Amino Acids (2009) 37:673–680
DOI 10.1007/s00726-008-0187-6

ORIGINAL ARTICLE

The effects of alanine ingestion on metabolic responses to exercise in cyclists

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Received: 10 July 2008 / Accepted: 24 September 2008 / Published online: 12 October 2008

Abstract The influence of alanine on plasma amino acid concentrations and fuel substrates as well as cycling performance was examined. Four solutions [6% alanine (ALA); 6% sucrose (CHO); 6% alanine and 6% sucrose (ALA–CHO); an artificially sweetened placebo (PLC)] were tested using a double-blind, randomised, cross-over design. During each trial, ten cyclists ingested 500 mL of test solution 30 min before exercise and 250 mL after 15, 30, and 45 min of exercise. Participants cycled for 45 min at 75% VO_2max followed by a 15-min performance trial. Blood was collected before beverage consumption and prior to the performance trial. Alanine concentration was increased ($p < 0.05$) by approximately tenfold for ALA and ALA–CHO and less than twofold for CHO and PLC. Alanine ingestion increased concentrations of most gluconeogenic amino acids. Overall, alanine supplementation tended to produce favourable metabolic effects, but did not influence performance.

Keywords Amino acids · Carbohydrate · Cycling · Athletes · Endurance

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Introduction

Exercise increases the demands of working muscles for fuel sources, yet endogenous energy stores of the muscles can supply only a fraction of the energy needed for athletic competition. Therefore, energy production from other endogenous (e.g., liver glycogen) and potentially exogenous fuel substrates (e.g., dietary carbohydrate) is necessary to avoid rapid fatigue. Dietary regimens that alter substrate utilization, to improve energy availability for working muscles, are major foci of sports nutrition.

Carbohydrate supplementation has been demonstrated to increase performance of several different types of physical activity by providing fuel to the muscles in the form of glucose. Exogenous carbohydrate reduces endogenous carbohydrate oxidation (Burelle et al. 1999), thereby promoting enhanced endurance performance (Below et al. 1995; Coggan and Coyle 1987; Febbraio et al. 2000; McConell et al. 1999). Much attention has recently turned to the potential roles of dietary amino acids and proteins in reducing fatigue and promoting muscle synthesis or decreasing catabolism (Ivy et al. 2003; Koopman et al. 2004; Niles et al. 2001).

Muscle protein turnover increases during strenuous physical activity (Dohm et al. 1987) and exercise produces an approximately two- to threefold increase in the flux of alanine into the bloodstream (Williams et al. 1998). Supplementation of alanine decreases the catabolism of essential amino acids under some conditions including various inborn errors of metabolism (Bodamer et al. 2000; Kelts et al. 1985; Wolff et al. 1985); however, its influence on amino acid catabolism during exercise has not been investigated. Ingestion of either carbohydrate or alanine can diminish post-exercise ketosis (Carlin et al. 1987; Koeslag et al. 1982), with the effects of alanine lasting

longer than those of glucose (Koeslag et al. 1982). Furthermore, ingestion of alanine or glucose can suppress serum concentrations of free fatty acids, presumably by serving as preferential fuel substrates (Carlin et al. 1987). Alanine oxidation increases during vigorous exercise (White and Brooks 1981), and human research has demonstrated that alanine oxidation accounts for approximately 10% of total energy production when consumed at the rate of 1 g/kg during prolonged submaximal exercise (Korach-Andre et al. 2002).

Overall, limited research is available regarding changes in metabolism during exercise with alanine administration and its potential effect on endurance exercise; therefore, the goal of this study was to examine the influence of alanine supplementation on metabolic responses to short term endurance cycling. To accomplish this, we compared the effects of beverages containing alanine, sucrose, alanine plus sucrose, and an artificially flavoured and sweetened placebo on concentrations of plasma amino acids and serum fuel substrates. We hypothesized that alanine, particularly when combined with dietary carbohydrate, would favourably affect concentrations of serum fuel substrates and amino acids. Furthermore, we incorporated a 15-min endurance trial after 45 min of vigorous submaximal exercise to determine if alanine supplementation would enhance short-term endurance exercise performance.

