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

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

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

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

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

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

450

451 We observed that whilst prolonged exercise per se reduced W' by ~25% (EX vs. CON), accelerated
452 endogenous carbohydrate oxidation during prolonged exercise did not exacerbate this effect (Figure
453 5C). This aligns with our study reporting no effect of a ~39% difference in muscle glycogen on W' (35),
454 and with a study reporting that carbohydrate ingestion during two hours of heavy-intensity cycling did
455 not mitigate exercise-induced loss of W' (8). These data suggest that durability of W' is not sensitive to
456 endogenous carbohydrate availability, at least in the context of the ~19% increase in endogenous
457 carbohydrate oxidation observed in EX+NH. It remains possible that larger between-trial differences in
458 endogenous carbohydrate oxidation could elicit effects on W', or that carbohydrate availability only
459 affects W' below threshold glycogen values not reached in these studies.

460

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



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474

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



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## References


1. **Jones AM**, **Burnley M**, **Black MI**, **Poole DC**, **Vanhatalo A**. The maximal metabolic steady state: redefining the 'gold standard.' *Physiol Rep* 7: 1–16, 2019. doi: 10.14814/phy2.14098.

2. **Jones AM**, **Wilkerson DP**, **DiMenna F**, **Fulford J**, **Poole DC**. Muscle metabolic responses to exercise above and below the "critical power" assessed using 31P-MRS. *Am J Physiol Regul Integr Comp Physiol* 294: R585–R593, 2008. doi: 10.1152/ajpregu.00731.2007.

3. **Black MI**, **Jones AM**, **Blackwell JR**, **Bailey SJ**, **Wylie LJ**, **McDonagh STJ**, **Thompson C**, **Kelly J**, **Sumners P**, **Mileva KN**, **Bowtell JL**, **Vanhatalo A**. Muscle metabolic and neuromuscular determinants of fatigue during cycling in different exercise intensity domains. *J Appl Physiol* 122: 446–459, 2017. doi: 10.1152/japplphysiol.00942.2016.

4. **Vanhatalo A**, **Fulford J**, **Dimenna FJ**, **Jones AM**. Influence of hyperoxia on muscle metabolic responses and the power-duration relationship during severe-intensity exercise in humans: A 31P magnetic resonance spectroscopy study. *Exp Physiol* 95: 528–540, 2010. doi: 10.1113/expphysiol.2009.050500.

5. **Jamnick NA**, **Pettitt RW**, **Granata C**, **Pyne DB**, **Bishop DJ**. An examination and critique of current methods to determine exercise intensity. *Sports Medicine* 50: 1729–1756, 2020. doi: 10.1007/s40279-020-01322-8.

6. **Clark IE**, **Vanhatalo A**, **Bailey SJ**, **Wylie LJ**, **Kirby BS**, **Wilkins BW**, **Jones AM**. Effects of two hours of heavy-intensity exercise on the power-duration relationship. *Med Sci Sports Exerc* 50: 1658–1668, 2018. doi: 10.1249/MSS.0000000000001601.

7. **Clark IE**, **Vanhatalo A**, **Thompson C**, **Wylie LJ**, **Bailey SJ**, **Kirby BS**, **Wilkins BW**, **Jones AM**. Changes in the power-duration relationship following prolonged exercise: estimation using conventional and all-out protocols and relationship with muscle glycogen. *Am J Physiol Regul Integr Comp Physiol* 317: R59–R67, 2019. doi: 10.1152/ajpregu.00031.2019.

8. **Clark IE**, **Vanhatalo A**, **Thompson C**, **Joseph C**, **Black MI**, **Blackwell JR**, **Wylie LJ**, **Tan R**, **Bailey SJ**, **Wilkins BW**, **Kirby BS**, **Jones AM**. Dynamics of the power-duration relationship during prolonged endurance exercise and influence of carbohydrate ingestion. *J Appl Physiol* 127: 726–736, 2019. doi: 10.1152/japplphysiol.00207.2019.

9. **Stevenson JD**, **Kilding AE**, **Plews DJ**, **Maunder E**. Prolonged cycling reduces power output at the moderate-to-heavy intensity transition. *Eur J Appl Physiol* 122: 2673–2682, 2022. doi: 10.1007/s00421-022-05036-9.

10. **Hamilton K**, **Kilding AE**, **Plews DJ**, **Mildenhall MJ**, **Waldron M**, **Charoensap T**, **Cox TH**, **Brick MJ**, **Leigh WB**, **Maunder E**. Durability of the moderate-to-heavy-intensity transition is related to the effects of prolonged exercise on severe-intensity performance. *Eur J Appl Physiol* 124: 2427–2438, 2024. doi: 10.1007/s00421-024-05459-6.

11. **Gallo G**, **Faelli EL**, **Ruggeri P**, **Filipas L**, **Codella R**, **Plews DJ**, **Maunder E**. Power output at the moderate-to-heavy intensity transition decreases in a non-linear fashion during prolonged exercise. *Eur J Appl Physiol* 124: 2353–2364, 2024. doi: 10.1007/s00421-024-05440-3.

12. **Maunder E**, **Seiler S**, **Mildenhall MJ**, **Kilding AE**, **Plews DJ**. The importance of 'durability' in the physiological profiling of endurance athletes. *Sports Medicine* 51: 1619–1628, 2021. doi: 10.1007/s40279-021-01459-0.




Downloaded from journals.physiology.org/journal/jappl at Auckland Univ of Tech (156.062.003.021) on October 27, 2026.

13. **Jones AM**. The fourth dimension: physiological resilience as an independent determinant of endurance exercise performance. *Journal of Physiology* 602: 4113–4128, 2024. doi: 10.1113/JP284205.

14. **Rothschild JA**, **Gallo G**, **Hamilton K**, **Stevenson JD**, **Dudley-Rode H**, **Charoensap T**, **Plews DJ**, **Kilding AE**, **Maunder E**. Durability of the moderate-to-heavy intensity transition can be predicted using readily available markers of physiological decoupling. *Eur J Appl Physiol* 125: 2911–2920, 2025. doi: 10.1007/s00421-025-05815-0.

15. **Hunter B**, **Maunder E**, **Jones AM**, **Gallo G**, **Muniz-Pumares D**. Durability as an index of endurance exercise performance: Methodological considerations. *Exp Physiol* 110: 1612–1624, 2025. doi: 10.1113/EP092120.

