J Nutr Sci Vitaminol, **58**, 36–44, 2012

Branched-Chain Amino Acid Supplementation during a 100-km Ultra-Marathon—A Randomized Controlled Trial

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(Received April 27, 2011)

Summary Ultra-marathon running is supposed to increase the parameters of skeletal muscle damage and impair renal function. The purpose of this study was to investigate the effect of branched-chain amino acid supplementation on skeletal muscle damage and renal function during a 100-km ultra-marathon. Twenty-eight athletes were randomly divided into two groups, one group using branched-chain amino acid supplementation (BCAA) and a control group (CON). The athletes in the BCAA group were supplemented with a total of 50 g of an amino acid concentrate including 20 g of BCAA. The intake of energy, antioxidants and parameters of both skeletal muscle damage and renal function were determined. Race time was not different between BCAA and CON when controlled for the personal best time in a 100-km ultra-marathon. Neither the intake of energy and antioxidants nor the parameters of skeletal muscle damage and renal function were different between BCAA and CON. We concluded that BCAA-supplementation before and during a 100-km ultra-marathon had no effect on performance, skeletal muscle damage or renal function.

Key Words athlete, nutrition, performance, protein, ergogenic supplement

Ultra-marathon running covering distances of more than the classic marathon distance of 42.195 km is of increasing popularity. In recent years, studies investigated athletes participating in ultra-marathons over 100 km (*1*) or even longer (*2*, *3*). Marathon and especially ultra-marathon running as an eccentric exercise may cause skeletal muscle damage resulting in an increase in myocellular enzymes such as plasma creatine kinase (*1–3*) and myoglobin (*1*). Skeletal muscle damage and the corresponding increase in myocellular enzymes in plasma may lead to rhabdomyolysis, thus causing an impairment of renal function (*4*), reflected by a decline in the post-race creatinine clearance (*5*) and even renal failure in ultra-marathoners (*6*).

The impairment of renal function may be dependent upon both the length and the intensity of a run performance. After a half-marathon, a limited and temporary decline in renal function without renal damage was described (*7*). In a marathon, renal function measured by using urine flow rates, creatinine clearance and protein excretion was not impaired (*8*). In ultra-marathoners, however, several reports about an impairment of renal function have been published (*4–6*). A net breakdown of body protein during endurance exercise has been shown and the mobilized amino acids were available for increased rates of oxidation and gluconeogenesis during an endurance performance (*9*). The increase of parameters of skeletal muscle damage during ultra-running might be due to a decrease in skeletal muscle mass as has been observed in ultra-marathoners (*10*).

In recent years, several studies showing a reduced increase in myocellular enzymes in cyclists during endurance performances after the combined ingestion of carbohydrates and protein have been reported (*11*, *12*). A carbohydrate-protein beverage during intense performance reduced the increase in plasma creatine kinase (*11*) and myoglobin (*12*). Among protein supplements, branched-chain amino acids (BCAA) are considered to enhance performance (*13*), to have beneficial effects in decreasing exercise-induced skeletal muscle damage (*14*, *15*) and to promote muscle-protein synthesis (*15*). During prolonged endurance exercise, the oxidation of BCAA is increased several-fold (*16*). In contrast to other amino acids, BCAA are unique in that they are principally metabolize extrahepatically in the skeletal muscle tissue (*17*). The oral intake of BCAA might have beneficial effects on skeletal muscle in the state of an impaired renal function. During renal failure, BCAA-metabolism is impaired and an oral supplementation of BCAA can induce an improvement of appetite and nutritional status (*18*). In patients suffering chronic renal failure, the oral ingestion of amino acids increased the arterial concentration of amino acids (*19*), the flow of amino acids to all organs (*20*) and the amino acid uptake by the leg muscles compared to control subjects (*21*).

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The aim of the present study was to therefore to investigate whether BCAA-supplementation before and during a 100-km ultra-marathon might have beneficial effects on parameters of both skeletal muscle damage and renal function. We hypothesized that (i) a BCAA-supplementation would reduce the increase in parameters of skeletal muscle damage and (ii) lessen the impairment of renal function during an ultra-marathon performance.

METHODS

Subjects. The organizer of the 100 km Lauf in Biel, Berne, Switzerland, contacted all participants of the race in 2009 via a separate newsletter at the time of inscription to the race, in which they were asked to participate in the study. About 1,000 male Caucasian runners started in the race; a total of 30 male ultra-runners volunteered to participate in the study. Pre-race, 28 athletes appeared for the investigations; one athlete in the control group dropped out after 71 km due to medical problems. The athletes were informed of the experimental risks and gave their informed written consent. The study was approved by the Institutional Review Board for the Use of Human Subjects of the University of Berne, Switzerland.

