Attenuation of Delayed Onset Muscle Soreness with Acute Consumption of Essential Amino Acids

Original Research

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Abstract

Introduction: Prior studies of the acute benefits of protein supplementation have determined a benefit in improving post-exercise muscle anabolism and aiding the recovery of muscle function and performance. Previous acute protein supplement studies in post-exercise protein synthesis and anabolic intracellular signaling reported no attenuation in muscle damage or elevated muscle function. The aim of this study is to implement a specific content of essential amino acids with resistance and aerobic exercises to quantify the difference in strength, endurance, and flexibility during the delayed onset muscle soreness common with a new exercise protocol.

Methods: We enrolled 42 participants (22 EAA and 20 Controls) completed an hour-long aerobic and resistance exercise protocol including flexibility, resistance, and aerobic exercises for three consecutive days. The study participants were randomly assigned to the EAA (6.6g) per day (EAA + Gatorade) group or the control (Gatorade) group. The data was analyzed in a double-blinded format.

Results: Both groups improved the initial flexibility respectively throughout the three exercise days but were not significantly different ($p=0.32$) in the sit and reach. For the resistance/power activities, the EAA group improved in the repetitions for push-ups ($p=0.014$ vs 0.21) and dips (0.0002 vs 0.59) compared to the controls. The EAA group was faster although not statistically significant in the 20-meter sprint and improved in the 1.5-mile run during the third day ($P=0.002$ vs 0.48) compared to the control group.

Conclusions: The data in the results supports that acute ingestion of the essential amino acid supplements provides increased physical performance and decreases the DOMS symptoms in sedentary participants over the three-day trial period of exercise.

Key Words: Amino Acid Supplementation, Anaerobic and Aerobic Exercise

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Introduction

Delayed Onset Muscle Soreness (DOMS) typically appears in muscles within 24 hours after repeating high-intensity exercise in muscles that are unaccustomed to the contractile force. A major factor hindering sedentary patients from continuing an exercise regimen is the uncomfortable and often debilitating muscle soreness associated with increasing physical activity. It is believed that the damage to the myofibers and the inflammation that follows is the cause of the DOMS and may persist for several days. The pain and inflammation may also cause functional muscle impairment.[4, 18] An increase in inflammatory markers, such as cytokines that include the interleukins and tumor necrosis factor-alpha within the muscle and blood are indicators of muscle inflammation that can be quantified. Elevated concentrations of creatine kinase, lactate dehydrogenase and myoglobin in blood are biomarkers of cellular muscle damage after exercise that can also be quantified in the laboratory setting.[5, 17] Interestingly, exercise-induced muscle damage contributes to the pathways for muscle protein synthesis (MPS) and increases sarcomeres numbers in the muscle fiber.[18] The muscular mechanical stress that is induced by eccentric exercise attracts various cells to the site of damage to eventually repair and remodel the muscle tissue including neutrophils and pro-inflammatory macrophages secreting cytokines and

initiating myoblast production.[17] During this time, the rates of protein anabolism and catabolism increased. If there are not enough free amino acids available in the body during that time, the rate of degradation can exceed the synthesis and cause a negative net protein balance. In theory, the consumption of protein supplements during recovery should promote skeletal muscle anabolism and stimulate protein synthesis.[11] Levenhagen et al. found that the post-exercise administration of amino acids enhances the uptake of EAAs, which promotes post-exercise repair and synthesis of muscle proteins. Catabolism ensures proper levels of intracellular amino acids and supports the quality of muscle proteins. By removing damaged muscle proteins, their building blocks can be reused in the synthesis of new efficient muscle proteins. Increased consumption of EAAs shifts net protein balance and promotes muscle anabolism.[2]

RR Wolfe et al. have found that the oral consumption of the essential amino acids for protein formation is anabolic, while the non-essential amino acids are catabolic.[26] The essential amino acids are necessary for protein nutrition, whether obtained by eating proteins or by a formula of the essential amino acids. Prior studies demonstrate the acute benefits of protein supplementation on post-exercise muscle anabolism, which may aid in the recovery of muscle performance. However, when protein supplements have been provided in research studies, acute changes in post-exercise protein synthesis and anabolic intracellular signaling have not resulted in measurable reductions in muscle damage and enhanced recovery of muscle function. Limitations in prior study design and variability in markers of muscle damage potentially reduced the strength of the previous research evidence.

