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The Effect of Sodium Acetate Ingestion on the Metabolic Response to Prolonged Moderate-Intensity Exercise in Humans

Gordon I. Smith, Asker E. Jeukendrup, and Derek Ball

At rest, administration of the short-chain fatty acid acetate suppresses fat oxidation without affecting carbohydrate utilization. The combined effect of increased acetate availability and exercise on substrate utilization is, however, unclear. With local ethics approval, we studied the effect of ingesting either sodium acetate (NaAc) or sodium bicarbonate (NaHCO₃) at a dose of 4 mmol·kg⁻¹ body mass 90 min before completing 120 min of exercise at 50% VO_{2peak}. Six healthy young men completed the trials after an overnight fast and ingested the sodium salts in randomized order. As expected NaAc ingestion decreased resting fat oxidation (mean ± SD; 0.09 ± 0.02 vs. 0.07 ± 0.02 g·min⁻¹ pre- and post-ingestion respectively, $p < .05$) with no effect upon carbohydrate utilization. In contrast, NaHCO₃ ingestion had no effect on substrate utilization at rest. In response to exercise, fat and CHO oxidation increased in both trials, but fat oxidation was lower (0.16 ± 0.10 vs. 0.29 ± 0.11 g·min⁻¹, $p < .05$) and carbohydrate oxidation higher (1.67 ± 0.35 vs. 1.44 ± 0.22 g·min⁻¹, $p < .05$) in the NaAc trial compared with the NaHCO₃ trial during the first 15 min of exercise. Over the final 75 min of exercise an increase in fat oxidation and decrease in carbohydrate oxidation was observed only in the NaAc trial. These results demonstrate that increasing plasma acetate concentration suppresses fat oxidation both at rest and at the onset of moderate-intensity exercise.

Keywords: sodium salt, substrate oxidation, acetyl availability

The coordination of fat and carbohydrate oxidation at rest and during exercise is a complex process. Although changes in fat and carbohydrate availability are known to partially regulate substrate utilization (Costill et al., 1977; Coyle et al., 1997; Sidossis & Wolfe, 1996; Thiébaud et al., 1982; Wolfe et al., 1988), over the past 40 years, significant focus has been placed on whether intracellular levels of acetyl-CoA play a key role in determining rates of fat and/or carbohydrate oxidation.

It has been postulated that increases in acetyl-CoA leads to a decrease in carbohydrate oxidation through inhibition of the pyruvate dehydrogenase complex (Behal et al., 1993; Randle et al., 1963; Randle et al., 1994). More recent hypotheses, however, suggest that increased acetyl group availability decreases fat rather than carbohydrate oxidation by inhibiting fat translocation into the mitochondria through either an increase in malonyl-CoA (Båvenholm et al., 2000; Merrill et al., 1998; Turcotte et al., 2002; Winder & Hardie, 1996), which is known to inhibit carnitine palmitoyltransferase I *in vitro* (McGarry et al., 1978) and/or a decrease in free carnitine with the consequent production of acetylcarnitine (Long et al.,

1982; van Loon et al., 2001). Furthermore, increases in muscle acetyl-CoA and acetylcarnitine have been closely linked to the intensity whereby fat oxidation decreases during incremental exercise (van Loon et al., 2001).

Acetate is known to be quickly metabolized to acetyl-CoA in a number of different tissues and organs within the body, including skeletal muscle. The administration of acetate has been shown to markedly increase resting skeletal muscle acetyl-CoA and acetylcarnitine content with a resultant decrease in free carnitine (Evans et al., 2001; Howlett et al., 1999; Putman et al., 1995; Roberts et al., 2005).

Previous studies investigating the metabolic effect of acetate administration in humans have predominately been performed in the resting state, where an acetate-induced depression in fat utilization has been reported with no effect upon carbohydrate oxidation (Akanji et al., 1989; Sigler et al., 1983; Smith et al., 2007). Whether increased acetate availability also affects substrate utilization during exercise is, however, currently unclear, but elevations in acetyl group availability have been found to continue at the onset of exercise with (Putman et al., 1995) or without acetate continuing to being given (Roberts et al., 2005). Indeed, the small number of studies to examine the effect of acetate administration on substrate utilization during exercise have limited their focus to changes in muscle glycogen use, which has been reported to be unchanged during exercise (Putman et al.,

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1995; Roberts et al., 2005). However, the effect of acetate administration on fat utilization during exercise has not, to our knowledge, been studied to date.

Sodium acetate administration is, however, also well known to induce a metabolic alkalosis (Putman et al. 1995; Smith et al. 2007), which may in itself affect muscle glycogen utilization rates during exercise (Hollidge-Horvat et al., 2000), increase rates of lipolysis and plasma FFA availability (Hood et al., 1990; Straumann et al., 1992; Galloway & Maughan, 1996) and affect muscle acetyl-CoA and free carnitine contents (Hollidge-Horvat et al., 2000; Roberts et al., 2002), which have all been shown to occur following sodium bicarbonate administration. It is therefore critical when examining the metabolic effect of acetate to control for the confounding effect of changes in blood pH to be able to separate out the effect(s) of acetate on fat and carbohydrate utilization from the effect of simply inducing a metabolic alkalosis.

Therefore, the purpose of the current study was to elucidate the effect of acetate administration on substrate utilization during low-to-moderate intensity exercise. We hypothesized that providing acetate at a dose known to suppress fat oxidation (Smith et al., 2007) and also significantly increase acetyl group availability (Howlett et al., 1999; Evans et al., 2001) at rest would continue to suppress fat oxidation during exercise with no effect upon carbohydrate utilization compared with responses following consumption of an equimolar dose of sodium bicarbonate. To increase the chance of detecting an effect of acetate on fat metabolism during exercise we chose to examine an exercise intensity (50% $\text{VO}_{2\text{max}}$) that would be close to where the maximum rate of fat utilization would be observed (Venables et al., 2005).