Race. The 100 km Lauf took place between June 12 and 13, 2009. The runners started at 10:00 p.m. on June 12. Two-thirds of the course was on asphalt, and one-third was on unpaved roads. They had to climb a total altitude of 645 m. Throughout the 100 km there were 17 aid stations, at intervals of 5 to 10 km, with a variety of food and beverages. The organizer offered hypotonic sports drinks, tea, soup, caffeinated drinks, water, bananas, oranges, energy bars and bread. The athletes were allowed to be supported by a cyclist in order to have additional food and clothing, if necessary. At the start on June 12, the ambient temperature was 17˚C, which dropped to 10˚C in the night and rose to 25˚C on June 13. There was no rain or wind.

Study protocol. The participants volunteering for this investigation kept a comprehensive training diary, including recording their weekly training units in running, showing duration in minutes and distance in kilometres, from inscription to the study until the start of the race. In addition, they reported their number of finished 100-km ultra-marathons including their personal best time in a 100-km ultra-marathon.

Randomization of subjects. The athletes who agreed to participate were randomly assigned to the BCAA (supplementation with BCAA) or CON (control) group upon inscription to the study. In the case of an athlete withdrawing pre-race, the next athlete filled the gap. Up to the start of the race, a total of 2×15 athletes had volunteered for the investigation. Twenty-eight of the expected 30 athletes reported to the investigators at the race site, between 04:00 p.m. and 09:00 p.m. on June 12, 2009. The athletes in BCAA received, on the occasion of the pre-race measurements, a prepared package of amino acids in the form of a commercial brand of tablets. The composition of the product is presented in Table 1. These athletes ingested 12 tablets 1 h before the start of the race, and then 4 tablets at each of the 17 aid stations. The runners were supplied with a total of 80 tablets in the pockets of their race clothing. In total, they ingested 50 g of amino acids; 20 g were BCAA such as leucine, isoleucine and valine. The BCAA represented 40% of all amino acids in the supplement. During the race, they consumed food and fluids ad libitum at the aid stations; they also recorded the intake of nutrients and fluid at each aid station. The athletes were advised to refrain from the intake of any supplements during the race. Due to the manufacturer's concerns regarding the high calcium content of the placebo tablets which, in combination with an expected dehydration, could be harmful for the renal function of the athletes, we had to abandon a placebo control. Thus, the athletes randomly assigned to CON also consumed food and fluids ad libitum and recorded their nutrient and fluid intake, but did not receive any placebo tablets.

Table 1. Composition of the BCAA-supplement.

Amino acid	Per tablet (mg)	Absolute intake during the race (g)	Relative intake during the race (%)
L-Leucine*	125	10	19
L-Isoleucine*	62.5	5	9.5
L-Valine*	62.5	5	9.5
L-Ornithine	62.5	5	9.5
L-Arginine	62.5	5	9.5
L-Cysteine	50	4	7.6
L-Tyrosine	50	4	7.6
L-Lysine	31.25	2.5	4.8
L-Phenylalanine	31.25	2.5	4.8
L-Threonine	31.25	2.5	4.8
L-Histidine	31.25	2.5	4.8
L-Methionine	12.5	1	1.9
L-Tryptophan	12.5	1	1.9

*Branched-chain amino acids.

Measurements and calculations. On race day between 04:00 p.m. and 09:00 p.m., 28 participants appeared for the pre-race anthropometric measurements and the collection of urinary and blood samples. Upon arrival at the finish the same measurements were performed within 1 h of finishing, there being 27 finishers.

Anthropometric measurements and calculations. Body mass was measured using a commercial scale (Beurer BF 15, Beurer GmbH, Ulm, Germany) to the nearest 0.1 kg. Body height was determined using a stadiometer to the nearest 1 cm. Body mass index (kg/m^2) was calculated using body mass and body height. The percentage of body fat was calculated using the following anthropometric formula according to Ball et al. with percent body fat=$0.465+0.180\times(\Sigma 7SF)-0.0002406\times(\Sigma 7SF)^2+0.0661\times(age)$, where Σ7SF=sum of skin-fold thickness of pectoralis, axilla, triceps, sub scapular, abdomen, suprailiac and thigh (*22*). The skin-fold data were obtained using a skin-fold caliper (GPM-Hautfaltenmessgerät, Siber & Hegner, Zurich, Switzerland) and

recorded to the nearest 0.2 mm. One trained investigator took all the anthropometric measurements in order to eliminate inter-tester variability (*23*). The skin-fold measurements were taken once for the entire eight skin-folds and were then repeated twice more by the same investigator; the mean of the three times was then used for the analyses. The timing of the taking of the skin-fold measurements was standardized to ensure reliability, and the readings were performed after 4 s following Becque et al. (*24*).