Materials and methods

Subjects

Thirteen trained cyclists volunteered to participate in this study. All had been cycling for at least 1 year. Participants cycling less than 4 h per week were excluded. Three withdrew for personal reasons prior to completing all trials; therefore, data of ten subjects (nine males and one female) are presented. Table 1 presents the physical characteristics of the subjects who completed the study. Prior to initiating the study, all five participants were required to complete a health risk screening questionnaire and sign a written consent. The protocol was approved by the San Diego State University Institutional Review Board, the Human Research Protection Program at the University of California, San Diego, and the General Clinic Research Centre at the University of California, San Diego.

Table 1 Physical characteristics of the participants ($n = 10$)

Variable	Mean ± SD
Age (years)	34.7 ± 4.5
Height (m)	1.76 ± 0.12
Mass (kg)	76.2 ± 12.1
VO_2max (mL/kg/min)	52.6 ± 9.8

Research design

Initially, subjects reported to the laboratory for assessment of peak oxygen uptake (VO_2max) using a ramped exercise test and for familiarization with the exercise protocol. All exercise tests were performed on an electronically braked cycle ergometer (Lode, The Netherlands).

For each trial, subjects reported to the laboratory at approximately the same time of day, on four separate occasions, after an overnight (12 h) fast. Subjects abstained from alcohol, tobacco, and strenuous exercise for the previous 24 h and consumed a standardized dinner 12 h before arriving at the laboratory. Participants were asked to maintain a food diary for the 2 days prior to each trial. Each trial was separated by a minimum of 7 days to allow subjects to return to normal training routines.

Prior to each trial, subject's body mass was measured and a fasting pre-exercise blood sample was collected. Immediately afterward, subjects were given 500 mL of an aqueous test solution and rested for 30 min. Subsequently subjects warmed up at a workload equivalent to 65% of their VO_2max for 5 min. The workload was then increased to 75% of VO_2max for 45 min. At 15 and 30 min into exercise, the subjects were given 250 mL of the same beverage. At 45 min of exercise, an exercising blood sample was collected, followed by consumption of another 250 mL of the test solution. At this time, subjects commenced a 15-min performance trial with the cycle ergometer set in isokinetic mode. The amount of work completed during the 15-min time trial was used to assess performance.

The beverages tested contained (a) 6% alanine (ALA), (b) 6% sucrose (CHO), (c) 6% alanine plus 6% sucrose (ALA–CHO), or (d) an artificially flavoured and sweetened placebo (PLC). Alanine was obtained from Ajinomoto, USA, Inc. All beverages were prepared with the same flavour (orange) and sweetness, by using a non-caloric sweetener. All beverages contained sodium (458 mg/L) and potassium (104 mg/L). To maintain blinding of investigators, a person not involved in the exercise testing prepared and labelled the solutions. The trials were conducted using a randomized cross-over approach, with a double-blind design. To minimize thermal stress, the temperature in the laboratory was kept at 21 ± 1°C and the subjects were cooled with an electric fan during exercise.

At the end of each trial, subjects rated the flavour of the test solution using a 100 mm visual analogue scale. On one extreme of the scale (0 mm) was the descriptor "do not like at all". At the other extreme (100 mm) was the descriptor "like 100%". At the same time, subjects were also asked to rate their gastrointestinal discomfort during the trial as their

level of "upset stomach/cramping" using a 100 mm visual analogue scale. At one extreme (0 mm) was the descriptor "not at all upset" and at the other extreme (100 mm) was the descriptor "totally upset".

