

Glutamine-Antioxidant Supplementation Increases Body Cell Mass in AIDS Patients With Weight Loss: A Randomized, Double-Blind Controlled Trial

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ABSTRACT

Loss of body cell mass, the active functioning tissue of the body, commonly occurs in patients with human immunodeficiency virus (HIV) infection, and the extent of wasting is related to the length of survival. We evaluated the anabolic role of the amino acid L-glutamine (GLN) and antioxidants in a double-blind, placebo-controlled trial in 26 patients with >5% weight loss since disease onset. Subjects received GLN-antioxidants (40 g/d) in divided doses or glycine (40 g/d) as the placebo for 12 wk. Throughout the study, the subjects were seen weekly by a nutritionist, and body weight, bioelectric impedance assessment, and nutritional counseling were performed. Twenty-one subjects completed the study, and the groups were well matched. The 5 patients excluded from analysis all met a priori exclusion criteria. Over 3 mo, the GLN-antioxidant group gained 2.2 kg in body weight (3.2%), whereas the control group gained 0.3 kg (0.4%, $P = 0.04$ for difference between groups). The GLN-antioxidant group gained 1.8 kg in body cell mass, whereas the control group gained 0.4 kg ($P = 0.007$). Intracellular water increased in the GLN-antioxidant group but not in the control group. In conclusion, GLN-antioxidant nutrient supplementation can increase body weight, body cell mass, and intracellular water when compared with placebo supplementation. GLN-antioxidant supplementation provides a highly cost-effective therapy for the rehabilitation of HIV+ patients with weight loss. *Nutrition* 1999; 15:860-864. ©Elsevier Science Inc. 1999

Key words: glutamine, antioxidants, weight loss, wasting, human immunodeficiency virus (HIV), AIDS

INTRODUCTION

Significant weight loss commonly occurs in patients with human immunodeficiency virus (HIV) infection.¹ The extent of loss of body cell mass (BCM), which is the metabolically active tissue of the body, correlates with length of survival.¹ Attempts to reverse this erosion of protein-rich tissue with appetite stimulants, oral nutritional supplements, and enteral or parenteral nutrition have resulted in deposition of adipose tissue, with variable or no restoration of BCM.²⁻⁴ Recombinant human growth hormone (rhGH) administration resulted in gain in lean tissue, but the effects were not sustainable once treatment was terminated.⁶

It has been hypothesized that the conditionally essential amino acid, L-glutamine (GLN), may be rate limiting for repletion of

BCM.⁷ GLN is synthesized primarily in skeletal muscle and released into the circulation. Tissues that consume GLN, e.g., the immune system, gastrointestinal tract, kidney, and liver, extract GLN as needed from the circulation.⁸⁻¹¹ During stress and inflammation, consumption of GLN exceeds the ability of skeletal muscle to supply this amino acid. Blood and muscle GLN concentrations fall, and progressive muscle breakdown occurs in an attempt to satisfy the GLN demand.⁷

At present, standard parenteral nutrition solutions and most enteral feeding formulas do not contain GLN; therefore, repletion is not possible.

GLN plays a major metabolic role in the maintenance of visceral tissues. This amino acid is the primary fuel for enterocytes

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and colonocytes and for the body's immune tissue.^{12,13} It is used at sites of tissue repair and participates in renal acid-base balance.¹⁴ It also provides the glutamate necessary for glutathione production.¹⁵ The demand for GLN in all of these situations is greatly heightened during infection with HIV.⁷

Nutrients do not function in isolation. For example, all necessary amino acids and cofactors must be available to achieve protein synthesis. Moreover, the production of reactive oxygen species has been associated with AIDS wasting and weight loss.¹⁶ In an effort to provide an environment more favorable to anabolism, a mixture of antioxidant nutrients was provided along with glutamine.

This study was undertaken to determine whether GLN supplementation and select antioxidants could satisfy an increased GLN requirement as occurs with weight loss and thus reverse the loss of BCM in patients with AIDS.

METHODS

Study participants were men and non-pregnant women, with HIV infection and without active opportunistic infections, recruited from private-practice physicians who specialize in HIV care in Broward County, Florida. The study was approved by the Human Ethics Committee of Pompano Beach Community Hospital, and signed informed consent was obtained from each volunteer before enrollment, with written approval from each patient's physician. Eligible study patients were randomized to a placebo-control or an experimental group.

