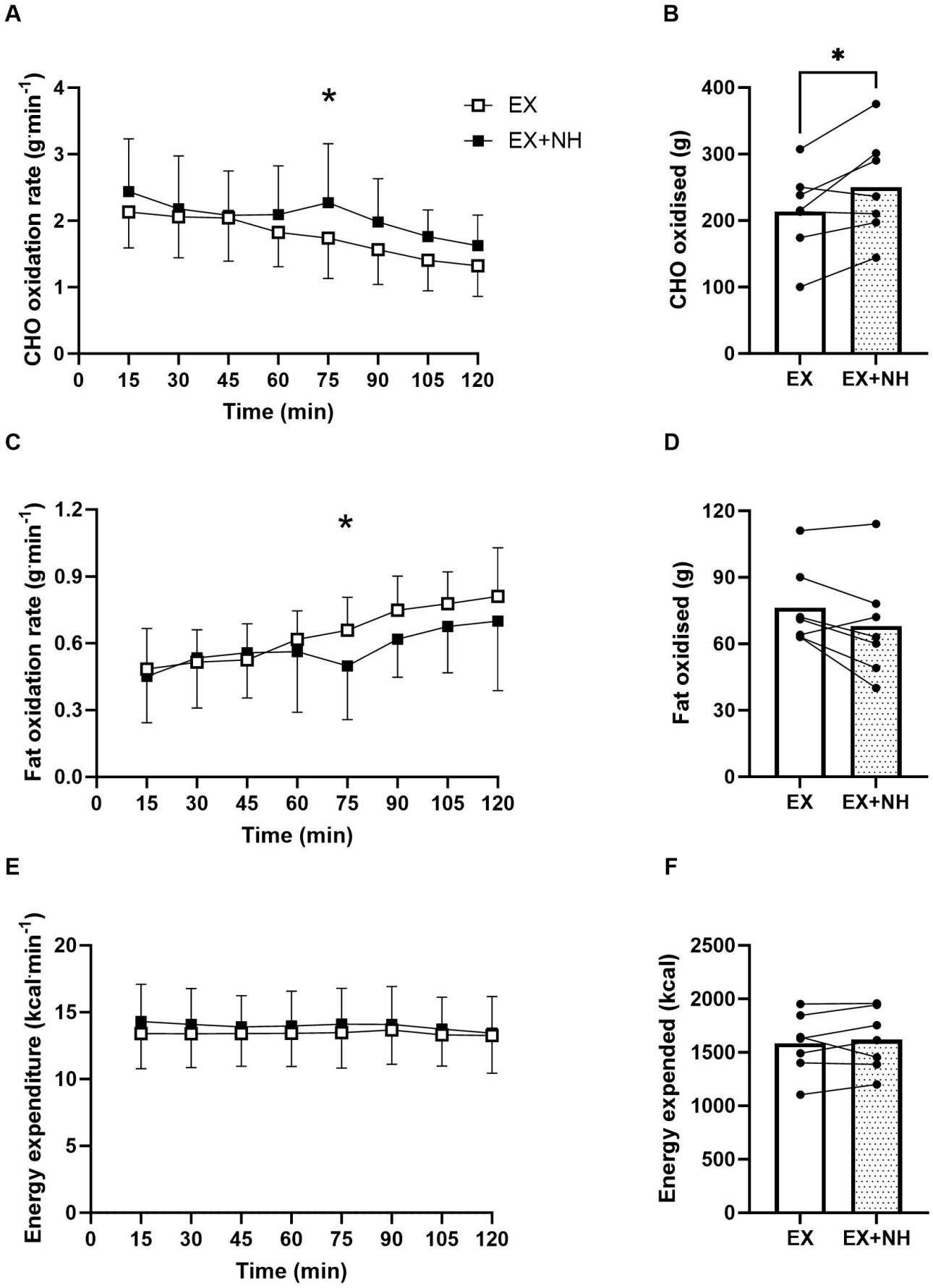
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715 ingestion and mild heat stress applied to the upper leg muscles during prolonged cycling. N = 7 due to
716 a technical fault with $\dot{V}CO_2$ measures in four participants. * denotes $P < 0.05$ between-trials.