Blood samples were collected from the antecubital region in the following: tubes containing heparin for analysis of plasma amino acids, free fatty acids, and 3-hydroxybutyrate; tubes containing potassium oxalate/sodium fluoride for analysis of glucose and lactate; tubes with no additive for analysis of insulin and blood urea nitrogen (BUN); and tubes containing EDTA for analysis of hematocrit and hemoglobin. Plasma amino acid analyses were performed using a dedicated amino acid analyzer (Beckman Model 6300, Fullerton, CA). Glutamate and asparagine concentrations were expressed as sums of the two amino acids. Free fatty acids were measured using a commercial kit purchased from Wako Chemicals GmbH (Neuss, Germany). Maughan's fluorimetric method was used to assess 3-hydroxybutyrate concentrations (Maughan 1981). Glucose and lactate were analyzed using YSI auto-analyzer (Yellow Springs, OH). Insulin was assessed by radioimmunoassay using a kit from Linco Research, Inc. (St Charles, MO). BUN was analyzed colourmetrically (Stanbio Labs, Boerne, TX).

All post-exercise values were corrected for plasma volume shifts using haemoglobin and hematocrit measured according to the method of Dill and Costill (1974). Hemoglobin was analyzed by the cyanmethemoglobin technique with a kit from Sigma Diagnostics (St Louis, MO), and hematocrit was determined using micro-capillary tube centrifugation. Dietary analyses were performed using Nutritionist Pro software (First DataBank, San Bruno, CA).

Statistical analyses

Statistical analyses were conducted using the Statistical Program for the Social Sciences (SPSS, version 10.0) computer software package. Data are presented as means ± standard deviation. A one-way analysis of variance was used to analyze the difference in performance between the trials. Repeated measures analysis of variance (four trials × two time-points) tests were used to analyze differences in biochemical variables. Paired comparisons *t* tests were employed as post-hoc tests where necessary. An alpha level of $p < 0.05$ was considered statistically significant.

Results

Dietary intake preceding each trial was similar for energy, protein, carbohydrate, and fat (Table 2). No significant differences in cycling performance were detected between the trials (Table 3). Table 4 indicates that participants preferred ($p < 0.05$) the CHO beverage compared to those solutions that contained alanine and tended to prefer the PLC beverage compared to ALA ($p < 0.05$) and ALA–CHO ($p < 0.10$). However, all participants reported that they remained blinded to test beverage identities throughout the study. No significant difference in gastro-intestinal discomfort during testing was reported between trials (Table 4).

Plasma amino acids

Fasting concentrations of plasma amino acids were not significantly different in the pre-exercise state among the trials (Table 5). The most striking change in plasma amino acid concentrations after exercise was the significant increase ($p < 0.05$) in alanine within each of the trials. While alanine supplementation clearly elevated post-exercise plasma concentrations of alanine (roughly tenfold for

Table 2 Dietary intake of participants for 2 days prior to each trial

Trial	Energy (kcal)	Protein (g)	Carbohydrates (g)	Fat (g)
ALA	2,744 ± 1,147	107 ± 28	332 ± 204	120 ± 88
CHO	2,449 ± 656	96 ± 26	311 ± 160	120 ± 111
ALA–CHO	2,345 ± 742	86 ± 12	285 ± 155	123 ± 120
PLC	2,471 ± 599	103 ± 30	298 ± 134	117 ± 92

Mean ± SD

Table 3 Performance at the end of exercise bouts with consumption of a test solutioin containing alanine, carbohydrate, alanine plus carbohydrate or placebo

Trial	Work Completed (kj)
ALA	219 ± 34
CHO	222 ± 39
ALA–CHO	218 ± 48
PLC	229 ± 36

Mean ± SD

Table 4 Ratings of flavor for test solutions and levels of gastrointestinal (GI) discomfort for each trial[1,2]

Trial	Flavor	GI Discomfort
ALA	39 ± 30[a]	26 ± 30
CHO	69 ± 25[b]	25 ± 25
ALA–CHO	44 ± 28[a,c]	26 ± 29
PLC	64 ± 22[b,c]	11 ± 21