16. **Bergström J**, **Hermansen L**, **Hultman E**, **Saltin B**. Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71: 140–150, 1967.

17. **Ørtenblad N**, **Nielsen J**, **Saltin B**, **Holmberg HC**. Role of glycogen availability in sarcoplasmic reticulum Ca2+ kinetics in human skeletal muscle. *Journal of Physiology* 589: 711–725, 2011. doi: 10.1113/jphysiol.2010.195982.

18. **Nielsen J**, **Holmberg HC**, **Schrøder HD**, **Saltin B**, **Ørtenblad N**. Human skeletal muscle glycogen utilization in exhaustive exercise: role of subcellular localization and fibre type. *Journal of Physiology* 589: 2871–2885, 2011. doi: 10.1113/jphysiol.2010.204487.

19. **Nielsen J**, **Cheng AJ**, **Ørtenblad N**, **Westerblad H**. Subcellular distribution of glycogen and decreased tetanic Ca2+ in fatigued single intact mouse muscle fibres. *Journal of Physiology* 592: 2003–2012, 2014. doi: 10.1113/jphysiol.2014.271528.

20. **Jensen R**, **Nielsen J**, **Ørtenblad N**. Inhibition of glycogenolysis prolongs action potential repriming period and impairs muscle function in rat skeletal muscle. *Journal of Physiology* 598: 789–803, 2020. doi: 10.1113/JP278543.

21. **Nielsen J**, **Dubillot P**, **Stausholm MLH**, **Ørtenblad N**. Specific ATPases drive compartmentalized glycogen utilization in rat skeletal muscle. *Journal of General Physiology* 154: 1–9, 2022. doi: 10.1085/jgp.202113071.

22. **Nielsen J**, **Jensen R**, **Ørtenblad N**. Assessments of individual fiber glycogen and mitochondrial volume percentages reveal a graded reduction in muscle oxidative power during prolonged exhaustive exercise. *Scand J Med Sci Sports* 34: e14571, 2024. doi: 10.1111/sms.14571.

23. **Mitchell EA**, **Martin NRW**, **Bailey SJ**, **Ferguson RA**. Critical power is positively related to skeletal muscle capillarity and type i muscle fibers in endurance-trained individuals. *J Appl Physiol* 125: 737–745, 2018. doi: 10.1152/japplphysiol.01126.2017.

24. **Gonzalez JT**, **Fuchs CJ**, **Betts JA**, **van Loon LJC**. Liver glycogen metabolism during and after prolonged endurance-type exercise. *Am J Physiol Endocrinol Metab* 311: E543–E553, 2016. doi: 10.1152/ajpendo.00232.2016.

25. **Gonzalez JT**, **Fuchs CJ**, **Smith FE**, **Thelwall PE**, **Taylor R**, **Stevenson EJ**, **Trenell MI**, **Cermak NM**, **van Loon LJC**. Ingestion of glucose or sucrose prevents liver but not muscle glycogen depletion during prolonged endurance-type exercise in trained cyclists. *Am J Physiol Endocrinol Metab* 309: E1032–E1039, 2015. doi: 10.1152/ajpendo.00376.2015.

26. **Stevenson EJ**, **Thelwall PE**, **Thomas K**, **Smith F**, **Brand-Miller J**, **Trenell MI**. Dietary glycemic index influences lipid oxidation but not muscle or liver glycogen oxidation during



Downloaded from journals.physiology.org/journal/jappl at Auckland Univ of Tech (156.062.003.021) on October 27, 2026.

exercise. *Am J Physiol Endocrinol Metab* 296: E1140–E1147, 2009. doi: 10.1152/ajpendo.90788.2008.

27. **Coyle EF**, **Hagberg JM**, **Hurley BF**, **Martin WH**, **Ehsani AA**, **Holloszy JO**. Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. *J Appl Physiol* 55: 230–235, 1983. doi: 10.1152/jappl.1983.55.1.230.

28. **Coyle EF**, **Coggan AR**, **Hemmert K**, **Lowe RC**, **Walters TJ**. Substrate usage during prolonged exercise following a preexercise meal. *J Appl Physiol* 59: 429–433, 1985.

29. **Coyle EF**, **Coggan AR**, **Hemmert K**, **Ivy JL**. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* 61: 165–172, 1986.

30. **Elghobashy ME**, **Richards AJ**, **Malekzadeh R**, **Patel D**, **Turner L V.**, **Burr JF**, **Power GA**, **Laham R**, **Riddell MC**, **Cheng AJ**. Carbohydrate ingestion increases interstitial glucose and mitigates neuromuscular fatigue during single-leg knee extensions. *Med Sci Sports Exerc* 56: 1495–1504, 2024. doi: 10.1249/MSS.0000000000003440.

31. **Dudley-Rode H**, **Zinn C**, **Plews DJ**, **Charoensap T**, **Maunder E**. Carbohydrate ingestion during prolonged exercise blunts the reduction in power output at the moderate-to-heavy intensity transition. *Eur J Appl Physiol* 125: 1349–1359, 2025. doi: 10.1007/s00421-024-05687-w.

32. **Norte BR**, **Slinn MM**, **Johnson KO**, **Mahon E**, **Shepherd SO**, **Strauss JA**, **Louis JB**. Graded carbohydrate ingestion up to 120 g·h−1 attenuates the reduction in critical power following 3 h of moderate-intensity exercise in a dose-dependent manner. *Scand J Med Sci Sports* 36: e70326, 2026. doi: 10.1111/sms.70326.

33. **Rothschild JA**, **Dudley-Rode H**, **Carpenter H**, **Smith ASM**, **Plews DJ**, **Maunder E**. Carbohydrate ingestion during prolonged exercise and net skeletal muscle glycogen utilization: a meta-analysis. *J Appl Physiol* 140: 76–87, 2026. doi: 10.1152/japplphysiol.00861.2025.

34. **Chambers ES**, **Bridge MW**, **Jones DA**. Carbohydrate sensing in the human mouth: Effects on exercise performance and brain activity. *Journal of Physiology* 587: 1779–1794, 2009. doi: 10.1113/jphysiol.2008.164285.