Analysis of blood samples. After venipuncture of an antecubital vein, two Sarstedt S-Monovettes (serum gel, 7.5 mL) for chemical and one Sarstedt S-Monovette (EDTA, 2.7 mL) (Sarstedt, Nümbrecht) for hematological analysis were drawn. Monovettes for serum were centrifuged at 3,000 $\times g$ for 10 min at 4°Celsius. The serum was collected and stored on ice. Blood samples were transported immediately after collection to the laboratory and analyzed within 6 h. In the venous blood samples, hemoglobin and hematocrit were measured using ADVIA® 120 (Siemens Healthcare Diagnostics, Deerfield, IL, USA). In the serum samples, creatinine, urea, sodium, potassium, plasma osmolality, creatine-kinase, myoglobin and albumin were measured using COBAS INTEGRA® 800 (Roche, Mannheim, Germany). Osmolality of plasma was determined using Fiske® Modell 210 Mikro-Osmometer (IG Instrumenten-Gesellschaft AG, Zurich, Switzerland).

Analysis of urinary samples. Before blood sampling, urine was collected in Sarstedt monovettes (10 mL). Creatinine, urea, sodium, potassium, specific gravity and osmolality were determined. Osmolality of urine was determined using a Fiske® Modell 210 Mikro-Osmometer (IG Instrumenten-Gesellschaft AG). Urine specific gravity was analyzed using a Clinitek Atlas® Automated Urine Chemistry Analyzer (Siemens Healthcare Diagnostics). Urinary creatinine and urea were measured using a COBAS INTEGRA® 800. Electrolytes in the urinary samples were determined using an ISE IL 943 Flame Photometer (GMI, Inc., Ramsey, MN, USA).

Calculations of serum and urine parameters. Fractional sodium excretion ($FENa^+$) was calculated according to the formula of Steiner (*25*) where

$FENa^+ = [Na^+]_{urine} \times [Creatinine]_{serum} / ([Na^+]_{serum} \times [Creatinine]_{urine}) \times 100$.

Fractional urea excretion (FEurea) was calculated according to the formula of Dole (*26*) where

$FEurea = [Urea]_{urine} \times [Creatinine]_{serum} / ([Urea]_{serum} \times [Creatinine]_{urine}) \times 100$.

Transtubular potassium gradient (TTPG) was calculated according to the formula of West et al. (*27*) where

$TTPG = [K^+]_{urine} \times osmolality_{serum} / [K^+]_{serum} \times osmolality_{urine}$.

Creatinine clearance (CCR) was calculated according to the formula of Gault et al. (*28*) where

CCR (mL/min) = (140 − age) × body mass (kg)/$Creatinine_{serum}$ (mg/dL) × 72.

Glomerular filtration rate (GFR) was calculated according to the formula of Levey et al. (*29*) where

GFR (mL/min/1.73 m^2) = $170 \times [Creatinine]_{serum}^{-0.999} \times [Urea]_{serum}/2.144^{-0.170} \times [Albumin]_{serum}^{+0.318} \times age^{-0.176}$.

Percentage change in plasma volume (%ΔPV) was calculated from pre- and post-exercise levels of hematocrit (Hct) and hemoglobin (Hb) following the equation of Strauss et al. (*30*) where

$\%\Delta PV = 100 \times (Hb_{pre}/Hb_{post}) \times (1 - Hct_{post}/1 - Hct_{pre}) - 100$.

Estimation of energy intake and energy expenditure. The athletes consumed food and drinks ad libitum during the race and recorded their intake of fluids and solid nutrition at each aid station. The investigator provided a prepared paper where every aid station with the food offered was indicated and the athletes could use a pencil to mark what they ingested. At the aid stations, liquids and food were prepared in a standardized manner. Ingestion of fluids, solid food and antioxidants such as vitamin C, vitamin E, and Coenzyme Q10 were estimated according to the reports of the athletes (*31*, *32*). Energy expenditure during the event was estimated using body mass and time spent running (*33*). As an example: A runner with a body mass of 78 kg running at a speed of 11.1 $km \cdot h^{-1}$ expended 14.9 $kcal \cdot min^{-1}$ resulting in a total energy expenditure of 541 (race time in min) × 14.9 = 8,061 kcal during the race.