Mean ± SD

Within a variable, values with different superscripts are significantly different ($p < 0.05$)

Table 5 Pre- and post-exercise concentrations (μmol/L) of plasma amino acids for ALA, CHO, ALA–CHO, and PLC trials

Variable	ALA Trial		CHO Trial		ALA–CHO Trial		PLC Trial	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
Alanine	381 ± 67	3,672 ± 1,384*,a	395 ± 100	617 ± 167*,b	401 ± 83	4,152 ± 1,224*,a	374 ± 99	488 ± 73*c
Arginine	87 ± 19	93 ± 19	86 ± 27	86 ± 26	92 ± 18	90 ± 18	85 ± 22	82 ± 17
Aspartate	6 ± 3	29 ± 19*,a	6 ± 3	5 ± 2b	4 ± 1	34 ± 23*,a	6 ± 3	4 ± 1b
Glutamine	679 ± 77	853 ± 76*,a	676 ± 80	708 ± 114b	702 ± 55	869 ± 88*,a	688 ± 83	654 ± 63b
Glutamate + Aspargine	104 ± 17	197 ± 46*,a	104 ± 23	98 ± 36b	106 ± 11	208 ± 39*,a	105 ± 20	91 ± 17*,b
Glycine	251 ± 44	334 ± 75*,a	257 ± 27	270 ± 45b	264 ± 44	353 ± 53*,a	256 ± 34	234 ± 31*,b
Histidine	85 ± 10	85 ± 11	90 ± 11	90 ± 20	88 ± 9	89 ± 8	84 ± 13	82 ± 16
Isoleucine	73 ± 17	65 ± 15*	82 ± 24	71 ± 19	78 ± 18	66 ± 15*	74 ± 15	65 ± 14*
Leucine	150 ± 30	133 ± 29*	159 ± 39	141 ± 33	155 ± 28	130 ± 22*	150 ± 26	133 ± 25*
Lysine	179 ± 19	178 ± 32	184 ± 29	183 ± 33	185 ± 19	177 ± 23	182 ± 21	167 ± 19*
Methionine	29 ± 4	30 ± 5	30 ± 6	31 ± 8	30 ± 4	31 ± 5	29 ± 6	28 ± 5
Ornithine	63 ± 13	55 ± 10*	67 ± 23	55 ± 15*	63 ± 16	54 ± 10*	66 ± 13	51 ± 8*
Phenylalanine	58 ± 6	50 ± 9*	57 ± 7	59 ± 12	58 ± 6	51 ± 7*	58 ± 13	56 ± 6
Proline	223 ± 96	340 ± 110*,a	204 ± 60	216 ± 58b	224 ± 72	356 ± 93*,a	183 ± 58	175 ± 64c
Serine	100 ± 21	182 ± 64*,a	107 ± 17	106 ± 23b	104 ± 18	194 ± 51*,a	104 ± 18	91 ± 16*,c
Taurine	62 ± 8	92 ± 19*,a	68 ± 11	85 ± 22*a	61 ± 7	94 ± 17*,a	66 ± 9	73 ± 14*,b
Threonine	132 ± 31	246 ± 78*,a	135 ± 30	136 ± 33b	133 ± 25	259 ± 68*a	132 ± 23	117 ± 22*b
Tyrosine	67 ± 7	68 ± 12	69 ± 12	67 ± 12	70 ± 5	68 ± 9	65 ± 8	63 ± 9
Valine	271 ± 48	252 ± 47	276 ± 60	249 ± 58*	275 ± 47	251 ± 40*	267 ± 40	234 ± 44*

Mean ± SD

*Denotes significant differences after exercise within a trial ($p < 0.05$)