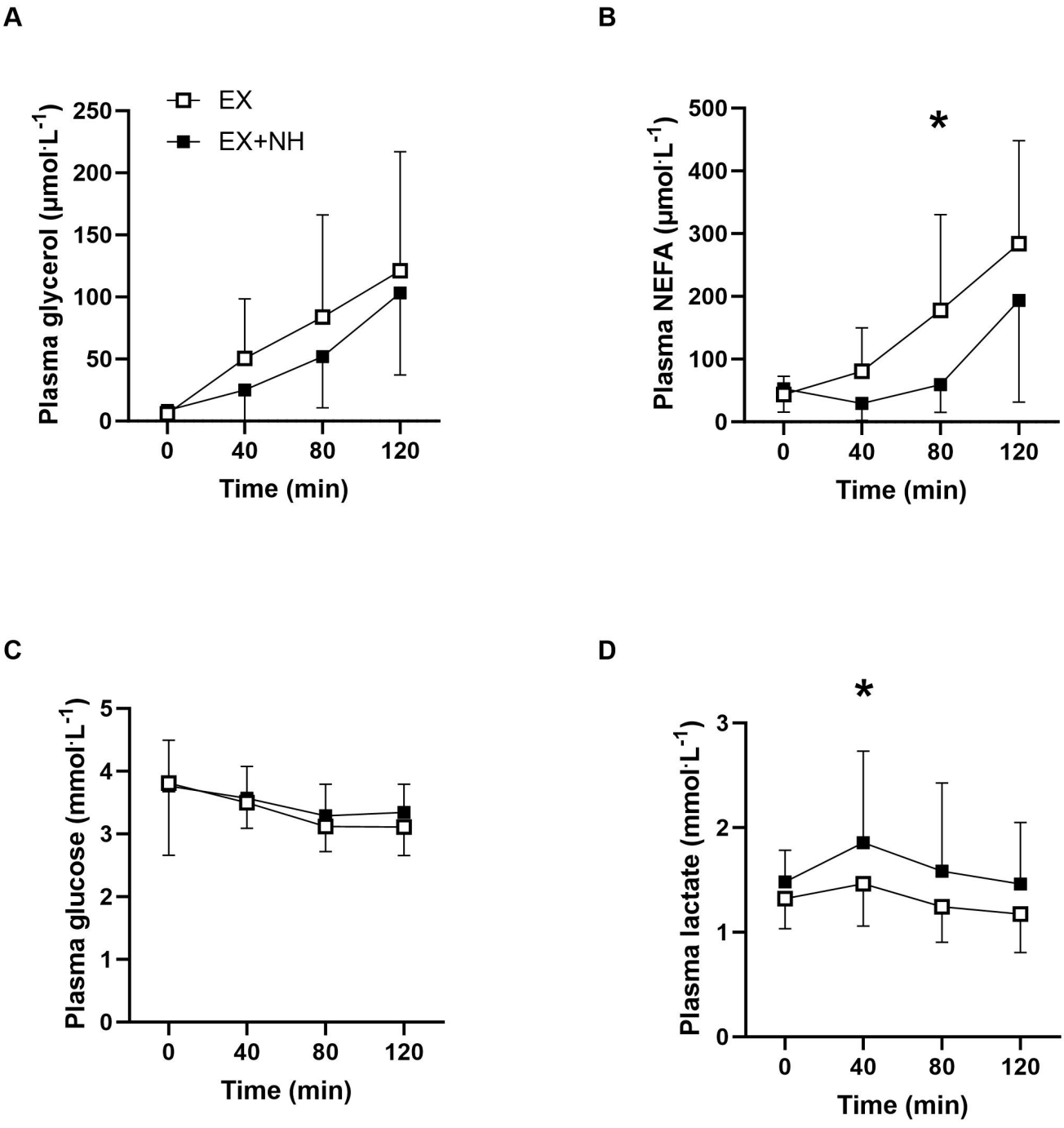
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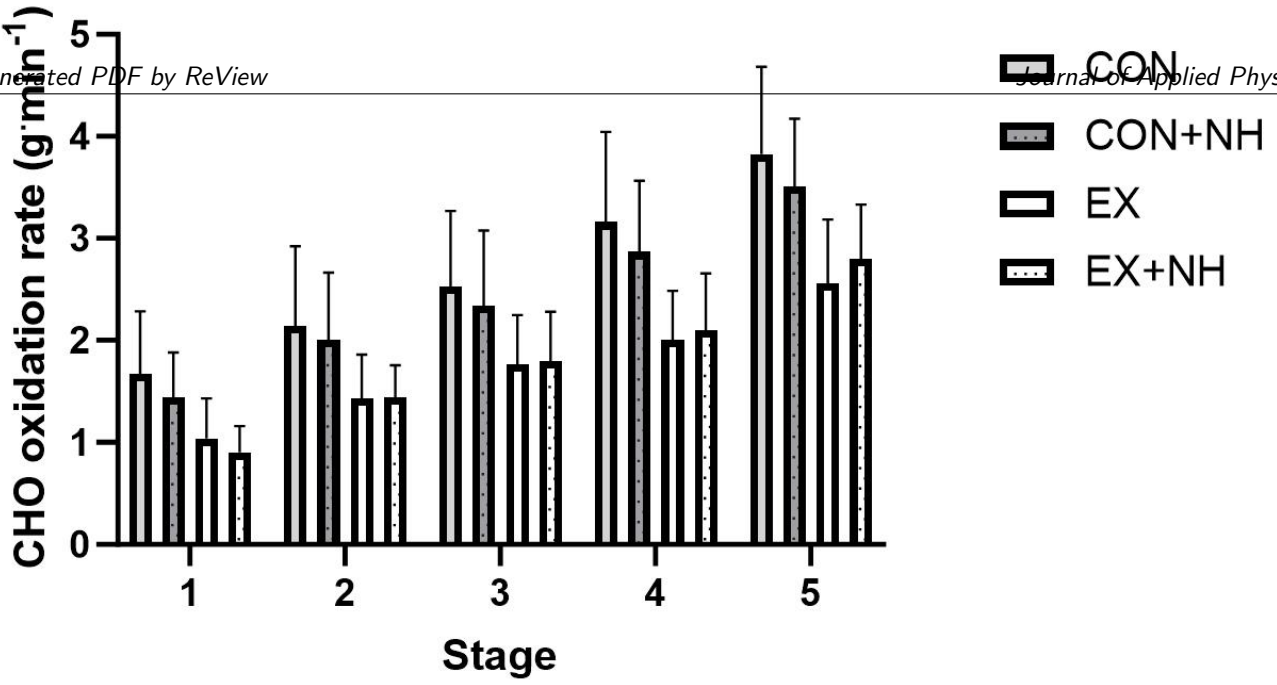
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