Effect of supplementation with a cysteine donor on muscular performance

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Lands, L. C., V. L. Grey, and A. A. Smountas. Effect of supplementation with a cysteine donor on muscular performance. *J. Appl. Physiol.* 87(4): 1381–1385, 1999.—Oxidative stress contributes to muscular fatigue. GSH is the major intracellular antioxidant, the biosynthesis of which is dependent on cysteine availability. We hypothesized that supplementation with a whey-based cysteine donor [Immunocal (HMS90)] designed to augment intracellular GSH would enhance performance. Twenty healthy young adults (10 men, 10 women) were studied presupplementation and 3 mo postsupplementation with either Immunocal (20 g/day) or casein placebo. Muscular performance was assessed by whole leg isokinetic cycle testing, measuring peak power and 30-s work capacity. Lymphocyte GSH was used as a marker of tissue GSH. There were no baseline differences (age, ht, wt, %ideal wt, peak power, 30-s work capacity). Follow-up data on 18 subjects (9 Immunocal, 9 placebo) were analyzed. Both peak power [13 ± 3.5 (SE) %, $P < 0.02$] and 30-s work capacity (13 ± 3.7%, $P < 0.03$) increased significantly in the Immunocal group, with no change (2 ± 9.0 and 1 ± 9.3%) in the placebo group. Lymphocyte GSH also increased significantly in the Immunocal group (35.5 ± 11.04%, $P < 0.02$), with no change in the placebo group (−0.9 ± 9.6%). This is the first study to demonstrate that prolonged supplementation with a product designed to augment antioxidant defenses resulted in improved volitional performance.

oxidative stress; exercise

OXIDATIVE STRESS contributes to the development of muscular fatigue (27). GSH is a major intracellular antioxidant, the biosynthesis of which depends on the intracellular availability of cysteine (1). Previous work has shown that supplementation with *N*-acetylcysteine can slow the onset of muscular fatigue (26). However, there are significant adverse effects with such treatment (18, 26), possibly related to elevations in extracellular cysteine (1, 24). Cysteine, in the form of glutamylcystine moieties, more readily enters into cells. Immunocal, a whey-based oral supplement with a relative abundance of glutamylcystine, has been shown to augment intracellular GSH concentrations in vitro (5). We hypothesized that if this would occur in vivo, then supplementation with Immunocal would improve muscular performance.

MATERIALS AND METHODS

Twenty healthy young adults (10 men, 10 women) were enrolled in the trial. After signing informed consent, subjects were randomly assigned to take either Immunocal (10 g/dose twice daily) or an equivalent amount of casein placebo for 3 mo (5 men and 5 women in each group; both Immunocal and casein placebo were provided by Immunotec Research, Vaudreuil, PQ). Subjects were supplied with a canister containing a 30-day supply. Subjects returned each month for a refill of their treatment. The change in weight of the canister was used as an indicator of compliance. Both subjects and principal investigators were blinded as to which supplement was given until the subject completed the trial.

Subjects were studied in the morning, 3–4 h after a standardized breakfast (1 glass of low-fat milk, 2 slices of unbuttered toast with jam, and 1 glass of juice). Subjects underwent the same measures at the beginning and end of the trial.

Subjects in stockinged feet had their height measured on a stadiometer, and their weight was recorded while they were lightly clothed and standing on an electronic balance. Weight was also expressed as a percentage of ideal weight for height and age (14). Triceps and subscapular skinfold thicknesses were used to assess their percentage of body fat (19).

Subjects had 10 ml of blood drawn from an antecubital vein into a heparinized syringe for analysis of lymphocyte GSH concentration on four occasions before each exercise session: twice, separated by 2–3 h 2 days before the study, and twice, separated by 2–3 h on the day of the study. The mean of the four measurements was used for pre- and postsupplementation comparison. Subjects also completed an activity questionnaire, which asked them to recall their activity on a typical day in the past week (3). Briefly, the questionnaire breaks the day into segments between meals. The time spent in moderate (e.g., walking) and vigorous (e.g., running, cycling) activity was calculated for each time period as well as the percentage of awake time spent being active.

Muscular ability was assessed during isokinetic cycling (20, 23). Although a 30-s isokinetic sprint is primarily glycolytic (25, 35), the total work performed during the sprint correlates strongly with aerobic capacity, as measured during progressive cycle ergometry testing (20, 23). The combination of sprint work capacity with lung function allows for the prediction of maximal progressive work capacity, whereas the short nature of the test allows for the evaluation of the independent contribution of peripheral skeletal muscle function to exercise limitation in both health and disease (20, 22, 23). A 30-s isokinetic sprint results in electrolyte flux, lactate generation, and increased oxygen consumption (25), conditions that should give rise to reactive oxygen species.