Methods

Subjects

Six healthy males ([mean \pm SD] age 24 ± 4 yr, height 1.81 ± 0.10 m, and body mass 73.92 ± 11.46 kg) volunteered to participate in this study. All the participants gave their written informed consent after the purpose, procedures, and possible health risks were explained to them. The Grampian research ethics committee approved all the experimental procedures used within this study.

Preliminary measures

Before completing the experiment each subject undertook two preliminary trials. The first was used to determine their peak oxygen uptake ($\text{VO}_{2\text{peak}}$) using a discontinuous, incremental cycle ergometer test to volitional exhaustion, performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The subjects' $\text{VO}_{2\text{peak}}$ and maximum power output during this incremental test were 53.8 ± 8.7 ml·kg⁻¹·min⁻¹ and 314 ± 45 W, respectively. At least two days later participants completed a practice trial where they completed 120 min of exercise at a workload calculated to elicit 50%

of $\text{VO}_{2\text{peak}}$. This second trial served to familiarize the subjects with the experimental procedures and provide confirmation that the calculated workload elicited the desired rate of oxygen consumption.

Experimental Design

Each subject performed two experimental trials at least 1 week apart, which provided an adequate washout period between trials. On each occasion NaAc or NaHCO₃ was ingested at rest before commencing cycling for 120 min at 119 ± 16 W (~50% $\text{VO}_{2\text{peak}}$); an intensity whereby fat oxidation is reported to be near maximal (Achten & Jeukendrup, 2004). The order of drink administration was subjected to a randomized, crossover, single-blind experimental design.

Diet and Activity Before Experimental Trials

Subjects were instructed to perform a prolonged bout of intense exercise approximately 60 min in duration 5 days before each visit to the laboratory in an attempt to minimize ¹³C glycogen stores. They were then instructed to record their dietary intake over the 2-day period immediately before their first experimental trial and then replicate this intake before their second visit. During the experimental period volunteers were instructed to avoid the ingestion of foodstuffs derived from carbohydrates from C4 plants (e.g., sugar cane and maize corn) to maintain a low background ¹³C enrichment in expired CO₂. Subjects reported to the laboratory at 8 a.m. following an overnight fast and were also instructed to consume 500 ml of tap water 1 hr before attending the laboratory.

Protocol

Upon entering the laboratory each subject was asked to void completely before their body mass was recorded. Subjects then rested for at least 60 min during which time two 4-min respiratory gas samples were collected after the expired air had passed through a mixing chamber (MLA245 Gas Mixing Chamber, ADInstruments Pty Ltd, Castle Hill, Australia), which was then connected to a Douglas bag. This arrangement allowed the simultaneous collection of small volumes of expired gas into 10 ml Exetainer tubes (Labco Ltd., High Wycombe, UK) directly from the mixing chamber while also collecting whole breaths in the Douglas bag. Preliminary studies revealed that the fraction of expired O₂ and CO₂ sampled directly from the mixing chamber took 1 min and 2.5 min to stabilize during exercise and at rest, respectively. The mouthpiece was therefore inserted at least 2 min before any respiratory collections were taken during exercise and 3 min before collections at rest.

Expired gas collected in Douglas bags was immediately analyzed for percentage oxygen and carbon dioxide using a gas analyzer (Servomex 1440, Servomex Group Ltd, East Sussex, UK), which was calibrated before,

during and immediately after each test using gases of known composition. The volume of the expired gas was measured using a dry gas meter (Harvard dry gas meter, Harvard Apparatus Ltd, Edenbridge, Kent, UK) with gas temperature recorded using a thermocouple situated within the dry gas meter (RS components NTA912, Corby, Northants, UK). Breath samples collected in the Exetainer tubes were later analyzed for ^{13}C content by continuous flow isotope ratio mass spectrometry (Europa Scientific, Crewe, UK). Before any respiratory gas calculations were performed the volume of gas collected in the Exetainer tubes was added to the measured expired volume collected in the Douglas bag.

Upon completion of the second resting gas sample a 21 g butterfly needle was introduced into a superficial vein on the dorsal surface of a warmed hand and a 5 ml blood sample obtained. Patency of the indwelling cannula was maintained throughout each trial by regular flushing with small volumes of 0.9% sterile saline.

Following the initial blood sample participants ingested 500 ml of a low-calorie lemon cordial drink (42 kJ/500 ml; 2.5 g of carbohydrate/500 ml) with a further 500 ml of the same drink given 45 min later. The beverages contained either NaAc (trihydrate) or NaHCO_3 at a dose of 2 mmol·kg⁻¹ body mass such that in total 4 mmol·kg⁻¹ body mass of the sodium salt was given in total during each trial. To quantify exogenous acetate oxidation [$^{1,2-^{13}\text{C}}$] sodium acetate at a dose of 2 mg·kg⁻¹ body mass was added to the sodium acetate drink giving final enrichments of $+342.6 \pm 15.4 \delta\text{‰}$ vs. Pee Dee Belemnite (PDB) measured using mass spectrometry (Europa Scientific Geo 20–20, Crewe, UK).

Subjects were instructed to consume the prepared drinks within 5 min. Blood and respiratory gases were sampled 90 min post-ingestion of the first drink. As soon as the subjects had provided their resting post-ingestion blood and gas samples they commenced cycling for 120 min with 2 min respiratory gas collections taken every 15 min and blood samples collected every 30 min. Heart rate was also recorded (Polar Electro, Finland) every 15 min during exercise.

Blood Analyses

Blood samples (5 ml) were dispensed into a tube containing EDTA (1.3 g/L) with an aliquot used to immediately determine whole blood glucose and lactate concentration (YSI 2300 STAT Plus, YSI Inc., Yellow Springs, OH). Approximately 4 ml of blood was then spun down with the plasma separated and frozen at -20°C and later used to determine free fatty acids (FFA; Wako FFA C test kit, Wako Chemicals, Richmond, USA) and acetate (Powers et al., 1995) concentration.