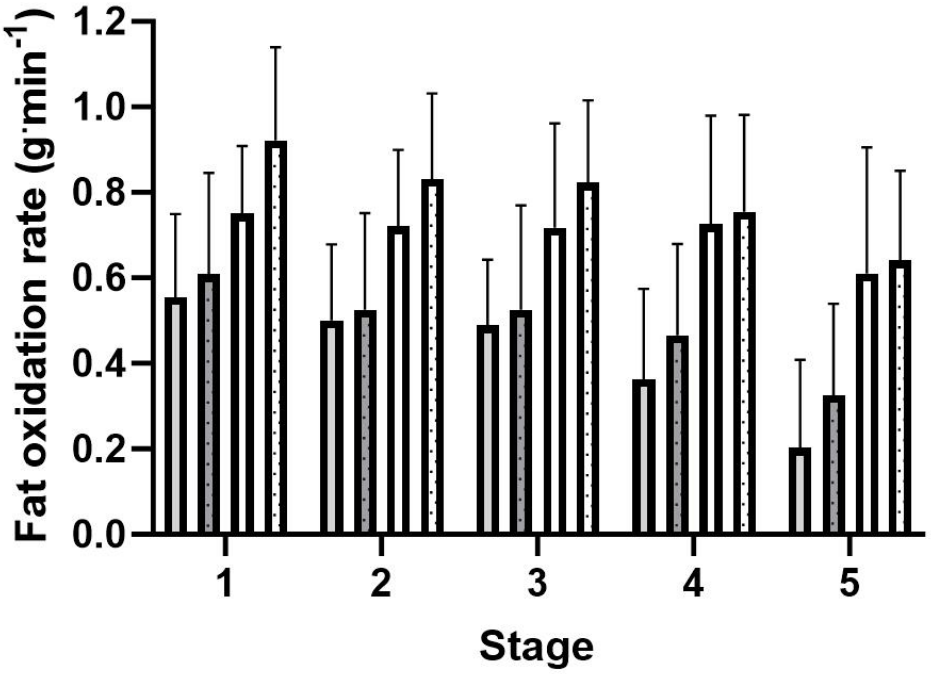
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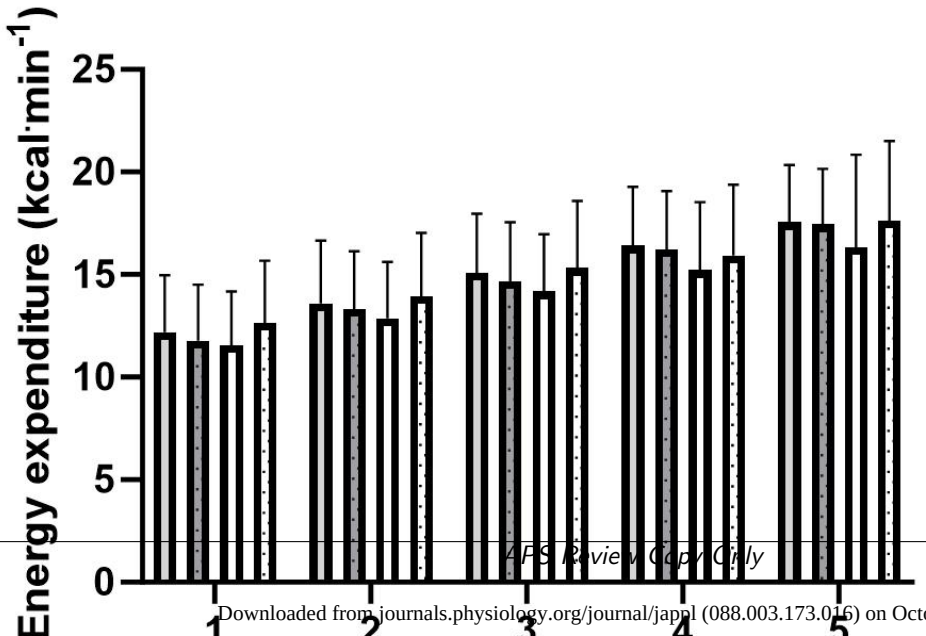
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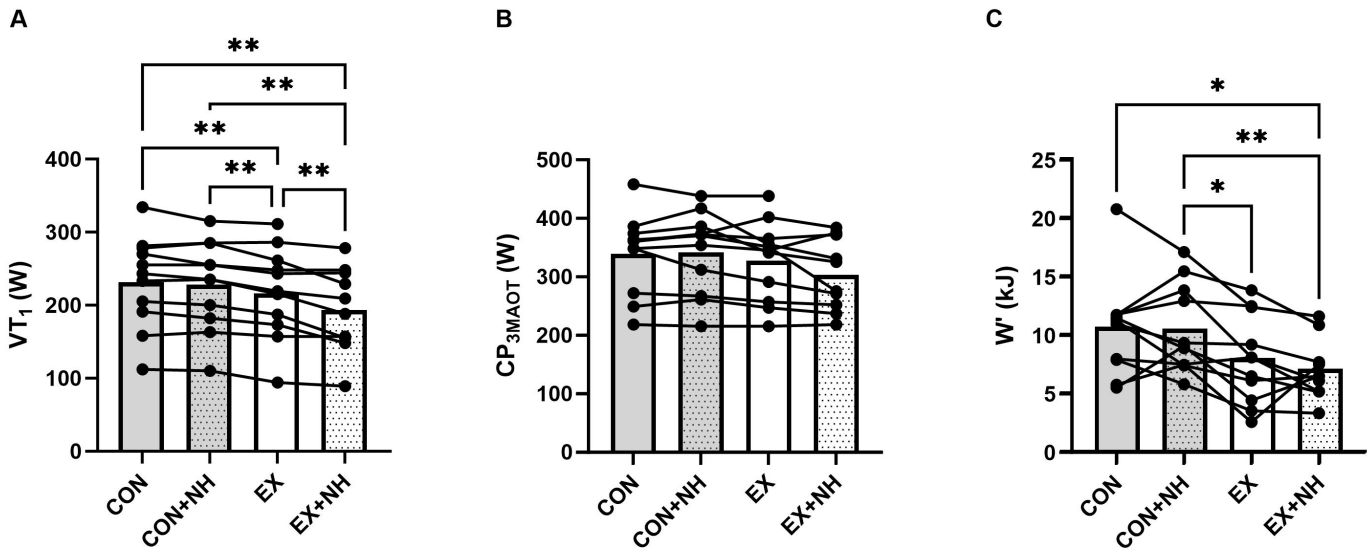