On a custom-designed cycle, which has been previously described (21), the subject made an all-out effort during 30 s of cycling at 60 rpm. During cycling, the strain on the flywheel axle strut is continuously monitored at 200 Hz. These data are subsequently analyzed to give the maximal power achieved (W) and the total work achieved (kJ). Both values were expressed as a percentage of predicted, using gender-specific equations on the basis of age and height (23).

Lymphocyte preparation. Blood was diluted in an equal amount of RPMI-1640 medium, and the resultant mixture

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was placed in a tube containing 4 ml of Ficoll-Hypaque and centrifuged at 400 *g* (1,400 rpm, IEC-7) for 30 min. The cells at the interface (90% lymphocytes) were removed by pipette and resuspended in 10 ml of 4°C RPMI-1640 and kept on ice. The suspension was then centrifuged at 450 *g* (1,800 rpm) in a 4°C centrifuge (IEC-PR6) for 10 min. After removal of the supernatant, the pellet was washed again in cold RPMI-1640. The pellet was resuspended in 4 ml of 1× PBS (pH 7.40), and a 0.2-ml aliquot was removed for automated cell counting (Coulter S-plus JR). The cell count was used to calculate the suspension volume required for a 1×10^6 lymphocyte aliquot.

Aliquots of appropriate volume were then centrifuged in prechilled tubes at 500 *g* (800 rpm, Eppendorf 5402) for 10 min at 4°C. The supernatant was removed, and the pellet was resuspended in 970 μl of cold, distilled water. To this, 30 μl of 30% 5-sulfosalicylic acid (SSA) were added to make a final concentration of 0.9% SSA, and the solution was incubated for 15 min on ice. The solution was then centrifuged at 5,000 *g* (8,000 rpm, Eppendorf 5402) for 10 min at 4°C. The supernatant was removed and stored at −70°C for later analysis of GSH.

GSH analysis. Total GSH in the 0.9% SSA extract was determined by the glutathione reductase recycling method of Tietze (33) adapted for the Cobas Mira spectrophotometer (Roche Diagnostics) (9). Briefly, the Cobas Mira pipettes 210 μl NADPH (0.3 mmol/l), 30 μl DTNB (6.0 mmol/l), and 95 μl of sample, standard, or 0.9% SSA into cuvettes. After a 4-min incubation at 37°C, 15 μl glutathione reductase (1.0 U/100 μl) are added, and the reaction is monitored every 24 s for 12 min. Under these conditions, the method is linear for GSH concentrations between 0.5 and 5 μmol/l. The instrument constructs a calibration curve by assaying known GSH standards, and from this the GSH concentration of the unknown is evaluated. Reproducibility for GSH at these concentrations is <2% (intra-assay coefficient of variation). Laboratory control mean value ($n = 7$) is 1.31 μmol/10^6 lymphocytes, with a range of 0.69–2.18 μmol/10^6 lymphocytes.

Data analysis. Statistical analysis was performed by using Statistica 5.1 for Windows (Statsoft). Data were expressed as means ± SE. The groups' baseline data were compared by unpaired *t*-testing. Changes from baseline within each group were assessed by paired *t*-testing, and the changes between the groups by unpaired *t*-testing. A P value <0.05 was considered as significant.

RESULTS

At baseline, there were no significant differences in age, height, weight, percent ideal body weight, or percent body fat (Table 1). Leg peak power and 30-s work were not significantly different at baseline, nor were lymphocyte GSH levels and the percentage of awake time spent being active (Table 2).

Table 1. *Subject characteristics*

	Supplemented	Control
Age, yr	23.7 ± 1.20	23.7 ± 1.24
Height, cm	164.7 ± 2.75	169.0 ± 2.80
Weight, kg	69.1 ± 7.02	65.1 ± 2.66
Percent ideal body weight, %	106.0 ± 7.03	101.9 ± 3.03
Percent body fat, %	21.9 ± 2.13	18.6 ± 2.08

Values are means ± SE; $n = 9$ subjects/group. Supplemented and control groups were administered Immunocal (20 g/day) or casein placebo, respectively.

Table 2. *Baseline parameters*

	Supplemented	Control
Peak power, %predicted	96.4 ± 6.73	91.4 ± 2.64
30-s Work, %predicted	81.3 ± 6.22	79.0 ± 3.27
GSH, μmol/10^6 lymphocytes	1.19 ± 0.10	1.45 ± 0.15
Time spent in activity, %awake time	49.7 ± 2.68	62.3 ± 5.78

Values are means ± SE; $n = 9$ subjects/group.

