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Effect of α-Lipoic Acid Combined With Creatine Monohydrate on Human Skeletal Muscle Creatine and Phosphagen Concentration

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α-lipoic acid has been found to enhance glucose uptake into skeletal muscle in animal models. Studies have also found that the co-ingestion of carbohydrate along with creatine increases muscle creatine uptake by a process related to insulin-stimulated glucose disposal. The purpose of this study was to determine the effect of α-lipoic acid on human skeletal muscle creatine uptake by directly measuring intramuscular concentrations of creatine, phosphocreatine, and adenosine triphosphate when creatine monohydrate was co-ingested with α-lipoic acid. Muscle biopsies were acquired from the vastus lateralis m. of 16 male subjects (18–32 y) before and after the experimental intervention. After the initial biopsy, subjects ingested 20 g · d^{-1} of creatine monohydrate, 20 g · d^{-1} of creatine monohydrate + 100 g · d^{-1} of sucrose, or 20 g · d^{-1} of creatine monohydrate + 100 g · d^{-1} of sucrose + 1000 mg · d^{-1} of α-lipoic acid for 5 days. Subjects refrained from exercise and consumed the same balanced diet for 7 days. Body weight increased by 2.1% following the nutritional intervention, with no differences between the groups. There was a significant increase in total creatine concentration following creatine supplementation, with the group ingesting α-lipoic acid showing a significantly greater increase ($p < .05$) in phosphocreatine (87.6 → 106.2 mmol · kg^{-1} dry mass [dm]) and total creatine (137.8 → 156.8 mmol · kg^{-1} dm). These findings indicate that co-ingestion of α-lipoic acid with creatine and a small amount of sucrose can enhance muscle total creatine content as compared to the ingestion of creatine and sucrose or creatine alone.

Key Words: antioxidant, muscle biopsy, sport nutrition, anaerobic metabolism

Introduction

α-lipoic acid (ALA) is produced endogenously and consumed in the diet (27). Once produced or ingested, it is promptly taken up by the cell and reduced to dihydrolipoate

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(DHLA) by intracellular reducing equivalents (i.e., NADH, NADPH) and contributes to cellular antioxidant defense (21, 24). α-lipoic acid has been found to affect carbohydrate metabolism through its involvement with the pyruvate dehydrogenase enzyme complex and by increasing glycolytic flux related to elevated concentration of oxidized pyridine nucleotides (27). Increased expression of glucose transporter proteins (GLUT4) and significantly increased muscle glucose uptake has also been found in experiments with ALA treatments (8, 19).

Oral supplementation with creatine monohydrate at a dose of 20 $g \cdot d^{-1}$ for a period of 5–7 days has been found to increase intramuscular creatine concentration (13, 16) and to delay the onset of neuromuscular fatigue (1, 2, 14). The improvement in exercise performance has been attributed to the temporal buffering of energy depletion due to higher initial (resting) phosphocreatine concentrations and to increased phosphocreatine and adenosine triphosphate (ATP) resynthesis rates (5, 12, 13). Greenhaff et al. (13) and Green et al. (12) suggest that an increase in muscle total creatine of ≥ 20 $mmol \cdot kg^{-1}$ dry mass (dm) is required to produce significant improvements in phosphocreatine resynthesis and exercise performance. These findings emphasize the necessity of maximizing creatine uptake and retention during supplementation to enhance skeletal muscle exercise capacity, which is important for athletes or those individuals with low endogenous stores, such as the elderly (4), or with neuromuscular diseases (31).

Green et al. (12) demonstrated that whole body creatine retention was 60% greater when creatine was consumed along with carbohydrate compared to creatine consumed alone. These authors attributed the improved cellular retention of creatine to a stimulatory effect of insulin on the creatine transporter protein (17). However, large doses of either carbohydrate or insulin are necessary to significantly improve creatine uptake (12, 26, 29). The recommended amount of carbohydrate to consume is about 100 g per 5 g of creatine, which is difficult to palate and potentially hazardous to diabetics or those with glucose intolerance.

The effects of orally supplemented α-lipoic acid on cellular metabolism are currently unclear. As well, the membrane transport (sarcolemma and mitochondria) of creatine into and within skeletal muscle is not completely understood. However, knowing that creatine uptake into skeletal muscle is influenced by insulin and simultaneous glucose transport and that α-lipoic acid can increase glucose disposal, it is possible that α-lipoic acid has the ability to enhance creatine absorption by mediating changes in membrane transport. Therefore, the purpose of this study was to determine if combining α-lipoic acid along with a standard creatine loading protocol would increase intramuscular concentrations of creatine and phosphocreatine to a greater extent. It was hypothesized that there would be an increase in intramuscular creatine and phosphocreatine associated with the experimental intervention, and that the combination of α-lipoic acid with creatine and a small amount of sucrose ($100 g \cdot d^{-1}$) would result in greater increases than creatine and sucrose or creatine alone.

