RESEARCH

Oral Creatine Supplements Lower Plasma Homocysteine Concentrations in Humans

WILLIAM J KORZUN

OBJECTIVE: To determine if oral creatine supplements will lower the concentration of total plasma homocysteine (tHcy).

SETTING/PARTICIPANTS: Apparently healthy volunteers, at least 19 years old, were recruited from the University of South Alabama and surrounding community.

DESIGN/INTERVENTION/MAIN OUTCOME: Participants took multi-vitamins daily for four weeks, then were randomly divided into two groups. The control group (C) continued to take multi-vitamins daily for an additional four weeks. The experimental group (EX) took multivitamins plus an amount of creatine each day equal to twice their daily creatinine excretion, for the additional four weeks. Total plasma homocysteine concentrations were measured in all participants at the beginning and at the end of the second four week interval.

RESULTS: There were no statistically significant differences between the two groups in age, initial tHcy, serum folate, erythrocyte folate, serum vitamin B_{12}, or creatinine excretion. After four weeks of creatine supplements, tHcy in EX changed by an average of –0.9 µmol/L (range: -1.8 to 0.0), compared to an average change of +0.2 µmol/L in C (range: –0.6 to 0.9) during the same four weeks. The difference in the changes in tHcy between the two groups was statistically significant (p <0.01).

CONCLUSION: Creatine supplements may be an effective adjunct to vitamin supplements for lowering tHcy.

ABBREVIATIONS: C = group of participants taking only multivitamins; EX = group of participants taking multivitamins plus creatine; Hcy = homocysteine; SAH = S-adenosylhomocysteine; SAM = S-adenosylmethionine; tHcy = concentration of total plasma homocysteine.

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INDEX TERMS: creatine; homocysteine; human.

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Hyperhomocysteinemia is a risk factor for the development of cardiovascular disease in humans.[1-4] Homocysteine (Hcy) is a by-product of metabolic pathways in which methyl groups are transferred from S-adenosylmethionine (SAM) to 'acceptor' substrates. These include the biosynthetic pathways for creatine, epinephrine, phosphatidylcholine, and the methylation of DNA and RNA. In most tissues, Hcy is eliminated by remethylation to methionine, which requires adequate intake of vitamin B_{12} and folic acid. The liver also has the capacity to eliminate Hcy by transsulfuration to cysteine, dependent on an adequate intake of vitamin B_6, and to remethylate Hcy to methionine utilizing betaine as the methyl donor.[5] Hcy that cannot be metabolized by these tissue pathways is exported to the blood and contributes to the total plasma homocysteine concentration (tHcy).[2] Dietary supplements containing the three vitamins required for the elimination of Hcy have been shown to reduce tHcy in a variety of settings.[6-8] However, the effect of obviating the homocysteine-generating pathways on tHcy has not been extensively studied.

Each day, the average individual loses, depending on muscle mass, 10 to 15 millimoles of creatine due to spontaneous degradation to creatinine. Biosynthesis of creatine, to replace that loss, involves two enzyme-catalyzed reactions. In the first reaction, the amidino group of arginine is transferred to glycine, forming guanidinoacetate and ornithine. In the second reaction, a methyl group is transferred from SAM to guanidinoacetate to form creatine and S-adenosylhomocysteine (SAH). SAH is subsequently hydrolyzed to adenosine and Hcy, thus generating one molecule of Hcy for each

molecule of creatine synthesized. It has been estimated that creatine biosynthesis could potentially account for up to 75% of daily Hcy production.[5] High levels of exogenously provided creatine can repress the synthesis of the enzyme that catalyzes the first step in creatine biosynthesis in chick embryo livers and in rat kidneys.[9,10] Furthermore, rats fed a creatine-enriched diet had decreased tHcy compared to controls.[11] Oral creatine supplements are commonly used by athletes to enhance performance.[12,13] Studies involving creatine supplements in healthy individuals have failed to produce reports of adverse effects.[12-17] Therefore, this study was undertaken to determine if oral creatine supplements could lower tHcy in healthy volunteers. While this study was in progress, it was reported that creatine supplementation produced a small but statistically insignificant decrease in tHcy in healthy women, with or without a concurrent program of resistance training.[18]

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the University of South Alabama. Volunteers claiming to be in good health were recruited from the University and surrounding community. A total of 19 subjects gave written, informed consent after the study design and procedures were verbally explained to them.

Pure creatine monohydrate ($C_4H_9N_3O_2 \cdot H_2O$, molecular weight = 149 g/mole) was purchased from Experimental and Applied Sciences, Golden CO. One-a-day multivitamins (Centrum) were purchased from a local retailer. Each vitamin tablet reportedly contained 100% of the current recommended daily allowances of folic acid (400 µg), vitamin B_{12} (6 µg), and vitamin B_6 (2 mg).