The study was a randomized, double-blind trial with block design to balance groups for gender; age = <40 or ≥40 y; antiviral treatment, i.e., any or none; nutritional status, 5–14% weight loss versus >14% weight loss; and mode of contracting the disease, i.e., intravenous versus non-intravenous drug use. The subjects were eligible if they had experienced ≥5% unintended loss of usual body weight since the onset of the illness or had a <90% standard creatinine/height index, reflecting loss of lean tissue.

Patients were included if they had not been on other protocols or received other experimental medications for at least 2 mo before the study and were medically stable. If the patients were receiving B₁₂ or folate, they must have received these vitamins for at least 1 mo before starting the trial. If using testosterone, they must have received the hormone for 4 mo or more before entering this trial and continued it at the same dosage throughout the trial. If the subjects had been using N-acetyl cysteine, they discontinued this supplement; they continued to take ascorbic acid supplements at the usual dosage if they were using this vitamin.

Exclusion criteria included the current use of more than 5.0 g/d of glutamine, cirrhosis of the liver, or renal failure. If the subjects reported chronic diarrhea (>2 loose stools/d), an evaluation was performed to exclude an infectious etiology. If diarrhea was non-infectious, the subject was entered into the trial. All patients completed the Willett Food Frequency Questionnaire¹⁷ before consultation with the nutritionist and at the completion of the study. The subjects were also instructed to keep a 3-d dietary record to evaluate initial and final dietary eating patterns, the consumption of vitamin, mineral, and other nutritional supplements, and alcohol and recreational drug use. The 3-d diary included 1 d during the weekend and 2 d during the week. Three-day diet records were analyzed with The Food Processor, version 7.0, nutrient analysis software (Esha Research, Salem, OR, USA). Because GLN administration has been associated with changes in mood,¹⁸ the subjects also completed a 30-item profile of mood assessment form from the Short Form (SF) 36 for patients with HIV to assess mood at the beginning and end of the study.¹⁹

Weekly for 3 wk before the initiation of the nutritional supplement, the subjects had a bioelectric impedance assessment

(BIA; Model 101Q, RJL Systems, Clinton, MI, USA) to determine body composition. The measurements were taken as instructed by the manufacturer, and body compartments were determined by the manufacturer's Fluid and Nutrition Program (3.1b). A mean was taken of these values to determine initial body composition; body weight (BW) was likewise determined from the three baseline weight measurements. All participants had intensive nutritional counseling with a registered dietitian to ensure stable and adequate nutrient intake. Measurement of BW, BIA determination, and nutritional counseling continued at weekly intervals throughout the study, unless the subject was hospitalized or on vacation.

The study duration was 12 wk. All subjects received a daily vitamin and mineral preparation equal to the recommended dietary allowances to ensure intake of these micronutrients. Glycine, 40.0 g/d, was given as placebo. The treatment group received GLN 40.0 g/d, along with selected antioxidant nutrients (ascorbic acid 800.0 mg/d, α -tocopherol 500.0 IU/d, β -carotene 27 000.0 IU/d, selenium 280.0 μ g/d, and N-acetyl cysteine 2400.0 mg/d; Cambridge Nutraceutical, Boston, MA, USA). The 40.0 g GLN dose was selected because open label studies providing 30.0 and 40.0 g GLN/d have demonstrated marked weight gain and improvement in BCM,²⁰ and a blinded pilot study using 20.0 g GLN/d failed to demonstrate consistent results.²¹ The supplements were taken daily in four divided doses.

Packets were dispensed at 14-d intervals, and used packets were returned to monitor compliance.

Statistical Analysis

The primary objective of this study was to determine whether GLN-antioxidant supplementation was related to a gain in BW and/or BCM. The secondary objective was to determine whether this nutrient supplement was related to changes in CD4 count, mood, or dietary intake.

Only patients who completed the study were evaluated. At the initiation of the study, criteria were established that excluded randomized patients from analysis if the following occurred: the subjects were unable to consume the nutrient supplements; the subjects were unable or unwilling to participate in the periodic evaluations during the protocol (failure to be seen for an interval of more than 2 wk or have tests performed); individuals who sustained an intercurrent illness that prevented the ingestion of the supplement for 5 d or more; subjects who, for medical reasons, had a major change in their antiretroviral therapy during the course of the study; and individuals who sustained an acute major catastrophic illness or injury and could not continue in the study.