Within a variable, values with different superscript(s) are significantly different between trials at that time point ($p < 0.05$)

both ALA and ALA–CHO trials), its concentration also rose significantly ($p < 0.05$) following exercise in the CHO (~1.6-fold) and PLC (~1.3-fold) trials. It is notable that the plasma alanine concentration was significantly higher ($p < 0.05$) at the end of the exercise period for the CHO trial compared to the PLC trial. Alanine ingestion, with or without carbohydrate, increased ($p < 0.05$) post-exercise concentrations of plasma glutamine, aspartic acid, and proline. Furthermore, when compared to the PLC trial, CHO produced higher ($p < 0.05$) post-exercise plasma concentrations of glutamine and proline. When solutions containing alanine were fed, the plasma concentrations of glycine, serine, and threonine, as well as the sum of glutamate plus asparagine, increased significantly ($p < 0.05$) by the 45th minute of exercise. Conversely, concentrations of these same amino acids decreased ($p < 0.05$) by that time for the PLC trial and remained unchanged ($p > 0.05$) by feeding the CHO solution. Plasma concentrations of the branched chain amino acids (leucine, isoleucine, and valine) tended to decrease after exercise for all trials. However, the drops in leucine and isoleucine concentrations were not statistically significant ($p > 0.05$) during the CHO trial and the decrease in valine was not statistically significant ($p > 0.05$) during the ALA trial. Plasma lysine concentrations decreased ($p < 0.05$) during the PLC trial, but were maintained during the other trials. Phenylalanine concentrations were decreased ($p < 0.05$) following exercise during the trials in which alanine was fed, but not the CHO or PLC trials. Ornithine concentrations decreased ($p < 0.05$) in every trial but no significant differences among trials were observed. Taurine increased ($p < 0.05$) during all the trials except for PLC. Concentrations of arginine, histidine, methionine, and tyrosine remained unchanged ($p > 0.05$) after exercise across all trials.

Biochemical responses

Fasting concentrations of glucose, insulin, lactate, free fatty acids, and 3-hydroxybutyrate in plasma/serum were similar ($p > 0.05$) in the fasted, pre-exercise state among the trials (Table 6). Plasma glucose concentrations tended to rise during the CHO trial and fall during the PLC trial resulting in a significant difference after 45 min of exercise. No changes in plasma glucose concentrations were detected after exercise in the two trials in which alanine was consumed. In addition, serum concentrations of insulin decreased significantly ($p < 0.05$) during the ALA and PLC trials, in which no exogenous sources of carbohydrate

Table 6 Pre- and post-exercise concentrations of 3-hydroxybutyrate, free fatty acids, glucose, insulin, lactate, and BUN in plasma for ALA, CHO, ALA–CHO, and PLC trials

Variable	ALA Trial		CHO Trial		ALA–CHO Trial		PLC Trial	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
3-Hydroxybutyrate (nmol/mL)	3.6 ± 2.4	3.5 ± 1.8	2.7 ± 2.6	2.6 ± 1.1	2.5 ± 1.3	2.4 ± 1.0	3.4 ± 2.3	3.2 ± 1.6
Free Fatty Acids (mmol/L)	0.42 ± 0.17	0.41 ± 0.18[a]	0.39 ± 0.15	0.36 ± 0.14[a]	0.41 ± 0.20	0.33 ± 0.12[a]	0.43 ± 0.16	0.56 ± 0.27[b]
Glucose (mg/dL)	81 ± 17	81 ± 9	80 ± 17	102 ± 28[a]	82 ± 17	82 ± 20	81 ± 14	73 ± 19[b]
Insulin (μU/mL)	9.2 ± 4.9	5.5 ± 3.6*[,a]	8.6 ± 2.4	12.6 ± 10.0[b]	8.0 ± 2.6	9.0 ± 6.5	7.9 ± 2.4	4.7 ± 2.1*
Lactate (mg/dL)	13.0 ± 7.3	35.9 ± 20.3*	13.8 ± 7.1	39.1 ± 12.7*[,a]	12.4 ± 4.4	40.5 ± 15.7*[,a]	14.2 ± 5.5	28.0 ± 9.6*[,b]
BUN (mg/dL)	13.6 ± 2.2	14.4 ± 2.9	13.6 ± 3.2	13.3 ± 3.7	13.1 ± 2.5	13.8 ± 2.9	13.8 ± 3.5	12.2 ± 2.8