35. **Main E**, **Yoon SH**, **James SL**, **Mellor KM**, **Brick MJ**, **Leigh WB**, **Maunder E**. Low carbohydrate availability reduces power output at the moderate-to-heavy transition, impairs efficiency, and increases median power frequency during cycling in females. .

36. **Hawley JA**, **Burke LM**, **Angus DJ**, **Fallon KE**, **Martin DT**, **Febbraio MA**. Effect of altering substrate availability on metabolism and performance during intense exercise. *British Journal of Nutrition* 84: 829–838, 2000. doi: 10.1017/S0007114500002440.

37. **Leckey JJ**, **Burke LM**, **Morton JP**, **Hawley JA**. Altering fatty acid availability does not impair prolonged, continuous running to fatigue: evidence for carbohydrate dependence. *J Appl Physiol* 120: 107–113, 2016. doi: 10.1152/japplphysiol.00855.2015.

38. **Murray R**, **Bartoli WP**, **Eddy DE**, **Horn MK**. Physiological and performance responses to nicotinic-acid ingestion during exercise. *Med Sci Sports Exerc* 27: 1057–1062, 1995.

39. **Torrens SL**, **Areta JL**, **Parr EB**, **Hawley JA**. Carbohydrate dependence during prolonged simulated cycling time trials. *Eur J Appl Physiol* 116: 781–790, 2016. doi: 10.1007/s00421-016-3333-y.



Downloaded from journals.physiology.org/journal/jappl at Auckland Univ of Tech (156.062.003.021) on October 27, 2026.

40. **Bergström J**, **Hultman E**, **Jorfeldt L**, **Pernow B**, **Wahren J**. Effect of nicotinic acid on physical working capacity and on metabolism of muscle glycogen in man. *J Appl Physiol* 26: 170–176, 1969.

41. **Heath EM**, **Wilcox AR**, **Quinn CM**. Effects of nicotinic acid on respiratory exchange ratio and substrate levels during exercise. *Med Sci Sports Exerc* 25: 1018–1023, 1993.

42. **Febbraio MA**, **Carey MF**, **Snow RJ**, **Stathis CG**, **Hargreaves M**. Influence of elevated muscle temperature on metabolism during intense, dynamic exercise. *Am J Physiol Regul Integr Comp Physiol* 271: R1251–R1255, 1996. doi: 10.1152/ajpregu.1996.271.5.R1251.

43. **Starkie RL**, **Hargreaves M**, **Lambert DL**, **Proietto J**, **Febbraio MA**. Effects of temperature on muscle metabolism during submaximal exercise in humans. *Exp Physiol* 84: 775–784, 1999.

44. **Burnley M**, **Doust JH**, **Vanhatalo A**. A 3-min all-out test to determine peak oxygen uptake and the maximal steady state. *Med Sci Sports Exerc* 38: 1995–2003, 2006. doi: 10.1249/01.mss.0000232024.06114.a6.

45. **Vanhatalo A**, **Doust JH**, **Burnley M**. Determination of critical power using a 3-min all-out cycling test. *Med Sci Sports Exerc* 39: 548–555, 2007. doi: 10.1249/mss.0b013e31802dd3e6.

46. **Jeukendrup AE**, **Wallis GA**. Measurement of substrate oxidation during exercise by means of gas exchange measurements. *Int J Sports Med* 26: S28–S37, 2005. doi: 10.1055/s-2004-830512.

47. **Maunder E**, **King A**, **Rothschild JA**, **Brick MJ**, **Leigh WB**, **Hedges CP**, **Merry TL**, **Kilding AE**. Locally applied heat stress during exercise training may promote adaptations to mitochondrial enzyme activities in skeletal muscle. *Pflugers Arch* 476: 939–948, 2024. doi: 10.1007/s00424-024-02939-8.

48. **Van Beaumont W**, **Greenleaf JE**, **Juhos L**. Disproportional changes in hematocrit, plasma volume, and proteins during exercise and bed rest. *J Appl Physiol* 33: 55–61, 1972. doi: 10.1152/jappl.1972.33.1.55.

49. **Frandsen J**, **Vest SD**, **Ritz C**, **Larsen S**, **Dela F**, **Helge JW**. Plasma free fatty acid concentration is closely tied to whole body peak fat oxidation rate during repeated exercise. *J Appl Physiol* 126: 1563–1571, 2019.

50. **Stallknecht B**, **Kiens B**, **Helge JW**, **Richter EA**, **Galbo H**. Interstitial glycerol concentrations in human skeletal muscle and adipose tissue during graded exercise. *Acta Physiol Scand* 180: 367–377, 2004. doi: 10.1111/j.1365-201X.2004.01264.x.

51. **Ahlborg G**, **Felig P**, **Hagenfeldt L**, **Hendler R**, **Wahren J**. Substrate turnover during prolonged exercise in man. *Journal of Clinical Investigation* 53: 1080–1090, 1974.

52. **Barrett AMS**, **Maunder E**. Prolonged running reduces speed at the moderate-to-heavy intensity transition without additional reductions due to increased eccentric load. *Eur J Appl Physiol* 125: 2897–2910, 2025. doi: 10.1007/s00421-025-05792-4.

53. **Nuuttila OP**, **Laatikainen-Raussi V**, **Vohlakari K**, **Laatikainen-Raussi I**, **Ihalainen JK**. Durability in recreational runners: effects of 90-min low-intensity exercise on the running speed at the lactate threshold. *Eur J Appl Physiol* 125: 697–705, 2025. doi: 10.1007/s00421-024-05631-y.




Downloaded from journals.physiology.org/journal/jappl at Auckland Univ of Tech (156.062.003.021) on October 27, 2026.

54. **Jentjens RLPG**, **Venables MC**, **Jeukendrup AE**. Oxidation of exogenous glucose, sucrose, and maltose during prolonged cycling exercise. *J Appl Physiol* 96: 1285–1291, 2004. doi: 10.1152/japplphysiol.01023.2003.

55. **Wallis GA**, **Rowlands DS**, **Shaw C**, **Jentjens RLPG**, **Jeukendrup AE**. Oxidation of combined ingestion of maltodextrins and fructose during exercise. *Med Sci Sports Exerc* 37: 426–432, 2005. doi: 10.1249/01.MSS.0000155399.23358.82.

