

# Effects of glucose, glucose plus branched-chain amino acids, or placebo on bike performance over 100 km

KLAVS MADSEN, DAVE A. MACLEAN, BENTE KIENS, AND DIRK CHRISTENSEN  
*Department of Physical Education, Odense University, DK-5230 Odense M;  
and Copenhagen Muscle Research Center, Rigshospitalet, August Krogh Institute,  
University of Copenhagen, DK-2100 Copenhagen, Denmark*

**Madsen, Klavs, Dave A. MacLean, Bente Kiens, and Dirk Christensen.** Effects of glucose, glucose plus branched-chain amino acids, or placebo on bike performance over 100 km. *J. Appl. Physiol.* 81(6): 2644–2650, 1996.—This study was undertaken to determine the effects of ingesting either glucose (*trial G*) or glucose plus branched-chain amino acids (BCAA; *trial B*), compared with placebo (*trial P*), during prolonged exercise. Nine well-trained cyclists with a maximal oxygen uptake of  $63.1 \pm 1.5$  ml  $O_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  performed three laboratory trials consisting of 100 km of cycling separated by 7 days between each trial. During these trials, the subjects were encouraged to complete the 100 km as fast as possible on their own bicycles connected to a magnetic brake. No differences in performance times were observed between the three trials ( $160.1 \pm 4.1$ ,  $157.2 \pm 4.5$ , and  $159.8 \pm 3.7$  min, respectively). In *trial B*, plasma BCAA levels increased from  $339 \pm 28$   $\mu\text{M}$  at rest to  $1,026 \pm 62$   $\mu\text{M}$  after exercise ( $P < 0.01$ ). Plasma ammonia concentrations increased during the entire exercise period for all three trials and were significantly higher in *trial B* compared with *trials G* and *P* ( $P < 0.05$ ). The respiratory exchange ratio was similar in the three trials during the first 90 min of exercise; thereafter, it tended to drop more in *trial P* than in *trials G* and *B*. These data suggest that neither glucose nor glucose plus BCAA ingestion during 100 km of cycling enhance performance in well-trained cyclists.

ammonia; glutamine; tryptophan; endurance exercise; well-trained athletes

ENERGY DEMAND AND FUEL SUPPLY are important factors that influence exercise performance. The glycogen stores in the body are small, and depletion of these stores may occur toward the end of endurance exercise. If the rate of fat utilization is insufficient to maintain the energy demand at this time, fatigue occurs (1). The benefits of glucose administration during prolonged exercise have been reported by many investigators (7, 8, 10, 24), resulting in the maintenance of blood glucose levels and, in some studies, a reduction in muscle glycogen utilization (15, 27).

Recently, branched-chain amino acids (BCAA) have been introduced into the etiology of limitation in prolonged exercise (25). It is well known that amino acid oxidation increases with prolonged exercise, but the actual contribution of amino acids to energy demand is very minimal (14). However, it has been suggested that the oxidation of proteins and particularly of the BCAA may be greater, or play a larger role, when muscle glycogen is limited (19). During prolonged exercise where depletion of muscle glycogen occurs, a reduced plasma BCAA concentration has been observed, possibly due to a greater uptake and utilization of BCAA by

glycogen-depleted muscles (3). In conjunction with the falling BCAA levels toward the end of exercise, an increase in the plasma concentration of tryptophan has been observed (5). Tryptophan might have an influence on central fatigue, since it is synthesized into serotonin in specific areas of the brain. Brain serotonin is known to play a role in pain, arousal, and mood (31). Tryptophan is transported across the blood-brain barrier via a specific transport mechanism that it shares with BCAA (6), so an increase in plasma concentration ratio of free tryptophan/BCAA has been proposed to contribute to fatigue during prolonged exercise.