Questionnaires of subjective feelings. In combination with the pre- and post-race measurements, the athletes were asked about their subjective feelings of muscle soreness, using a subjective scale from 0 (absolutely no muscle soreness) to 20 (highest subjective discomfort with muscle soreness). After the race, the athletes were asked whether they had performed the run as expected, worse than expected or better than expected.

Statistical analysis. The Shapiro-Wilk test was used to check for normality distribution. Data are presented as mean and standard deviation (±SD). Parametric- and non-parametric comparisons within (pre- vs. post-race) and between groups (differences of changes during the race between BCAA and CON) were performed as appropriate. For bivariate analysis we applied parametric tests (i.e. Student's *t*-test and the Pearson correlation) as variables were normally distributed (checked by the Shapiro-Wilk test). A residual analysis (i.e. checking for homoscedasticity and normality distribution) was performed after applying multiple linear regression modeling. Correlation analyses and linear multiple regression were used to investigate the effect of BCAA-supplementation on the parameters of muscle damage and renal function. In addition we calculated Cohen's f^2 as an appropriate effect size that can be applied in the context of multiple regressions to estimate the relative importance of the differences between BCAA and CON. By convention, Cohen's f^2 effect sizes of 0.02, 0.15, and 0.35 were determined as small, medium, and large, respectively (*34*). Statistical significance was set at $p<0.05$ for all tests.

RESULTS

The baseline characteristics of the pre-race anthropometric and training parameters (see Table 2) showed no differences between the athletes in BCAA and CON

Table 2. Comparison of pre-race characteristics of the subjects using unpaired *t*-tests.

	BCAA (n=14)	CON (n=14)
Age (y)	42.4±9.1	45.1±6.1
Body mass (kg)	72.1±6.4	75.1±5.6
Body height (m)	1.74±0.06	1.80±0.06
Body mass index (kg/m^2)	23.5±1.5	22.9±2.2
Percent body fat (%)	14.1±3.0	16.0±4.5
Years as active runner (y)	13.1±9.4	10.3±8.3
Average weekly amount of running (km)	81.6±21.8	60.0±16.2
Average weekly amount of running (h)	7.4±2.3	5.7±2.0
Average speed in running during training (km/h)	10.9±1.8	11.2±1.1
Number of finished marathons (n)	22.8±22.3	16.4±16.4
Personal best time in a marathon (min)	191±33	209±26
Number of finished 100-km ultra-marathons (n)	5.7±5.1 (n=10)	2.8±2.3 (n=8)
Personal best time in a 100-km ultra-marathon (min)	601±107	672±98

Results are presented as mean (±SD). No significant differences were found between BCAA and CON.

Table 3. Comparison of energy balance and nutrient intake of the subjects during the race.

	BCAA (n=14)	CON (n=13)
Energy expenditure (kcal)	7,160±844	7,485±621
Energy intake (kcal)	3,311±1,450	2,590±1,334
Energy balance (kcal)	−3,848±1,369	−4,894±1,641
Fluid intake (L)	8.48±3.17	7.88±3.50
Intake of carbohydrates (g)	755.7±354.8	608.8±326.4
Intake of protein (g)	79.9±12.7**	26.7±22.0
Intake of fat (g)	5.1±4.8	7.0±7.1
Intake of vitamin C (mg)	290.4±450.2	398.6±417.4
Intake of vitamin E (μg)	0.37±0.30	0.62±0.35
Intake of Coenzyme Q10 (μg)	1.60±2.11	2.14±1.98

Results are presented as mean (±SD). Athletes in BCAA ingested highly significantly more protein compared to CON. ** $p<0.01$ (unpaired *t*-tests were applied for between-group comparisons).

($p>0.05$).

Performance

The mean (±SD) finishing time of the 14 athletes in BCAA was 624.3±79.5 min, whereas the remaining 13 athletes in CON finished within 697.8±89.7 min. The mean difference of 73.6 min in the 100 km race time between the two groups was statistically significant ($p=0.033$). The corresponding 95% confidence limits of the race time difference were between 6.5 min and 140.6 min. The race time was significantly associated with the personal best time in a 100-km ultra-marathon in both BCAA and CON, with Pearson correlation coefficients of 0.77 and 0.81 ($p<0.05$ for both), respectively. The corresponding mean (95% CI) difference in the personal best time for a 100-km ultra-marathon between BCAA and CON was 71.0 (−33.2 to 175.1) min ($p=0.17$). Due to the similar mean differences in the race time and the personal best time in a 100-km ultra-marathon between BCAA and CON and the significant association between the race time and the personal best time in a 100-km ultra-marathon, we performed a linear regression controlling for the personal best time in a 100-km ultra-marathon as a potential confounder for the difference between the race times. The resulting mean (±SE) race time difference of 5.5±28.6 min no longer remained statistically significant when adjusted for the personal best time in a 100-km ultra-marathon.