Data were analyzed with SYSTAT, version 5.2 (SYSTAT, Evanston, IL, USA). All data were tested for normality of distribution. Within- and between-group differences were examined using *t* tests and non-parametric tests when appropriate (Wilcoxon and Mann-Whitney *U* test). BW and BCM were averaged over 4-wk intervals by using all available data points to handle missing data due to subject schedule conflicts. Absolute and relative values were compared over time by using repeated measures analysis of variance; analysis of covariance was used to control for calories and other specific nutrients.

RESULTS

Twenty-six subjects were enrolled in the trial, and 21 subjects completed the study. Dropouts occurred for the following reasons: 1 control subject was placed on protease inhibitors, 1 control subject died, and 2 control and 1 GLN subjects dropped out because of study conflicts. Despite the loss of 5 individuals, the groups were well matched (Table I); there were no individuals who had a past history of intravenous drug abuse, and all but 3 individuals were taking antiretroviral medication. CD4 counts

TABLE I.

PATIENT CHARACTERISTICS AT STUDY ENTRY MEAN (RANGE)	PATIENT CHARACTERISTICS AT STUDY ENTRY MEAN (RANGE)	
	Placebo-control	Glutamine-antioxidants
Number of subjects	9	12
Age (y)	42 (33-53)	40 (30-50)
Gender	8 M, 1 F	11 M, 1 F
Height (cm)	176.1 (157.5-188.0)	175.3 (157.5-188.0)
Weight (kg)	71.6 (49.3-87.9)	68.3 (53.1-82.8)
Weight loss (%)	5.2 (0.3-8.0)	10.7 (2.0-18.0)*
Body mass index (kg/m ²)	22.9 (19.9-24.9)	22.2 (19.5-25.5)
CD ₄ + T lymphocytes (cells/mm ³)	183 (13-364)	147 (1-327)
Medications Taken		
Antiretrovirals (n subjects)	8	10
Antiretrovirals taken (n drugs)	19	21
Protease inhibitors (n subjects)	4	6
Protease inhibitors taken (n drugs)	4	7
Testosterone (n subjects)	1	1

*P = 0.008.

were similar in the two groups, and the medications prescribed were comparable in each group (Table I).

Treatment with GLN resulted in a significant and sustained increase in BW of 2.2 kg over 3 mo (3.2%). In contrast, control patients initially gained weight, but then BW returned to prestudy values; at 12 wk, the weight gain was 0.3 kg (0.4%, P = NS; Fig. 1).

Similar to BW, the GLN-antioxidant group gained more than 1 kg in BCM during the first month, and this gain was sustained over

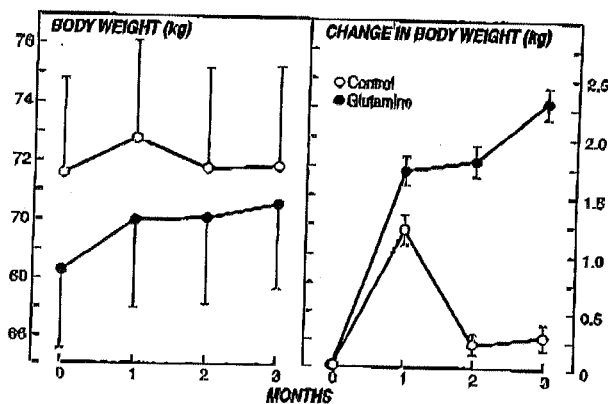


FIG. 1. The effect of placebo and L-glutamine-antioxidant supplementation on body weight (BW; left) and change in BW (right) over time (mean ± SEM). Using analysis of variance over time, an interaction was observed between time and treatment (P = 0.04). When the relative change over time was analyzed (ΔBW), there was a significant linear trend over time remaining (P = 0.03), with ΔBW at week 12 being significantly different at P = 0.04.

the 12-wk study period (Fig. 2), so the total gain in BCM averaged 1.8 kg. The control group initially gained BCM over the first month, but this could not be sustained; at 12 wk, the final gain in BCM was only 0.4 kg. Thus, at 12 wk, the treatment group had a significantly greater increase in both BCM (P = 0.007) and BW (P = 0.04) than did the control group.