56. **Gollnick PD**, **Piehl K**, **Saltin B**. Selective glycogen depletion pattern in human muscle fibres after exercise of varying intensity and at varying pedalling rates. *Journal of Physiology* 241: 45–57, 1974. doi: 10.1113/jphysiol.1974.sp010639.

57. **Gollnick PD**, **Armstrong RB**, **Saubert CW**, **Sembrowich WL**, **Shepherd RE**, **Saltin B**. Glycogen depletion patterns in human skeletal muscle fibers during prolonged work. *Pflugers Arch* 344: 1–12, 1973. doi: 10.1007/BF00587437.

58. **Hulston CJ**, **Venables MC**, **Mann CH**, **Martin C**, **Philp A**, **Baar K**, **Jeukendrup AE**. Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Med Sci Sports Exerc* 42: 2046–2055, 2010. doi: 10.1249/MSS.0b013e3181dd5070.

59. **Talanian JL**, **Galloway SDR**, **Heigenhauser GJF**, **Bonen A**, **Spriet LL**. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol* 102: 1439–1447, 2007. doi: 10.1152/japplphysiol.01098.2006.

60. **Talanian JL**, **Holloway GP**, **Snook LA**, **Heigenhauser GJF**, **Bonen A**, **Spriet LL**. Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle. *Am J Physiol Endocrinol Metab* 299: E180–E188, 2010. doi: 10.1152/ajpendo.00073.2010.

61. **Maunder E**, **Plews DJ**, **Wallis GA**, **Brick MJ**, **Leigh WB**, **Chang WL**, **Stewart T**, **Watkins CM**, **Kilding AE**. Peak fat oxidation is positively associated with vastus lateralis CD36 content, fed-state exercise fat oxidation, and endurance performance in trained males. *Eur J Appl Physiol* 122: 93–102, 2022. doi: 10.1007/s00421-021-04820-3.

62. **Frandsen J**, **Vest S**, **Larsen S**, **Dela F**, **Helge JW**. Maximal fat oxidation is related to performance in an Ironman triathlon. *Int J Sports Med* 38: 975–982, 2017. doi: 10.1055/s-0043-117178.

63. **Cook MD**, **Myers SD**, **Blacker SD**, **Willems MET**. New Zealand blackcurrant extract improves cycling performance and fat oxidation in cyclists. *Eur J Appl Physiol* 115: 2357–2365, 2015. doi: 10.1007/s00421-015-3215-8.

64. **Cook MD**, **Myers SD**, **Gault ML**, **Edwards VC**, **Willems MET**. Dose effects of New Zealand blackcurrant on substrate oxidation and physiological responses during prolonged cycling. *Eur J Appl Physiol* 117: 1207–1216, 2017. doi: 10.1007/s00421-017-3607-z.




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683 **Figure legends**

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

688

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

694

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

699

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

705

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

712

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

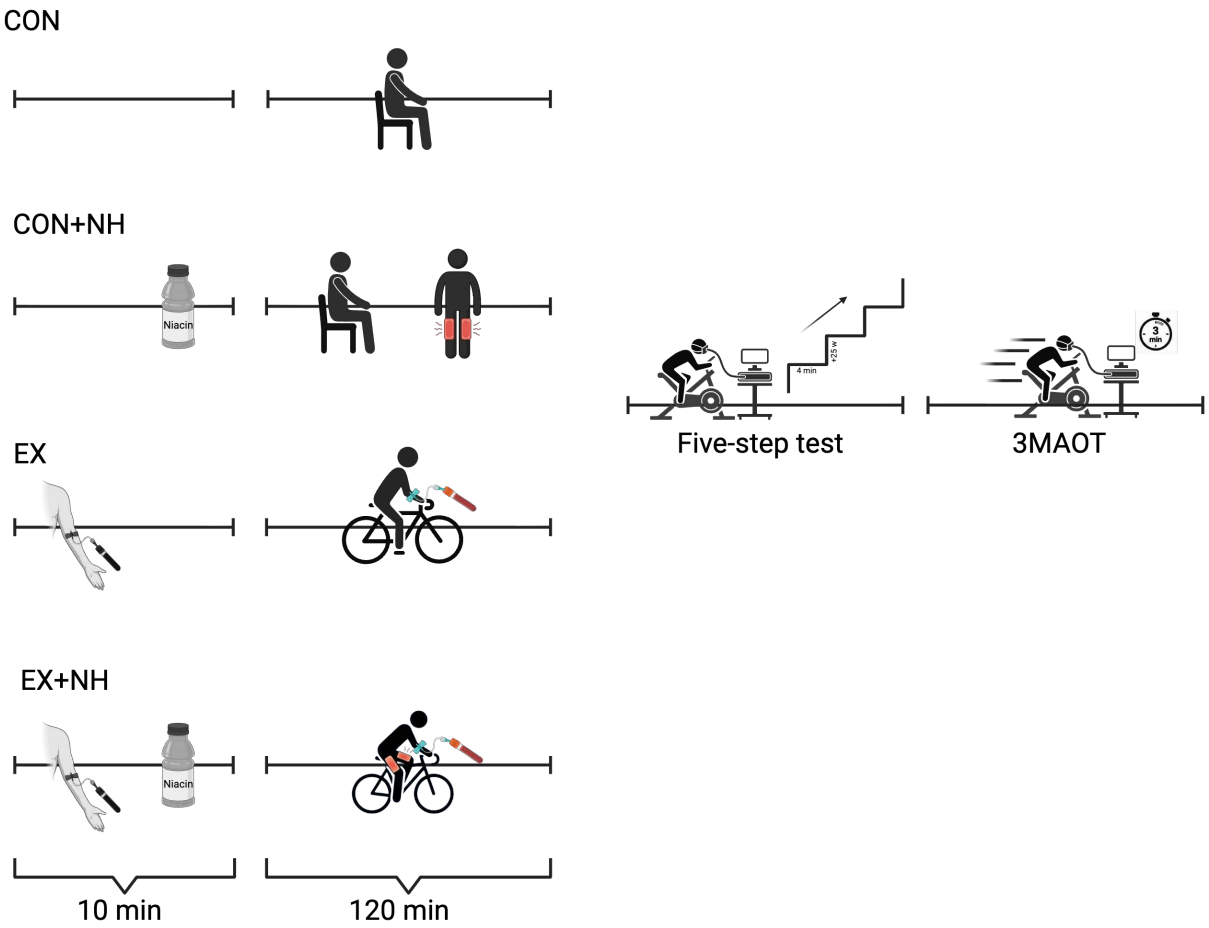


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715 ingestion and mild heat stress applied to the upper leg muscles during prolonged cycling. N = 7 due to
716 a technical fault with $\dot{V}CO_2$ measures in four participants. * denotes $P < 0.05$ between-trials.