On the basis of this hypothesis, Blomstrand et al. (4) in a field study tried to reduce the availability of tryptophan to the brain by giving a BCAA solution during prolonged exercise. Running performance in a marathon (42.2 km) was improved for the “slower” runners when BCAA was taken during the race; however, there was no effect on the performance in the “faster” runners. It should be noted that in the study by Blomstrand et al. no control group was used, and a change in performance was measured as a subject’s ability to run an interval late in the race faster than he or she did earlier in the race. In a recent study by van Hall et al. (28), the researchers failed to find any performance effect of BCAA ingestion during prolonged exercise and, furthermore, they also showed that ingestion of tryptophan had no negative effect on endurance performance. Therefore, it is still an open question whether BCAA supplementation can affect performance, especially in well-trained athletes with higher glycogen stores than in untrained individuals (26) and with a higher potential for lipid utilization (17). In addition to these considerations, supplementation of glucose during prolonged exercise could spare the muscle glycogen stores, and thus the oxidation of BCAA might be negligible. It is therefore possible that glucose administration is just as effective as the administration of glucose plus BCAA as far as the “tryptophan effect” is concerned.

The aim of this study was to investigate whether the administration of glucose or glucose plus BCAA during a controlled laboratory cycling event lasting 2.5–3 h (100 km) would enhance performance in comparison with the administration of only water in well-trained athletes.

## MATERIALS AND METHODS

Nine well-trained male subjects volunteered to take part in this study. They were experienced bicyclists or triathletes, aged  $26.9 \pm 1.1$  yr, weighing  $78.9 \pm 1.9$  kg, and with a maximal  $O_2$  uptake ( $\dot{V}O_{2\text{max}}$ ) of  $63.1 \pm 1.5$  ml  $O_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ .

The subjects were fully informed about the nature of the experiments and what was required of them before they volunteered to take part in this study. The experimental protocol was approved by the local Ethics Committee.

After three to five preliminary tests in which the subjects underwent  $\dot{V}O_{2\max}$  tests and were familiarized with the laboratory equipment and procedures, the subjects were required to complete three 100-km bicycle experiments separated by 7 days. During these trials, the subjects were encouraged to complete the 100 km as fast as possible on their own bicycles connected to a magnetic brake (Olimpionic-Cicl training, Politecnica 80, Italy). The three tests were carried out with supplements of either 1) glucose (*trial G*), 2) glucose plus BCAA (*trial B*), or 3) placebo (*trial P*), the order being determined in a Latin square design. Exercise training by each subject 3 days before the three trials was controlled and identical in training time and intensity. Food intake was controlled, and the same amount of energy and carbohydrates was consumed on an individual basis during the 3 days before the three experimental trials.

The subjects reported to the laboratory after a 4-h fast. They were weighed, and then a catheter was introduced into a dorsal hand vein to obtain blood samples while the subjects were cycling. The catheter was flushed with a 0.9% sodium chloride solution after each blood sample. After the subjects had rested supine for ~20 min, a venous blood sample was obtained. The subjects completed a 5-min warm-up period equivalent to 60%  $\dot{V}O_{2\max}$ , and then they were asked to keep an intensity of 70%  $\dot{V}O_{2\max}$ . This intensity was an appropriate guideline for each subject at the start of the 100 km and was maintained for the first 15 km. Thereafter, each subject chose their own intensity. A minicomputer (Colli no. 1 cartone) simultaneously registered speed and distance. The subjects were not informed about their cycling speed and exercise time until the whole experiment was finished; only the covered distance was visible for the subjects. Previously we have investigated the reproducibility of the 100-km performance test and found that the coefficient of variation was 3.5% (unpublished observations). With the nine subjects selected for this study, the minimal difference for statistical significance is 3.5 min. Before and during each experimental trial, glucose, glucose plus BCAA, or placebo were administered in a double-blind design. In *trial G*, the subjects ingested a 5% carbohydrate solution: 87.5 g of maltodextrins and 87.5 g of glucose, 1.5 g of sodium cyclamate, and 10 g of lemon in 3.5 liters of water. In *trial B*, the subjects ingested the same solution as in *trial G*, with the addition of 18 g of BCAA: 50% valine, 35% leucine, and 15% isoleucine. In *trial P*, the subjects ingested 1.5 g of sodium cyclamate and 10 g of lemon in 3.5 liters of water. The taste of the three solutions was indistinguishable, and the subjects as well as investigators were unaware of the composition of the solutions that the subject were given. The quantity and timing for ingestion of the solutions were as follows: 600 ml of the given solution immediately before exercise, 200 ml after 15 min of exercise, 350 ml after 35 min, and then 350 ml every 30 min.

