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# Effects of reduced free fatty acid availability on skeletal muscle PDH activation during aerobic exercise

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G. J. F. HEIGENHAUSER,<sup>2</sup> AND LAWRENCE L. SPRIET<sup>1</sup>

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**Stellingwerff, Trent, Matthew J. Watt, G. J. F. Heigenhauser, and Lawrence L. Spriet.** Effects of reduced free fatty acid availability on skeletal muscle PDH activation during aerobic exercise. *Am J Physiol Endocrinol Metab* 284: E589–E596, 2003. First published November 10, 2002; 10.1152/ajpendo.00418.2002.—This study investigated the effect of reduced free fatty acid (FFA) availability on pyruvate dehydrogenase activation (PDHa) and carbohydrate metabolism during moderate aerobic exercise. Eight active male subjects cycled for 40 min at 55%  $\dot{V}O_{2\text{ peak}}$  on two occasions. During one trial, subjects ingested 20 mg/kg body mass of the antilipolytic drug nicotinic acid (NA) during the hour before exercise to reduce FFA. Nothing was ingested in the control trial (CON). Blood and expired gas measurements were obtained throughout the trials, and muscle biopsy samples were obtained immediately before exercise and at 5, 20, and 40 min of exercise. Plasma FFA were lower in the NA trial ( $0.13 \pm 0.01$  vs.  $0.48 \pm 0.03$  mM,  $P < 0.05$ ), and the respiratory exchange ratio (RER) was increased with NA ( $0.93 \pm 0.01$  vs.  $0.89 \pm 0.01$ ,  $P < 0.05$ ), resulting in a  $14.5 \pm 1.8\%$  increase in carbohydrate oxidation compared with CON. PDHa increased rapidly in both trials at exercise onset but was  $\sim 15\%$  higher ( $P < 0.05$ ) throughout exercise in the NA trial ( $2.44 \pm 0.19$  and  $2.07 \pm 0.12$  mmol·kg wet muscle<sup>-1</sup>·min<sup>-1</sup> for NA and CON at 40 min). Muscle glycogenolysis was  $15.3 \pm 9.6\%$  greater in the NA trial vs. the CON trial but did not reach statistical significance. Glucose 6-phosphate contents were elevated ( $P < 0.05$ ) in the NA trial at 30 and 40 min of exercise, but pyruvate and lactate contents were unaffected. These data demonstrate that the reduction of exogenous FFA availability increased the activation of PDH and carbohydrate oxidation during moderate aerobic exercise in men. The increased activation of PDH was not explained by changes in muscle pyruvate or the ATP/ADP ratio but may be related to a decrease in the NADH/NAD<sup>+</sup> ratio or an epinephrine-induced increase in calcium concentration.

pyruvate dehydrogenase activity; nicotinic acid; carbohydrate and fat oxidation

FAT AND CARBOHYDRATE are the primary fuels for exercise. The exact mechanisms that dictate the relative contribution of each of these substrates at a given

exercise intensity are still not elucidated (40, 46). Numerous laboratories, including our own, have examined the effects of increasing fat availability on whole body and skeletal muscle carbohydrate metabolism (11, 14, 15, 17, 29, 30, 37, 43). The administration of Intralipid and heparin has been commonly used to acutely increase plasma free fatty acid (FFA) concentrations (17, 37, 43). The findings from our laboratory (15, 29, 30) demonstrated that muscle glycogen use was decreased between exercise power outputs of 40–85% maximal oxygen uptake ( $\dot{V}O_{2\text{ max}}$ ) and that pyruvate dehydrogenase activation (PDHa) was decreased at 40–65%  $\dot{V}O_{2\text{ max}}$  in the high-fat condition. This decreased glycogenolysis, glycolysis, and carbohydrate oxidation appeared to be mediated through reductions in the accumulation of free P<sub>i</sub>, ADP, and AMP, important regulators of glycogen phosphorylase and PDHa.

In contrast, relatively few studies have examined the effects of reduced fat availability on skeletal muscle carbohydrate metabolism during exercise. Previous human studies have shown that oral nicotinic acid (NA) supplementation decreases exogenous FFA availability and fat oxidation and increases carbohydrate oxidation, as indicated by an increased respiratory exchange ratio (RER) (6, 16, 19, 20, 22, 25, 28, 42). However, only Bergström et al. (3) examined muscle metabolism directly with muscle biopsies and showed increased glycogen depletion with NA ingestion. No studies have examined the effects of reduced FFA availability on the activation and regulation of PDH.

In our previous work (8, 29), increasing FFA availability decreased PDHa, possibly by increasing mitochondrial NADH and pyruvate at rest and during the 1st min of exercise. We argued that the elevated NADH stimulated PDH kinase (PDK) and that reduced pyruvate resulted in less PDK inhibition, together resulting in less activation of PDHa at the onset of exercise. It is currently unknown whether decreasing FFA availability through NA ingestion would do the opposite and activate PDH to a greater degree than in the control trial.

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Therefore, the purpose of the present study was to administer NA to decrease FFA availability to the exercising muscle and examine whole body carbohydrate oxidation and, for the first time, the activation of PDH during moderate exercise. Several muscle metabolites believed to regulate PDHa were also measured in an attempt to explain any changes in PDHa. We hypothesized that NA ingestion would increase the activation of PDH and that increased pyruvate levels would explain the greater PDHa and reliance on carbohydrate metabolism.