The purpose of this study is to compare the acute consumption of the essential amino acids formulated drink in sedentary individuals while completing a consecutive three-day exercise protocol (Vivissential exercise protocol) of stretching, resistance, and aerobic exercise. We aim to compare essential amino acid drinks and a placebo control in a double-blinded study to measure ability to attenuate the conditions of DOMS common with the beginning of resistance and exercise protocols. We hypothesize that the group with elevated essential amino acids will present with lower reported levels of soreness and enhanced performance of the tasks in the hour-long protocol.

Methods

Participants

We enrolled Forty-two young, previously sedentary participants (20 EAA and 22 placebo) who completed the double-blinded study. The study was approved by the Human Subjects Committee Internal Review Board at Eastern New Mexico University (IRB #1819011), and all experimental procedures conformed to the Declaration of Helsinki. All the participants that volunteered were men (EAA 8, Placebo 7) and women (EAA 14, Placebo 13) between the ages of 18-50. Before participating in the study, they did not exercise three or more times a week or more than 90 minutes a week. We excluded participants who used tobacco, were pregnant, or were diagnosed with hypertension. Participants who had a body mass index over forty, were on hormone replacement medication, or were diagnosed with diabetes were also excluded from the study. All participants read and signed the IRB-approved consent form agreement to the measurements before the screening visit.

Sample size estimations were calculated based on the expected differences in the marker of muscle damage (CK) between the pre-exercise concentrations compared to the three day post exercise blood draws.[6, 12] Assuming an alpha-error probability of 0.05, it was determined that the sample size (n=12) per group would achieve a 95% statistical power.

Protocol

Participation took place over four separate visits. For the first visit, the participants anthropometric measurements at this physical screening visit, arriving fasted 10-12 hours before arrival. BMI (Body Mass Index,) and percent body fat were measured through bioimpedance using an Omron Fat Loss Monitor (HBF-306C). Triglycerides, HDL (High-Density Lipoprotein), total cholesterol were measured from capillary blood samples obtained through finger prick (CardioChek Portable Blood Test System, PTS Panels, for cholesterol; Subsequently, participants were randomly prescribed the form of treatment as placebo (carbohydrate drink only) or treatment (carbohydrate drink with 6.6g of Vivissential EAAs) by using an Excel chart randomizer. The essential amino acid formulation is present in Table 1. Prior to the three-day consecutive exercise visits, the participants were familiarized with the exercise protocols.

On three consecutive days we collected exercise data, we asked the participants to refrain from the consumption of caffeine, NSAIDS and alcohol for at least 12 hours prior to their visit to avoid confounding variables. After a brief walking warm-up, the participants followed the Vivissential exercise fitness test that can be found in Table 2. The Vivissential exercise test combines measures of upper and lower limb flexibility, isometric muscle strength (hand grip),

isotonic muscle contractions to failure (push-ups, dips and chin-ups). Additional testing of anerobic burst speed in the form of the 20-meter sprint and an endurance measured run of 1.5 miles was performed. This is the first research protocol in a sedentary group to include multiple muscle groups in the arms, legs and torso over multiple days commonly associated with the development of DOMS in a sedentary population starting exercise protocols. Participants in a placebo group were drinking a 20 oz Gatorade drink during each exercise visit. Participants assigned to the treatment group were drinking a 20 oz Gatorade drink with 6.6 g of the Vivissential amino acid powder added to it. This was a double-blind study as neither the participants nor the attending exercise researchers were aware of the group's assignment when collecting data. Groups were revealed their assignment once all the data were collected prior to analysis of performance. At the end of the exercise on visit 2, 3 and 4 the participants were given a questionnaire to assess a Rating of Perceived Exertion using a modified Borg Scale (0-10), a Visual Analog Scale (VAS) using a scale of 0-10cm with 0 measuring no soreness and 10 equaling sever soreness.[7] The questionnaire also mapped 13 regions of soreness including the neck, triceps, biceps, forearms, hands, pectoralis, abdominals, gluteus, back, thighs (Quads), back of legs (hamstrings), claves and feet. All participants were asked to compare their RPE and VAS compared to the baseline at the beginning of the exercise protocol on visit 2 (day 1 of exercise). At the end of day 1 and 3 of exercise a 3mL blood sample was collected in EDTA vacutainers and processed for the measurement of plasma creatine kinase (CK). The blood was aliquoted in 500 uL contained and stored at -80 ^{o}C and later measured for CK using a Human CK ELISA Kit (Abcam), and a microplate spectrophotometer and absorbance was read at 450 nm.