Throughout the exercise, expired gas samples, heart rate (HR), and blood samples were obtained at *minutes* 10, 30, 60, 90, 120, 150 and at termination of exercise.

**Analyses.** Expired air was sampled in a Douglas bag.  $O_2$  and  $CO_2$  contents were determined with a paramagnetic oxygen analyzer (Servomex OA 184) and an infrared  $CO_2$  analyzer (Bechman LB2). Expired gas volume was measured with a 130-liter Tissot spirometer. HR was obtained with a Sporttester PE 3000 (Polar Electro KY, Finland).

Each blood sample was separated into sodium-heparinized vacutainer tubes for analyses of amino acids, ammonia,

glycerol, free fatty acids (FFA) (1 ml of blood was transferred into a tube containing ethylene glycol-bis( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid), glucose, and lactate. Blood samples were analyzed for lactate by an enzymatic lactate analyzer (Yellow Springs Instruments lactate analyzer model 23L), and glucose was determined spectrophotometrically with hexokinase and glucose-6-phosphate dehydrogenase (2). Plasma was separated by centrifugation at 4°C. Glycerol in plasma was analyzed by using an enzymatic method (Boehringer Mannheim) adjusted to fluorometric assays. Fatty acids in plasma were determined fluorometrically as described by Kiens et al. (18). Plasma amino acids were measured in duplicate by prior derivatization with phenylisothiocyanate and high-performance liquid chromatography (16). Ammonia was measured on the Kodak Ektachem DT60 (Eastman Kodak, Rochester, NY). Hemoglobin was determined with a spectrophotometer (Hemocue, Helsingborg, Sweden) to document changes in plasma volume.

**Statistics.** The data from the three trials were compared using a two-way analysis of variance for repeated measures. When a significant main effect and/or interaction occurred, the location of pairwise differences between mean values was identified by using a Student-Newman-Keuls multiple-range test. *P* values <0.05 were taken to indicate statistical significance. All data are reported as means  $\pm$  SE.