## METHODS

**Subjects.** Eight active male subjects volunteered to participate in this study. No subject was taking any medications or engaging in aerobic exercise more than two times per week. Their mean ( $\pm$ SE) age, weight, and  $\dot{V}O_{2\max}$  were  $24.4 \pm 1.3$  yr,  $75.5 \pm 4.7$  kg, and  $53.8 \pm 2.3$  ml $\cdot$ kg $^{-1}\cdot$ min $^{-1}$ , respectively. All subjects were informed of the experiment protocol, and the possible associated risks of the study were explained to subjects both orally and in writing before written informed consent was obtained. The ethics committees of the University of Guelph and McMaster University approved the study.

**Preexperimental protocol.** Subjects performed a continuous and incremental test to exhaustion on a cycle ergometer (LODE Instrument, Groningen, The Netherlands). A 3-day dietary recall over 2 weekdays and 1 weekend day was used to assess their normal diet. Their calculated percent ( $\pm$ SE) macronutrient breakdown of carbohydrate, fat, and protein was  $52 \pm 2$ ,  $33 \pm 1$ , and  $15 \pm 1\%$ , respectively.

Subjects visited the laboratory on three occasions. On the first visit, subjects completed a practice ride for 40 min with NA supplementation and no blood or muscle sampling. The purposes of the practice trial were to familiarize the subject with the protocol, confirm the subject's tolerance to oral NA supplementation, and to confirm the exercise power output of  $\sim 55\%$   $\dot{V}O_{2\max}$ . The mean ( $\pm$ SE) absolute power output for the trials was  $141 \pm 7$  W. Before all visits, subjects had abstained from any intense athletic activities and had consumed a normal diet during the preceding day. Each of the trials was conducted  $\geq 1$  wk apart and subjects reported to the laboratory after an overnight ( $>10$  h) fast.

**Experimental protocol.** During the two experimental trials, an indwelling catheter was inserted into an antecubital vein for blood sampling and was kept patent with an isotonic saline drip. Four incisions were made over the vastus lateralis muscle of one leg under local anesthesia (2% Lidocaine, no epinephrine) for later muscle biopsy sampling.

Subjects then consumed either NA or nothing (CON) in randomized fashion during the 60 min before exercise. Blood samples were obtained immediately before supplementation at  $-60$  and  $-30$  min, immediately before the start of exercise (0 min), and at 5, 10, 20, 30, and 40 min during the exercise protocol. Immediately before exercise, subjects underwent a resting muscle biopsy on a bed. The muscle sample was immediately frozen in liquid nitrogen. Subjects then moved to the cycle ergometer and started cycling at their predetermined power output. Additional muscle samples were taken at 5, 20, and 40 min of cycling. Fewer than 30 s elapsed between cessation of exercise, obtaining the muscle biopsy, and recommencing cycling. Muscle samples remained in liquid N<sub>2</sub> until analysis. Expired pulmonary gases (Quinton Q-plex 1; Quinton Instruments, Seattle, WA) were collected during 7–9, 17–19, 27–29, and 37–39 min for the measurement of expired fractions of O<sub>2</sub>, CO<sub>2</sub>, and ventilation. Whole

body fat and carbohydrate oxidation rates were calculated using the nonprotein RER (34).

**Drug administration.** During the NA trial, subjects ingested a total of 20 mg NA/kg body mass (BM) in three individual doses: 10 mg/kg BM 60 min before exercise, 5 mg/kg BM 30 min before exercise, and 5 mg/kg BM immediately before cycling, as previously described (19). NA was obtained from C. E. Jamieson, Toronto, Canada. The dosing period was spaced over 1 h to minimize adverse symptoms. All subjects experienced the normal side effects of NA ingestion, which included flushing (a reddening of the skin due to peripheral vasodilation over most of the body), tingling sensations, and a sensation of heat, which started  $\sim 15$ – $20$  min after the first dose and subsided during the final 30 min of rest before exercise. No subjects experienced stomach or gastrointestinal upset. Although we do not believe that these symptoms affected the whole body or skeletal muscle responses to exercise, we cannot completely rule this out.

**Analyses.** A small piece of frozen wet muscle ( $\sim 10$ – $15$  mg) was removed from the larger muscle piece while submerged in liquid N<sub>2</sub> for the determination of PDHa, as described by Putman et al. (36). The remainder of the muscle sample was freeze-dried, dissected free of all visible blood and connective tissue, and powdered for subsequent metabolite and glycogen analyses. An aliquot of freeze-dried muscle ( $\sim 10$ – $12$  mg) was extracted with 0.5 M perchloric acid (PCA) containing 1 mM EDTA and neutralized with 2.2 M KHCO<sub>3</sub>. This extract was used for the measurement of creatine (Cr), phosphocreatine (PCr), ATP, lactate, and glucose 6-phosphate (G-6-P) by enzymatic spectrophotometric assays (2, 18) and acetyl-CoA and acetylcarnitine with radiometric measures (7). Pyruvate and citrate were analyzed fluorometrically (32). Muscle glycogen content was measured from a second aliquot of freeze-dried muscle ( $\sim 4$ – $6$  mg) from resting and 40-min biopsy samples. All muscle measurements were normalized to the highest total Cr measured among the eight biopsies from each subject.