Sixteen of the 21 individuals completed both the initial and final food frequency questionnaires (76%), 18 of 21 (86%) completed the initial 3-d food records, but only 43% returned the final 3-d records. From the available data, dietary intake did not show any significant difference between baseline and the end of the study. (From the food frequency questionnaire data, n = 16, calories were 2256.0 versus 2376.0 kcal/d; mean value baseline versus end of study; protein 101.5 versus 106.9 g/d, carbohydrate 298 versus 322.0 g/d, fat 75.4 versus 76.6 g/d; P = NS.) The gain in BCM in the GLN-antioxidant group continued to be significant after controlling for calories, protein, carbohydrate, or fat intake. This was true when using data derived from either the initial and final food frequency questionnaires or the food records. Initial fat mass was similar in the two groups (Table II) and did not change over the course of the study. Total body water tended to rise in both groups. However, this rise was due primarily to an increase in intracellular water (ICW) in the GLN group, which rose 1.7 L over the 12-wk study period (Table II). CD4 counts remained stable throughout the study in both groups (mean ± SD: 206 ± 164 and 140 ± 115 cells/mm³ for the control and GLN groups, respectively) at the end of the study.

The SF30 assessment of mood score was initially 93% of optimal in both groups (raw scores were 120 ± 15 points for the control group versus 112 ± 24 points for the GLN group, P = NS), and this assessment did not change in either group, as measured at the end of the study (120 ± 21 versus 110 ± 18, P = NS).

No side effects to the supplements were reported or observed.

DISCUSSION

This study demonstrates for the first time that the provision of a specific nutrient supplement, when coupled with nutritional counseling, can improve weight and restore BCM. Patients who received the combination of GLN and antioxidants for 12 wk increased BW an average of >2 kg and BCM by 1.8 kg. In contrast, control subjects initially gained weight but were unable

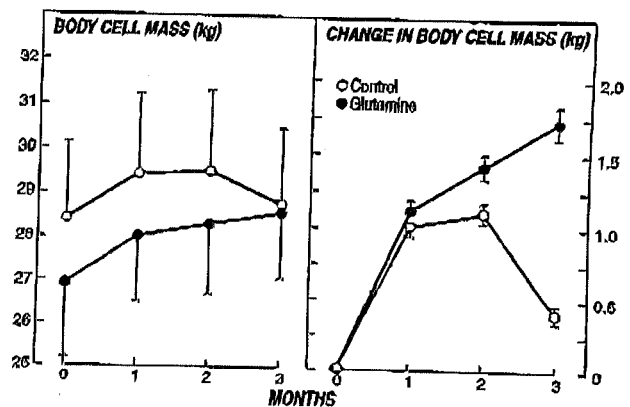


FIG. 2. The effect of placebo and L-glutamine-antioxidant supplementation on body cell mass (BCM; left) and relative changes in BCM (right) over time (mean ± SEM). By analysis of variance, there was a statistically significant interaction between time and treatment (P < 0.01). The relative changes in BCM were significant at week 12 (P = 0.007).

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TABLE II.
MEASUREMENTS TAKEN BEFORE AND AFTER 12 WK OF NUTRIENT SUPPLEMENTATION (MEAN \pm SD)

	Placebo-control		Glutamine-antioxidants	
	Before	After	Before	After
Weight (kg)	71.6 \pm 11.2	71.9 \pm 11.2	68.3 \pm 8.3	70.6 \pm 8.9
Body cell mass (kg)	28.4 \pm 6.0	28.8 \pm 6.1	26.9 \pm 4.4	28.6 \pm 4.6*
Intracellular water (L)	25.9 \pm 5.4	26.5 \pm 5.5	25.6 \pm 4.7	27.3 \pm 5.0*
Extracellular water (L)	17.3 \pm 2.9	17.0 \pm 2.8	16.7 \pm 1.3	16.4 \pm 1.8
Total body water (L)	43.3 \pm 8.0	43.5 \pm 7.9	42.3 \pm 5.0	43.8 \pm 5.7†
Fat mass (kg)	11.9 \pm 4.6	11.9 \pm 4.8	10.6 \pm 3.4	10.9 \pm 2.7

* $P < 0.001$ versus baseline.† $P = 0.01$ versus baseline.

to maintain the increased BW. At 12 wk, these individuals demonstrated a non-significant gain of 0.3 kg in BW and 0.4 kg in BCM.

