Immune and Metabolic Effects of Arginine in the Surgical Patient

JOHN M. DALY, M.D., JOHN REYNOLDS, M.B., F.R.C.S.(I), ARLEEN THOM, M.D., LINDA KINSLEY, B.S.N., MARIANNE DIETRICK-GALLAGHER, M.S.N., JIAN SHOU, M.D., and BRUCE RUGGIERI, M.S.

Arginine enhances immune function and promotes nitrogen retention in animal models, but its immunomodulatory effects in surgical patients are unknown. This randomized, prospective trial evaluated the immune and metabolic effects of supplemental L-arginine (25 g/day, n = 16) or isonitrogenous L-glycine (43 g/day, n = 14) in 30 cancer patients undergoing major operation. Two groups of patients received either arginine or glycine for 7 days after surgery as a supplement to a graduated enteral diet. Nitrogen balance was measured daily, and immune parameters were determined both before and after surgery, on Days 1, 4, and 7. The T-lymphocyte response to concanavalin A (con A) and PHA and dual marker phenotype analysis of lymphocyte (CD2, CD4, CD4/DR, CD8, CD8/DR) and macrophage (M3/DR) subsets were determined. Mean age, degree of preoperative weight loss, disease stage, number of perioperative transfusions, and calorie and nitrogen intake were similar for the groups studied. Mean daily nitrogen balance (−2.3 g/day in the arginine group *vs.* −3.9 g/day in the glycine group) was not significantly different between the two groups, but positive mean nitrogen balance was achieved only in the arginine group between Days 5 and 7 after surgery. Supplemental arginine significantly enhanced the mean T-lymphocyte response (stimulation index) to con A from 45 ± 26 on postoperative Day 1 to 72 ± 47 and 87 ± 49 on postoperative Days 4 and 7, compared with the values of 29 ± 15, 27 ± 20, and 33 ± 34 in the glycine group at the same time points, respectively. Supplemental arginine increased mean CD4 phenotype (% T-cells) on postoperative Days 1 and 7 from 25 ± 9 to 43 ± 14, compared with the values of 30 ± 14 and 29 ± 13 in the glycine group ($p < 0.05$). The beneficial effect of arginine on the immune system appeared distinct from its more moderate effect on nitrogen metabolism. As a nutrient substrate, arginine was nontoxic, and may benefit surgical patients who are at increased risk of infection.

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Reprint requests and correspondence: John M. Daly, M.D., Department of Surgery, the University of Pennsylvania School of Medicine, 3400 Spruce Street, Philadelphia, PA 19104.

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From the Division of Surgical Oncology, the Department of Surgery, the University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

MAJOR OPERATION and injury results in weight loss, negative nitrogen balance, and a variable degree of immune dysfunction. Perioperative nutritional support can usually accomplish positive nitrogen balance and ameliorate weight loss.[1] When immune dysfunction is caused by malnutrition, nutritional intervention can also reverse the anergic state, while giving priority to the improvement of weight, serum and tissue protein, and immune function.[1,2] When immunosuppression is a result of the trauma itself, however, nutritional support has generally not been helpful in reversing the dysfunction. In a study of 34 patients undergoing elective abdominal aortic aneurysmectomy, O'Mahony et al. administered a balanced amino acid solution intravenously to these patients.[3] No improvement in lymphocyte mitogenic responsiveness or in phenotype subset analysis was noted. Although generic balanced nutrient formulas have not been helpful in this situation, experimental data has suggested that pharmacologic doses of a single amino acid, arginine, has specific immunostimulatory effects that may be of value in the perioperative period.