One subject on placebo withdrew from the trial, citing headaches within the first few weeks. One subject on Immunocal had follow-up exercise results that were technically unacceptable. Results on follow-up data are reported for the remaining 18 subjects. The supplemented group showed significant increases in both measurements of muscle performance and lymphocyte GSH levels. No such differences were seen in the control group, and the differences between groups were all significant (Table 3, Fig. 1). The supplemented group also spent more time in activity, whereas there was no change in the control group. Although body weight did not change significantly in either group, the supplemented group had a decrease in their percent body fat. Both groups ingested ~90% of their supplement (Immunocal: 91.9 ± 5.8%; placebo: 90.6 ± 11.65%).

DISCUSSION

We demonstrated that ingestion of Immunocal, at a dose of 20 g/day, resulted in a 35.5% increase in circulating lymphocyte GSH concentrations. At the same time, supplemented subjects were able to generate more power to perform more work during a 30-s maximal effort. γ-Glutamyl amino acids can be transported into cells. In the case of glutamylcystine, this can effectively increase cellular GSH concentrations (2).

Immunocal is a bovine whey protein concentrate produced by a proprietary lenient technique involving microfiltration and low-temperature pasteurization of milk. Whey protein consists of several compounds, including albumin, lactoferrin, and α-lactalbumin, which are rich in cystine (the oxidized form of cysteine) residues. Albumin and lactoferrin are also rich in glutamylcystine, which is easily transported into cells, making it a more readily available substrate for GSH biosynthesis (4, 5). Immunocal contains 2.5% cystine, compared with 0.3% for casein.

Table 3. *Percent change from baseline*

	Supplemented	Control
Weight	−1.0 ± 0.57	0.2 ± 0.94
Percent body fat	−4.8 ± 1.96*†	5.1 ± 3.26
Peak power	13.3 ± 3.48*†	1.6 ± 3.00
30-s Work	12.7 ± 3.66*†	0.9 ± 3.10
Lymphocyte GSH	35.5 ± 11.04*†	−0.9 ± 9.64
Time spent in activity	13.7 ± 5.59*	4.7 ± 13.86

*Significantly different from baseline: percent body fat, $P < 0.05$; peak power, $P < 0.01$; 30-s work, $P < 0.02$; lymphocyte GSH, $P < 0.01$; time spent very active $P < 0.01$; time spent in activity, $P < 0.04$.
†Significantly different from control: percent body fat, $P < 0.02$; peak power, $P < 0.02$; 30-s work, $P < 0.02$ lymphocyte GSH, $P < 0.02$.

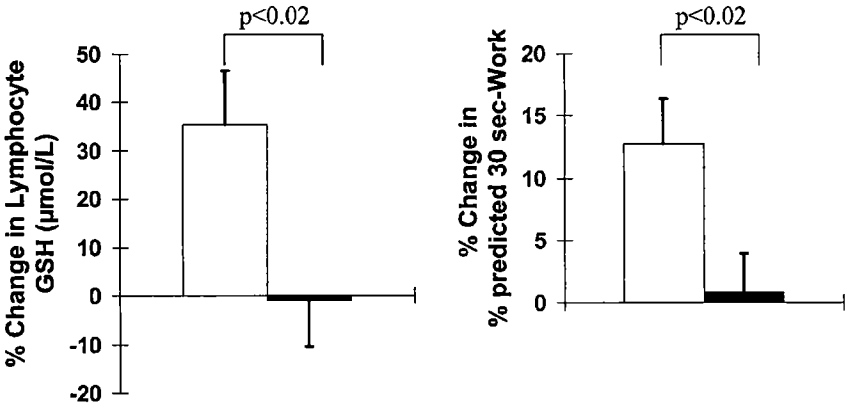

Fig. 1. Percent change from baseline in lymphocyte GSH levels (*left*) and predicted 30-s leg work capacity (*right*) in Immunocal (open bars) and control group (filled bars). Error bars, SE. Immunocal group was significantly different from baseline ($P < 0.01$) and from control group ($P < 0.02$) for lymphocyte GSH levels. Immunocal group was significantly different from baseline ($P < 0.02$) and from control group ($P < 0.02$) for 30-s work.

We utilized lymphocyte GSH concentrations as a marker of tissue GSH levels, as evidence from animal studies has suggested that this could track tissue levels, in response to both L-2-oxothiazolidine-4-carboxylic acid, a cysteine precursor, and buthionine sulfoximine, an inhibitor of the first, rate-limiting step in GSH synthesis, γ-glutamylcysteine synthase. Although a small trial of L-2-oxothiazolidine-4-carboxylic acid raised lymphocyte GSH levels, it also resulted in elevations in plasma cysteine and adverse effects (24). Although some patients did complain of bloating and occasional queasiness while on Immunocal, no other complaints were noted. This study, then, is the first demonstration of a well-tolerated oral supplement that could effectively raise tissue GSH concentrations.