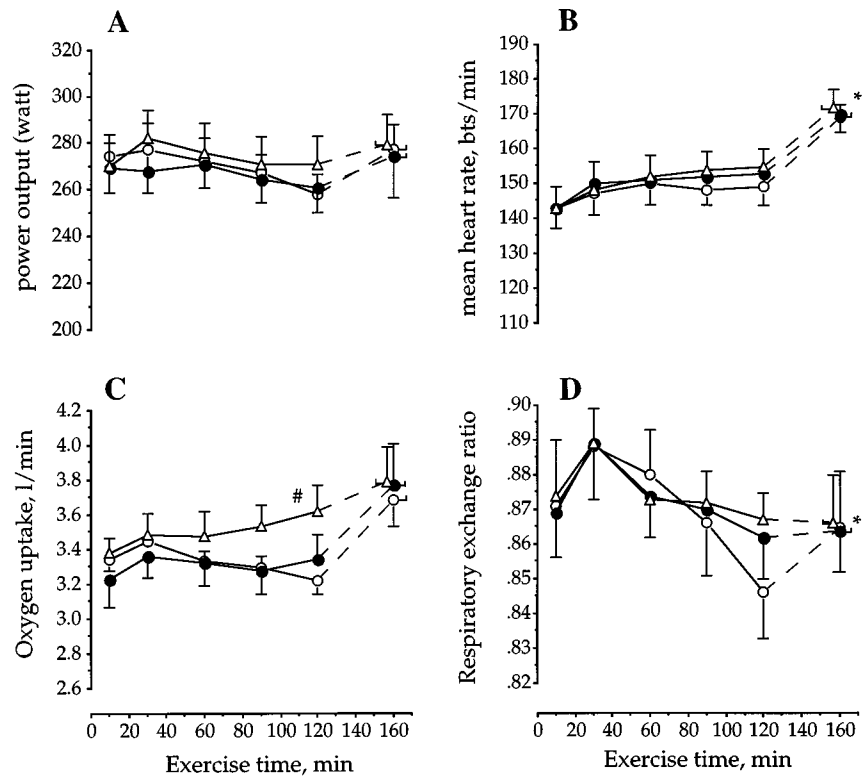
## RESULTS

No significant differences were observed among trials in performance time for the 100-km cycling exercise. The exercise time was  $160.1 \pm 4.1$  min in *trial G*,  $157.2 \pm 4.5$  min in *trial B*, and  $159.8 \pm 3.7$  min in *trial P* (not significant). The power output during the exercise period was remarkably constant, although the subjects were unaware of both power output and exercise time (Fig. 1). There were no significant differences among the three trials. The preselected mean workload during the first 15 km was  $271 \pm 1$  W, and this corresponded very well to the total mean workload of  $270 \pm 1$  W. As presented in Fig. 1, exercise elicited an average oxygen uptake ( $\dot{V}O_2$ ) of  $3.26 \pm 0.07$  l/min after 10 min of exercise and remained constant thereafter. Mean  $\dot{V}O_2$  (*minutes* 10–120) was  $3.50 \pm 0.06$  l/min in *trial B*,  $3.31 \pm 0.06$  in *trial G*, and  $3.39 \pm 0.04$  in *trial P*, and there was a significant time effect in *trial B* compared with *trials G* and *P* ( $P < 0.05$ ). The average  $\dot{V}O_2$  corresponded to slightly less than 70% of  $\dot{V}O_{2\max}$ . There was a gradual rise in HR with time for all trials (Fig. 1). The average HR during *trials B*, *G*, and *P* was  $154 \pm 3$ ,  $154 \pm 3$ , and  $151 \pm 2$  beats/min, respectively.

There was a steady decrease in respiratory exchange ratio (RER) with increasing duration of exercise ( $P < 0.01$ ), except for the final measurements performed during the finishing spurt (Fig. 1). The RER values were similar in all three trials, although they appeared to decrease in a more pronounced manner in *trial P* in the late exercise period, where RER declined to a value of  $0.846 \pm 0.013$  at *minute* 120, compared with  $0.862 \pm 0.012$  in *trial G* and  $0.867 \pm 0.008$  in *trial B*, respectively ( $P = 0.08$ ).

Blood glucose concentrations increased significantly at the beginning of the exercise period in *trial G*; thereafter, the blood glucose levels stabilized (Fig. 2). In *trial B*, blood glucose levels remained unchanged

Fig. 1. Average effect (A), heart rate (B), O<sub>2</sub> uptake (C), and respiratory exchange ratio (D) during 100-km bicycling in glucose trial (G; ●), branched-chain amino acids (BCAA) + glucose trial (B; △), and placebo trial (P; ○). Data are means ± SE of 9 subjects. \*Significant time effect ( $P < 0.01$ ). #Significant difference from trials P and G (time effect from minute 10 to 120;  $P < 0.05$ ).