Table 1. Calwood Nutritional Overtime formulation of the essential amino acids for 6.6 g of power added to the 20 oz bottle of Gatorade.

Essential Amino Acid – Vivissential formulation		
Substance	**mg**	**Percent Concentration**
L-histidine	321	5.2%
L-isoleucine	406	6.6%
L-leucine	635	10.3%
L-lysine	470	7.6%
L-methionine	635	10.3%
L-phenylalanine	635	10.3%
L-threonine	283	4.6%
L-tryptophan	148	2.4%
L-valine	470	7.6%
L-arginine	2172	35.2%
sodium	220	3.3%
chloride	152	2.3
potassium	55	0.8%

Table 2. Vivissentials Physical Fitness Test

Activity	
Sit and Reach	distance (cm)
Shoulder flexibility	distance (cm)
Isometric Handgrip	Weight (lbs.)
Sprint 20m	time (sec)
Push-ups	repetitions
5-minute rest	drink ¼ bottle
Chin-ups or Flex arm hang	repetitions or seconds

5-minute rest	drink ¼ bottle
Dips	repetitions
10-minute rest	drink ¼ bottle
1.5-mile run	time (min)
	drink ¼ bottle

Statistical Analysis

Within statistical variance between time points was assessed with a repeated measure ANOVA with a Tukey's post hoc analysis comparing Days 1, 2, and 3 in all exercise measurements. Variations in the delta between days and the group physical differences were assessed by an unpaired T-test. $P < 0.05$ was considered significant. Data are presented as means $\pm$ SD (Standard Deviation). The effect size for each measurement was calculated by determining Cohen's *d*.

Results

Comparison of the anthropometric data gathered from each group yielded no significant difference between the treatment EAA participants and the Placebo participants (Table 3).

Table 3. Physical characteristics of overall experimental groups displayed as averages $\pm$ SD; no categories were statistically different between groups. (BMI- Body Mass Index, BF%- percent Body Fat)

Participant Physical Profiles (Screening Visit)			
	EAA (Avg + SD)	Placebo (Avg + SD)	p=
Age	24.6 $\pm$ 7.0	24.3 $\pm$ 7.1	0.89
Height (in)	64.3 $\pm$ 2.8	65.5 $\pm$ 3.8	0.84
Weight (lbs.)	163.1 $\pm$ 37.8	162.3 $\pm$ 35.3	0.94
HDL (mg/dL)	63.3 $\pm$ 26.5	61.7 $\pm$ 15.3	0.61
Triglycerides (mg/dL)	159.0 $\pm$ 103	170 $\pm$ 127	0.17
Total Cholesterol (mg/dL)	160.3 $\pm$ 59.5	155.6 $\pm$ 48.3	0.13
LDL (mg/dL)	55.1 $\pm$ 30	38.6 $\pm$ 11.9	0.64
SBP (mmHg)	114.3 $\pm$ 8	113.7 $\pm$ 9.2	0.85
DBP (mmHg)	72.3 $\pm$ 5.1	72.25 $\pm$ 6.0	0.85
HR (bpm)	68.66 $\pm$ 9.3	63.3 $\pm$ 6.4	0.08
BMI	26.7 $\pm$ 6.4	27.1 $\pm$ 6.1	0.86
%BF	27.4 $\pm$ 7.0	26.6 $\pm$ 8.0	0.73