Subjective feelings of muscle soreness and performance

In BCAA, the subjective feeling of muscle soreness increased from 0.9±2.2 pre-race to 11.3±4.3 post-race ($p<0.05$); in CON from 0.4±1.0 pre-race to 9.4±4.6 post-race ($p<0.05$). The changes between BCAA and CON were not different ($p>0.05$). When the athletes were asked, post-race, whether they had completed the race as expected, better than expected or worse than planned, no differences were found between BCAA and CON ($p>0.05$).

Fluid intake and energy turnover

The athletes in BCAA consumed 8.5±3.2 L of fluids during the run, the runners in the CON 7.9±3.5 L ($p>0.05$). Energy intake, energy expenditure and energy balance were not different between the two groups ($p>0.05$) (see Table 3). The athletes in BCAA ingested significantly more protein compared to CON ($p<0.05$). Protein intake in BCAA was related neither to post-race plasma urea (Pearson $r=-0.40$, $p=0.15$) nor to the change in plasma urea (Pearson $r=-0.08$, $p=0.78$). The energy deficit was related to the decrease in body mass in BCAA (Pearson $r=0.7$, $p=0.003$). The additional effect of the BCAA-supplementation on the association between the loss of body mass and the energy deficit however was not relevant (Cohen's $f^2=0.018$). In BCAA, body mass decreased by 1.8±1.6 kg, in CON by 1.9±2.0 kg ($p>0.05$). No associations between race time and changes in body mass have been observed ($p>0.05$).

Changes in blood parameters

Table 4 represents the pre- and post-race changes in the values of blood parameters. Hematocrit, hemoglobin, sodium, potassium, albumin and total protein showed no changes ($p>0.05$). Creatine kinase, creatinine, urea, osmolality and myoglobin increased highly significantly in the two groups ($p<0.001$). The changes from post- to pre-race (Δ) were no different between the two groups ($p>0.05$). The post-race values for creatine kinase, serum urea and myoglobin were 2,637±1,278%, 175±32%, and 14,548±8,522% higher than the pre-race values in BCAA; and 2,749±1,962%, 168±38%, and 13,435±10,724% in CON ($p<0.001$),

Table 4. Comparison of changes in blood parameters during the race within and between BCAA and CON.

	BCAA			CON			Difference between Δ	
	Pre-race	Post-race	Δ Post-race − Pre-race	Pre race	Post race	Δ Post-race − Pre-race	Δ CON − Δ BCAA	Δ CON − Δ BCAA#
Hematocrit (%)	43.2±1.6	43.2±2.0	0.0±1.9	43.3±2.3	43.4±3.8	0.07±2.8	0.07±0.9	0.46±1.0
Hemoglobin (g/L)	14.7±0.7	14.6±0.9	−0.07±0.64	14.6±0.7	14.6±1.1	0.05±0.8	0.12±0.28	0.10±0.3
Sodium (mmol/L)	139.0±1.8	138.9±2.6	−0.14±3.91	139.1±1.3	140.8±2.0	1.69±2.28	1.83±1.24	1.60±1.39
Potassium (mmol/L)	4.1±0.3	4.3±0.5	0.17±0.61	4.1±0.2	4.3±0.3	0.15±0.25	0.03±0.18	0.01±0.21
Creatine kinase (U/L)	168.3±61.7	4,582±3,150	4,414±3,107**	157.8±74.5	3,861±2,357	3,703±2,340**	−711±1,065	−657±1,193
Creatinine (μmol/L)	76.8±11.1	91.8±21.0	14.9±16.5*	77.8±8.7	94.7±11.7	16.8±13.2*	1.8±5.8	1.0±6.5
Urea (mmol/L)	6.2±1.4	10.6±2.1	4.4±1.6**	5.9±1.5	9.5±1.6	3.6±1.5**	−0.83±0.6	1.0±0.7
Myoglobin (μg/L)	50.2±17.8	6,933±4,208	6,883±4,206**	43.8±13.0	5,709±4,053	5,665±4,049**	−1,218±1,591	−126±1,628
Osmolality (mosmol/kg H_2O)	293.4±3.2	304.6±6.7	11.2±6.9**	295.2±3.7	305.2±5.7	10.6±6.0**	−1.1±2.5	−0.4±2.8
Albumin (g/L)	46.0±1.7	45.0±2.5	−1±2.7	45.0±2.3	45.5±2.4	0.5±3.0	1.5±1.1	1.6±1.2
Total protein (g/L)	73.1±3.5	73.9±4.3	0.8±4.5	73.2±3.6	73.8±5.2	0.6±6.4	−0.17±2.1	0.06±2.4
Plasma volume change (%)	0	0.8±7.6*	0.8±7.6	0	0.0±10.0	0.0±10.0	−0.8±3.4	−1.4±3.8