To begin the study, each subject was given a bottle of multivitamins and was instructed to take one tablet every day for the duration of the study. They were also instructed to refrain from using creatine supplements until further notice. After four weeks, a fasting blood sample was collected by venipuncture and processed as described below. Each subject then collected and delivered a 24-hour urine specimen. The subjects were randomly assigned to either a control group (C) (n = 10) or an experimental group (EX) (n = 9), and their 24-hour creatinine excretion rates were measured. During the next four weeks, those in EX ingested, in addition to the multivitamins, a single daily dose of creatine monohydrate, added to the room temperature or chilled beverage of their choosing, equal to twice their daily creatinine excretion on a molar basis. Each subject in EX was provided a bottle of creatine monohydrate and a plastic scoop with a calibration mark to indicate the daily dose of creatine monohydrate for that subject. The daily dose of creatine monohydrate for each subject in EX was determined with the following formula:

(Daily dose of creatine monohydrate) = (2) x (24-hour creatinine excretion rate) x (1.319)

The 24-hour creatinine excretion is expressed in g/24 hrs., the factor of 1.319 represents the ratio of the molecular weight of creatine monohydrate (149g/mole) to the molecular weight of creatinine (113 g/mole), and the factor of 2 is used to insure an excess of creatine ingestion relative to metabolic demand. For example, subject 001 had a creatinine excretion rate of 1.17 g/24 hours, and was given a scoop calibrated for 3.1 g of creatine monohydrate. The dose of creatine ingested by subjects in EX ranged from 2.1 to 5.5 grams per day. During the second four weeks, those in C continued to take only the multi-vitamins. A second fasting blood sample was then collected from each subject at the end of the second four week period.

After an overnight fast, blood samples were collected by venipuncture into three commercially available evacuated tubes. One tube contained tripotassium EDTA, one contained lithium heparin, and the third contained no additive. Immediately after completion of the venipuncture, 100 µL of the EDTA-whole blood was mixed with 1.0 mL of folate lysis reagent (Abbott product # 9C13-60) containing ascorbic acid and guanidine hydrochloride, incubated at room temperature in the dark for 90 minutes, and then stored at -70 °C. The remainder of the EDTA specimen was centrifuged at 1500 x g at 4 °C for ten minutes, and the plasma then aliquoted and stored at -70 °C. The subject's hematocrit was determined from the heparinized sample. The additive-free blood was allowed to clot in the dark at room temperature for 30 minutes, centrifuged at 1500 x g at 4 °C for ten minutes, and the serum aliquoted and stored at -70 °C. Upon receipt, the 24-hour urine samples were mixed, the volumes measured, and aliquots were stored at -70 °C.

All frozen samples for a given analyte were analyzed on the same day, immediately after thawing at room temperature and mixing. All samples from a given subject were analyzed in the same run. Immediately prior to analysis, thawed hemolysates for erythrocyte folate levels, and urines for creatinine measurement were diluted with the protein diluents recommended by the manufacturers of the analyzers with which those tests were performed.

Serum folate, erythrocyte folate, and serum vitamin B_{12} were measured with an AxSYM System (Abbott Diagnostics), tHcy was measured with an IMx Analyzer (Abbott Diagnostics), hematocrits were measured with a NOVA 1 Analyzer (NOVA Biomedical), and urine creatinine concentrations were measured with a Dimension Analyzer (Dade-Behring). All analyses were performed with reagents, calibrators, and controls from the instrument manufacturers and according to their published instructions. Erythrocyte folate results were corrected for the subjects' hematocrits and serum folate concentrations.

The statistical significance of the differences between C and EX was determined with the Mann-Whitney U test.[19] Differences were considered to be significant if p was <0.05.

RESULTS

Eight volunteers in C and eight volunteers in EX completed the study, with the results presented in Table 1. There were no statistically significant differences between the C and EX groups in any of the measured parameters, except for the change in tHcy. The tHcy decreased in seven out of eight subjects in EX, with four subjects showing a decrease of 18% to 27%. Only three out of eight subjects in C showed decreases in tHcy, ranging from 1% to 9%. The difference between the C and EX groups in the changes in tHcy, was statistically significant, utilizing the Mann-Whitney U test (p <0.01). The means (and CVs) of the three control materials for the tHcy assay, during a 21 week period that spanned the course of this study, were 6.7 µmol/L (5.2%), 12.3 µmol/L (3.3%), and 25.7 µmol/L (2.8%). Among the eight subjects in EX, there was no significant correlation between their initial tHcy and the magnitude of the decrease in tHcy after four weeks of creatine use (Spearman rank-order correlation coefficient = 0.17, p >0.1). There was no significant correlation between serum B_{12}, serum folate, or erythrocyte folate levels after the first four weeks, and subsequent changes in tHcy during the next four weeks (Spearman rank-order correlation coefficients: 0.23, -0.24, and –0.18, respectively, p >0.1 for each).