The study shows the acute consumption of Vivissential amino acid drink during exercise attenuated the condition of DOMS in previously sedentary subjects. All data in Table 4 show the differences between day 1 and 2 and 1 and 3. To measure the differences in leg flexibility, the Sit and Reach measurements technique indicated a significant improvement by both groups. The EAA group had a 1.36 $\pm$ 2.96 cm improvement ($p=0.04$) and the Placebo group improved by 2.35 $\pm$ 3.1 cm ($p=0.004$). There was no significant difference between the groups ($p=0.312$). For upper body flexibility was measured the Shoulder Flex distance. The EAA group improved by 1.59 $\pm$ 6.8 cm, although not significantly ($p=0.19$) while the Placebo group significantly improved by 6.42 $\pm$ 8.2 cm ($p=0.001$). The first day one measurements of flexibility shown in the Placebo group to be randomly lower in both the Sit and Reach (6.5 $\pm$ 5.9 cm EAA 11.1 $\pm$ 8.2 cm) and the Shoulder Flexibility (40.6 $\pm$ 9.6 cm vs EAA 43.3 $\pm$ 11.3 cm) respectively. The groups were statistically different on the day one measurement on the Sit and Reach ($p=0.048$) but not significantly different on the first Shoulder Flexibility measurements ($p=0.414$)

Table 4. A summary of the study group exercise measurements. The table is of the mean average delta ± standard deviation between day 1 and 2 and 1 and 4 of the exercise protocols for each group. The effect size is shown as the measure of Cohen's *d*.

Participant Exercise Trial Differences (Day 1 vs Day 2)						
	EAA (n= 22)	p=	**Placebo (n= 20)**	p=	EAA vs Placebo	Cohen's *d*
Sit and Reach (cm)	0.83 ± 2.32	0.11	1.41 ± 2.41	***0.016***	0.43	-0.241
Shoulder Flex (cm)	0.22 ± 5.6	0.8	3.3 ± 5.3	***0.010***	0.07	-0.582
Handgrip right (kg)	-0.31 ± 3.14	0.66	1.43 ± 4.11	0.138	0.14	-0.424
20 M sprint (sec)	-0.032 ± 3.43	0.47	0.036 ± 0.29	0.58	0.38	-0.232
Push-ups (# to failure)	1.86 ± 5.6	0.136	0.4 ± 6.14	0.77	0.42	0.238
Chin-ups (# to failure)	0.0 ± 3.0	0.88	0.14 ± 2.6	0.89	0.58	-0.055
Flex arm hang (sec)	2.34 ± 5.4	0.148	1.24 ± 3.38	0.11	0.58	0.323
Dips (reps)	1.36 ± 2.8	***0.032***	-1.45 ± 4.1	0.13	***0.012***	0.687
1.5-mile run (min)	-0.184 + 0.64	0.19	0.114 ± 0.72	0.48	0.16	-0.414

Participant Exercise Trial Differences (Day 1 vs Day 3)						
	EAA (n= 22)	p=	**Placebo (n=20)**	p=	EAA vs Placebo	Cohen's *d*
Sit and Reach (cm)	1.36 ± 2.96	***0.04***	2.35 ± 3.1	***0.004***	0.312	-0.318
Shoulder Flex (cm)	1.59 ± 6.8	0.19	6.42 ± 8.2	***0.001***	***0.044***	-0.589
Handgrip right (kg)	1.41 ± 3.2	0.051	2.82 ± 4.53	***0.01***	0.245	-0.313
20 M sprint (sec)	0.013 ± 0.28	0.32	0.09 ± 0.38	0.28	0.44	-0.211
Push-ups (# to failure)	4.72 ± 8.3	***0.014***	2.55 ± 8.7	0.21	0.41	0.250
Chin-ups (# to failure)	1.0 ± 4.9	0.88	0.28 ± 2.49	0.88	0.74	0.286
Flex arm hang (sec)	5.7 ± 8.7	0.16	1.26 ± 4.62	0.08	0.15	0.973
Dips (reps)	3.14 ± 6.5	***0.0002***	-1.05 ± 5.73	0.59	***0.038***	0.731
1.5-mile run (min)	-0.55 ± 0.7	***0.002***	-0.031 ± 1.23	0.48	0.102	-0.422