Calculations

The isotopic enrichment in the breath samples was expressed as δ per mil difference between the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and a known laboratory reference standard according to the formula of Craig (1957).

$$\delta \text{ } ^{13}\text{C} = ((^{13}\text{C}/^{12}\text{C}_{\text{sample}}/^{13}\text{C}/^{12}\text{C}_{\text{standard}})-1) \cdot 10^3$$

per mil

The $\delta \text{ } ^{13}\text{C}$ was then related to an international standard (PDB). Acetate oxidation was calculated according to the following formula:

$$\text{Acetate oxidation} = \text{VCO}_2 \cdot ((\delta \text{ Exp} - \delta \text{ Exp}_{\text{bkg}}) / (\delta \text{ Exp} - \delta \text{ Exp}_{\text{bkg}}))(1/k)$$

Where $\delta \text{ Exp}$ is the ^{13}C enrichment of expired air following ingestion of NaAc, $\delta \text{ Ing}$ is the ^{13}C enrichment of the ingested solution, $\delta \text{ Exp}_{\text{bkg}}$ is the ^{13}C enrichment of expired air at the matched time-point during the NaHCO_3 trial, and k is the volume of CO_2 (in L) produced by the oxidation of 1 g acetate (2 mol/1 mol = 44.8 L of $\text{CO}_2/136.08 \text{ g} = 0.329 \text{ L of } \text{CO}_2/\text{g}$). At rest we used a correction factor of 54% (Schneiter, Di Vetta, Jequier, and Tappy, 1995) to account for loss of oxidized ^{13}C within the NaHCO_3 pool and during isotopic exchange reactions in the tricarboxylic acid cycle.

During exercise, however, acetate recovery in the breath is transiently greater than 100% (van Loon et al., 2003) before reaching a plateau in breath CO_2 enrichment reflecting the true rate of acetate oxidation after 30 min of exercise (van Hall, 1999; van Loon et al., 2003). As such, without the use of a correction factor during the early phase of exercise acetate oxidation would be overestimated. We therefore used a correction factor of 115% at the 15-min time point during exercise as employed by van Loon et al., (2003). It should be noted that applying slightly different correction factors (i.e., 100–140%) had minimal effect upon the calculated rates of fat and carbohydrate oxidation at this time and did not influence the overall conclusions presented within this study.

Carbohydrate and fat oxidation rates were calculated according to Frayn (1983) at rest and Jeukendrup and Wallis (2005) during exercise with the assumption of negligible protein oxidation. During the NaAc trial fat and carbohydrate utilization was calculated after the VO_2 and VCO_2 values were corrected to account for oxidation of the ingested acetate as previously described by Akanji et al., (1989). Briefly the oxidation of 1 mol of NaAc is known to consume 2 mol of O_2 (44.8 L) and liberate 1 mol of CO_2 (22.4 L; Akanji et al., 1989). These values were subtracted from the VO_2 and VCO_2 at the corresponding time point to give the “nonacetate” VO_2 and VCO_2 from which post-ingestion fat and carbohydrate utilization was based. For example, if at rest the uncorrected (i.e., measured) VO_2 and VCO_2 is assumed to be 0.30 and 0.24 L·min⁻¹, respectively with a corresponding acetate oxidation rate of 2 mmol·min⁻¹ then 0.0896 L·min⁻¹ O_2 and 0.0448 L·min⁻¹ CO_2 would be deducted from the total volumes leaving a nonacetate VO_2 and VCO_2 of 0.2104 and 0.1952 L·min⁻¹, respectively with the corrected volumes then used to calculate fat and carbohydrate oxidation rates. Total energy expenditure was calculated according to Jeukendrup and Wallis (2005) with energy expenditure attributable to oxidation of the ingested acetate calculated as described by Akanji et al.

(1989) assuming 876.13 kJ produced for every 1 mol of acetate oxidized.

Statistical Analysis

After testing for normality of distribution the data (blood metabolite, substrate utilization rates, energy expenditure, breath ^{13}C enrichment and heart rate during exercise) were analyzed using a two-way ANOVA with repeated measures (trial \times time) with substrate utilization and energy expenditure data obtained during the resting and exercising phases analyzed separately. Where a significant interaction was found, Tukey's post hoc testing was performed to identify significant mean differences. To examine whether blood glucose declined at a similar rate during exercise in both trials linear regression analysis was used with the slopes compared using a paired student t test. Results are presented as mean \pm SD in text and tables and for reasons of clarity as mean \pm SEM in the figures. Differences were considered significant at the $p < .05$ level.

Results

Cardiorespiratory Measures and Ratings of Perceived Exhaustion

Respiratory data (VO_2 , VCO_2 and RER) including uncorrected and corrected rates in the case of the acetate trial are presented in Table 1. Briefly, despite the exercise bouts being performed at the same workload (119 ± 16 W), cadence (NaAc, 68 ± 13 vs. NaHCO_3 , 67 ± 13 revs $\cdot\text{min}^{-1}$) and ambient temperature (21.4 ± 1.7 vs. 20.5 ± 1.6 $^\circ\text{C}$; NaAc and NaHCO_3 trials, respectively), the mean uncorrected VO_2 was significantly higher ($p < .05$) during exercise following NaAc ingestion (1.89 ± 0.14 vs. 1.82 ± 0.17 $\text{L}\cdot\text{min}^{-1}$; NaAc and NaHCO_3 trials, respectively). As such, the 120-min exercise bout required on average $49 \pm 2\%$ of $\text{VO}_{2\text{peak}}$ following NaAc ingestion and $47 \pm 3\%$ $\text{VO}_{2\text{peak}}$ following NaHCO_3 ingestion. Heart rate increased to 130 ± 17 (NaAc) bpm and 123 ± 9 (NaHCO_3) bpm throughout exercise ($p = .01$) with a similar increase observed in both trials ($p = .96$).

Blood Metabolites

Plasma acetate concentration was similar before ingestion of both sodium salts (Figure 1) with no change either at rest or during exercise following NaHCO_3 ingestion. In contrast, NaAc ingestion resulted in a significant increase in plasma acetate concentration from 0.13 ± 0.12 – 1.38 ± 0.61 $\text{mmol}\cdot\text{L}^{-1}$ 90 min after ingestion ($p < .01$). Following 30 min of exercise the plasma concentration of acetate had returned to baseline levels, remaining at ~ 0.07 $\text{mmol}\cdot\text{L}^{-1}$ for the last 90 min of the exercise bout.