Venous whole blood was placed in a heparinized tube, and a portion was immediately deproteinized in a 1:5 ratio with 0.6% (wt/vol) PCA. This PCA extract was stored at  $-20^{\circ}\text{C}$  and later analyzed for glycerol, lactate, and glucose (2). A second portion of blood was immediately centrifuged, and 400  $\mu\text{l}$  of plasma were added to 100  $\mu\text{l}$  of NaCl and incubated at  $56^{\circ}\text{C}$  for 30 min to inactivate lipoprotein lipase activity. The plasma was stored at  $-20^{\circ}\text{C}$  and later analyzed for FFA with a colorimetric assay (Wako NEFA C test kit; Wako Chemicals, Richmond, VA). A final portion of blood was centrifuged and the plasma used for the determination of insulin by radioimmunoassay (Coat-a-Count Insulin test kit, Diganostics Products, Los Angeles, CA).

**Calculations.** Free ADP (ADP<sub>f</sub>) and AMP (AMP<sub>f</sub>) contents were calculated by assuming equilibrium of the creatine kinase and adenylate kinase reactions (13). Specifically, ADP<sub>f</sub> was calculated using the measured ATP, Cr, and PCr values, an estimated H<sup>+</sup> concentration, and the creatine kinase constant of  $1.66 \times 10^9$ . The H<sup>+</sup> concentration was estimated from the measured lactate and pyruvate contents as described by Sahlin et al. (38). AMP<sub>f</sub> was calculated from the estimated ADP<sub>f</sub> and measured ATP content using the adenylate kinase equilibrium constant of 1.05. Free inorganic phosphate (P<sub>if</sub>) was calculated by adding the estimated resting free phosphate of 10.8 mmol/kg dry mass (dm) (13) to the difference in PCr content ( $\Delta[\text{PCr}]$ ) minus the accumulation of G-6-P between rest and the given exercise time points.

Whole body rates of carbohydrate and fat oxidation (g/min) were calculated during exercise from the rates of CO<sub>2</sub> pro-

Table 1.  $\dot{V}O_2$  and respiratory responses during 40 min of exercise at  $\sim 55\%$   $\dot{V}O_{2\text{ peak}}$  following ingestion of NA or no supplementation

Measure	Trial	Time, min			
		10	20	30	40
$\dot{V}O_2$	CON	2.21 ± 0.11	2.22 ± 0.11*	2.28 ± 0.12*	2.36 ± 0.11*
	NA	2.15 ± 0.10	2.24 ± 0.11*	2.26 ± 0.11*	2.28 ± 0.10*
$\dot{V}_E$	CON	51.7 ± 2.7	52.5 ± 2.6	52.9 ± 2.7	55.1 ± 2.8
	NA‡	54.1 ± 2.9	55.0 ± 3.2	56.2 ± 3.0	56.0 ± 3.0

Values are means ± SE expressed as l/min;  $n = 8$ . NA, nicotinic acid; CON, no supplementation;  $\dot{V}O_2$ , oxygen uptake;  $\dot{V}_E$ , expired ventilation. \*Significantly different from 10 min of same condition ( $P < 0.05$ ); ‡trial effect of NA significantly different from CON ( $P < 0.05$ ).

duction ( $\dot{V}CO_2$ ) and  $O_2$  consumption ( $\dot{V}O_2$ ) by use of the non-protein RER values according to the following equations (34) carbohydrate oxidation

$$= 4.585 \dot{V}CO_2 \text{ (l/min)} - 3.226 \dot{V}O_2 \text{ (l/min)}$$

$$\text{fat oxidation} = 1.695 \dot{V}O_2 \text{ (l/min)} - 1.701 \dot{V}CO_2 \text{ (l/min)}$$

**Statistics.** All data are presented as means ± SE. A two-way repeated-measures ANOVA (treatment × time) was used to determine significant differences between treatments. When a significant  $F$ -ratio was obtained, post hoc analysis was completed using a Student-Newman-Keuls test. A single-tailed paired  $t$ -test was used to determine the net glycogen utilization between trials. Statistical significance was accepted at  $P < 0.05$ .

## RESULTS

**Respiratory measures.** There were no differences in  $\dot{V}O_2$  over time in either trial or between trials (Table 1). Percent  $\dot{V}O_{2\text{ peak}}$  in both trials averaged  $56.4 \pm 0.6\%$ . Ventilation was higher ( $P < 0.05$ ; trial effect) in NA compared with CON (Table 1). RER was significantly higher ( $0.93 \pm 0.01$  vs.  $0.89 \pm 0.01$ ,  $P < 0.05$ ) in the NA trial compared with control (Fig. 1). NA ingestion resulted in a  $14.5 \pm 1.8\%$  increase ( $P < 0.05$ ) in total carbohydrate oxidation and a  $32.6 \pm 3.9\%$  reduction ( $P < 0.05$ ) in fat oxidation (Fig. 2).