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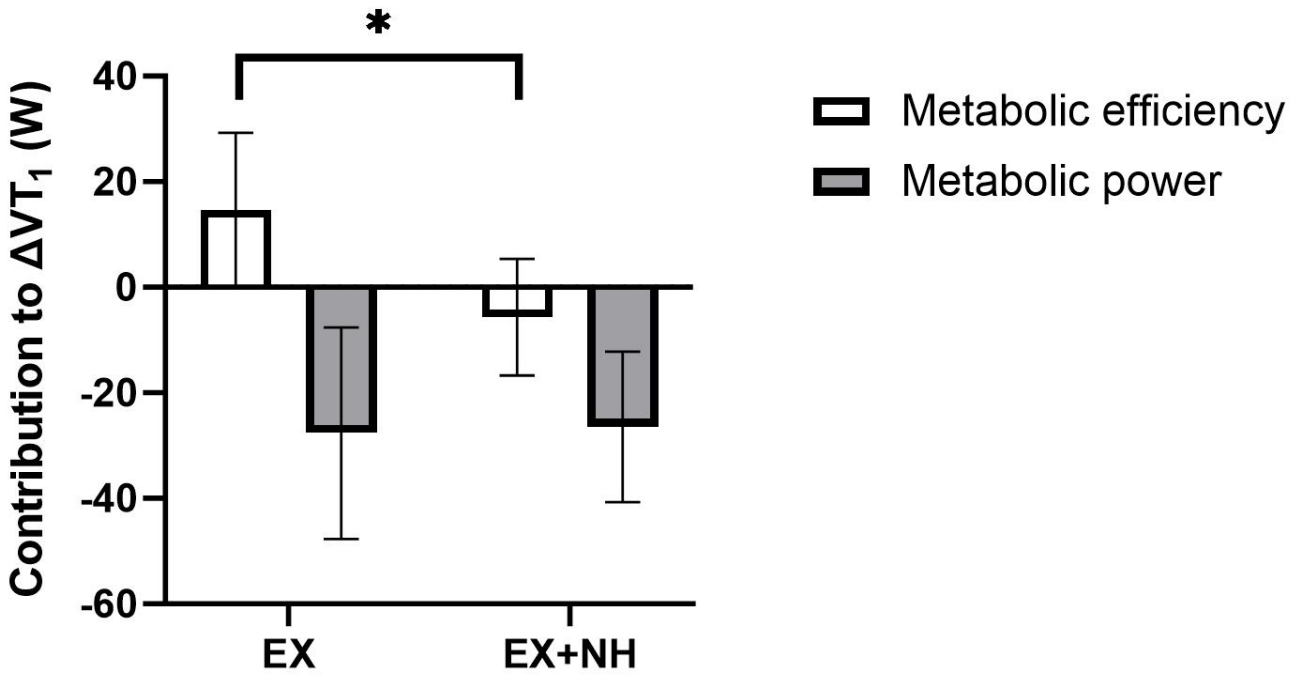
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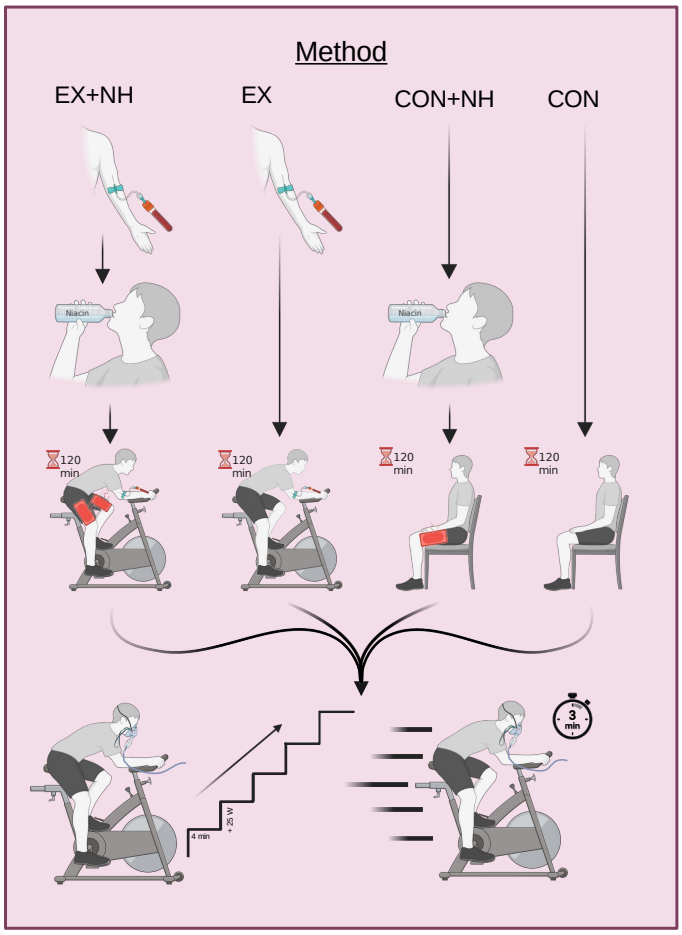