There was no difference between trials in the blood concentration of glucose and lactate (Table 2) before ingestion of the sodium salts. As shown in Table 2, blood glucose was significantly higher 90 min post-ingestion

of NaHCO_3 compared with NaAc ingestion ($p < .05$). Blood glucose then declined at a similar rate (0.007 ± 0.003 vs. 0.006 ± 0.002 $\text{mmol}\cdot\text{L}^{-1}\cdot\text{min}^{-1}$, respectively; $p = .70$) in both trials. However, as a consequence of the differing glycemic responses following sodium salt ingestion at rest the concentration of blood glucose remained lower ($p < .05$) during the last 60 min of exercise during the NaAc trial. Blood lactate was similar between trials at rest. Exercise produced an increase in blood lactate concentration that was apparent over the first 30 min of exercise ($p < .001$) and the magnitude of the lactate response was similar between trials ($p = .81$).

Plasma FFA concentration was similar before sodium salt ingestion. There was an increase in FFA concentration observed at rest but only following NaHCO_3 ingestion ($p < .05$; Figure 1). There was a contrast in the responses over the first 30 min during exercise where the plasma FFA concentration significantly increased in the NaAc trial ($p < .05$) but in the NaHCO_3 trial they returned to basal levels. FFA concentration remained above basal levels throughout exercise in the NaAc trial. In contrast, in the NaHCO_3 trial FFA concentration only increased above basal levels after 90 min of exercise. As a result of the different lipemic responses between trials plasma FFA concentration was found to be significantly higher in the NaAc compared with the NaHCO_3 trial after 90 min of exercise ($p < .01$).

Breath ^{13}C enrichment

Breath ^{13}C enrichment, as shown in Supplemental Figure 1, was similar before ingestion of both sodium salts. NaHCO_3 ingestion had no effect upon breath enrichment, remaining at about -26.10 $\delta\text{‰}$ vs. PDB at rest and during exercise ($p > .05$). In contrast, ingestion of NaAc substantially increased breath enrichment to $+26.23 \pm 7.91$ $\delta\text{‰}$ vs. PDB after 90 min at rest ($p < .01$). Breath enrichment then decreased throughout the exercise bout in the NaAc trial but remained significantly higher compared with the matched time points in the NaHCO_3 trial over the first 105 min of exercise. From the breath enrichment data it was calculated that $62.1 \pm 8.4\%$ of the ingested NaAc was oxidized during exercise.

Substrate Utilization and Energy Expenditure at Rest and During Exercise

There was no difference in the rate of fat or carbohydrate oxidation at rest between trials before ingestion of the sodium salts (Table 3) with NaHCO_3 having no effect upon resting substrate utilization. In contrast, NaAc consumption reduced the rate of fat oxidation by $\sim 30\%$ 90 min after ingestion of the sodium salt such that it was significantly lower compared with the matched time point in the NaHCO_3 trial. NaAc ingestion did not, however, affect the rate of carbohydrate utilization at rest (Table 3). Resting energy expenditure (Table 3) was similar between trials ($p = .18$). Sodium salt ingestion

Table 1 Respiratory Data at Rest and During Exercise Before and After Sodium Acetate and Sodium Bicarbonate Ingestion

Time (min)	Sodium Salt	VO ₂ (l·min ⁻¹)	VCO ₂ (l·min ⁻¹)	RER
Pre ingestion	Uncorrected NaAc	0.30 ± 0.05	0.24 ± 0.06	0.81 ± 0.06
	NaHCO ₃	0.27 ± 0.03	0.21 ± 0.03	0.78 ± 0.04
Post ingestion	Uncorrected NaAc	0.33 ± 0.08	0.26 ± 0.08	0.76 ± 0.06
	Corrected NaAc	0.26 ± 0.06	0.22 ± 0.07	0.83 ± 0.07
	NaHCO ₃	0.31 ± 0.05	0.24 ± 0.04	0.78 ± 0.02
15	Uncorrected NaAc	1.81 ± 0.15	1.63 ± 0.17	0.90 ± 0.04
	Corrected NaAc	1.64 ± 0.15	1.57 ± 0.17	0.94 ± 0.04
	NaHCO ₃	1.72 ± 0.07	1.54 ± 0.15	0.90 ± 0.03
30	Uncorrected NaAc	1.84 ± 0.16	1.59 ± 0.19	0.86 ± 0.04
	Corrected NaAc	1.70 ± 0.20	1.52 ± 0.20	0.89 ± 0.04
	NaHCO ₃	1.78 ± 0.21	1.54 ± 0.17	0.88 ± 0.03
45	Uncorrected NaAc	1.87 ± 0.16	1.58 ± 0.19	0.85 ± 0.05
	Corrected NaAc	1.78 ± 0.17	1.54 ± 0.19	0.86 ± 0.05
	NaHCO ₃	1.79 ± 0.19	1.54 ± 0.17	0.86 ± 0.02
60	Uncorrected NaAc	1.87 ± 0.15	1.58 ± 0.16	0.84 ± 0.03
	Corrected NaAc	1.81 ± 0.17	1.55 ± 0.17	0.85 ± 0.03
	NaHCO ₃	1.82 ± 0.16	1.57 ± 0.13	0.86 ± 0.03
75	Uncorrected NaAc	1.91 ± 0.12	1.60 ± 0.15	0.84 ± 0.04
	Corrected NaAc	1.86 ± 0.13	1.57 ± 0.15	0.84 ± 0.04
	NaHCO ₃	1.79 ± 0.14	1.55 ± 0.12	0.87 ± 0.03
90	Uncorrected NaAc	1.94 ± 0.13	1.61 ± 0.16	0.83 ± 0.03
	Corrected NaAc	1.90 ± 0.14	1.59 ± 0.16	0.83 ± 0.03
	NaHCO ₃	1.88 ± 0.18	1.61 ± 0.16	0.86 ± 0.03
115	Uncorrected NaAc	1.92 ± 0.13	1.59 ± 0.14	0.83 ± 0.02
	Corrected NaAc	1.89 ± 0.14	1.57 ± 0.14	0.83 ± 0.02
	NaHCO ₃	1.88 ± 0.20	1.59 ± 0.15	0.85 ± 0.04
120	Uncorrected NaAc	1.96 ± 0.14	1.60 ± 0.14	0.81 ± 0.02
	Corrected NaAc	1.94 ± 0.14	1.58 ± 0.14	0.82 ± 0.02
	NaHCO ₃	1.87 ± 0.18	1.57 ± 0.13	0.84 ± 0.03