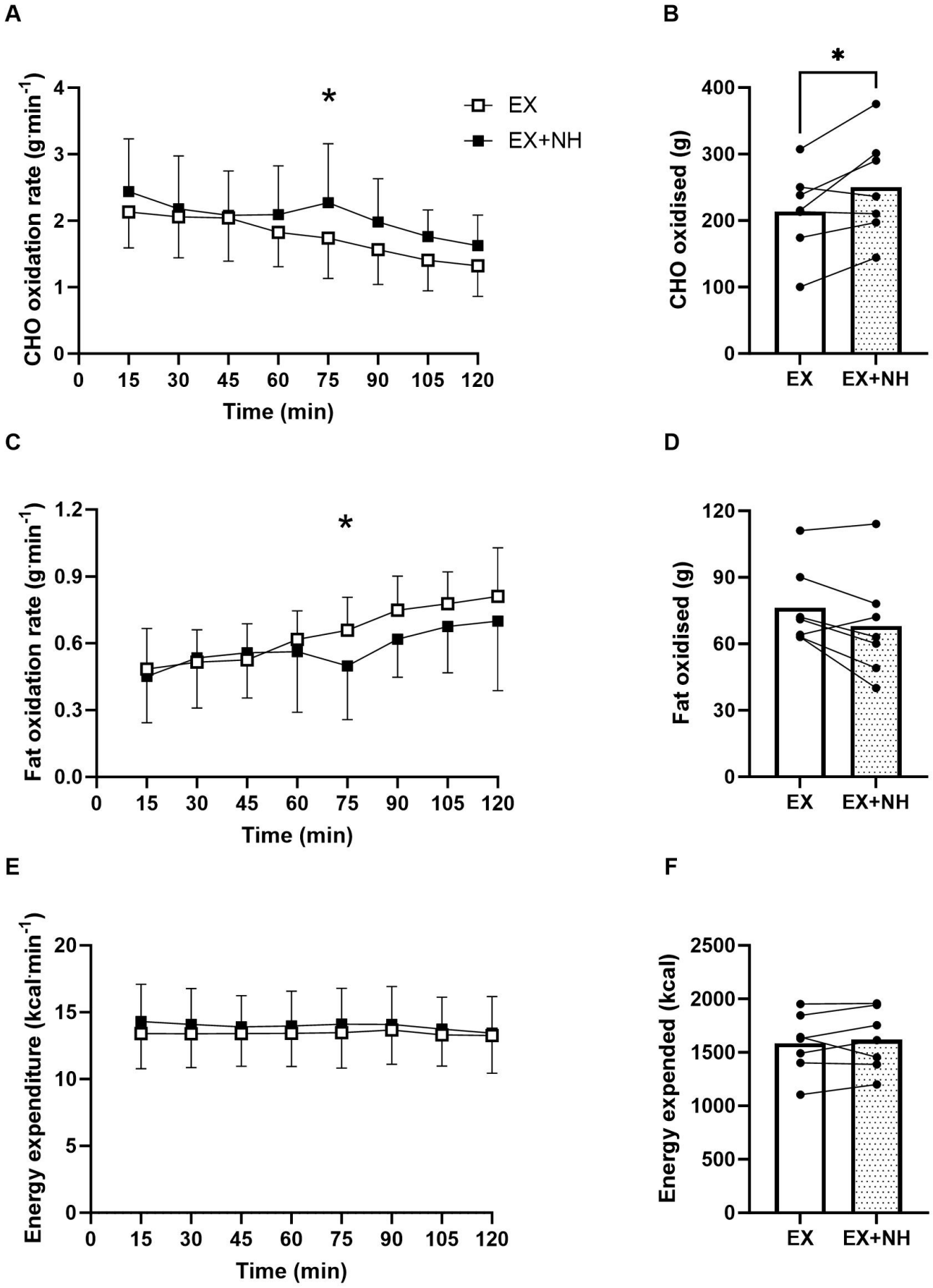


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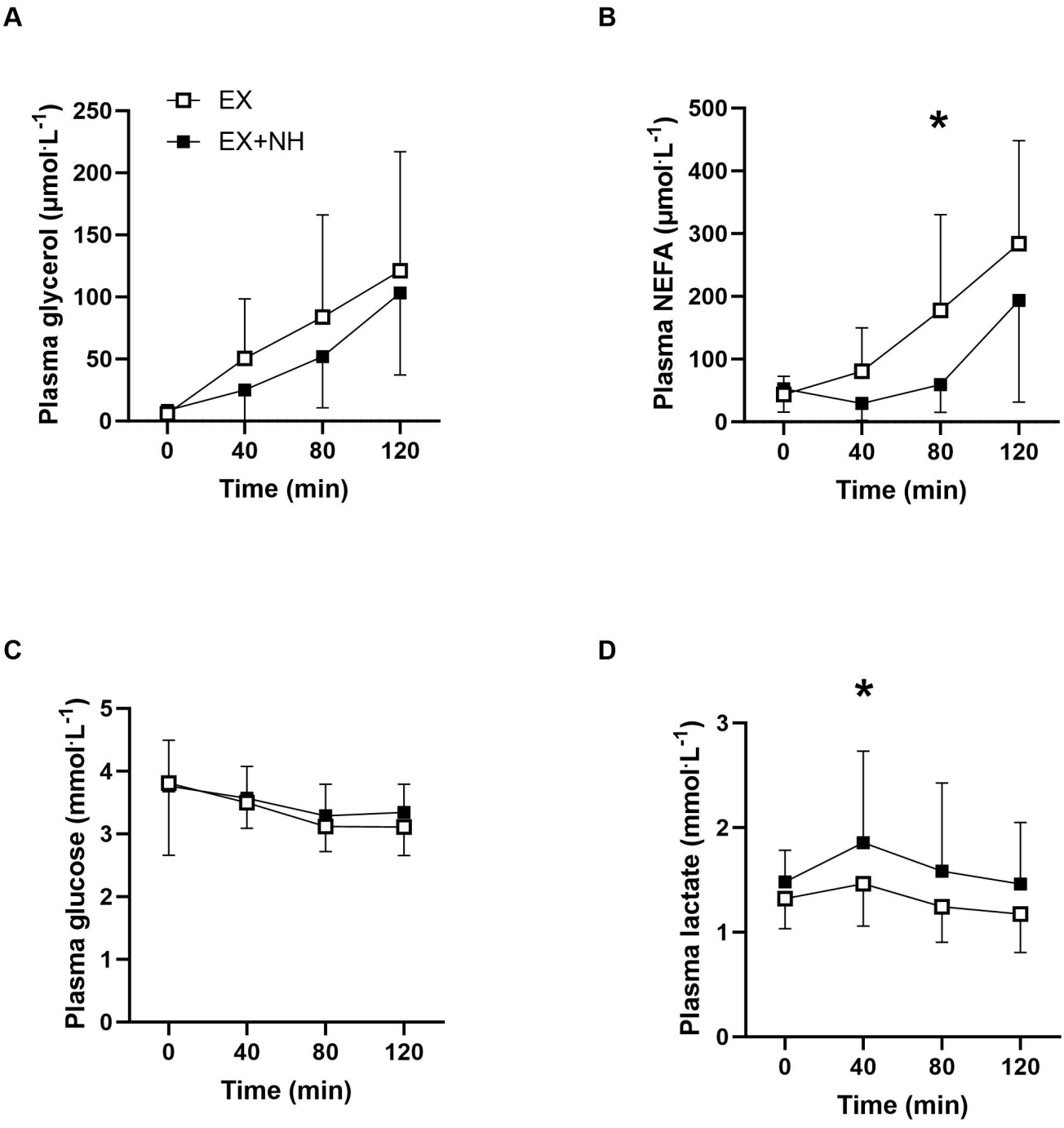



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