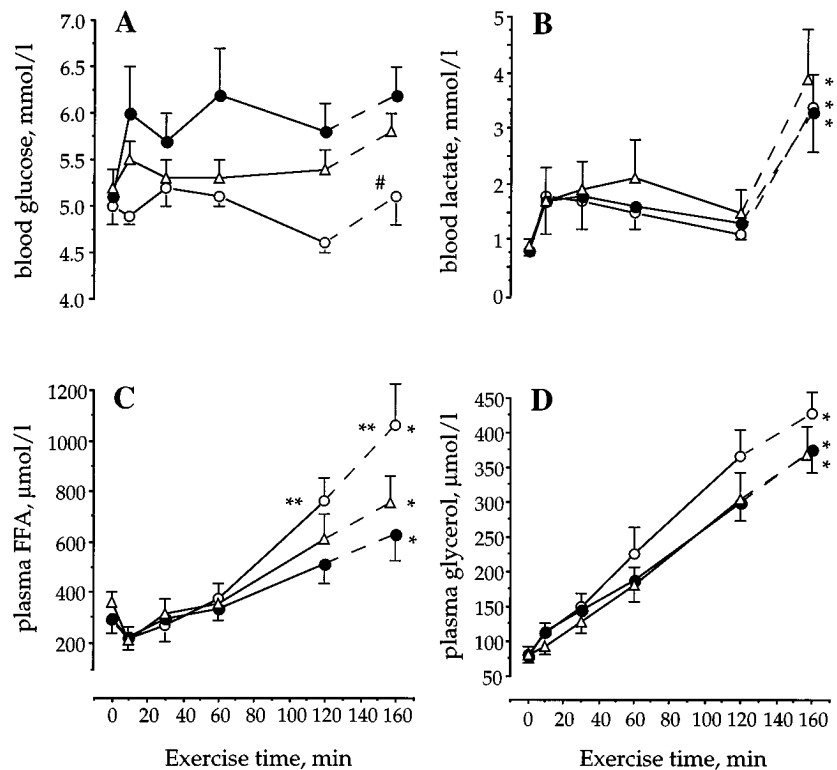


throughout the entire exercise period. In trial P, glucose levels were identical with those in trial B in the first part of the exercise period but declined in the late phases of exercise ( $P < 0.05$ ) to  $4.6 \pm 0.1$  mmol/l. After 10 min of exercise, blood lactate concentrations increased to  $1.8 \pm 0.5$  mmol/l in all three trials, a level

that was maintained throughout the exercise period, except during the finishing spurt where blood lactate concentrations reached  $3.4 \pm 0.6$  mmol/l (Fig. 2,  $P < 0.01$ ).

Blood glycerol concentrations increased ( $P < 0.01$ ) similarly in all three trials from  $81 \pm 11$   $\mu$ mol/l at rest

Fig. 2. Blood glucose (A), blood lactate (B), plasma free fatty acids (FFA; C), and plasma glycerol (D) as a function of time during exhaustive bicycling in trial G (●), trial B (△), and trial P (○). Data are means ± SE of 9 subjects. \*Significant time effect ( $P < 0.01$ ). \*\*Significant difference from trials G and B at 120 min and at end of exercise ( $P < 0.05$ ). #Significant difference from trials G and B ( $P < 0.05$ ).



to peak values of  $370 \pm 34 \mu\text{mol/l}$  in *trials G* and *B* and to  $429 \pm 30 \mu\text{mol/l}$  in *trial P* (Fig. 2). Plasma FFA concentrations increased similarly in all three trials (Fig. 2,  $P < 0.01$ ). In *trials G* and *B*, plasma FFA concentrations were lower than in *trial P* from *minute 120* to the end of exercise ( $P < 0.05$ ).

All three trials demonstrated significantly increasing plasma ammonia concentrations during exercise (Fig. 3). Plasma ammonia levels were significantly higher for *trial B* compared with *trials G* and *P* ( $P < 0.05$ ), and a significant time effect appeared ( $P < 0.05$ ).

The BCAA supplementation resulted in a significant increase in the venous plasma BCAA concentrations (*trial B*) throughout the exercise period ( $P < 0.01$ ; Fig. 3). In *trial B*, the plasma total and essential amino acid concentrations were both significantly elevated above the levels shown in *trials G* and *P*. However, the significant increases in total and essential amino acid levels were related to the increase in the BCAA level. The total amino acids minus BCAA and the essential amino acids minus BCAA were not significantly different among the three trials (Fig. 3).