To compare the changes in resistance, exercise the following muscle contraction exercises were measured including isometric handgrip, push-up, chin-ups, flex arm hang time and triceps dips. Both groups improved from day 1 to day 3 in hand strength using the isometric handgrip strength although only the Placebo group was significant (EAA p=0.051 and Placebo p =0.001). The EAA group's initial Handgrip strength was higher (39.7 kg) compared to the Placebo group (38.3 kg), yet not significant p=0.87). The EAA group improved by 4.72 ± 8.3 push-ups from Day 1 to 3 (p=0.01) while the Placebo improved by 2.55 ± 8.5 (p=0.21). Neither group improved significantly in the measurements of Chin-ups or Flex-arm hang time over the three days of exercise. Only the EAA group improved significantly in Triceps Dips 3.14 ± 6.5 (p = 0.0002) and the Placebo group decreased in number by 1.05 ± 5.73 (p= 0.59).

To evaluate fast burst and endurance the participants completed a timed 20-meter sprint and a 1.5-mile run. The EAA group improved by 0.31 ± 3.14 seconds between day 1 and 2 but did not change between day 1 and 3 (0.013 ± 0.28

sec). The Placebo group time on day 2 was 0.063 ± 0.29 slower and 0.09 ± 0.38 sec slower on day 3, however there is no significant differences between the groups or exercise visit days. While assessing the muscles endurance in a 1.5 mile run in an in-door track, the EAA group improved by 0.55 + 0.7 min (p=0.002) by day 3 and the Placebo group decreased by 0.031 ± 1.23 min (p=0.48). The day 1 initial times for the EAA group was 15.47 ± 3.0 min and the Placebo group times were 16.2 ± 4.2 min (p=0.537 between groups). To measure the muscle degradation biomarkers produced following the exercise protocol, the subjects acutely ingesting the amino acid supplement had decreased levels of plasma CK although not significantly different (data not shown).

In a post exercise questionnaire, we assessed the participants' opinion of RPE for each exercise day, soreness (VAS) level and number regions that were perceived to be sore. Data is present in Table 5. The EAA group RPE on the first day of exercise intensity on a scale of 0-10 to be less than the Placebo group (p=0.0009). There were no significant differences between the groups or between exercise days in perceived intensity on the last day of exercise. On a scale of 0-10, the level of soreness was not different between the groups (EAA 6.64 ± 0.35 and Placebo 6.67 ± 0.70) on the last day. Additionally, the regions of soreness were also not significantly different (EAA 4.0 ± 0.6 and Placebo 4.1 ± 0.36).

Table 5. Analysis of the post-questionnaire given to all participants after the study. The patients were asked to rank each question on a scale of 0-10 with 10 being the very, very hard and 0 being rest or very, very easy. The number of sore regions is a summation of the number of anatomical regions listed in the questionnaire that the patient wrote down were sore on the last day of exercise.

	REP Modified Borg-First Exercise Session (0-10)	RPE Modified Borg- 3rd Exercise Day (0-10)	Soreness (VAS) on the 3rd Exercise Day (0-10)	Likelihood of Continuing exercising (0-10)	Number of sore regions (13 possible)
Placebo	6.83 ± 0.35	6.21 ± 0.51	6.67 ± 0.70	7.42 ± 0.79	4.0 ± 0.6
EAA	6.14 ± 0.52	6.50 ± 0.52	6.64 ± 0.35	7.64 ± 0.53	4.1 ± 0.36
p =	***0.0009***	0.1817	0.8956	0.4316	0.6255

Discussion

The present study's primary finding is that the randomly assigned EAA group participants had significant improvement over the three days of exercise compared to the placebo group within exercises of strength and endurance but not flexibility. The data in the results supports the claim that acute ingestion of this essential amino acid supplement decreases the DOMS symptoms accompanied by decreased commencement of a new exercise regime. Not all exercises produced an attenuated response in the control group. Muscle function and performance recovery are shown to be improved potentially from the anabolic benefits of protein supplementation.[25] In a 2014 review, the authors state that the physical performance test in clinical research usually involves cycling or running to exhaustion at a given exercise intensity or over a set distance and timed to completion.[16] Muscle function tests include isometric, isokinetic, or dynamic measures of muscle strength, like one-repetition maximum (1RM), or muscular endurance measured by repetitions performed over a defined range of motion and resistance. The dips exercise in our study showed a 30% improvement for the EAA group between day 1 and 3 over exercise. Serum or plasma creatine kinase (CK) and urinary 3-methylhistidine are often used as indirect biomarkers of muscle protein damage or catabolism. Although our measurements did not show any differences in CK in a three-day exercise trial, according to multiple studies, CK, an intramuscular enzyme, is an accurate indicator of muscle damage.[2, 6, 13, 20]