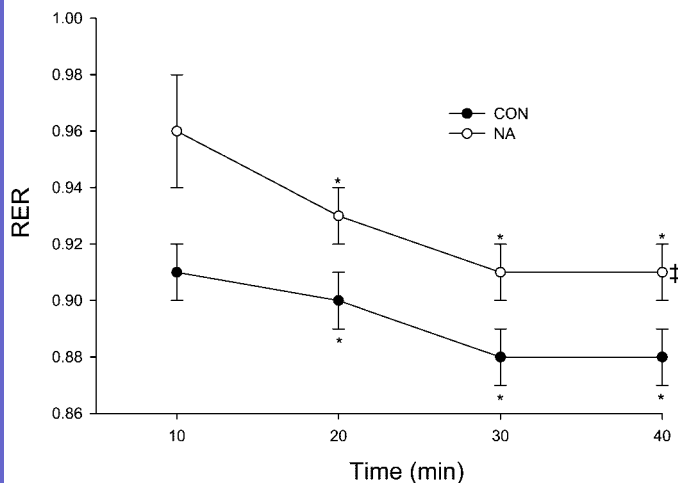


Fig. 1. Respiratory exchange ratio (RER) during 40 min of exercise at  $\sim 55\%$  peak  $O_2$  uptake ( $\dot{V}O_{2\text{ peak}}$ ) following the ingestion of nicotinic acid (NA) or no supplementation (CON). Values are means ± SE;  $n = 8$ . \*Significantly different from 10 min of same condition ( $P < 0.05$ ); ‡trial effect of NA significantly different from CON ( $P < 0.05$ ).

**PDHa.** NA supplementation had no effect on resting PDHa (Fig. 3). PDHa increased to higher levels in the NA trial throughout exercise ( $P < 0.05$ ; trial effect). Specifically, increases in the NA trial were  $\sim 10$ , 10, and 15% higher than CON at 5, 20, and 40 min of exercise, respectively.

**Muscle metabolites.** PCr was similar at rest and decreased ( $P < 0.05$ ) to the same extent during exercise in both trials (Table 2). The muscle content of ATP was unaffected by prior NA ingestion or exercise in both trials (Table 2). ADP<sub>f</sub> and AMP<sub>f</sub> were not different between trials at rest and increased ( $P < 0.05$ ) similarly throughout exercise (Table 2). Calculated free  $P_i$  ( $P_{if}$ ) increased ( $P < 0.05$ ) during exercise but was not different between trials (Table 2).

There was no difference in the pre- or postexercise glycogen contents between treatments (Table 3). Net glycogen utilization was  $15.3 \pm 9.6\%$  greater [not significant (NS);  $P = 0.0625$ ] in the NA trial compared with CON. Resting muscle G-6-P contents were similar between trials but increased ( $P < 0.05$ ) more in the NA trial compared with the CON trial following 20 and 40 min of exercise (Table 3). Muscle pyruvate and lactate were not different at rest between trials and increased

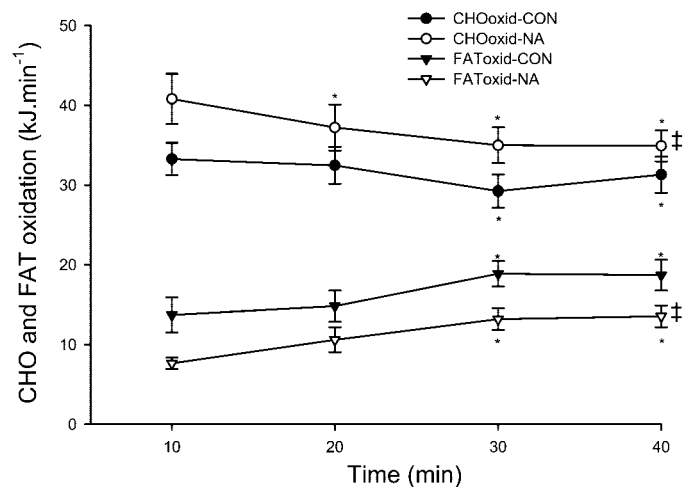


Fig. 2. Calculated carbohydrate (CHO<sub>oxid</sub>) and fat oxidation rates (FAT<sub>oxid</sub>) during 40 min of exercise at  $\sim 55\%$   $\dot{V}O_{2\text{ peak}}$  following the ingestion of NA or no supplementation (CON). Values are means ± SE;  $n = 8$ . \*Significantly different from 10 min of same condition ( $P < 0.05$ ); ‡trial effect of NA significantly different from CON ( $P < 0.05$ ).

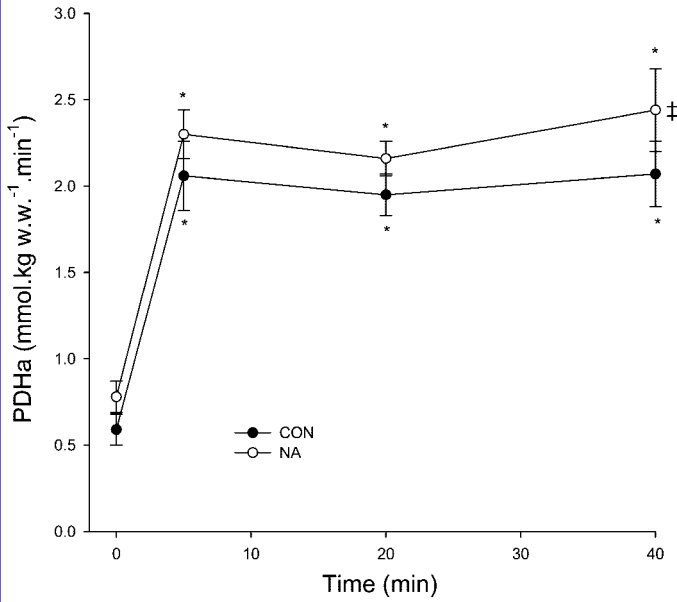


Fig. 3. Pyruvate dehydrogenase activation (PDHa) at rest and during 40 min of exercise at ~55%  $\dot{V}O_{2peak}$  following the ingestion of NA or no supplementation (CON). Values are means  $\pm$  SE;  $n = 8$ . \*Significantly different from 0 min of same condition ( $P < 0.05$ ); ‡trial effect of NA significantly different from CON ( $P < 0.05$ ).