In 1978, Barbul et al. noted that, in rats, supplemental dietary arginine reduced the thymic involution that occurs after injury.[4] A significant increase in thymocyte content and thymocyte activation to mitogens occurred in animals receiving supplemental arginine. Seifter et al. demonstrated that arginine improved the survival of injured rodents.[5] Supplemental dietary arginine lessened the post-injury loss of weight and accelerated the increase in wound-breaking strength and the rate of collagen deposition after injury. Arginine has been studied in several

stress models demonstrating improvement in host immunity.[6] A diet containing arginine as 2% of the total nonprotein calories significantly increased animal survival after 30% body surface burn, improved delayed cutaneous hypersensitivity responses, and improved local bacterial containment of subdermal staphylococcal injections.[7] The etiology of these metabolic and immunologic effects is unclear. Arginine stimulates growth hormone secretion as well as other hormones. Theoretically, this neurohumoral response could be responsible for both immune and metabolic effects. However, recent evidence suggests that arginine alone has direct effects on macrophages and lymphocytes that enhance their responsiveness to antigenic stimuli.[8]

Despite numerous studies of a variety of animal models demonstrating the efficacy of arginine-supplemented diets in reducing the catabolic response to major trauma, sepsis, and injury, and in improving the immune response after a variety of adverse stimuli, few studies of this sort have been conducted in humans. Elsair et al. demonstrated improvement of nitrogen balance during 3 days after cholecystectomy had been performed in patients receiving intravenous (I.V.) arginine (15 g/day).[9] Barbul et al. studied normal human volunteers given oral arginine hydrochloride supplements (30 g/day) for 1 week, and noted a significant increase in peripheral blood mean lymphocyte blastogenic response to con A and PHA.[10] However, the immunologic effects of arginine in injured humans have not been reported.

The purpose of this study, therefore, was to evaluate the effects of arginine supplementation on the immune, metabolic, and endocrine parameters in a population of surgical patients known to be at high risk of developing complications during the perioperative period.

Materials and Methods

Adult patients with gastrointestinal (G.I.) malignancies who were to undergo major operation were eligible for entry into the study if they had 1) no history of intestinal disease, 2) no history of insulin-dependent diabetes mellitus, 3) normal renal function (serum creatinine level below 2 mg/ml and blood urea nitrogen level below 30 mg/ml), 4) no previous intestinal resection, 5) no previous abdominal or pelvic radiotherapy, 6) no preoperative evidence of infection (temperature greater than 37.6 C, white blood cell count greater than 10,000 cells/ml^3, or bacteremia, 7) no steroids or anti-immune medication, and 8) body weight not greater than 130% of ideal body weight. Baseline studies on all patients consisted of complete history and physical examination. Preoperative laboratory studies included measurement of serum urea nitrogen, serum glutamic oxyloacetic transaminase, lactic dehydrogenase, albumin, total protein, uric acid, calcium, phosphorus, bilirubin, alkaline phosphatase, and creatinine levels. A complete blood count, as well as prothrombin and partial thromboplastin times, were also obtained. General assessment of nutritional status included measurements of height, body weight, and a description of usual body weight and the percentage of usual body weight and ideal body weight. All patients gave informed consent to this study, which was approved by the institutional review board of the University of Pennsylvania School of Medicine. Patients were stratified based on the extent of their weight loss (less than 10% of usual body weight *vs.* greater than or equal to 10% of usual body weight) and the blood transfusions they had received during their operation (none *vs.* greater than or equal to one). Patients were then randomized to one of two treatment groups. Group 1 patients received enteral alimentation with a modular diet (Table 1) supplemented with 25 g of L-arginine daily, whereas Group 2 patients received the same modular diet with L-glycine (43 g/day) as an isonitrogenous control supplement.

TABLE 1. *Enteral Diet**

	g/L	% Total kcal
Protein	45	16.5
Carbohydrate	200	73.4
Fat	12.2	10.1
(1.09 Kcal/ml)		

* Nutrisource Modular Diet, Sandoz Nutrition, Minneapolis, MN. Diets supplemented each day with either 25 g of L-arginine or 43 g of L-glycine.

At the time of the operation, all patients underwent procedures as indicated by their primary site and stage of disease. A needle-catheter jejunostomy was placed in the proximal jejunum. In the recovery room, an abdominal roentgenogram was obtained after injecting 20 ml of meglumine diatrizoate in order to document that the catheter tip was in the proper location and that no leaks were present. Using an infusion pump, infusion of a 5% dextrose and water solution was then begun through the catheter jejunostomy at a rate of 30 ml per hour. Blood and fluid replacement was continued intravenously as clinically indicated for each patient.