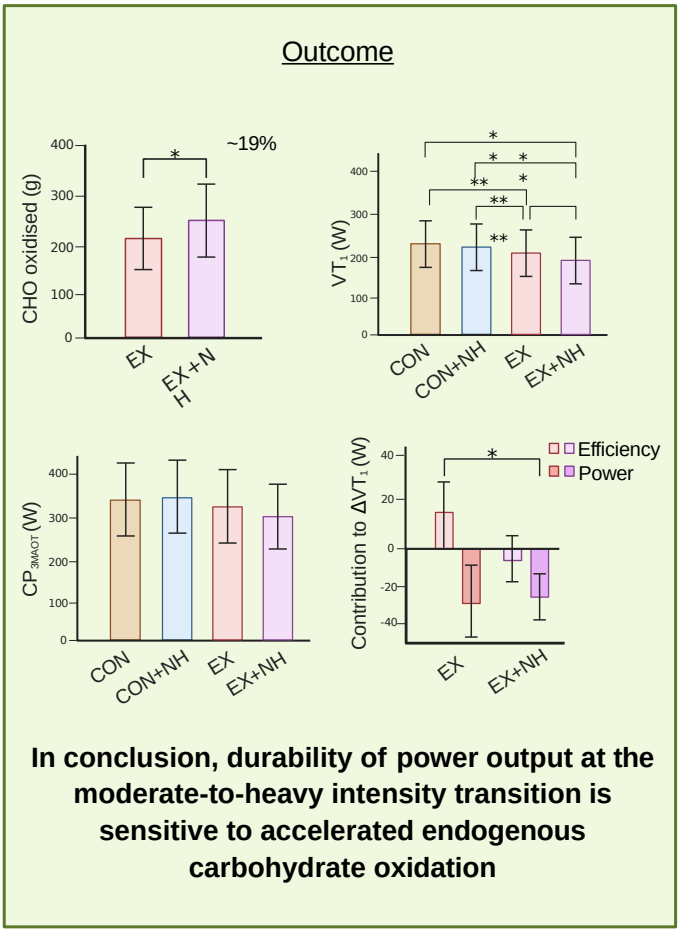
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Accelerated endogenous carbohydrate oxidation reduces durability





In conclusion, durability of power output at the moderate-to-heavy intensity transition is sensitive to accelerated endogenous carbohydrate oxidation

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