Mean ± SD

*Denotes significant differences after exercise within a trial ($p < 0.05$)

Within a variable, values with different superscript(s) are significantly different between trials at that timepoint ($p < 0.05$)

were provided. Furthermore, post-exercise insulin concentrations were higher ($p < 0.05$) for the CHO trial compared to the ALA trial. The post-exercise concentrations of plasma lactate were significantly higher ($p < 0.05$) than baseline in all the trials. However, the post-exercise concentrations of lactate were significantly higher ($p < 0.05$) during the ALA, CHO, and ALA–CHO trials compared to the PLC trial. Plasma free fatty acid concentrations did not change significantly ($p > 0.05$) with exercise during any trial, but were higher at the post-exercise time point for PLC compared to all other trials. Plasma concentrations of 3-hydroxybutyrate remained stable throughout all trials and did not differ among trials after 45 min of exercise. BUN was not significantly ($p > 0.05$) changed during any trial.

Discussion

Alanine produced in the muscle can be transported through the blood to provide substrate for gluconeogenesis in the liver. Under normal circumstances, this requires that the diet and/or endogenous metabolism of protein/amino acids provide the sources of nitrogen for the alanine produced. Since alanine can serve as a significant exogenous energy source during exercise (Korach-Andre et al. 2002), we reasoned that the provision of alanine as a dietary supplement would serve as an alternate resource for the alanine "pool" and therefore prevent a decrease in circulating concentrations of other amino acids. Consistent with this hypothesis, alanine supplementation, with or without exogenous carbohydrate, augmented the exercise-induced increase in plasma alanine concentrations. Furthermore, the exogenous supply of carbohydrate tended to prevent the exercise-induced drop in plasma concentrations of gluconeogenic amino acids. In addition, alanine consumption, with or without carbohydrate, not only prevented the decrease in concentrations of many of these amino acids, but actually produced elevations in their concentrations. However, when alanine was included in the test beverage, the concentrations of the branched chain amino acids decreased to a degree similar to that which occurred for the PLC trial. Although, carbohydrate intake alone prevented a statistically significant exercise-induced drop in the concentrations of both leucine and isoleucine, the non-significant decrease was similar in magnitude to that detected during the other trials and is primarily explained by a greater variation in responses among subjects for plasma concentrations of these amino acids. Overall, alanine supplementation prevented the drop in gluconeogenic amino acids other than the branched chain amino acids. A similar "sparing" effect was not observed for ketogenic amino acids.

Since this is the first study to our knowledge that has addressed the effects of alanine supplementation before and during exercise, on plasma concentrations of amino acids, it is not possible to directly compare the results to other studies. However, Korach-Andre et al. (2002) examined the flux of exogenous ^{13}C and ^{15}N labeled alanine (73.7 ± 2 g) in healthy men during a 180-min cycling trial performed at a low intensity (53 ± 2% of VO_2max). The concentration of ^{15}N urea, measured in urine and sweat, was very low compared to the amount of ingested alanine that was decarboxylated and excreted as CO_2. This suggests that the amino-nitrogen of the alanine could have been incorporated in the synthesis of non-essential amino acids and protein. Although plasma amino acid concentrations were not measured in that study, the nitrogen conservation, along with the increase in gluconeogenic amino acid concentrations observed in this study, support

the possibility that alanine enhances gluconeogenic amino acid production. Thus, during exercise, alanine may promote gluconeogenesis in two ways. One is through the use of alanine as a substrate for glucose production via the glucose–alanine cycle. The other is as a donor of amino nitrogen in the production of gluconeogenic amino acids. Nevertheless, alanine decarboxylation and oxidation during exercise may be due to more than the simple conversion of alanine to glucose in the liver (Korach-Andre et al. 2002; White and Brooks 1981; Williams et al. 1998; Wolfe et al. 1984).