Methods

Experimental Design

Subjects were randomly assigned to ingest creatine monohydrate ($4 \times 5 g \cdot d^{-1}$) alone, creatine monohydrate ($4 \times 5 g \cdot d^{-1}$) plus a small amount of sucrose (4×25 g

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· d^{-1}), or creatine monohydrate (4 × 5 g · d^{-1}) plus sucrose (4 × 25 g · d^{-1}) plus α-lipoic acid (4 × 250 mg · d^{-1}, racemic mixture [R and S] Sigma, St. Louis, MO, USA) for 5 days. Prior to supplementation each subject was measured for body weight and had a muscle biopsy taken from his vastus lateralis m. under local anesthetic. These same measures were performed again following 5 days of ingesting the respective supplement treatments. The muscle tissue was analyzed fluorometrically for creatine, phosphocreatine, and adenosine triphosphate concentrations (25, 31). All subjects agreed not to exercise during the course of the study and also to consume the same balanced diet for each day of the study plus 2 days prior to supplementation.

Subjects

Twenty males 18–32 years of age were recruited from the university population. No subject was diabetic or had a history of impaired glucose metabolism. Those subjects selected had not supplemented with creatine monohydrate within the previous 6 weeks, which is greater than the time (4 weeks) required for skeletal muscle creatine concentrations to return to baseline (10). All subjects were recreational weightlifters, training on average three times per week, and all agreed not to exercise during the 7 days of the study. Subjects were not permitted to exercise during supplementation because this has been found to be a stimulant for creatine absorption into skeletal muscle (16). Four subjects dropped out of the study due to discomfort experienced during the baseline biopsy procedure. The protocol for this study was approved by the Human Ethics Review Board at the University of Saskatchewan. Each subject signed an informed consent form and was free to withdraw from the study at any time.

Food diaries indicated that all subjects were non-vegetarians and ate fish, chicken, or red meat a minimum of five times per week. As well, subjects were instructed to consume the same balanced diet for the duration of the study plus 2 days prior (2 d control → 5 d of the study) to control the amount of creatine consumed from the habitual diet plus the addition of the supplement intervention. Subject characteristics are presented in Table 1.

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Table 1 Subject Characteristics (Mean ± Standard Error)

Group	*n*	Age (y)	Height (cm)	Weight (kg)
CR	6	24 ± 0.8	185 ± 1.9	85 ± 4.9
CRS	6	22 ± 0.4	178 ± 1.1	79 ± 2.3
CRSLA	4	23 ± 0.8	180 ± 1.2	81 ± 3.4

Note. CR = Subjects consumed 4 × 5 g · d^{-1} creatine monohydrate for 5 d. CRS = Subjects consumed CR and 4 × 25 g · d^{-1} of sucrose for 5 d. CRSLA = Subjects consumed CRS plus 4 × 250 mg · d^{-1} α-lipoic acid for 5 d.

Supplementation

Subjects were randomly assigned (double-blind) to one of three groups. One group ingested 4×5 g · d^{-1} of creatine monohydrate (CR) alone. A second group ingested 4×5 g · d^{-1} of creatine monohydrate and 4×25 g · d^{-1} sucrose (CRS), while a third group ingested 4×5 g · d^{-1} creatine monohydrate and 4×25 g · d^{-1} sucrose plus 4×250 mg · d^{-1} of α-lipoic acid (CRSLA). The respective daily supplement intervention was ingested every 4–6 hours mixed with approximately 250 ml of water for 5 days. Sucrose was used in two of the experimental groups to disguise the taste of α-lipoic acid, and the group consuming only creatine was included to ensure that the amount of sucrose added did not influence creatine uptake greater than creatine monohydrate alone (Control). This amount of carbohydrate combined with creatine is much less than the amount recommended (~100 g / 5 g creatine dose) to increase muscle creatine uptake during creatine loading. The different supplements (except CR) had the same appearance, taste, and texture and were indistinguishable from one another. Therefore, only two experimental groups were completely blinded (CRS & CRSLA), and the third group (CR) served as a control for the CRS group, since we wanted to know if the amount of sucrose included to blind CRS and CRSLA affected muscle creatine content.