At 11:00 a.m. on the first postoperative day, jejunostomy patients were randomly assigned to receive either an arginine supplemented enteral diet or a glycine supplemented enteral diet. The solutions were formulated by one of the authors (L.K.) and provided daily to the nursing staff for administration to patients. The formulas were coded and the investigators were blinded until after data analysis was complete. Diets were isocaloric and nearly iso-osmolar, with identical protein and fat content provided in a chemically defined, liquid form. The progression of jejunostomy feeding infusion rates is shown in

TABLE 2. *Jejunostomy Feeding Schedule*

Day	Concentration	Amount (ml/hour)
0	0.5 W	30
1	0.5	50
2	0.5	60
3	0.75	60
4	1.0	70
5	1.0	80
6	1.0	80
7	1.0	80

Table 2. This diet was intended to provide approximately 25 kcal/kg per day by the fourth postoperative day. G.I. symptomatology was assessed and recorded daily. If moderate to severe symptoms of nausea, vomiting, abdominal cramping, or diarrhea occurred, the infusion was discontinued for 8 to 12 hours and then resumed, when possible, at the next lower infusion rate. Adverse symptoms were managed as clinically indicated, with diarrhea and cramping treated with enteral codeine or tincture of opium. When adverse symptoms occurred, they were recorded for each day: nausea or vomiting that required antimetics, diarrhea of 3–5 loose bowel movements per day, abdominal cramping or bloating that limited ambulation and was unrelated to flatus, pain that was relieved only by medication and was distinct from incisional pain and fever, and an oral temperature of more than 38.5 C. All patients were managed with sump tube nasogastric drainage, with the tube being removed when clinically indicated. Other than clear liquids taken orally, patients received no nutrition during the first 7 postoperative days. Progression from clear liquids to regular diet occurred as clinically indicated. All patients received I.V. fluids (5% dextrose and 0.5% normal saline solution) and other electrolytes as clinically indicated.

Body weight of all patients was assessed daily. All I.V. and enteral intake was measured and calorie and nitrogen intake was calculated. Urine was collected for each 24-hour period; urine nitrogen content was measured by the Kjeldahl technique, and creatinine was measured by an automated system (Technicon, Tarrytown, NY). Urine nitrogen levels were corrected for each 24-hour period by using urine creatinine excretion, and values were corrected for changes in serum urea nitrogen levels. No attempt was made to collect the fecal output. In the absence of diarrhea, a significant amount of stool was not excreted until oral intake was resumed after Day 7.

Immune Function Studies

Blood was collected at 8:00 a.m. on the day before operation and on the first, fourth, and seventh postoperative days. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. Cells were washed three times with Hank's balanced salt solution (HBSS), resuspended in culture medium, and counted. Cell viability was determined by trypan blue dye exclusion.

Freshly isolated PBMC were suspended at a concentration of 1×10^6/ml in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% heat inactivated pooled normal blood type A serum, 2mM L-glutamine, 100 μ/ml penicillin and 100 μg/ml steptomycin. PBMC were cultured at a concentration of 1×10^6/ml in flat-bottomed microtitre plates with/without con A (Sigma %; 5 μg/ml, St. Louis, MO) or phytohemagglutinin (Sigma; 10 μg/ml). The cultures were incubated in a humidified incubator at 37 C in 5% CO_2 for 3 days. Eight hours before harvesting, 1 μCi of ^{3}H-thymidine was added to each well, and the radioactivity in the pellets counted in a liquid scintillation counter (Tricarb, Model 500, Packard, Sterling, VA). Data is expressed as a stimulation index (SI) where

$$SI = \frac{\text{mean counts per minute stimulated lymphocytes (triplicates)}}{\text{mean counts per minute unstimulated lymphocytes}}$$

The Leu series of monoclonal antibodies, including anti-Leu^5 (CD2), anti-Leu^3 (CD4), anti-Leu^2 (CD8), and anti-Leu M^3 was used in these studies (Becton Dickinson, Sunnyvale, CA). These antibodies are directly conjugated with fluorescein isothiocyanate (FITC). Antibody recognizing the HLA-DR framework was directly conjugated with phycoerythrocein and was used in combination with all the fluoresceinated antibodies for dual-marker subset analysis. Details of the cell surface targets of these antibodies and the population identified are shown in Table 3.