Note. Values are mean ± SD. Corrected NaAc values reflect the measured VO₂ and VCO₂ (i.e., uncorrected NaAc values) after the contribution from acetate oxidation is deducted.

significantly increased energy expenditure ($p = .03$) with no difference between the trials in the magnitude of the response ($p = .58$).

Both fat and carbohydrate oxidation increased during the transition from rest to exercise in both trials. However, fat utilization was significantly lower ($p < .05$) and carbohydrate utilization higher ($p < .05$) after 15 min of exercise following NaAc compared with NaHCO₃ ingestion (Figure 2). At this time the energy expenditure attributable to fat oxidation was on average 5.01 ± 2.50 kJ·min⁻¹ lower during the NaAc trial with the oxidation

of exogenous acetate supplying 3.24 ± 0.40 kJ·min⁻¹ of energy at this time. During this period of exercise, acetate oxidation rate more than doubled from 0.09 ± 0.03 – 0.22 ± 0.03 g·min⁻¹ in the transition from rest to 15 min of exercise. Over the next 15 min, the rate of fat oxidation increased and carbohydrate oxidation decreased in the NaAc trial, such that, after 30 min of the exercise there was no difference in either fat or carbohydrate utilization between trials (Figure 2); These changes in substrate utilization also coincided with a significant decrease ($p = .01$) in acetate oxidation (Figure 2). Thereafter, a significant

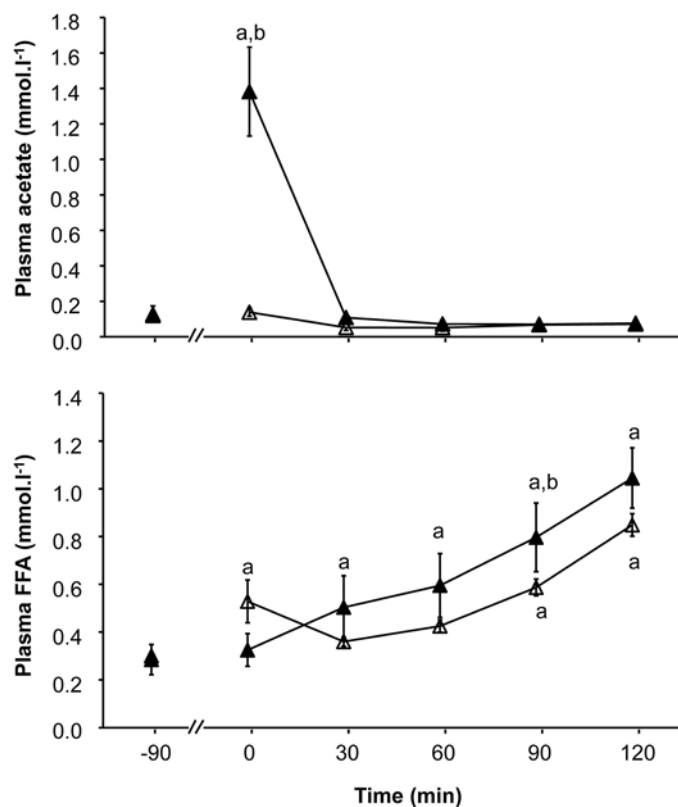


Figure 1 — Plasma acetate and FFA concentration following ingestion of sodium acetate (triangle with fill) and sodium bicarbonate (triangle with no fill). Data are mean \pm SEM. Significant difference denoted by: a = $p < .01$ vs. preingestion (-90 min); b = $p < .01$ between trials at matched time points.

Table 2 The Effect of Sodium Acetate and Sodium Bicarbonate Ingestion on Blood Glucose and Lactate Concentration

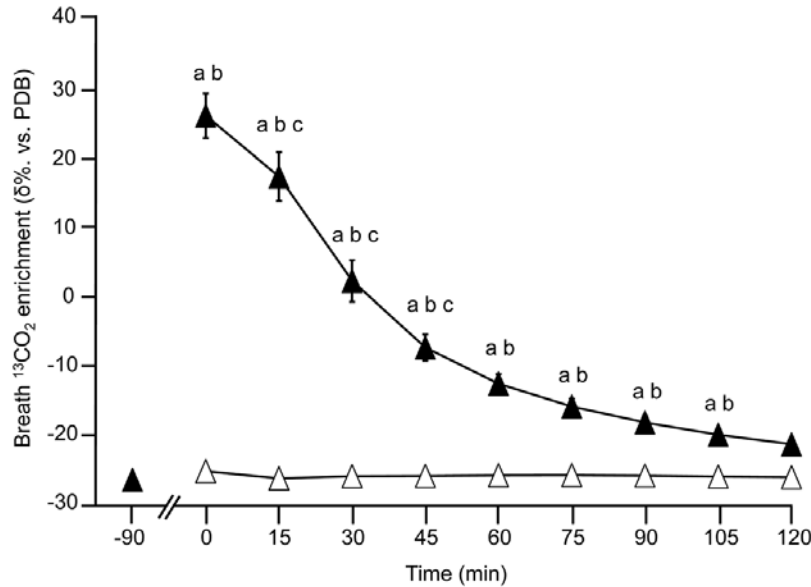
Time (min)	Sodium Salt	Blood Glucose (mmol.l ⁻¹)	Blood Lactate (mmol.l ⁻¹)
Preingestion	NaAc	4.15 \pm 0.22	0.50 \pm 0.09
	NaHCO ₃	4.26 \pm 0.19	0.41 \pm 0.10
Preexercise	NaAc	4.03 \pm 0.20 ^b	0.64 \pm 0.14
	NaHCO ₃	4.38 \pm 0.43	0.50 \pm 0.10
30	NaAc	3.87 \pm 0.23	1.05 \pm 0.38 ^{a,c}
	NaHCO ₃	4.16 \pm 0.40	0.94 \pm 0.27 ^{a,c}
60	NaAc	3.57 \pm 0.29 ^{a,b}	0.85 \pm 0.23
	NaHCO ₃	4.04 \pm 0.40	0.69 \pm 0.18
90	NaAc	3.43 \pm 0.34 ^{a,b}	0.78 \pm 0.20
	NaHCO ₃	3.84 \pm 0.44	0.65 \pm 0.10
120	NaAc	3.21 \pm 0.43 ^{a,b}	0.84 \pm 0.21
	NaHCO ₃	3.59 \pm 0.45 ^a	0.66 \pm 0.15