Recent studies have focused on branch-chain amino acids (BCAA) as a sufficient supplementation in attenuating muscle soreness.[8, 9, 14] There are three branched-chain EAAs: leucin, isoleucine, and valine. However, there are mixed results regarding BCAA supplementation and its effects on decreasing post-exercise muscle damage. When compared to carbohydrate-only supplementation, Kephart et al. found that BCAAs combined with carbohydrates (3 g/d L-leucine, 1 g/d L-isoleucine, and 2 g/d L-valine with 2 g of CHO) did not reduce the levels of serum myoglobin, a muscle damage biomarker, nor perceived soreness following three days of high-intensity resistance training in healthy, trained males. However, in this study, the participants consumed the assigned supplements after the bouts of exercise, which may be why the results were similar between the groups.[10] The timing of taking supplements plays a significant role in muscle recovery after exercise. The 9.6 grams per day of BCAA supplement administered to fifteen young men before or after exercise showed that the BCAA supplement before exercise was more beneficial in attenuating DOMS

and exercise-induced muscle damage than taking the supplement after the eccentric exercise.[19] In a double-blind trial of low-protein mixed macronutrient beverages with the BCAA Leucine supplemented, Churchward-Venne et al. concluded that muscle protein synthesis rates are increased with a high concentration of leucine by measuring myofibrillar protein.[3] Leucine is essential for inhibiting Sestrin-2 for the amino-acid-dependent MTORC1 activation in muscle cells protein synthesis at the lysosome. In the study, the investigators gave a high concentration (5.0 g total leucine) plus 6 grams of whey protein supplementation to supply an increase in myofibrillar protein synthesis in young men and elevated leucine levels in the blood amino acids, including Arginine for inhibition of TSC1 and TSC1 at the ribosome and to allow and to allow Rheb $_{GTP}$ to bind $mTORC_1$ and activate it for protein synthesis. A complete balance of essential amino acids would be necessary to increase myofibrillar protein synthesis and improve the available amino acids for anabolic balance decreased DOMS. In comparison, another study used 0.1860 grams of methionine, which made up 3.1% of the 6 grams of EAAs used.[1] Higher levels of methionine help produce new proteins in the damaged skeletal muscles after an exercise session. Another ingredient that is crucial to look at is arginine. Arginine, the main ingredient on the list of Vivissential EAA formulas, is needed to stimulate muscle anabolism.[15] Also, L-arginine and L-citrulline are precursors for nitric oxide synthesis and can enhance blood flow and nutrient delivery to working muscles, which is a potential ergogenic effect. Even though arginine is naturally synthesized in the body, physical activity increases the demand for it, which can only be met by supplementation.