Significant changes after supplementation were observed for the following amino acids: BCAA (valine, leucine, and isoleucine), arginine, tryptophan, tyrosine, and glutamine (glutamine and tryptophan are presented in Table 1). Plasma glutamine, tyrosine, tryptophan, and arginine concentrations were elevated ( $P < 0.01$ ) during exercise in *trial P*. Plasma glutamine levels were greater for *trial B* than for *trials G* and *P* ( $P < 0.01$ ; treatment and time effect), and in contrast to *trial P*, plasma tyrosine concentrations remained unchanged during *trials B* and *G* ( $P < 0.01$ ; *trials B* and *G* vs. *trial P*, treatment and time effect). Plasma trypto-

phan concentrations increased more in *trial P* compared with *trial B* ( $P < 0.05$ ), and plasma arginine concentrations increased more for *trial B* than for *trial P* ( $P < 0.05$ ). There were no significant differences between treatments for any other plasma amino acids.

## DISCUSSION

**Performance.** A variety of investigations have shown that carbohydrate feedings during prolonged exercise can delay fatigue and improve cycling performance (7, 8, 10, 24). This is accomplished primarily by the maintenance of blood glucose levels and a reduction in muscle glycogen utilization. These effects are most important toward the end of exercise, as exhaustion approaches and glycogen is depleted. The majority of investigators who have examined the effects of exogenous carbohydrate supplementation have utilized a time-to-exhaustion test. This is a useful test to search for explanations regarding fatigue, but the observation that carbohydrate ingestion improved time to exhaustion does not necessarily mean that it would improve performance in other exercise situations or when exhaustion is not reached.

In the present study, a controlled laboratory test was used to mimic a competition situation where a given distance (100 km) was to be covered as fast as possible. In this case, each subject selected his own exercise intensity and cycled for  $\sim 2.5$  h on flat terrain. It was demonstrated under these conditions that carbohydrate supplementation, of the magnitude that has been shown to increase performance under exhaustive exercise conditions, did not increase performance compared with placebo. One reason for this finding is that the

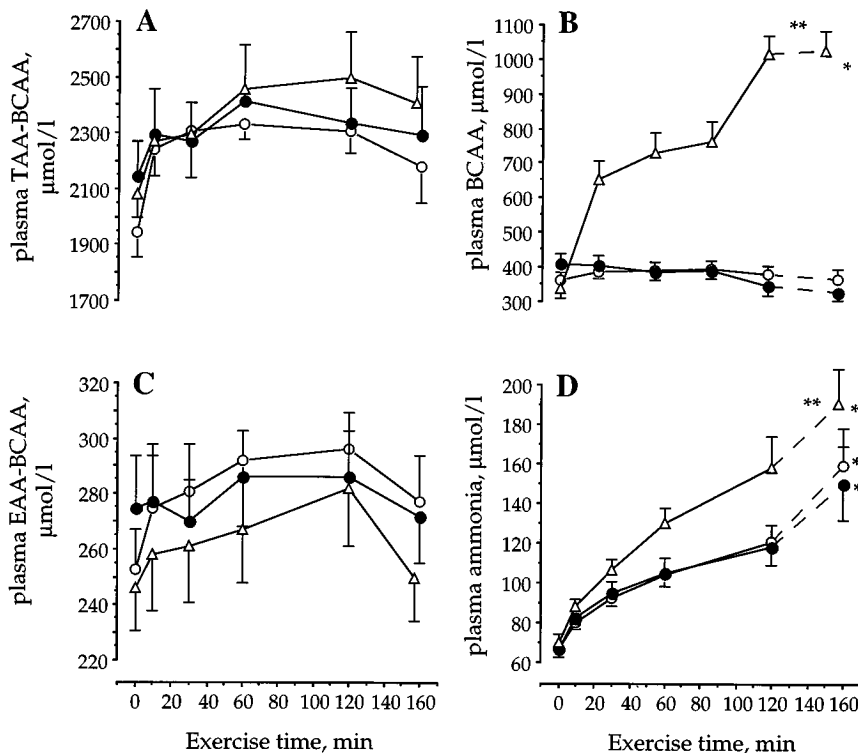


Fig. 3. Plasma total amino acids (TAA)-BCAA (A), BCAA (B), essential amino acids (EAA)-BCAA (C), and ammonia (D) before and during 100 km of bicycling in *trial G* (●), *trial B* (△), and *trial P* (○). Data are means  $\pm$  SE of 9 subjects. \*Significant time effect ( $P < 0.01$ ). \*\*Significant difference from *trials G* and *P* ( $P < 0.01$ ; treatment and time effect).