Note. Values are mean \pm SD. Preexercise value 90 min post-ingestion of the sodium salts.

^a $p < 0.05$ vs. preingestion.

^b $p < 0.05$ vs. between trials at matched time points.

^c $p < 0.05$ vs. preceding time point.



Supplemental Figure 1 — Breath enrichment kinetics at rest and during exercise following ingestion of sodium acetate (triangle with fill) and sodium bicarbonate (triangle with no fill) before exercise. Data are mean \pm SEM. Significant difference denoted by: a = $p < .05$ vs. preingestion (-90 min); b = $p < .05$ between trials at matched time points; c = $p < .05$ vs. preceding time point.

Table 3 Effect of Sodium Acetate and Sodium Bicarbonate Ingestion on Substrate utilization and Energy Expenditure at Rest

Trial	Time	Fat oxidation	CHO oxidation	Energy expenditure
		(g·min ⁻¹)	(g·min ⁻¹)	(kJ·min ⁻¹)
NaAc	Preingestion	0.09 \pm 0.02	0.15 \pm 0.11	5.97 \pm 1.07
	Post-ingestion	0.07 \pm 0.02 ^a	0.17 \pm 0.12	6.55 \pm 1.65 ^b
NaHCO ₃	Preingestion	0.10 \pm 0.02	0.09 \pm 0.04	5.29 \pm 0.62
	Post-ingestion	0.11 \pm 0.03	0.11 \pm 0.02	6.19 \pm 1.06 ^b

Note. Data are mean \pm SD. In the NaAc trial, energy expenditure also includes a contribution from acetate oxidation.

^a $p < 0.05$ between trials at matched time points.

^bSignificant main effect of time ($p < .05$) with no difference between trials ($p = .18$) at matched time points.

increase in fat oxidation and significant decrease in carbohydrate oxidation was only observed during the final 75 min of exercise in the NaAc trial (Figure 2). Despite the difference in the temporal rates of fat and carbohydrate oxidation there was no difference between trials in the total amount of fat (50.6 ± 7.2 g vs. 49.4 ± 11.3 g NaAc and NaHCO₃ trials, respectively; $p = .79$) and carbohydrate (146.5 ± 33.4 g vs. 150.2 ± 23.8 g during the NaAc and NaHCO₃ trials, respectively; $p = .74$) oxidized over the entire 120 min exercise bout.

Energy expenditure (Figure 2) increased over time during exercise in both trials ($p < .001$), however, energy expenditure was slightly but significantly higher throughout exercise during the NaAc compared with the NaHCO₃ trial ($p < .05$). The energy derived during exercise from

oxidation of the ingested acetate contributed to $3.5 \pm 0.7\%$ of the total energy expenditure over the entire 120 min of exercise.

Discussion

The purpose of the current study was to examine whether perturbing substrate utilization at rest by ingesting sodium acetate, would influence substrate utilization during a subsequent bout of prolonged exercise. In this study we observed that NaAc ingestion significantly reduces the rate of fat oxidation at rest compared with a counterbalanced salt (NaHCO₃) trial and this supports those studies previously examining the metabolic effects of acetate administration at rest (Akanji et al., 1989; Burnier et al.,