TABLE 3. *Lymphocyte and Monocyte Subset Analysis*

Target Antigens	Target Population	HLA-DR	HLA-DR-bearing cells
Leu 5	Total T-lymphocytes	Leu 3–HLA-DR	Activated inducer/helper T-cells
Leu 3	Inducer/helper T-cells Class II, restricted	Leu 2–HLA-DR	Activated suppressor/cytotoxic T-cells
Leu 2	Suppressor/Cytotoxic T-cells Class I, restricted	Leu M3–HLA-DR	Antigen-presenting monocytes
Leu M3	Monocytes		

TABLE 4. *Patient Characteristics (Mean ± standard deviation)*

	Arginine	Glycine
Number of patients	16	14
Age (yrs)	66 ± 8	62 ± 10
Male/Female	12/4	12/2
Body Weight (kg)	70 ± 15	79 ± 20
% Usual Weight	91 ± 0.1	92 ± 0.1
% Ideal Weight	105 ± 15	112 ± 21
>10% Weight Loss	10 of 16 pts.	7 of 14 pts.
Blood Transfusions	13 of 16 pts.	10 of 14 pts.

TABLE 5. *Diagnosis and Stage of Disease (Numbers of Patients)*

	Arginine Group	Glycine Group
Diagnosis of Disease		
Esophageal	8	6
Gastric	3	2
Pancreatic	3	4
Colorectal	2	1
Melanoma	0	1
Stage of Disease		
I	3	2
II	3	3
III	6	8
IV	2	1

Peripheral blood mononuclear cells (1×10^6) were incubated with an appropriate concentration of antibody diluted in phosphate buffered saline containing 2% fetal bovine serum and 0.1% sodium azide for 45 minutes at 4 C. The stained cells were washed three times and analyzed in a flow microfluorimeter (Ortho Cytofluorograf System, 50HH, Ortho Diagnostics Instruments, MA). A minimum of 1000 lymphocytes and 200 monocytes were analyzed in each specimen. Lymphocyte data is expressed as a percentage of cells in each population relative to total lymphocytes. HLA-DR on cells is described as a percentage of cells from each specific cell population.

Endocrine Measurements

Plasma insulin, plasma glucagon, serum cortisol, serum growth hormone, and serum somatomedin C were measured by radioimmunoassay (RIA), as previously described.[11-13]

Statistical analysis of metabolic endpoints, such as daily nitrogen balance (gram per day) and serum hormonal measurements, was performed using one-way analysis of variance. Analysis of immune function studies was made through the use of one-way analysis of variance and nonparametric testing methods, using the Mann-Whitney and Wilcoxan tests. Data are reported as the mean ± standard deviation. Statistical significance was determined at the $p < 0.05$ level.

Results

Patient Characteristics

Characteristics of the patients upon entry into the study were similar for the two groups, reflecting the homogeneity of the patient population that was studied, as shown in Table 4. The mean age, percentage of usual body weight, and number of patients who had lost greater than 10% of their body weight were similar for both groups. In addition, the clinical and pathologic diagnoses and stages of disease were similarly distributed for the arginine and glycine-supplemented groups, as shown in Table 5. The most common operative procedure was esophagogastrectomy, which was performed in eight of 16 patients who received arginine and in six of 14 patients who received glycine. Transfusions were given intraoperatively or within the first postoperative day in 13 of 16 patients who received arginine and in ten of 14 patients who received glycine, as shown in Table 4.