Results are presented as means (±SD) for within-group comparisons and as means (±SE) for the comparison of changes (Δ) between groups using unpaired *t*-tests.
*$p<0.05$; **$p<0.001$. No differences were found when the changes (Δ) between BCAA and CON were compared.
#Difference between changes (Δ) additionally controlled for race time by using multiple regression analysis with the blood parameter change as independent (outcome) variable. The dependent model variables consisted of the predictor variable of interest (i.e. BCAA vs. Con) and race time as a potential confounding variables.

Table 5. Comparison of urine parameters and renal function between BCAA and CON.

	BCAA			CON			Difference between Δ	
	Pre-race	Post-race	Δ Post-race − Pre-race	Pre-race	Post-race	Δ Post-race − Pre-race	Δ CON − Δ BCAA	Δ CON − Δ BCAA#
Creatinine (μmol/L)	6,100±3,527	13,726±7,931**	7,662±9,202	6,862±4,212	18,949±7,538**	12,087±7,948	4,424±3,324	4,638±3,718
Urea (mmol/L)	251.8±152.9	428.1±133.3*	176.3±155.8	247.2±134.4	454.3±106.6*	207.1±110.5	30.8±52.4	22.5±58.5
Sodium (mmol/L)	150.6±64.0	75.8±44.3**	−74.8±51.7	133.1±73.4	54.9±41.6**	−78.2±62.8	−3.4±22.1	−7.9±24.6
Potassium (mmol/L)	43.6±33.1	129.5±47.8**	85.9±74.5	40.0±23.9	163.3±55.8**	123.6±50.6	37.6±18.8	47.7±20.5
Ratio Potassium : Sodium	0.27±0.17	2.69±2.02**	2.41±2.01	0.32±0.17	5.87±6.37**	5.57±6.37	3.2±1.8	4.4±1.9
Urine specific gravity (g/mL)	1.015±0.009	1.024±0.007**	0.0089±0.007	1.015±0.007	1.027±0.004**	0.012±0.006	0.004±0.003	0.004±0.003
Osmolality (mosmol/kg H_2O)	565.5±298.2	863.7±158.3**	298±212	527.3±319.6	878.5±161.0**	351±244	52.9±88.8	3.8±96.4
Protein (mg/L)	44.4±23.7	380.6±588.9**	336±595	47.5±35.5	328.4±229.1**	280±217	55.3±175.3	136.6±192.2
Fractional sodium excretion (%)	1.72±1.08	0.48±0.48**	−1.23±1.23	1.39±0.85	0.24±0.24**	−1.15±0.76	0.09±0.4	0.4±0.4
Fractional urea excretion (%)	53.5±11.8	33.7±15.8*	−19.8±20.7	52.1±11.3	26.9±11.6*	−25.2±14.8	−5.5±7.0	−4.9±7.8
Transtubular potassium gradient (ratio)	25.7±26.9	87.7±40.0**	61.9±29.2	22.5±20.1	113.1±54.2**	90.2±44.2	28.7±14.3	37.2±15.4
Creatinine clearance (mL/min)	114.4±19.3	96.0±22.1*	−18.4±17.4	114.0±13.1	92.3±11.5*	−21.7±14.8	−3.3±6.2	−3.4±7.0
Glomerular filtration rate (mL/min)	103.3±16.2	81.3±18.4**	−22.0±13.6	100.9±11.7	76.9±10.6**	−24.0±15.4	−2.0±5.6	−1.4±6.2

Results are presented as mean (±SD) for within-group comparisons and as mean (±SE) for the comparison of changes (Δ) between groups using unpaired *t*-tests.
*$p<0.05$; **$p<0.001$. No significant differences were found when the changes (Δ) between BCAA and CON were compared.
#Difference between changes (Δ) additionally controlled for race time by using multiple regression analysis with the urine parameter change as independent (outcome) variable. The dependent model variables consisted of the predictor variable of interest (i.e. BCAA vs. Con) and race time as a potential confounding variables.

respectively. The increases were not different between the groups ($p>0.05$). In BCAA, race time was significantly and positively correlated to the increase in plasma urea concentration (Pearson $r=0.56$, $p=0.038$), but not in CON (Pearson $r=-0.30$, $p=0.3$). The corresponding effect size f^2 for the observed difference between race time and the change in plasma urea concentration between the groups was 0.23. The changes in the parameters of skeletal muscle damage and post-race creatine kinase were not related to race time in the two groups ($p>0.05$).