Muscle Biopsy and Analysis

Percutaneous needle biopsies were obtained from the distal third of the vastus lateralis muscle using a 5-mm Stille needle (Micrins, New York, NY, USA) under local anesthetic with 1% lidocaine (Smith-Kline Beecham, Toronto, ON, Canada) and with suction applied via a 60-cc syringe (9). Immediately after the muscle sample was excised, it was exposed to the room air for 60 s and then placed in a plastic vial and frozen in liquid nitrogen (28). The average wet mass obtained during the biopsy was 85 mg. Samples were lyophilized overnight at ultralow temperature and pressure and then stored at –80 °C.

Freeze-dried muscle samples were powdered in a controlled environment, with 15% to 30% relative humidity to prevent rehydration of the muscle sample. Powdering was done manually using 7-cm curved tissue forceps and all connective tissue, and fat was dissected away from the powdered muscle. After powdering, 5 to 10 mg of tissue was weighed and placed into a 2-ml vial for extraction with perchloric acid (31).

Muscle metabolites were extracted with 0.5 M perchloric acid containing 1 mM of EDTA at a ratio of 800 μL to every 10 mg of powder for 15 min on ice, while periodically vortexing. Samples were then centrifuged in a pre-cooled centrifuge (4 °C) for 5 min at 15,000 rpm. The supernatant was weighed into another 2-ml vial and neutralized with 2.2 M $KHCO_3$ added at a volume equal to one fifth the mass of the extract supernatant. The subsequent metabolite assays were performed using methods previously described (15, 25). Neutralized extracts were prepared for spectrophotometric determination of ATP, phosphocreatine (PCr), and free creatine (Cr) using a Hitachi F2500 spectrofluorometer (Chromabec, Montreal, PQ) at an excitation wavelength of 340 nm and an emission wavelength of 445 nm. Coefficients of variation for the ATP, PCr, and Cr assays using this machine were determined using the repeated measurement of 100 samples and found to be 2.05%, 3.78%, and 3.08%, respectively.

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Statistical Analysis

A two-way analysis of variance (ANOVA) procedure (group × time) was used to determine if there were any differences between groups for any of the dependant variables (body weight; Cr, PCr, ATP, and total creatine [TCr] = Cr + PCr). Whenever a significant *F* value was evident, Tukey post hoc tests were performed to identify where significance occurred. Significance was accepted at $p < .05$. All values are expressed as mean ± standard error.

Results

There were no significant differences among groups for any dependant variable at baseline. Body weight increased significantly ($p < .05$) by 1.7 kg (+2.1%) for CRSLA, by 1.6 kg (+2.0%) for CRS, and 1.8 kg (+2.1%) for CR, with no significant difference between groups. There were no changes for free creatine concentration or adenosine triphosphate concentration from pre to post or differences between groups. There was a significant interaction ($p < .05$) for the ANOVA for phosphocreatine concentration, with the change demonstrated following CRSLA treatment (+18.9 mmol · kg^{-1} dry mass) being significantly greater than the other groups. The ANOVA for total creatine also indicated a significant interaction ($p < .05$), with the change from pre to post test following CRSLA being significantly greater than the other groups (138 mmol · kg^{-1} dm → 157 mmol · kg^{-1} dm). Table 2 contains all group means ± standard errors.

Discussion

This is the first study to investigate the effect of oral ingestion of α-lipoic acid on creatine concentration in human skeletal muscle during creatine supplementation. Because the link between glucose disposal and creatine absorption has been established, we reasoned that α-lipoic acid would facilitate creatine uptake greater than when creatine was ingested alone. Indeed, those subjects in the present study who supplemented with α-lipoic acid experienced the greatest increase in muscle total creatine concentration.

Muscle creatine content increases with the oral ingestion of creatine; however, there is large individual variation in the change in total creatine concentration that occurs (5, 16, 20). The cellular transport of creatine across the sarcolemma occurs via a specific sodium-dependant transporter that is enhanced by insulin through stimulation of sodium-potassium ATPase pump activity (23). Green et al. (12) demonstrated that large doses of carbohydrate (CHO) consumed along with creatine augmented creatine uptake into skeletal muscle greater than creatine alone and attributed this to elevated plasma insulin concentrations. As well, this supplement combination (CM + CHO) has been found to reduce individual variation in the increase in total creatine resulting from acute loading doses (6, 12). Robinson et al. (26) and Steenge et al. (29) have found that supraphysiologic levels of insulin are necessary to augment creatine absorption, and therefore suggest that ~100 g of carbohydrate must be consumed along with each 5-g dose of creatine. However, a recent study by these same authors demonstrated that muscle total creatine increased to the same extent when creatine was consumed with 50 g of protein and 47 g of carbohydrate as compared to creatine consumed with 97 g of carbohydrate (30).