( $P < 0.05$ ) similarly between trials during exercise (Table 3).

Acetyl-CoA contents were similar between trials at rest and at 5 and 20 min of exercise but were higher ( $P < 0.05$ ) in the NA trial by 40 min of exercise (Table 3). There was no difference in resting citrate between trials, and citrate increased similarly during exercise (Table 3). After the 60-min NA supplementation period, resting acetylcarnitine concentrations were significantly lower in NA compared with CON (Table 3). Acetylcarnitine increased similarly upon the commencement of exercise, and there was no difference between treatments during exercise.

**Blood measurements.** Resting plasma glycerol and FFA were similar between trials before NA ingestion (Fig. 4). At rest, plasma FFA was lower in the NA trial

following 30 and 60 min of supplementation vs. CON and remained at very low levels during exercise. Once exercise commenced, plasma glycerol increased in both trials but to a higher level ( $P < 0.05$ ) in the CON trial by 20 min and beyond (Fig. 4). Plasma FFA increased ( $P < 0.05$ ) in the CON trial after exercise onset but remained below resting values ( $P < 0.05$ ) during exercise in the NA trial (Fig. 4).

Plasma lactate was similar at rest and during the supplementation period between trials (Table 4). During exercise, plasma lactate increased ( $P < 0.05$ ) in both NA and CON, and the concentration was greater ( $P < 0.05$ ) in NA than in CON at 5, 10, and 20 min of exercise.

Throughout supplementation and exercise, blood glucose was greater in NA ( $P < 0.05$ ; trial effect) than in CON. In both trials, blood glucose peaked at 10 min of exercise and returned to basal levels for the remainder of exercise. There was no difference in plasma insulin during the 60-min supplementation period between NA and CON (Table 4). Plasma insulin decreased ( $P < 0.05$ ) during exercise, but there was no difference between NA and CON.

**DISCUSSION**

This study investigated the effect of reduced FFA availability on PDH and carbohydrate metabolism during moderate aerobic exercise. The administration of NA decreased plasma FFA to low levels at rest and during exercise. NA ingestion did not affect PDHa at rest, but acetylcarnitine was markedly reduced. In support of our first hypothesis, the reduced exogenous FFA availability resulted in increased carbohydrate oxidation and a greater PDHa during exercise. Contrary to our second hypothesis, the PDHa could not be explained by changes in muscle pyruvate or the ATP-to-ADP ratio (ATP/ADP) but may be related to a decreased mitochondrial NADH-to-NAD<sup>+</sup> ratio (NADH/NAD<sup>+</sup>) or an epinephrine-induced increase in calcium concentration.

*Reduced FFA availability increases PDHa.* The novel finding of this study is that reduced FFA availability

Table 2. High-energy phosphate contents at rest and during 40 min of exercise at ~55%  $\dot{V}O_{2 peak}$  following ingestion of NA or no supplementation

Measure	Trial	Time, min			
		0	5	20	40
PCr	CON	76.5 $\pm$ 2.0	50.5 $\pm$ 3.5*	54.3 $\pm$ 1.7*	51.2 $\pm$ 3.6*
	NA	79.0 $\pm$ 2.0	53.6 $\pm$ 2.3*	49.4 $\pm$ 3.6*	52.4 $\pm$ 2.5*
ATP	CON	25.2 $\pm$ 0.6	24.8 $\pm$ 0.4	23.8 $\pm$ 0.5	23.5 $\pm$ 0.9
	NA	24.2 $\pm$ 1.1	23.2 $\pm$ 0.6	24.2 $\pm$ 1.0	23.2 $\pm$ 1.0
ADP <sub>f</sub>	CON	97.5 $\pm$ 6.5	189.9 $\pm$ 20.0*	164.4 $\pm$ 12.4*	191.9 $\pm$ 18.0*
	NA	88.4 $\pm$ 6.4	161.5 $\pm$ 10.5*	195.1 $\pm$ 20.1*	179.7 $\pm$ 18.8*
AMP <sub>f</sub>	CON	0.37 $\pm$ 0.05	1.51 $\pm$ 0.30*	1.12 $\pm$ 0.15*	1.65 $\pm$ 0.33*
	NA	0.31 $\pm$ 0.04	1.10 $\pm$ 0.13*	1.64 $\pm$ 0.30*	1.46 $\pm$ 0.29*
P <sub>if</sub>	CON	10.8	35.7 $\pm$ 3.3	32.5 $\pm$ 1.0	38.5 $\pm$ 3.3
	NA	10.8	34.6 $\pm$ 2.4	38.7 $\pm$ 4.3	36.0 $\pm$ 3.5

Values are means  $\pm$  SE;  $n = 8$ . PCr, phosphocreatine; ADP<sub>f</sub>, AMP<sub>f</sub>, and P<sub>if</sub>, free ADP, AMP, and P<sub>i</sub>, respectively. All values are expressed as mmol/kg dry mass except for ADP<sub>f</sub> and AMP<sub>f</sub>, which are expressed as  $\mu$ mol/kg dry mass. \*Significantly different from 0 min of same condition ( $P < 0.05$ ). Resting P<sub>if</sub> of 10.8 assumed from Dudley et. al. (13).