The basis for the determination of BCM was the measurement of reactance and resistance using BIA.²² This technique has increasingly been accepted in the evaluation of HIV+ subjects, and measurements derived from this analysis have been correlated with length of survival, total body water, and BCM in patients with AIDS.²³ We found minimal changes in fat mass and extracellular water in both groups. However, there was a significant increase in BCM and ICW in the group receiving GLN-antioxidant supplementation.

The supplement studied contained two classes of active ingredients: GLN, an amino acid, and antioxidant nutrients (vitamins C and E, β -carotene, selenium, and N-acetyl cysteine). We cannot exclude the role that antioxidants may play in this response, but there is a strong physiologic basis to suspect that glutamine is the primary nutrient responsible for this effect.⁷ Open label studies using similar doses of GLN produced virtually the same results as observed in the present study.²⁰ In addition, no reports have been forthcoming describing changes in BW or body composition associated with antioxidant therapy alone.

Isotopic studies in subjects with HIV wasting have shown a preferential reduction in muscle protein, a failure to sustain an elevated rate of protein synthesis, and a significant increase in GLN release into the blood stream.²⁴ By providing exogenous GLN, the increased GLN demands were presumably satisfied and possibly skeletal muscle protein synthesis was supported.²⁵ Additional kinetic measurements are required to delineate the specific mechanisms involved and also determine the role of the antioxidant nutrients in these subjects.

Accumulation of lean tissue has also been observed in patients with AIDS wasting after rhGH administration.²⁶ Studies performed over 12 wk of rhGH administration showed a gain in lean tissue to approximately the same degree as that seen with subjects receiving GLN in this study.

Cost has become a consideration in the care of chronically ill patients. Based on the cost of rhGH at \$1000/wk versus GLN-antioxidant supplement at \$31/wk, the cost for anabolism of 1.0 kg of BCM to a person with HIV wasting using rhGH is approximately \$9230. The cost for a similar kilogram of BCM using GLN-antioxidant supplementation is \$220.

We did not monitor the study subjects who received GLN after termination of the trial, and we do not know the effects of GLN withdrawal on body composition. Protein kinetic studies have demonstrated increased proteolysis and synthesis rates in asymptomatic HIV+ patients, with increased GLN release in symptomatic individuals.²⁴ This finding and the results of the present trial suggest that glutamine is a critical nutrient in this disease, and continuous provision of this amino acid seems warranted.

Although the BW of the two groups was similar at the beginning of the study, the group receiving the active treatment at the beginning of the study weighed about 3.0 kg less than the control group, and they had lost a greater percentage of their BW during the course of their illness. Could the response to nutritional therapy be different in these two groups? Other studies have suggested that the more favorable responses to nutritional therapy in patients with HIV-wasting occur in patients with minimal weight loss.²⁷ This would favor a weight gain in our control not our treatment group. The control subjects were offered GLN-antioxidant supplementation upon completion of the blinded trial, and four subjects received the supplement in an open label fashion. Over the ensuing 12 wk, these individuals gained a mean of 2.1 kg BW and 1.3 kg BCM while on GLN-antioxidant supplementation.

Larger multicenter studies are needed to determine whether GLN-antioxidants will support BCM and reduce the incidence of infection over the long term, as has been observed in other populations.²⁸⁻³⁰ This study should serve as a catalyst to evaluate further the impact of GLN-antioxidant supplementation, a cost-effective combination, on the long-term morbidity and mortality in patients with HIV infection.

SUMMARY

This randomized, double-blind, placebo-controlled trial demonstrates for the first time that supplementation of the amino acid GLN and the provision of adequate antioxidants and nutritional counseling to subjects with HIV wasting can improve BW and restore BCM. This low-cost and low-risk supplement may be the preferred method of initial nutritional support in patients with weight loss of $>5\%$. Larger trials are needed to evaluate the clinical impact of this approach on reducing opportunistic infection and possibly long-term mortality.

REFERENCES

1. Kotler DP, Tierney AR, Wang J, Pierson RN Jr. Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. *Am J Clin Nutr* 1989;50:444
2. Von Roenn JH, Armstrong D, Kotler DP, et al. Megacal acetate in patients with AIDS-related cachexia. *Ann Intern Med* 1994;121:393
3. Chlebowski RT, Beull G, Grosvenor M, et al. Long-term effects of early