Previous research has suggested that prolonged exercise may ultimately lead to a reduction in plasma alanine concentration (Medelli et al. 2003; Rennie et al. 1981; Struder et al. 1995). In our research, alanine concentrations increased after a shorter exercise bout. This has been supported by previous research examining the effects of short-term exercise (20 min) on alanine metabolism (Bergstrom et al. 1985). It is not clear at what point the apparent reversal of the change in alanine concentrations occurs and which factors are responsible. However, it is likely that enhanced catabolism of alanine, helps to meet energy demands and to produce non-essential amino acids.

Alanine, which is considered the most gluconeogenic amino acid, and lactate, which is the end product of anaerobic glycolysis are transported from the peripheral tissues, via the circulation, to the liver. Here it is used as substrates for gluconeogenesis. In this study, both concentrations of alanine and lactate increased significantly in all trials. The concentration of alanine is increased via enhanced rates of transamination reactions. During exercise, alanine efflux, primarily from amino acids rather than liberation from protein, is approximately doubled (Williams et al. 1998). In addition, Williams et al. (1998) also suggest that alanine, rather than glutamine, serves as the main nitrogen carrier from the muscle to the liver during moderate intensity exercise. Wasserman et al. (1988) showed that exercise, in dogs, enhanced the conversion of alanine to glucose by intrahepatic mechanisms. Furthermore, these authors also showed increases in transmembrane transport within the hepatocyte and the release of substrates from extrahepatic tissues is stimulated by exercise (Wasserman et al. 1988). According to White and Brooks (1981), exercise enhances oxidative decarboxylation of alanine, although it does not appear that all oxidized alanine was first converted to glucose.

In this study consumption of CHO generated the expected increase in post-exercise serum insulin concentration. The addition of alanine along with the carbohydrate feeding abolished this effect. This difference is likely due to the stability of plasma glucose concentrations during the ALA–CHO trial; whereas, plasma glucose was elevated after exercise by feeding CHO alone. Similar carbohydrate only feeding regimens have also produced higher insulin concentrations during exercise (Davis et al. 1990). In contrast, a study by McConell et al. (2000), demonstrated a decrease in serum concentration of insulin when subjects consumed a carbohydrate supplement immediately before the start of exercise**.** During the control trial of that study, endogenous glucose production continued at the same rate throughout the bout of exercise; however, in the carbohydrate trial, glucose production was significantly suppressed at 22.5 min into exercise until the end of the trial but remained above resting levels. This, combined with the similar increase in the glucose clearance rate for both trials (McConell et al. 2000), offers an explanation for the detection of a lower concentration of glucose in the blood for the PLC trial in our study. The absence of a similar result for the ALA trial may be due to a glucose-sparing effect.

As reviewed by Noakes (2000), it has been postulated that the capacity to produce ATP (primarily from fat and carbohydrate) is the essential factor for determining athletic performance during endurance events. The catabolism of proteins provides an important source of energy, albeit to a lesser degree than fat or carbohydrate, for the muscle during exercise (Rennie et al. 1981). Previous research has suggested that the utilization of amino acids as an energy source can be reduced by alanine consumption under some circumstances (Kelts et al. 1985; Wolff et al. 1985). We also suspected that alanine could spare other fuel substrates, particularly glucose, during exercise. Therefore, it was hypothesized that alanine supplementation would enhance exercise performance at a level similar to that which occurs with the provision of exogenous carbohydrate. Furthermore, it was also hypothesized that an increase in performance by an alanine–carbohydrate feeding at twice the overall energy intake would be greater than the increase in performance by feedings of either alanine or a carbohydrate alone. While the metabolic shifts that occurred in this study could favour enhanced performance (i.e., higher concentrations of some blood-borne fuel substrates), this exercise protocol failed to elicit a significant difference in performance of the trained cyclists with treatment of ALA, CHO or the ALA–CHO combination. Interestingly, at the end of 45 min of exercise, plasma free fatty acid concentrations were lower for the groups supplied with exogenous fuels compared to PLC, suggesting that lipolysis may have been decreased. This suggests that alanine and carbohydrate may serve as preferred fuel substrates rather than "additional" energy substrates. Sparing fat utilization during exercise is not typically considered beneficial for performance.