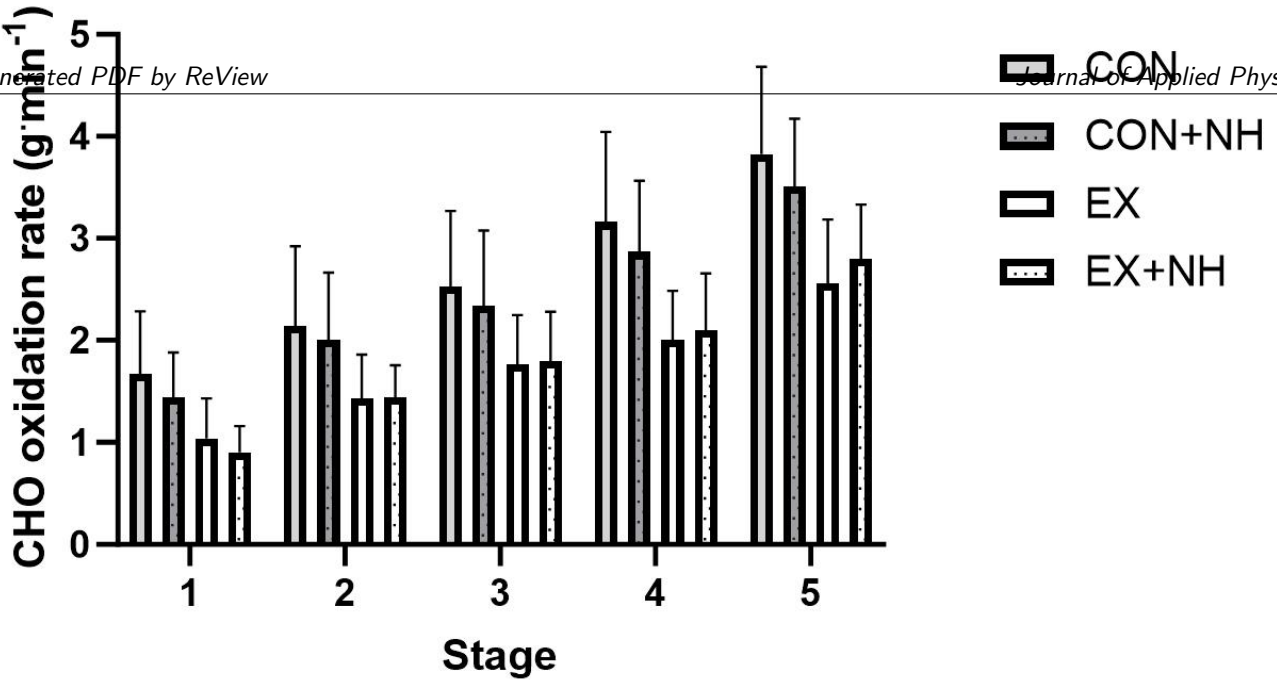



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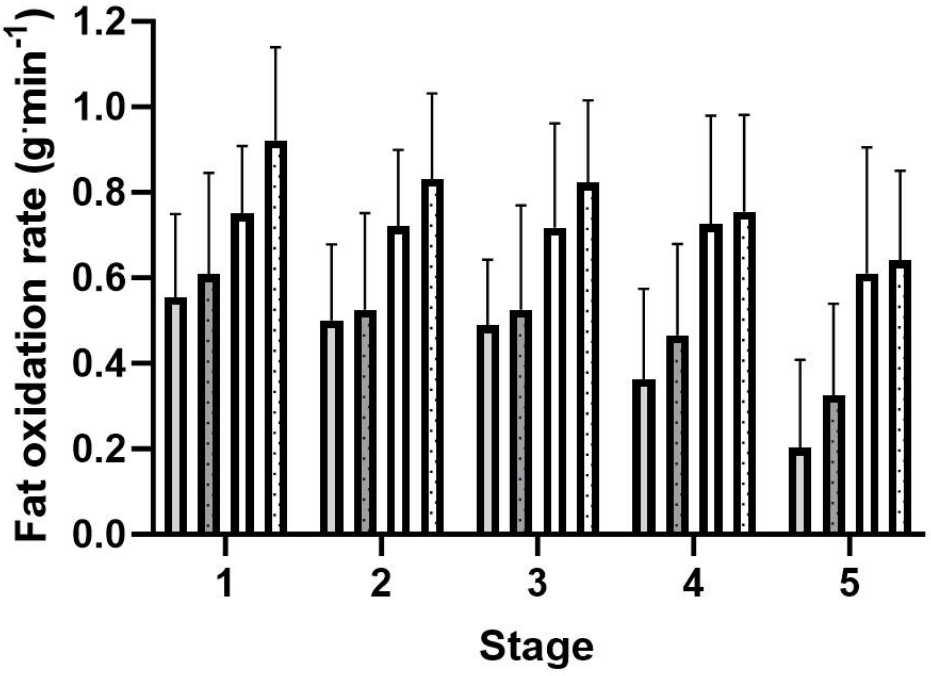