DISCUSSION

The results of this study suggest that creatine supplements, in modest doses, may lower tHcy in humans. The doses of creatine required to provide twice the daily demand for creatine in the study subjects ranged from 2.1 to 5.5 g per day. Athletes typically ingest 2 to 5 g per day as a maintenance dose following several days of a 20 to 30 g per day loading dose when using creatine as a performance-enhancing supplement. The only published reports of seriously adverse effects of creatine supplements involve an individual who had pre-existing focal segmental glomerulosclerosis, and individuals who consumed ≥20 g per day for at least four weeks.[20-22]

The changes in tHcy seen in EX after four weeks of creatine supplementation, compared to the changes in tHcy seen in C, support the hypothesis that exogenous creatine may suppress endogenous creatine biosynthesis sufficiently to lower Hcy production and tHcy. However, given the analytical imprecision of the assay for tHcy as seen in the CVs of the controls, and published estimates of the biological variation in tHcy ranging from 7.0% to 9.4%, only half of the sub-

Table 1. Study results*

Characteristic	Control group	Creatine group
Gender	3 males, 5 females	4 males, 4 females
Age (years)	31 ± 13 (21 – 58)	29 ± 8 (22 – 47)
Serum folate (nmol/L)	31.6 ± 3.6 (29.0 – 39.9)	30.9 ± 1.2 (29.4 – 32.8)
Erythrocyte folate (nmol/L)	1006 ± 352 (609 – 1588)	993 ± 218 (528 – 1225)
Serum vitamin B-12 (pmol/L)	459 ± 163 (259 – 757)	367 ± 59 (274 – 435)
Creatinine excretion (mmol/day)	10.9 ± 3.4 (6.5 – 16.9)	11.2 ± 4.2 (6.6 – 17.6)
Initial tHcy (µmol/L)	6.2 ± 1.2 (4.0 – 7.6)	7.1 ± 1.8 (4.8 – 10.3)
Change in tHcy (µmol/L)†	0.2 ± 0.5 (-0.6 – 0.9)	-0.9 ± 0.7 (-1.8 – 0.0)

* Except for gender, results are expressed as mean ± SD (range).
† Difference between control and creatine groups is statistically significant (Mann-Whitney U test, p <0.01)

jects in EX experienced a larger decrease in tHcy than what could be explained by random variation.[23] Part of the explanation for these findings may be that all subjects had fairly low tHcy at the onset of the study. It may be that hepatic remethylation and transsulfuration in some subjects were already at a rate commensurate with Hcy production; and the decrease in creatine biosynthesis had no effect on the export of Hcy into the plasma. There were also several variables in this study that were not controlled that may have affected the results.

One factor that may have influenced the results was that some of the subjects may not have achieved high enough creatine levels in vivo to completely suppress endogenous creatine synthesis. Creatine, in the amounts consumed by the subjects in this study, is not completely soluble in some fluids at or below room temperature. Some of the dose may not have completely dissolved in the beverage chosen by the subject as the vehicle for the creatine, but may have remained at the bottom or adhered to the side of the container in which the creatine and the beverage were mixed. Also, some of the dose may have been ingested in the solid form, rather than in solution. It has been recently reported that creatine administered in a solid form is absorbed, but that lower peak plasma concentrations may result, compared to when the same dose is ingested as a solution.[24] Furthermore, it was assumed that the subjects correctly followed the instructions for participation in the study; however, their compliance was not verified.

Another potentially significant variable in this study was the dietary habits of the subjects. Subjects were not restricted in the amounts or types of food they consumed. They were simply asked to not alter their eating habits during the study. Methionine, in excess of what is required for essential methylation reactions and protein synthesis, is converted to Hcy as the methyl group of SAM is transferred to glycine.[5,25] An inadvertent increase in dietary methionine could potentially offset the effect of reduced creatine synthesis on tHcy. This effect of methionine intake is confounded, however, by the potential interindividual variation in the daily turnover of polyamines. Methionine and SAM are consumed in the biosynthesis of spermidine and spermine; however, homocysteine is not generated by this pathway of SAM utilization.[25] The level of choline in the diet may also have impacted the results because choline is a precursor to betaine, which can drive the remethylation of Hcy independently of the cobalamin and folate dependent methionine synthase reaction. Thus, the inconsistent response of the subjects to creatine ingestion may be partly due to variations in dietary intake of choline and methionine, relative to the metabolic demand for SAM in pathways other than creatine synthesis.

In contrast to the results of this study, Steenge found an insignificant decrease in tHcy in females after eight weeks of creatine supplements with or without concurrent resistance training.[18] This discrepancy in results cannot be explained by the inclusion of male subjects in the present study because three of the four subjects in EX with significant decreases in tHcy were female. The authors of the earlier study suggest that the level of methionine in the diets of their subjects may have maintained their tHcy levels in spite of the potential effect of the creatine supplements.

CONCLUSION

The results of this study suggest that oral creatine supplements may lower tHcy in some individuals. This study should be repeated with a larger number of subjects and with more rigorous control over the diets of the subjects and the mode of delivery of the creatine. Furthermore, the study should be extended to include subjects with hyperhomocysteinemia, particularly those whose tHcy is not normalized by vitamin supplementation.

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