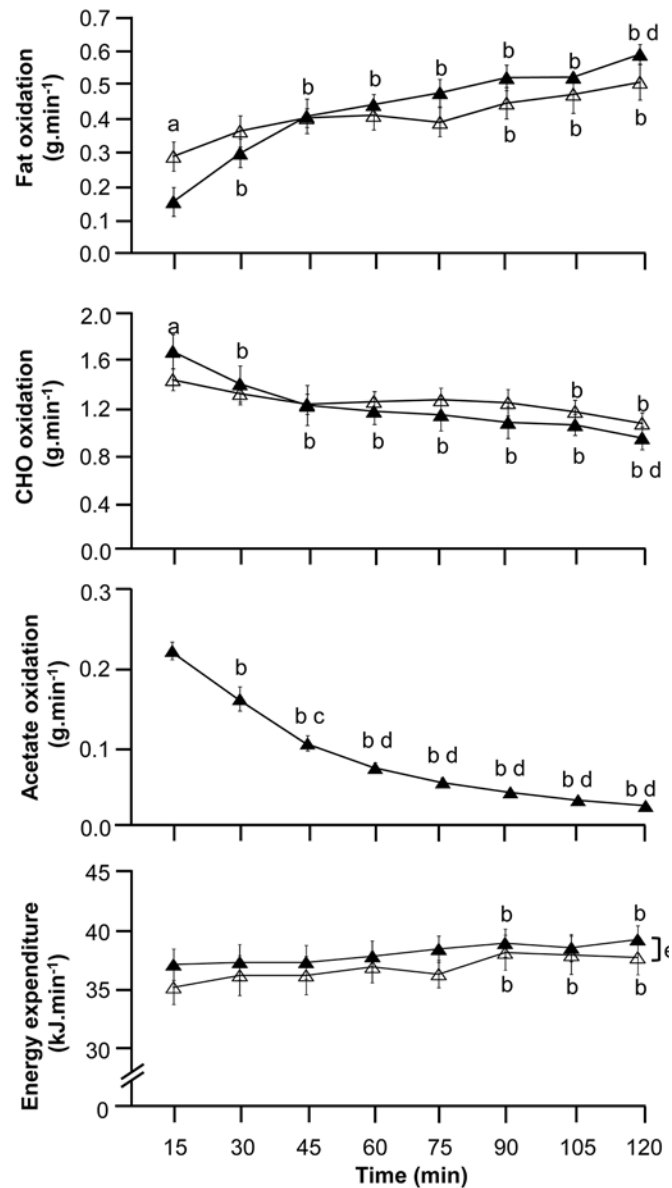


Figure 2 — Rates of fat, carbohydrate (CHO), and acetate oxidation (sodium acetate trial only) and total energy expenditure during exercise following ingestion of sodium acetate (triangle with fill) and sodium bicarbonate (triangle with no fill) at rest. Data are mean \pm SEM. Significant difference denoted by: a = $p < .05$ vs. matched time point during bicarbonate trials; b = $p < .05$ vs. 15 min; c = $p < .05$ vs. 30 min. d = $p < .05$ vs. 45 min. e = ANOVA revealed main effect of time ($p < .05$)

1992; Sigler et al., 1983; Smith, et al., 2007). At the same time carbohydrate utilization was found to be unaffected. The current results therefore add further support to the concept that under conditions where acetate availability is increased, such as following moderate ethanol ingestion, increased consumption of dietary fiber or during renal dialysis where acetate is used to help maintain acid-base balance substrate utilization will be perturbed.

We, however, extend these well-known observations at rest by finding that the suppression in fat oxidation continues at the onset and early stages of prolonged exercise. The suppression in fat utilization was, however, found to

be transient in nature with no difference between trials after 30 min of exercise. The brief nature of this response presumably reflects changes in exogenous acetate oxidation, which was highest in the first 15 min of exercise with the majority of this substrate oxidized within 30 min; the period whereby plasma acetate had returned to baseline.

At rest, the effect of acetate ingestion on substrate utilization differed slightly from the results we have previously presented (Smith et al., 2007). In our earlier study we reported that with sodium acetate ingestion there was no change in the total amount of fat and carbohydrate oxidized over a 180-min period and that acetate

oxidation had replaced the alkalosis-induced stimulation in fat oxidation observed following sodium bicarbonate ingestion. In contrast, we found in the current study that fat oxidation was lower after acetate oxidation compared with the preingestion rate. This difference is probably attributable to 2 key differences between the two studies. First, twice the amount of acetate was ingested in the current compared with our previous study (i.e., 4 vs. 2 mmol·kg⁻¹, respectively) and secondly, in the current study we only measured substrate oxidation 90 min following ingestion of the first dose of acetate in the resting state. This would correspond to the time when ¹³CO₂ breath enrichment was at its peak in our previous study (Smith et al., 2007).

It is currently unclear why increasing acetate availability inhibits fat utilization both at rest and during the early phase of exercise although it has been suggested that this could be due to an acetate-induced reduction in plasma FFA availability (Crouse et al., 1968). However, consistent with our previous findings at rest (Smith et al., 2007) we did not observe a decline in FFA concentration following NaAc ingestion although acetate did appear to prevent the rise in plasma FFA concentration (Smith et al., 2007) normally observed when inducing a metabolic alkalosis (Hood et al., 1990; Galloway & Maughan, 1996). Furthermore, plasma FFA concentration increased at the onset of exercise in the NaAc trial suggesting that plasma FFA availability may not have been a limiting factor to fat oxidation at this time as the rate of appearance must have been greater than the rate of uptake from the circulation. As such, it seems unlikely that plasma FFA availability is the primary mechanism responsible for the suppressed rate of fat oxidation observed both at rest and during the onset of exercise. Circumstantial evidence suggests that the decrease in fat oxidation was attributable to a decline in free carnitine availability. Carnitine availability has been postulated to regulate long-chain fatty acid entry into the mitochondria and thus represents a major limiting factor in the ability to oxidize fatty acids (Stephens et al., 2007). Increases in muscle acetylcarnitine content, which lead to a stoichiometric decrease in free carnitine content, have been reported in resting skeletal muscle following infusion of acetate at a dose equivalent to that given in the current study (Evans et al., 2001; Howlett, et al., 1999). Furthermore, the increase in muscle acetylcarnitine content has been found to persist at the onset of exercise with (Putman et al., 1995) or without acetate continuing to being given (Roberts et al., 2005). In addition, the magnitude of the change in muscle acetylcarnitine and free carnitine content reported following administration of the same dose of acetate (4 mmol·kg body mass⁻¹) as given in the current study (Evans et al., 2001; Howlett et al., 1999) is similar to that observed during high-intensity exercise where fat oxidation is markedly decreased compared with moderate intensity exercise (van Loon et al., 2001). The muscle carnitine content in this study was also similar to that found in individuals with myopathic carnitine deficiency (Engel & Angelini 1973; Di Mauro, et al.,

1980; Siliprandi et al., 1989) a condition where resting fat utilization is impaired.