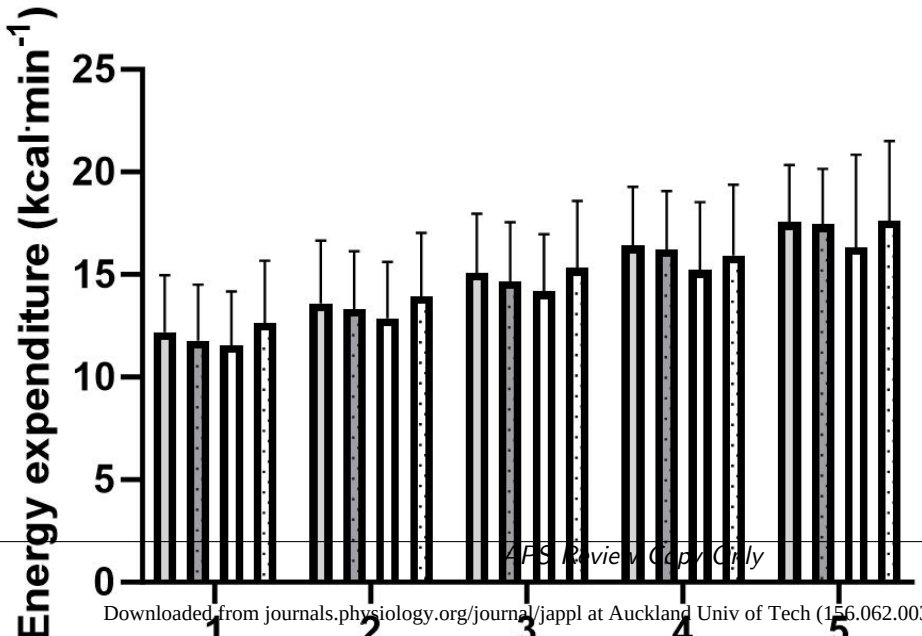

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B

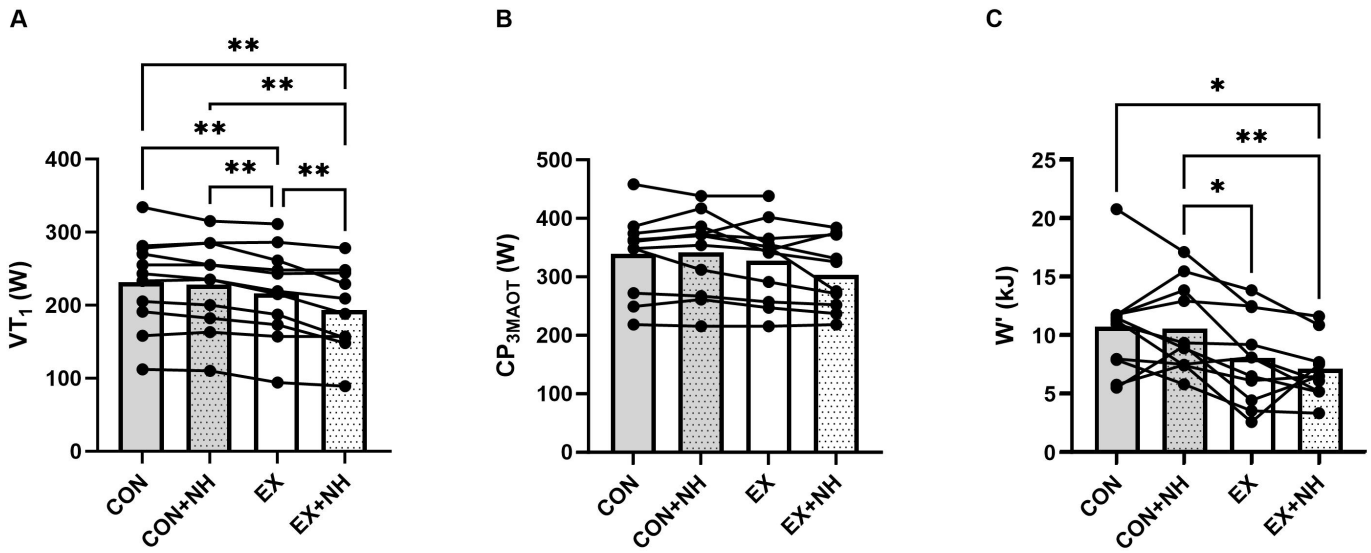



C



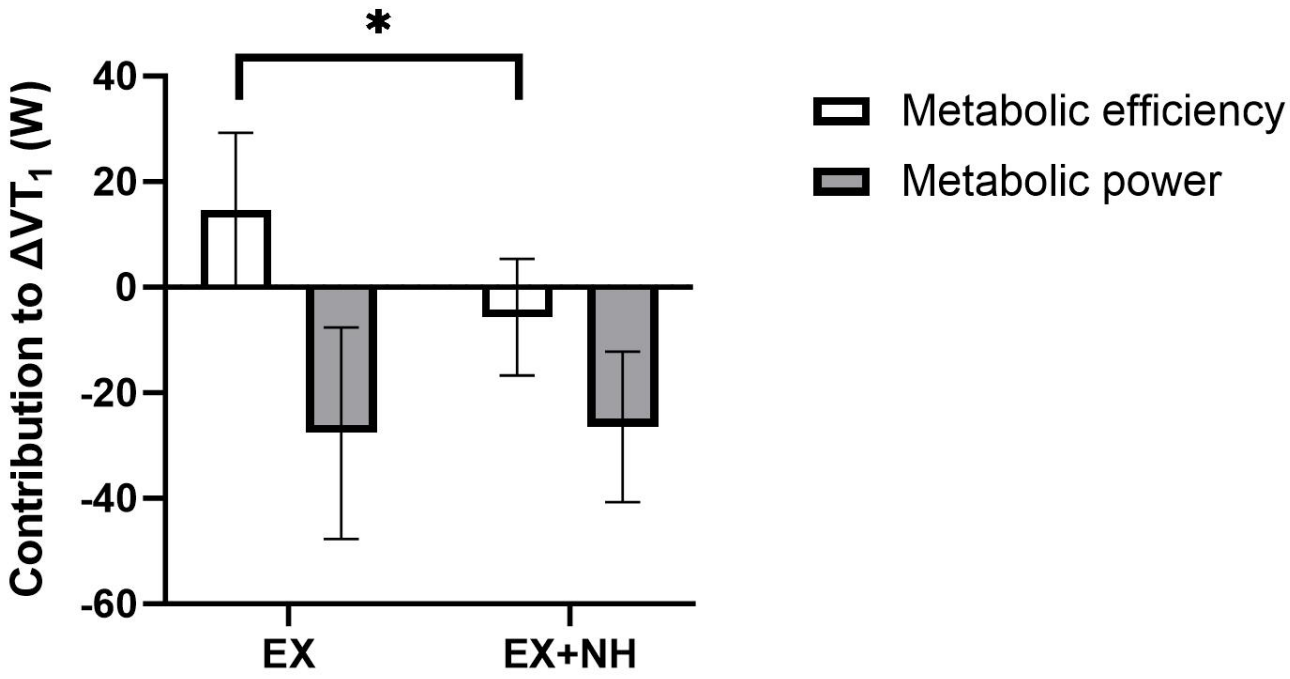


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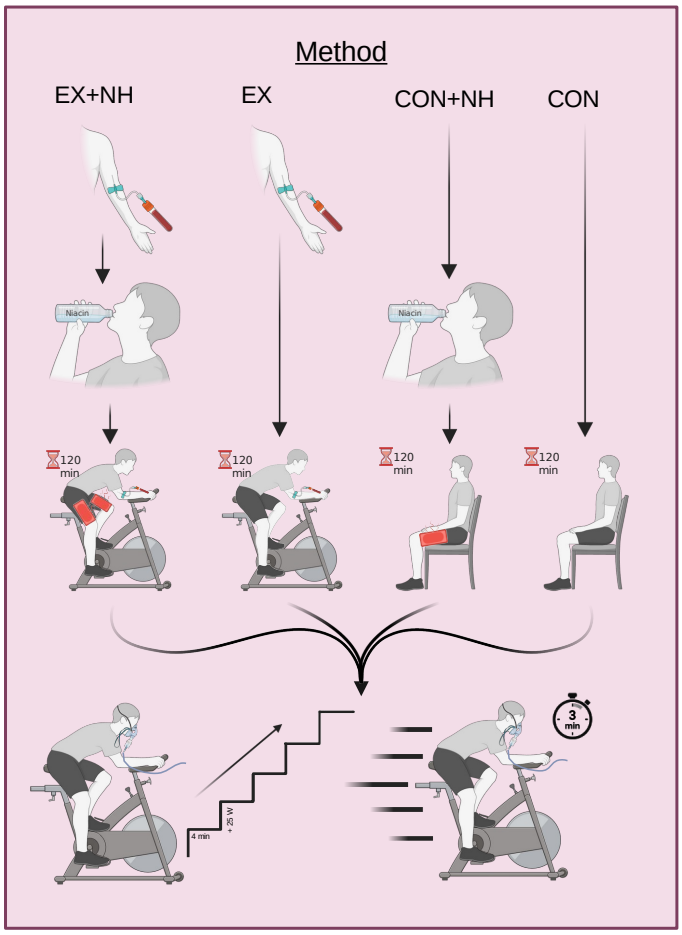