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Table 2 Intramuscular Concentrations of ATP, Cr, PCr, and TCr Measured Before and After 5 Days of Ingesting 4 × 5 g · d^{-1} Creatine Monohydrate (CR), CR Plus 4 × 25 g · d^{-1} of Sucrose (CRS), or CRS Plus 4 × 250 mg · d^{-1} α-Lipoic Acid (CRSLA)

	ATP		Cr		PCr		TCr	
Variable	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CR	22.5 (1)	21.1 (2)	40.5 (5)	46.2 (6)	89.5 (3)	88.9 (6)	130.0 (6)	135.1 (5)
CRS	23.1 (2)	21.6 (2)	52.8 (6)	57.0 (5)	85.1 (5)	83.6 (4)	137.9 (7)	140.6 (5)
CRSLA	21.5 (1)	21.6 (1)	50.2 (5)	50.6 (6)	87.6 (4)	106.2 (6)†‡	137.8 (9)	156.8 (9)†‡

Note. Values are means ± standard error in mmol · kg^{-1} dry mass. Results are given for pre and post supplementation for 5 days. †Significant difference from pretest value (within), $p < .05$; ‡significant difference from other posttest values (between), $p < .05$.

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Nevertheless, the increase in muscle creatine was attributed to the increase in plasma insulin that resulted from the addition of the other macronutrients included with the creatine supplement. Our findings suggest that α-lipoic acid could be used to enhance creatine uptake. This may be important for individuals with glucose intolerance who would want to avoid large increases in insulin levels.

The magnitude of change in phosphocreatine and total creatine concentration found in the present study is markedly lower for the CRS and CR groups, but similar for the CRSLA group compared to that reported by others in which subjects consumed creatine alone (1, 5, 16, 18). Subjects in the Harris et al. (16) and Hultman et al. (18) studies experienced an increase of about 20% in total creatine, and the ratio of phosphocreatine to creatine decreased after supplementation. However, in the present study subjects in the CRS and CR groups only experienced an increase of about 5% in total creatine, with little or no change in phosphocreatine. This change is similar to the modest change reported by Odland et al. (22), who also found no change in phosphocreatine concentration after 3 days of 20 $g \cdot d^{-1}$ combined with exercise. Vandenberghe et al. (32) also reported a smaller increase (6%) in PCr concentration than previous findings (16, 18) after 4 days of loading creatine (20 $g \cdot d^{-1}$) alone. Because these authors used ^{31}P-NMR to assess muscle metabolite content, there are no values for free creatine and total creatine. The increase in phosphocreatine and total creatine for the CRSLA group was +18 $mmol \cdot kg^{-1}$ dm for phosphocreatine and +19 $mmol \cdot kg^{-1}$ dm for total creatine. This 17% increase in total creatine is similar to most other acute loading studies involving direct measurements of muscle creatine concentration (1, 5, 16, 18). Subjects consuming creatine alone in the Green et al. (12) study increased total creatine to approximately the same level as the subjects in the present study (mean post TCr = 144 $mmol \cdot kg^{-1}$ dm), although those consuming creatine plus CHO in the Green et al. (12) study increased total creatine to 152 $mmol \cdot kg^{-1}$ dm. It is important to note that the change in muscle PCr and TCr exhibited by the CRSLA subjects in the present study was less than that reported in other studies (12, 16, 18), which may have been related to the starting muscle creatine levels.

Baseline muscle creatine concentrations exhibited by subjects in the present study were approximately 10% higher (~135 $mmol \cdot kg^{-1}$ vs. ~125 $mmol \cdot kg^{-1}$) than initial values reported by many other studies (1, 5, 16, 18). These high initial muscle creatine levels may have been due to high animal meat diets as indicated by the food records or may have been due to subjects incorrectly reporting their history of creatine use. Regardless, these high initial values are a limitation of this study and may have been the reason for the lack of significant increase in PCr and TCr experienced by the CRS and CR groups.

In conclusion, creatine supplementation caused a significant increase in body mass and total creatine concentration, and α-lipoic acid supplementation increased muscle phosphocreatine and total creatine concentration greater than creatine and a small amount of sucrose or creatine alone in this population. These findings are of interest to individuals wishing to elevate muscle creatine content and anaerobic energy status in an attempt to improve athletic performance or to prevent age-related or disease-related impairment in creatine retention or metabolism. Future studies are needed to determine if these findings would be the same in creatine-naïve subjects (i.e., lower initial creatine) and to determine the minimal effective dose of α-lipoic acid necessary to significantly increase muscle total creatine concentration and if this translates into improved exercise performance.

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