Table 1. Plasma glutamine and tryptophan at rest and during 100 km of bicycling in glucose trial, BCAA + glucose trial, and placebo trial

	Rest	10 min	30 min	60 min	120 min	End
Glutamine						
<i>P</i>	468 ± 19	532 ± 23	583 ± 16	600 ± 14	649 ± 32	566 ± 30
<i>G</i>	543 ± 27	569 ± 32	567 ± 29	628 ± 39	628 ± 42	633 ± 49
<i>B*</i>	529 ± 23	590 ± 37	613 ± 28	691 ± 48	761 ± 47	762 ± 52
Trypto- phan						
<i>P</i>	9 ± 2	8 ± 1	9 ± 1	10 ± 1	14 ± 2	14 ± 1
<i>G</i>	9 ± 1	10 ± 2	10 ± 1	10 ± 2	12 ± 2	12 ± 2
<i>B†</i>	10 ± 2	9 ± 2	9 ± 1	7 ± 1	9 ± 1	11 ± 1

Values are means ± SE of 9 subjects. P, placebo trial; G, glucose trial; B, branched-chain amino acids (BCAA) + glucose trial. \*Significant difference from trials P and G ( $P < 0.01$ ; treatment and time effect). †Significant difference from trial P ( $P < 0.05$ ; time effect).

subjects were well trained and could maintain substantial energy production from fat oxidation during all three exercise bouts. Another explanation for the lack of beneficial effect of ingesting glucose in the present study could be the fact that our subjects were studied during exercise performed 4 h after a meal, whereas most previous studies showing a beneficial effect of carbohydrate supplementation have been performed by using subjects who were fasted overnight. As a result, at the end of the 100-km test, muscle glycogen and blood glucose were not limiting. For example, the RER was not different among trials and only declined slightly during the exercise period.

The present findings are important as they illustrate that, under simulated competitive exercise conditions where performance is measured by the time it takes to cover a certain distance, traditional carbohydrate supplementation does not always increase performance. Therefore, caution must be used when assuming that carbohydrate administration will help increase performance in all types of endurance exercise.

In recent years, the use of BCAA during prolonged exercise has become more popular. One reason for this is based on the hypothesis that consumption of these amino acids may prevent or delay central fatigue (25). Blomstrand et al. (4) investigated this hypothesis in a field study where BCAA were given to marathon runners. They reported that exercise performance was increased in "slow" but not in "fast" runners. In the present study, BCAA administration resulted in a large increase in the BCAA levels in the plasma as well as a decrease in the tryptophan/BCAA ratio (Fig. 4), yet no significant increase in performance was observed. Furthermore, plasma tryptophan concentrations increased ~50% during trial P, whereas the changes were attenuated in trial B. Davis et al. (9) found that glucose supplementation during prolonged cycling attenuated the observed increase in the free tryptophan/BCAA ratio. However, in the present study, BCAA plus glucose supplementation seem to be more effective in preventing the increase in plasma tryptophan compared with glucose supplementation alone. Plasma FFA can displace tryptophan from albumin and increase the free

portion in the plasma, and plasma concentrations of FFA and free tryptophan have been highly correlated (9). Because FFA was higher in the late phase of exercise for the trial P compared with trials B and G, the increase in free tryptophan might be even more pronounced in trial P. However, these data are unable to support the hypothesis that a maintained or decreased plasma free tryptophan/BCAA ratio could delay central fatigue and increase performance during prolonged exercise. Instead, the data support van Hall et al. (28), who showed either that manipulation of tryptophan supply to the brain has no additional effect on serotonergic activity or that manipulation of serotonergic activity functionally does not contribute to mechanisms of fatigue during prolonged exercise.