In the study, there was not a group to consume the Vivissential formulation of amino acids mixed in water only. Calwood Nutritional does supply multiple flavors of their essential amino acids without adding sugars. However, the Vivissential formulation was added with carbohydrates from the Gatorade flavor of choice from the participants. Multiple studies showed reduced perceived muscle soreness and muscle degradation biomarkers after acute consumption of carbohydrate with protein supplement drinks but did not see improvements in performance.[13, 20, 21] Millard-Stafford *et al.* compared the effects of carbohydrate-only and carbohydrate-plus-protein drinks on recovery in eight runners following a 21-kilometer run plus treadmill run to fatigue (RTF) at 90% VO_2. Runners who consumed carbohydrates with protein drinks reported lower muscle soreness than those consuming just carbohydrate drinks, but plasma CK and performance were similar regardless of the supplement consumed.[13] Romano-Ely et al. compared the effects of a carbohydrate–protein–antioxidant beverage (CHOPA) to an isocaloric carbohydrate-only only (CHO) beverage on time to fatigue and muscle damage in fourteen male cyclists. Cyclists consuming a CHOPA drink reported lower muscle soreness and lower plasma CK levels, but there was no difference in performance between the groups.[20] Rowlands et al. similarly found reduced CK levels and lower perceived leg soreness in cyclists who consumed protein-enriched carbohydrate drinks after 2.5 hours of high-intensity interval cycling compared to cyclists who only consumed carbohydrate drinks. However, both groups performed proportionally well.[21] Our results show an improvement in the resistance performance on day three of the EAA group with a 2.7% time decrease compared to the placebo group in the endurance 1.5-mile run. A few studies also found improvement in post-exercise muscle function following carbohydrate with protein supplementation.[22, 23] Like our study, all the studies mentioned tested post-exercise muscle damage, soreness, and muscle function. However, none of them provided the results of improvement in all three aspects following a carbohydrate plus protein drink ingestion. It is conceivable that the carbohydrates could increase the crosstalk activity of the PI3K, AKT, and mTOR activity. Wilburn et al. show that the IRS1-AKT-PI3K may improve MTOR1 muscle protein anabolic activity. They were unable to measure the direct changes in this mechanism, with measurements of p70S6K Thr^{89} phosphorylation and the IRS1 pathway AKT-phosphorylation, causing increased MTOR activity simultaneously. Wilburn et al. suggest both mechanisms may be necessary, but the timing postexercise when these independent molecular pathways may be stimulated, and the nutrient availability needs to be investigated.[24] With the carbohydrates and the elevated concentrations of L-Arg and L-Met in the Vivissential formulation, these cellular pathways may improve performance. However, as seen in Table 5, our studies of young participants showed no significant difference in the number, region, and severity of muscle soreness following the Vivissential exercise protocol administered for three days. More investigations have been called to show if a greater significant difference would be shown in an older population where DOMS may be significantly elevated in a more sedentary group of individuals with increased sarcopenia compared to the younger group from this study.

A potential study limitation study could include the timing and handling of the plasma samples to measure Creatine Kinase. A couple of the plasma samples were hemolyzed in the post-exercise collection on day three and removed from the analysis. The loss of the samples would have decreased the power of the statistical differences. A secondary measurement of Lactate Dehydrogenase from the plasma samples would give a secondary measure of potential muscle damage. Muscle flexibility was also not consistent between the groups as the EAA group already had a higher flexibility, as seen in the Sit-and-Reach and Shoulder flexibility on day one. The These groups were chosen randomly, and the exercise investigators were blinded to the assigned supplement. Thus, the differences in flexibility were unexpected. In

the exercise protocol, the isometric handgrip MVC and the Chin-ups showed no significant differences between the groups. The placebo group did improve in the isometric handgrip from day one to three. However, this might have been just from effort and efficiency improvement as the participants became more familiar with the exercise protocol. The Chin-ups were exceedingly difficult for both groups and may show a lack of upper body strength in this sedentary population. For future studies using this protocol, the flex-arm hang to failure, dips, and push-ups may be better indicators of changes in resistance strength training for a sedentary group. Alternative exercises of isotonic muscle contractions instead of using the individual's weight for resistance would include triceps pull downs, supine bench press and biceps curls as 50% of the 1 RM until task failure could be a more controlled exercise to test the potential performance changes due to the EAA supplementation. However, the applied exercise protocol in this study was useful to compare multiregional muscle exercise performance without needing an expensive weight machine.

Conclusions

In conclusion, this randomized and double-blinded study of high doses of essential amino acids during the beginning of a training protocol was able to improve exercise performance outcomes in young sedentary individuals. These positive results across multiple physical tasks were seen during the presumed period of pain in joints and muscles associated with DOMS. The study indicates the potential need for supplementation with all of the essential amino acids to be used to attenuate decline in performance during the onset of DOM and not just the BCAAs. The EAA + Gatorade group did not have a difference in the VAS or number of sore regions despite the improvement in performance. Future investigations should measure the ability to attenuate DOMS in an older sedentary population where the prescription of resistance and aerobic exercise is important to decrease rates of sarcopenia and improve strength and mobility. In the older sedentary group, a consideration supplementation of higher concentrations of the essential amino acids including the branch chain amino acids of leucine, isoleucine or valine.

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Conflict of Interest

No conflict of interest.

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