Changes in urinary parameters and renal function

Urinary creatinine, urea, potassium, the potassium-to-sodium ratio, specific gravity, osmolality, protein, and transtubular potassium gradient increased in both groups ($p<0.05$) (see Table 5), whereas sodium, fractional sodium excretion, fractional urea excretion, creatinine clearance and glomerular filtration rate decreased ($p<0.05$). The changes from post- to pre-race (Δ) were not different between the two groups ($p>0.05$). In BCAA, race time was significantly and negatively related to the decrease in glomerular filtration rate (Pearson $r=-0.58$, $p=0.03$), but not in CON (Pearson $r=0.39$, $p=0.18$). Post-race fractional urea excretion was related to race time in BCAA (Pearson $r=0.55$, $p=0.04$), but not in CON (Pearson $r=-0.45$, $p=0.10$). The increase in plasma urea was significantly and negatively related to the decrease in glomerular filtration rate in both BCAA (Pearson $r=-0.69$, $p=0.007$) and CON (Pearson $r=-0.87$, $p<0.0001$). The change in plasma urea was also significantly and negatively related to post-race fractional urea excretion in both BCAA (Pearson $r=-0.79$, $p=0.001$) and CON (Pearson $r=-0.64$, $p=0.018$).

DISCUSSION

The aim of the present study was to investigate whether BCAA-supplementation before and during a 100-km ultra-marathon might show beneficial effects on parameters of both skeletal muscle damage and renal function. We hypothesized that BCAA-supplementation would lead to a reduced increase in the parameters of skeletal muscle damage, and a decreased impairment of renal function. In contrast to our hypothesis, the changes in neither the parameters of skeletal muscle damage nor renal function were different between BCAA and CON.

Firstly, we hypothesised that BCAA-supplementation might lower the post-race increase in parameters of skeletal muscle damage. However, we found no differences in the increase in either creatine kinase or myoglobin between BCAA and CON. In marathon runners, post-race creatine kinase was significantly elevated among faster runners compared to slower ones, and elevations of creatine kinase drawn 24 h after the marathon were inversely related to finishing times (*35*). Skenderi et al. described 39 runners in the Spartathlon, a 246 km ultra-run, which the subjects completed within 33.3±0.5 h (*3*). The finishing time was not correlated to the post-race creatine kinase activity, as it was in the present study. The degree of skeletal muscle damage is, however, strictly related to trainability. Ultrastructural examination of muscle biopsies revealed evidence of muscle changes consistent with endurance exercise training (*36*). Although pre-race creatinine kinase showed no significant difference between the two groups, previous races in the past months might have had a potential 'protective' effect against serious muscle damage after an ultra-marathon (*37*). However, BCAA supplementation may have beneficial effects on markers of skeletal muscle. Tang showed in male swimmers that BCAA supplementation for 2 wk attenuated the increase in urinary urea nitrogen, hydroxyproline, and 3-methylhistidine after intense swimming (*38*).

Apart from the markers of skeletal muscle damage, the subjective feeling of muscle soreness after the race was not different between BCAA and CON. However, it has been shown that BCAA supplementation can ameliorate soreness from eccentric exercise (*39*, *40*). Presumably the timing of asking immediately post-race was too early in the present study. In untrained male subjects, ratings of perceived soreness were lower at 24 h post-exercise with BCAA-supplementation before eccentric exercise (*14*). Furthermore, in untrained females, muscle soreness was significantly lower after two and three days with BCAA-supplementation (*39*). When non-weight-trained males consumed BCAA before and after an intense eccentric exercise, muscle soreness was reduced 48 h and 72 h after exercise (*40*).

Secondly, we hypothesised that BCAA-supplementation would cause a reduced impairment of renal function. However, no differences between BCAA and CON could be found. Creatinine clearance, glomerular filtration rate, fractional sodium excretion and fractional urea excretion decreased, reflecting reduced renal function (*1*, *8*) in both BCAA and CON. The concentrations of urea in plasma and urine were not different between BCAA and CON. In BCAA, race time was correlated to the change in plasma urea, the change in glomerular function and the change in post-race fractional urea excretion. However, the change in urea was significantly and negatively related to both the decrease in glomerular filtration rate and post-race fractional urea excretion between BCAA and CON. Since there were no differences between BCAA and CON, we assume that the additional intake of BCAA had no effect on renal function.