Although the present study shows that BCAA supplementation has no effect on performance in well-trained subjects under the present conditions, we cannot rule out the possibility that BCAA ingestion could still be beneficial during more prolonged exercise or in untrained individuals, when carbohydrate availability is more likely to be limiting. However, the marked differences in the tryptophan/BCAA ratio would seem to rule out this mechanism, regardless of the role of carbohydrate availability.

**Amino acid metabolism and ammonia.** It is well established that skeletal muscle produces ammonia during both prolonged submaximal as well as short-term intense exercise (13, 22, 29). The ammonia can be produced by either the deamination of BCAA or the deamination of AMP to IMP as one of the steps of the purine nucleotide cycle. Previous studies have suggested that the majority of the ammonia produced during a prolonged submaximal exercise bout comes from the deamination of BCAA (21, 22, 29, 30).

The rate of appearance of BCAA in arterial plasma occurs rather quickly after ingestion. This has been attributed to the low activity of the BCAA aminotransferase enzyme, the first enzyme in the pathway of BCAA degradation in the liver (12). As a result, ingested BCAA selectively escape uptake by the liver and are preferentially removed by skeletal muscle (11). It has been demonstrated previously that cycling exercise

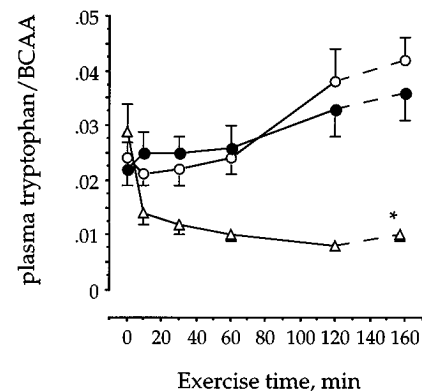


Fig. 4. Plasma tryptophan/BCAA ratio before and during 100 km of bicycling in trial G (●), trial B (△), and trial P (○). Data are means ± SE of 9 subjects. \*Significant difference from trials G and P ( $P < 0.01$ ; treatment and time effect).

after BCAA administration results in significantly higher venous plasma ammonia and glutamine levels compared with placebo (20). Similarly, MacLean et al. (21) demonstrated that after BCAA supplementation working skeletal muscle removed more BCAA from the plasma and released higher amounts of both ammonia and glutamine compared with control. In the present study, *trial B* was characterized by significantly higher circulating BCAA levels as well as significantly higher venous plasma ammonia and glutamine levels during exercise compared with *trials P* and *G*. These findings are consistent with those of others and suggest that the greater venous plasma ammonia and glutamine levels during exercise for the *trial B* were a result of a greater uptake of BCAA by the working muscle and a greater release of ammonia and glutamine by the muscle.

It is generally accepted that changes in the venous plasma ammonia levels qualitatively reflect changes in muscle ammonia production. In the present study, the subjects exercised for 2.5 h, and the venous plasma ammonia levels for all three trials steadily increased. These data suggest that, even during very long-term exercise, muscle ammonia production continues to increase. In *trial B*, not only did the venous plasma ammonia levels continue to increase throughout the experiment but the venous plasma ammonia levels were significantly higher compared with *trials G* and *P*. These data strongly suggest that the exercising muscle continued to remove and utilize BCAA because of their high circulating levels, resulting in even greater muscle ammonia production.

**Conclusions.** This study clearly demonstrates that the ingestion of glucose or glucose plus BCAA does not enhance performance during a 100-km time trial in well-trained athletes. Similarly, BCAA supplementation resulted in a dramatic decrease in the tryptophan/BCAA ratio compared with placebo and glucose supplementation, yet there was no increase in performance or indication that central fatigue was reduced or altered. Finally, BCAA supplementation resulted in significantly greater plasma ammonia levels during exercise, and it appears that muscle ammonia production continues to increase as exercise progresses, even during very long-term work.

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Address for reprint requests: K. Madsen, Dept. of Physical Education, Odense Univ., Campusvej 55, DK-5230 Odense M, Denmark.

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