Table 3. Muscle metabolite data at rest and during 40 min of exercise at ~55%  $\dot{V}O_2$  peak following ingestion of NA or no supplementation

Measure	Trial	Time, min			
		0	5	20	40
Glycogen	CON	375.9 ± 16.7	ND	ND	225.3 ± 18.6*
	NA	414.4 ± 12.9	ND	ND	241.2 ± 25.8*
G-6-P	CON	0.60 ± 0.21	1.63 ± 0.26*	1.11 ± 0.33*†	1.17 ± 0.22*†
	NA	0.58 ± 0.10	2.08 ± 0.35*	2.22 ± 0.47*	1.89 ± 0.39*
Pyruvate	CON	0.13 ± 0.03	0.30 ± 0.05*	0.43 ± 0.09*	0.30 ± 0.05*
	NA	0.13 ± 0.02	0.36 ± 0.06*	0.45 ± 0.11*	0.39 ± 0.06*
Lactate	CON	7.0 ± 1.2	25.2 ± 4.0*	19.9 ± 3.7*	14.2 ± 2.8*
	NA	5.0 ± 0.8	21.0 ± 3.2*	21.8 ± 3.2*	14.2 ± 2.6*
Acetyl-CoA	CON	7.8 ± 1.1	14.6 ± 1.3*	14.6 ± 1.0*	14.2 ± 1.0*†
	NA	5.6 ± 1.4	16.4 ± 0.6*	16.2 ± 1.2*	18.1 ± 2.2*
Citrate	CON	1.44 ± 0.12	1.54 ± 0.15*	1.74 ± 0.15*	1.70 ± 0.26*
	NA	0.99 ± 0.05	1.72 ± 0.27*	1.64 ± 0.21*	1.66 ± 0.19*
Acetylcarnitine	CON	4.9 ± 0.5†	11.1 ± 1.0*	11.4 ± 0.9*	11.9 ± 1.3*
	NA	2.6 ± 1.1	11.1 ± 0.7*	12.8 ± 1.1*	13.2 ± 1.9*

Values are means ± SE;  $n = 8$ . G-6-P, glucose 6-phosphate; ND, no determination. All values are expressed as mmol/kg dry mass except for acetyl-CoA, which is expressed as  $\mu\text{mol/kg}$  dry mass. \*Significantly different from 0 min of same condition ( $P < 0.05$ ); †significantly different from corresponding time point for NA ( $P < 0.05$ ).

increased PDHa during moderate aerobic exercise. PDH is covalently regulated by PDH phosphatase, which dephosphorylates and activates PDH to its active, a form, and PDH kinase, which phosphorylates and inhibits PDH to its inactive, b form (24, 26, 39). In turn, several allosteric modulators regulate the activities of PDH phosphatase and PDK. Specifically, at rest, PDK is stimulated by high ratios of acetyl-CoA/CoA, ATP/ADP, and NADH/NAD<sup>+</sup>, keeping PDH mainly in the inactive, b form. Conversely, during exercise, PDK is inhibited by pyruvate and low ratios of ATP/ADP, and the phosphatase is acutely stimulated by Ca<sup>2+</sup> moving PDH to the a form (for reviews see Refs. 24, 39).

The increase in PDHa in the NA trial cannot be explained by changes in pyruvate and the ATP/ADP ratio, as the exercise-induced changes were similar between trials. We were surprised by the lack of increase in muscle pyruvate in the NA trial, but the expected increase due to the increased glycolytic flux was either not present or too small to detect. Although there was a marginal increase in acetyl-CoA at the end of exercise in the NA treatment, which might suggest a downregulation of PDHa by the kinase, previous studies suggest that changes in PDHa during exercise are independent of the acetyl-CoA/CoA ratio (10, 23, 36). Our laboratory has previously shown that NADH/NAD<sup>+</sup> is increased at rest and under conditions of increased fat availability, resulting in decreased PDHa (8, 29). Specifically, Odland et al. (29) estimated mitochondrial NADH content with the whole muscle technique when FFA availability was increased and found increased [NADH] at rest and in the 1st min of exercise and a decreased PDHa. This finding is supported by *in vitro* work, which suggests that PDK is stimulated by NADH and inhibited by NAD<sup>+</sup> (35). In the present study, the NADH/NAD<sup>+</sup> ratio may also have been a factor but was not measured. In this study, the reverse situation may have occurred, with decreasing FFA and

decreasing mitochondrial [NADH] with this reduced redox state increasing PDHa by inhibiting the kinase. Regardless, there is still no consensus as to what role the redox state plays with regard to PDHa during exercise, and until there is a clearly established method to measure the mitochondrial redox state, these issues will not be resolved.

Watt et al. (45) have suggested that PDHa may also be mediated by epinephrine-induced cAMP effects on either the phosphatase or the kinase through increases in mitochondrial calcium concentration. It is well established that increases in Ca<sup>2+</sup>, pyruvate, and ADP contribute to the activation of PDH at the onset of exercise (9, 23, 24, 31, 36). When epinephrine was added to the perfusion medium during *in vitro* work in isolated rat heart, higher intramitochondrial concentrations of Ca<sup>2+</sup> (12) were reported as well as increased PDHa (27). Similarly, another study demonstrated increased sarcoplasmic calcium release by  $\beta_2$ -adrenergic stimulation of mouse skeletal muscle (5). These data suggest that increased epinephrine, which has been reported in similar studies involving NA and exercise (19, 22), may increase Ca<sup>2+</sup> and ultimately increase PDHa via activation of the PDH phosphatase. In a study with a similar NA dose and exercise power output, NA ingestion caused a 43% increase in epinephrine concentration (22). Hawley et al. (19) also found 34 and 43% increases in epinephrine concentration in their NA trial vs. high-carbohydrate and high-fat diet trials, respectively, at the end of 20 min of cycling at 80%  $\dot{V}O_{2\text{max}}$ . Like Watt et al., the present study also indirectly supports a role for epinephrine increasing calcium concentration in the NA trial and ultimately upregulating PDHa. However, this hypothesis needs to be directly tested in human skeletal muscle.