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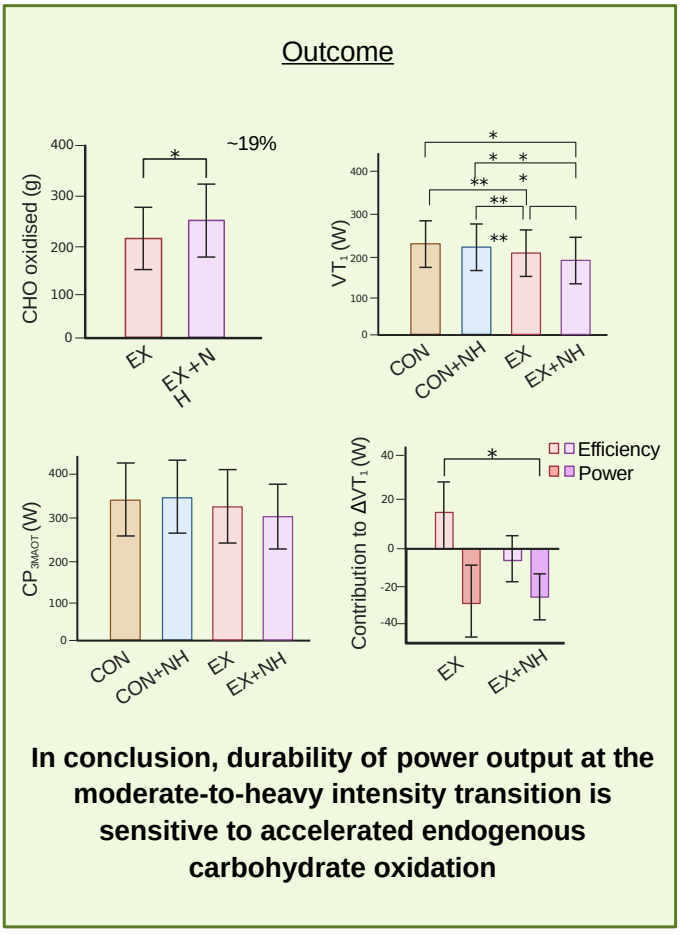





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# Accelerated endogenous carbohydrate oxidation reduces durability




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



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