Another explanation for the lack of a performance-enhancing effect of any of the treatments relative to PLC is the relatively short duration (1 h) of exercise performed by

our participants, which were trained cyclists. It is possible that under different conditions of testing (i.e., longer bout of submaximal exercise prior to the performance test, a longer performance test, use of sedentary subjects or a test of exercise to exhaustion), the experimental treatments would have improved performance. Although carbohydrate feedings have been demonstrated to enhance performance of exercise lasting 1 h (Below et al. 1995), other research has suggested that exogenous carbohydrate does not enhance exercise of a relatively short duration (McConell et al. 1999). A primary explanation for the purported performance enhancing effect of dietary carbohydrate consumption is that fatigue related to the lack of exogenous fuel hinges upon the notion that exercise must be performed for an adequate duration at an intensity sufficient to reduce endogenous fuel (e.g., glycogen) availability. These conditions could increase reliance upon exogenous energy consumption. It is therefore possible that a better test of our hypothesis that ALA, CHO, and ALA–CHO can enhance performance would include a longer exercise bout. An alternative explanation is that by comparing our feeding trials to a sweetened placebo, we may have produced a placebo effect that could have diminished our chances of observing a treatment effect.

Another interesting result of this study is the differential influence of the treatments on glutamine status. The ingestion of ALA and ALA–CHO led to increased glutamine concentration during exercise. This is a potentially important issue given the role of glutamine in the maintenance of normal immune system function in athletes (Walsh et al. 1998) and since prolonged exercise lowers plasma glutamine status (Van Hall et al. 1998) and plasma concentrations of glutamine are lower in overtrained versus control athletes (Parry-Billings et al. 1992). It is not possible to determine the full implications of the alanine-induced elevations in glutamine concentration from this study. However, these data suggest that the influence of alanine on glutamine metabolism and symptoms of overtraining should be investigated.

Exercise performance is affected by many factors. These factors can be biochemical in nature or simply external circumstances or stimuli that influence the outcome. Participants in this study tended to prefer the flavour of the PLC and CHO beverages versus the solutions containing alanine (Table 4). Furthermore, although no statistically significantly differences in gastrointestinal discomfort were detected, participants tended to report a higher rating for discomfort during the trials when alanine and/or carbohydrates were ingested in comparison to the PLC trial (Table 4). However, since no significant difference was detected, the likelihood that discomfort was sufficient to confound the performance tests is diminished. Of note, gastrointestinal discomfort has been reported previously following ingestion of 100 g of alanine (Koeslag et al. 1985), which is slightly more than the total (75 g) consumed by our subjects.

Overall, this research suggests that alanine supplementation (with or without carbohydrate) can prevent exercise-induced decreases in many gluconeogenic amino acids and promote a metabolic profile that could be favourable for performance, however it failed to enhance 1 h of endurance cycling in trained cyclists. Further investigation is necessary to determine the effects of alanine supplementation during more prolonged exercise and performance testing regimens. Future research is also needed to determine the dose response to supplementation of alanine and to determine if exogenous alanine can prevent exercise-induced protein catabolism.

Acknowledgments This study was partially supported by GCRC Grant No. NIH-MO1 RR00827.

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