*Effects of reduced FFA availability on whole body carbohydrate oxidation.* The present dose of 20 mg/kg BM significantly reduced fat availability to be oxidized by the working muscles. This NA dose resulted in

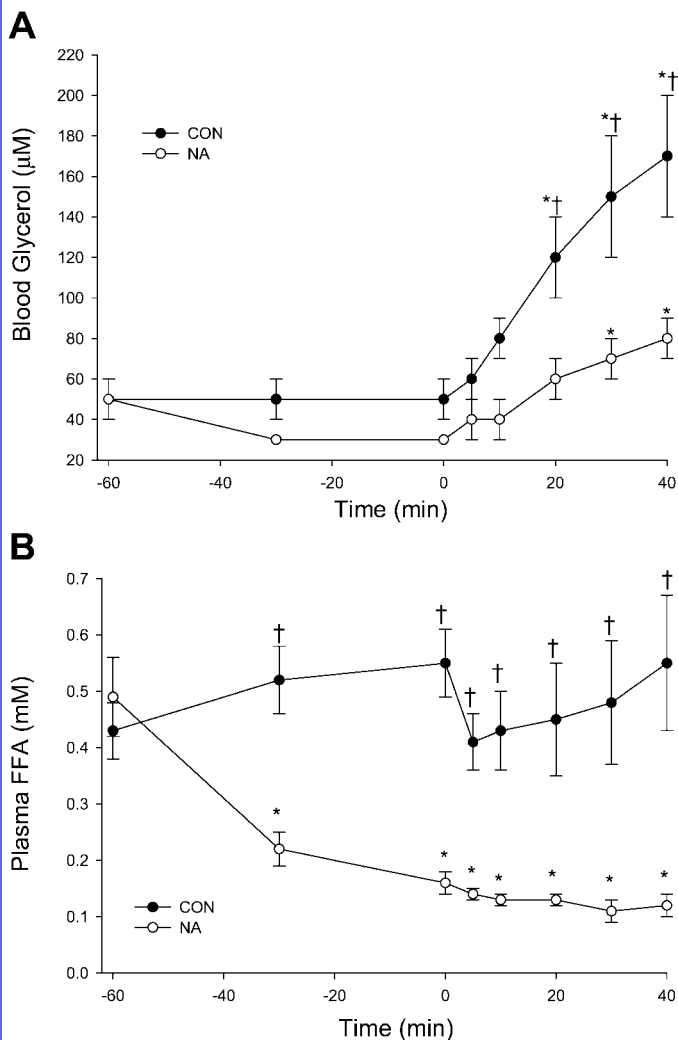


Fig. 4. Plasma glycerol (A) and free fatty acids (FFA; B) at rest and during 40 min of exercise at  $\sim 55\% \dot{V}O_{2peak}$  following the ingestion of NA or no supplementation (CON). Values are means  $\pm$  SE;  $n = 8$ . \*Significantly different from preingestion ( $-60$  min) of same condition ( $P < 0.05$ ); †significantly different from corresponding time point for NA ( $P < 0.05$ ).

reduced FFA availability, and this reduced FFA availability increased whole body carbohydrate oxidation by  $\sim 15\%$  and decreased fat oxidation by  $\sim 30\%$  during exercise. All of these findings are consistent with pre-

vious NA studies, which have reported similar shifts in RER after NA ingestion and during exercise for 30–60 min at similar power outputs ( $\sim 50\text{--}70\% \dot{V}O_{2max}$ ) (3, 20, 22, 25, 28). Howlett et al. (22) reported average RER values of  $0.81 \pm 0.01$  in the control trial and  $0.88 \pm 0.01$  in the NA trial between 30 and 60 min of exercise at a similar intensity of  $\sim 60\%$  of  $\dot{V}O_{2max}$ . It should be noted that that study used female subjects, and their findings of slightly increased carbohydrate metabolism under conditions of reduced FFA availability may be due to a sex difference. It has been reported that females rely more heavily on fat oxidation than males do at a given exercise intensity, which may have resulted in their being more affected by the NA (21, 41).

*Reduced FFA availability on muscle glycogenolysis and glycolysis.* There exists only one other study in obtaining muscle biopsy samples when exogenous FFA availability was reduced during exercise, and the only muscle parameter measured was glycogen content (3). In that study, Bergström et al. (3) reported a significant  $\sim 20\%$  increase in net glycogen utilization in the NA trial compared with controls after 45–60 min of exercise at a  $\dot{V}O_2$  of  $\sim 1.1$  l/min. In the present study, we did not find a significant difference in glycogen depletion between CON and NA treatments at a  $\dot{V}O_2$  of  $\sim 2.2$  l/min, even though there was a nonsignificant 15% increase in muscle glycogen depletion in the NA trial (CON:  $\Delta$ glycogen of  $\sim 150$  glycosyl units vs. NA:  $\Delta$ glycogen of  $\sim 173$  glycosyl units, NS,  $P = 0.063$ ). It is likely that a significant effect would have been found if we had extended our exercise trial length to 60 min, as in the trial of Bergström et al.