One might argue that the additional intake of amino acids in the state of renal impairment might deteriorate renal function. Excessive protein intake of >3 g·kg body mass^{-1}·day^{-1} may have detrimental effects on renal function. However, these subjects ingested only ~1.1 g·kg body mass^{-1} (BCAA) and ~0.35 g·kg body mass^{-1} (CON) during the race, respectively. Acute intakes of BCAA-supplements of ~10–30 g·day^{-1} seem to be without ill effect (*41*).

Our athletes ingested amino acids as a pre-race load of 12 g and then 4 g at each aid station during the race. The total time of supplementation was between 12 and 13 h. This time period might have been too short to

show an effect of BCAA-supplementation on performance. In a recent study, an amino acid supplementation of 4 wk showed substantial effects (*42*). Shimomura et al. could demonstrate that the ingestion of 5 g of BCAA 15 min prior to seven sets of 20 squats per set reduced the onset of muscle soreness and muscle fatigue for several days after exercise (*43*). However, van Hall et al. (*44*) reported no increase in performance when BCAA were ingested during exercise. The duration of the BCAA-supplementation might have also been too short to show an effect on creatine kinase. Ohtani et al. showed a decrease in post-exercise creatine kinase compared to pre exercise when athletes ingested three times per day 2.2 g of a mixture of amino acids for 1 mo (*42*). In addition, BCAA supplementation for 3 wk during and following resistance training increased serum testosterone levels and decreased both cortisol and creatine kinase levels in resistance-trained males (*45*). Therefore, pre-race BCAA supplementation may increase subsequent competitive performance when ingested for a longer period of time.

Apart from BCAA, antioxidants such as vitamin C, vitamin E and Coenzyme Q10 might have an effect on skeletal muscle damage during performance (*46*). We determined in these subjects the intake of vitamin C, vitamin E and Coenzyme Q10; however, we found no differences between BCAA and CON. Although the long-term dietary supplementation with both vitamin C and E ameliorated muscle functional decrements subsequent to eccentric muscle contraction (*47*), ultra-marathoners (*48*) seemed to benefit from the intake of antioxidants regarding skeletal muscle damage.

The finding that the athletes in BCAA were significantly faster could not be attributed to the BCAA-supplementation but was rather affected by the pre-race experience of the subjects. Although the athletes were randomly assigned into the two groups and no statistically significant differences regarding anthropometry, training or pre-race experience were found between BCAA and CON, a potential discrepancy due to the personal best time in a 100-km ultra-marathon occurred. The personal best time in an ultra-endurance performance was shown to be an important variable regarding performance in ultra-endurance races (*49*). Thus, adjusting for personal best times resulted in a non significant difference in race time between BCAA and CON. However, these ultra-endurance athletes were running through the night and athletes in BCAA achieved a faster race time compared to athletes in CON. Portier et al. showed in offshore sailors racing for 32 h that BCAA reduced the feeling of fatigue (*50*). Therefore, BCAA-supplementation in the present athletes might have had beneficial effects regarding fatigue while running during the night. It has also been shown that BCAA supplementation lowers perceived exertion during moderate endurance performance (*51*). During exhaustive exercise, BCAA supplementation also showed an increase in resistance to fatigue (*52*). These beneficial effects might have been responsible for the fact that the present athletes ran faster during the night.

The finding that subjective feeling of muscle soreness and performance showed no differences between BCAA and CON supported the negative results in laboratory parameters and effect on race time. Performance might, however, also be influenced by pre-race nutrition (*53*). Unfortunately, we did not compare pre-race diet. Furthermore, the markers of skeletal muscle damage such as creatine kinase and myoglobin may remain elevated up to 7 d after a marathon (*54*). Therefore, these parameters should be measured not only immediately after the race but also in the recovery phase (*14*). It has been shown that BCAA-supplementation reduced changes in lactate dehydrogenase 2 h 5 d and in creatine kinase from 4 h to 5 d post exercise (*12*). The determination of glomerular filtration rate using creatinine is limited due to the fact that serum concentration of creatinine is influenced by tubular secretion, meat intake, exercise and muscle mass (*55*).

To summarize, BCAA-supplementation before and during a 100-km ultra-marathon showed no effect on subjective feeling of muscle soreness or biochemical parameters of either skeletal muscle damage or renal function. In future studies, ultra-marathoners should be supplemented for a longer time period pre-race with BCAA in order to show an improved ultra-endurance performance.

Acknowledgments

Mary Miller from England helped us in the translation.

Author disclosures

The authors have no conflict of interest and received no external funding.

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