Based on the finding that oxidation of exogenous acetate accounted for ~65% of the difference in fat oxidation between trials it might be expected that there would be an increase in carbohydrate oxidation following NaAc administration to meet the energy demands of exercise. However, we found that the increase in carbohydrate utilization was significantly greater than that required to simply replace the remaining ~30% decrease in fat oxidation suggesting that, in contrast to the response observed at rest (Akanji et al., 1989; Burnier et al., 1992; Smith et al., 2007), acetate may stimulate carbohydrate catabolism during exercise. Although we cannot identify the mechanism(s) behind this phenomenon from the present results it is plausible that this effect is due to changes in muscle AMP content. The enzyme that sets the upper limit of glycogen utilization is glycogen phosphorylase and AMP content has been implicated in posttransformational allosteric regulation of this enzyme (Dyck et al., 1996; Parolin et al., 2000). Indeed, acetate infusion has been shown to significantly increase AMP content in a variety of tissues including skeletal muscle (Davis & Davis-van Thienen, 1977; Zydowo et al., 1993; Spydevold et al., 1976) presumably as activation of 1 mol of acetate to acetyl-CoA by acetyl-CoA synthetase stoichiometrically consumes 1 mole ATP and produces 1 mol of AMP. Corroborating evidence suggests that an increase in AMP content occurs *in vivo* within man where metabolites of adenine nucleotide degradation (for example, hypoxanthine and uric acid) have been found to increase in the plasma during acetate administration (Puig & Fox, 1984; Tekkanat et al., 1988).

Although the initial perturbation in substrate utilization was reversed 30 min into the exercise bout there continued to be a significant increase in fat oxidation and a reciprocal decrease in carbohydrate oxidation over last 75 min of exercise in the NaAc trial; changes in substrate utilization that were not replicated over the same time period during the NaHCO₃ trial. There are several possible reasons for the different metabolic responses late in exercise. Firstly, there was a higher plasma fatty acid concentration 90 min into the exercise bout during the NaAc trial. Increasing FFA delivery has previously been shown to lead greater fat utilization and a down-regulation and therefore sparing of endogenous carbohydrate stores (Hargreaves et al., 1991; Odland et al., 1998). The increase in fatty acid availability during exercise was unexpected in light of the putative inhibitory effect that acetate has upon lipolysis at rest (Crouse et al., 1968). This finding does, however, support the results of Ball and Green (2001) where a greater glycerol concentration was found compared with their placebo trial (sodium chloride ingestion) after 45 min of exercise undertaken at 60% VO_{2max}.

Alternatively, the increase in fat oxidation and decline in carbohydrate utilization late in exercise in the NaAc trial could have been due to greater glycogen utilization at the start of exercise, such that the availability

of endogenous carbohydrate was lower toward the end of exercise necessitating an increased contribution from fats to meet the energy demand. However, this is an unlikely explanation as the difference in carbohydrate oxidation over the first 30 min of exercise was calculated to equate to 4.8 ± 6.1 g of glucose, or $<2\%$ of total resting muscle glycogen stores based on 1.6–1.8 g glycosyl U·kg wet weight⁻¹ in human skeletal muscle (Bangsbo et al., 1992; Putman et al., 1995; van Loon et al., 2001) and 20 kg muscle mass. Finally, the lower blood glucose concentration in the sodium acetate trial might have led to the reduction in carbohydrate oxidation in the NaAc trial. This too seems unlikely in light of evidence that even when muscle glycogen concentration is low, glucose oxidation is unaffected provided lipolysis is not impaired (Zderic et al., 2004). Furthermore the decline in blood glucose concentration was not different between trials suggesting a similar rate of utilization and none of the subjects displayed signs of hypoglycemia (i.e., blood glucose <2.5 mmol·l⁻¹) at the end of the exercise bout in the acetate trial.

There are a number of limitations in the current study. First, we did not measure blood gases to confirm that the change in blood pH was similar between the NaAc and NaHCO₃ trials, however, based on similar studies performed by our group (Smith et al., 2007) and others (Putman et al., 1995) the same degree of metabolic alkalosis would be expected. Secondly, in the current study the sodium salts were ingested rather than administered by infusion, as reported in the majority of previous studies (i.e., Akanji et al., 1989; Putman et al., 1995; Evans et al., 2001), which could raise questions concerning the bioavailability of acetate. It has been shown that acetate is limited by its intestinal absorption rate, which becomes saturated at concentrations above 40 mmol·l⁻¹ in humans (Schmitt et al., 1976), a concentration that is substantially lower than the acetate concentration of the ingested drinks (~300 mmol·l⁻¹). However, in our earlier study we found the peak acetate concentration occurred 45 min after consumption of the acetate drink (Smith et al., 2007) and as the NaAc drinks were given 90 and 45 min before the exercise the majority of the ingested acetate should have been absorbed before the onset of exercise. Thirdly, in the current study we chose to use NaHCO₃ rather than NaCl in our control arm. However, we believe that to accurately determine the effect of acetate on metabolism it was important to control for changes in pH as a means to separate out the effect(s) of acetate on fat and carbohydrate utilization from the effect of simply inducing a metabolic alkalosis. Finally, we have proposed that the reduction in fat oxidation following acetate administration was a result of increased acetyl group availability and subsequent elevated acetylcarnitine formation as previously observed by others following infusion of acetate (Howlett et al., 1999; Evans et al., 2001; Roberts et al., 2005). However, without measuring the muscle free carnitine and acetylcarnitine content we have no direct evidence that the ingestion of acetate, at

a dose administered in the current study, would result in changes in acetylation of carnitine that coincided with a level that could inhibit the suppression of fat oxidation at rest and during the early phase of exercise. Elucidating the temporal relationship between the two events should therefore be a key focus of future studies examining the metabolic effect of acetate administration.

In conclusion, ingestion of sodium acetate significantly reduced fat oxidation at rest without affecting carbohydrate utilization. When exercise was performed immediately after the rest period, there was continued suppression in fat oxidation for the first 15 min. Oxidation of exogenous acetate did not completely account for the decrease in fat utilization at this time, and as a consequence, carbohydrate oxidation was significantly higher after 15 min during the NaAc trial. By 30 min into exercise, these effects had been reversed, with no difference in substrate utilization between trials; events that coincided with plasma acetate concentration returning to baseline levels in the NaAc trial. Over the final 75 min of exercise, there was increased utilization of fatty acids and lower carbohydrate oxidation in the acetate trial only—possibly due to increased fatty acid availability in the NaAc compared with the NaHCO₃ trial.

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This study was supported by a University of Aberdeen research studentship (GIS). The authors have no relevant conflicts of interest to disclose.

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