A possible explanation for the increased G-6-P found in the present study may have involved increased glucose uptake into the muscle after NA ingestion. Blood glucose was elevated ( $P < 0.05$ ; trial effect) by 10% during exercise after NA supplementation, although this has not been a consistent finding (1, 6, 19, 22, 33). Previous studies in exercising humans (22) and dogs (4) have reported greater whole body glucose disappearance with NA supplementation. Therefore, the combined effects of a significant  $\sim 10\%$  increase in blood glucose, a possible increase in glucose disappearance, and an  $\sim 15\%$  increase in net glycogen utilization may have accounted for the significant increases in

Table 4. Blood metabolite concentrations at rest and during 40 min of exercise at  $55\% \dot{V}O_{2peak}$  following ingestion of NA or no supplementation

Measure	Trial	Time, min							
		-60	0	5	10	20	30	40	
Lactate, mM	CON	1.05 $\pm$ 0.06	1.02 $\pm$ 0.09	1.92 $\pm$ 0.16*†	2.44 $\pm$ 0.27*†	2.52 $\pm$ 0.24*†	2.47 $\pm$ 0.24*	2.20 $\pm$ 0.22*	
	NA	0.96 $\pm$ 0.10	1.18 $\pm$ 0.09	2.79 $\pm$ 0.39*	3.76 $\pm$ 0.42*	3.52 $\pm$ 0.54*	2.99 $\pm$ 0.38*	2.54 $\pm$ 0.29*	
Insulin, pM	CON	12.7 $\pm$ 2.7	14.9 $\pm$ 3.9	20.2 $\pm$ 3.9*	13.2 $\pm$ 2.8	10.2 $\pm$ 2.9*	10.1 $\pm$ 3.2*	9.7 $\pm$ 3.1*	
	NA	23.9 $\pm$ 5.2	22.3 $\pm$ 3.0	17.8 $\pm$ 4.2*	11.5 $\pm$ 3.3*	13.4 $\pm$ 3.5*	15.5 $\pm$ 4.1*	11.8 $\pm$ 3.7*	
Glucose, mM	CON	4.16 $\pm$ 0.15	4.13 $\pm$ 0.09	4.35 $\pm$ 0.10*	4.38 $\pm$ 0.06*	4.30 $\pm$ 0.06	4.15 $\pm$ 0.10	4.17 $\pm$ 0.09	
	NA†	4.33 $\pm$ 0.20	4.98 $\pm$ 0.20	4.95 $\pm$ 0.13*	5.08 $\pm$ 0.22*	4.82 $\pm$ 0.18	4.76 $\pm$ 0.16	4.52 $\pm$ 0.07	

Values are means  $\pm$  SE;  $n = 8$ . \*Significantly different from rest ( $-60$  min) of same condition ( $P < 0.05$ ); †significantly different from corresponding time point for NA ( $P < 0.05$ ); ‡trial effect of NA significantly different from CON ( $P < 0.05$ ).



muscle G-6-P content following 30 and 40 min of exercise during the NA trial.

**Acetyl group availability.** A novel finding of this investigation was that acetylcarnitine was significantly reduced at rest following 60 min of NA ingestion. It was expected that the NA ingestion would have no effect on resting acetylcarnitine levels or would actually cause an increase. The reduction in FFA availability should have increased the reliance on carbohydrate in the resting muscle. Accordingly, an increased flux through glycolysis leading to greater pyruvate production and an overproduction of acetyl-CoA may lead to an increased production of acetylcarnitine. This has been shown to occur at the onset of exercise (23), when the production of pyruvate and acetyl-CoA is severalfold higher than during rest. Interestingly, the present results are in agreement with previous work from our laboratory (44), where a carbohydrate load (1 g carbohydrate/kg BM) ingested 1 h before exercise also caused a decrease in plasma FFA and resulted in reduced muscle acetylcarnitine content at rest. The reason for this decrease is not readily apparent, and the significance of this finding at rest remains to be elucidated but does not appear to affect the subsequent response to exercise (44). Acetylcarnitine normally functions as a buffer for acetyl-CoA and is formed when there is an overproduction of acetyl units in the muscle and is degraded to provide acetyl units when the supply is reduced. The primary fuel source for energy at rest is through fat oxidation, resulting in the production of acetyl-CoA units via  $\beta$ -oxidation. Possibly by limiting exogenous FFA availability, during NA ingestion, inadequate acetyl units and reducing equivalents (NADH) were being provided to meet the energy needs of the electron transport chain in resting muscle. This, in turn, could have caused a decreased resting acetylcarnitine level. A decreased resting pyruvate level might have been expected during NA ingestion if it were limiting the production of acetyl-CoA through PDH, but this did not occur.

In summary, the administration of NA decreased plasma FFA availability at rest and during exercise. The activation of PDH was unaffected by low FFA availability at rest, but acetylcarnitine was markedly reduced. In support of our hypothesis, the reduced exogenous FFA availability resulted in a significant increase in RER and a significantly greater activation of PDH during exercise. Contrary to our hypothesis, the increased PDHa was not explained by changes in muscle pyruvate or the ATP/ADP ratio but may be related to a decreased NADH/NAD<sup>+</sup> ratio or an epinephrine-induced increase in calcium concentration.

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