Of more import are the functional findings. Subjects on Immunocal were able to generate greater power and increased the amount of work they could achieve during an all-out 30-s sprint. We have previously demonstrated that leg muscle 30-s isokinetic work output is a significant factor contributing to progressive exercise performance in patients with cystic fibrosis (20) and patients after lung transplant (22), independent of the effect of pulmonary impairment. Similar results have also been demonstrated in healthy adults (23). The ability to perform exercise has a significant impact on quality of life (10). Our results suggest then that the improvements in volitional performance that we measured in the laboratory can have a direct impact on functional ability. In this regard, it is intriguing that the subjects on Immunocal increased the percentage of time they spent being active.

Oxidative stress is associated with strenuous muscular contraction, leading to fatigue (8, 13, 15, 16, 28, 31). However, as pointed out by both Reid and colleagues (26) and Sen (27), although biochemical parameters of oxidative stress can be altered by supplementation, it has been difficult to demonstrate improvement in muscular performance. Animal models of muscular fatigue have demonstrated a beneficial effect of pretreatment with *N*-acetylcysteine (29, 32). Reid and co-workers were the first to demonstrate that pretreatment with intravenous *N*-acetylcysteine could increase force output of the tibilais anterior in humans when electrically stimulated to fatigue at low frequencies. A recent study reported that the time to voluntary task failure (inability to maintain 80% of maximal transdiaphragmatic pressure while breathing against a resistive load) in healthy humans could be increased by the use of intravenous *N*-acetylcysteine (34).

N-acetylcysteine can serve to maintain adequate stores of GSH through several mechanisms, including supplying cysteine for GSH biosynthesis and directly scavenging reactive oxygen species. Because *N*-acetylcysteine does not cross the sarcolemma or increase blood total GSH concentrations but does reduce blood GSH oxidation after exercise (28), it is likely that the results of Reid and co-workers (26) were due to *N*-acetylcysteine's free radical scavenging effects. The prevention of free radical-induced muscular dysfunction by free radical scavenging most likely explains the results of recent animal studies of diaphragm fatigue (32). However, other potential effects, such as improved blood flow or increased central nervous system respiratory drive, could also contribute (12). Unfortunately, *N*-acetlycysteine is associated with a number of adverse effects that detract from its utility as an ergogenic aid. These include blurred vision, dysphoria, and gastrointestinal discomfort. In the study by Travaline and co-workers (34), four subjects were premedicated with diphenhydramine and ranitidine to prevent the development of adverse effects.

The exact mechanism(s) of how Immunocal improved muscular performance is unclear. The most obvious mechanism would be an increase in intracellular glutathione levels, leading to a decrease in oxidant-induced muscular dysfunction. Our patients increased the percentage of time spent in moderate-to-vigorous activity, so that a central effect leading to increased activity and improved neural regulation of muscular function cannot be excluded. Many of the subjects reported a sense of feeling more energetic. This feeling could relate to central mechansims but could also relate to a decrease in muscular damage from antioxidant protection, as muscle soreness and sarcolemma permeability have been linked to oxidative stress (30). Our activity questionnaire provides us with time spent in activity but does not describe how that time was spent. However, this enhanced activity could have led to a training effect.

Subjects on Immunocal had a decrease in their percentage of body fat while maintaining their weight. Although this result sounds almost too good to be true, in healthy subjects plasma concentrations of cysteine and glutamine have been prognostic of subsequent changes in lean body mass (17). In patients with wasting disorders, such as cancer and human immunodeficiency virus infections, these values are reduced early on, preceding overt cachexia (7, 11).

The biochemical changes seen in wasting disorders have led to the concept of a low cyst(e)ine-glutamine syndrome. In this model, hepatic catabolism of cyst(e)ine to sulfate leads to the generation of hydrogen ions, which remove bicarbonate through buffering. Bicarbonate is required for the first rate-limiting step in the conversion of ammonium to urea. Removal of bicarbonate promotes ammonia's conversion to glutamine, thus conserving nitrogen in the amino acid pool. Our results are consistent with this model of cysteine metabolism. The change in redox state resulting from augmentation of glutathione stores could also alter gene expression to promote muscle growth (6). This suggests that supplementation with a cysteine donor may favorably influence body composition toward increased muscle mass. We do not believe that the changes we saw in body composition and muscle function were simply due to augmented protein intake, as the casein-supplemented group did not demonstrate these changes.

In conclusion, supplementation of healthy young adults with a whey-based oral supplement augmented lymphocyte GSH concentrations, while increasing muscular performance in these subjects. Aside from its potential as an ergogenic aid, such supplementation may have particular benefit in patients with persistent inflammatory conditions.

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