Metabolic Studies

As shown in Figures 1 and 2, in terms of caloric intake and nitrogen intake during the first 7 postoperative days, mean nutrient intake increased similarly in both groups. As shown in Table 6, mean total daily caloric intake was 1372 ± 549 calories in the arginine-supplemented group compared with 1389 ± 574 calories in the glycine-supplemented group. Mean nitrogen intake was nearly identical in both groups (15.3 ± 6.4 *vs.* 15.0 ± 5.6 g per day). There was no significant difference in mean cumulative nitrogen balance (−2.3 ± 6.5 g *vs.* −3.9 ± 7.0 g) in the arginine- and glycine-supplemented groups, respectively. Mean daily nitrogen balance became positive after Day 5 in the arginine group, whereas it remained negative in

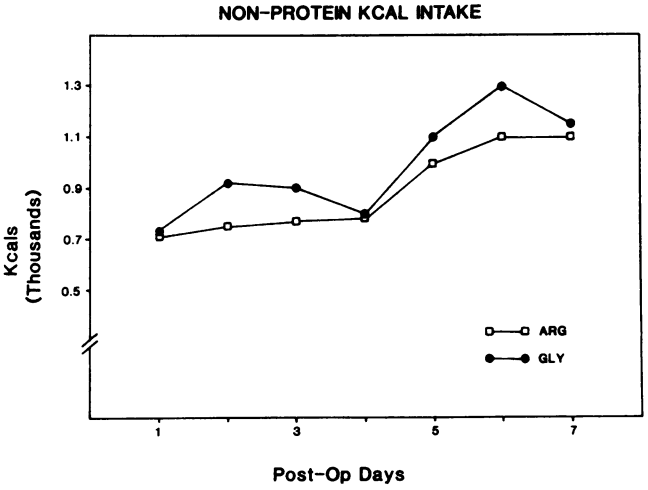

FIG. 1. Nonprotein kcal intake increased similarly in both arginine and glycine groups during the postoperative period as flow rate and formula concentration was increased.

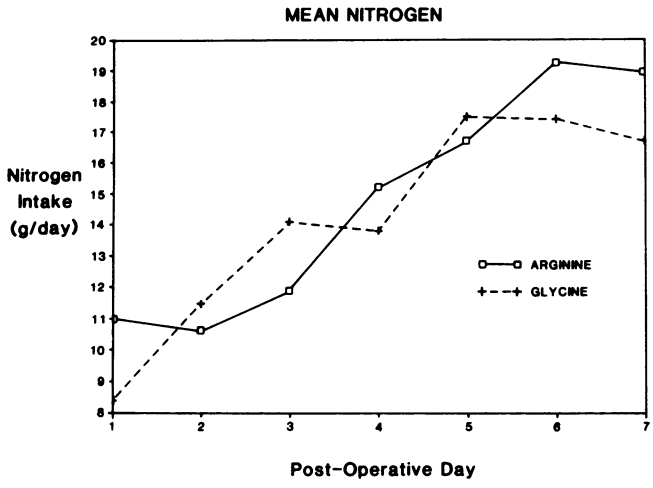

FIG. 2. Nitrogen intake increased similarly in both the arginine and glycine groups. The high initial levels were due to the administration of 25 g of L-arginine or 43 g of L-glycine daily to each patient.

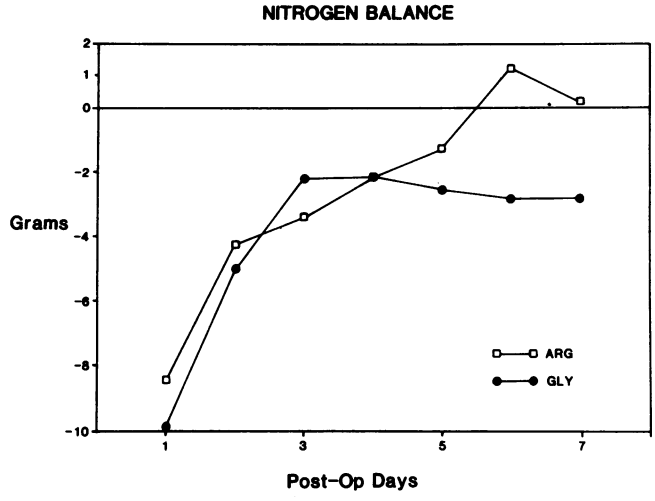

FIG. 3. As dietary intake increased, nitrogen balance improved in both groups. However, only in the arginine group was positive nitrogen balance achieved during postoperative Days 5–7.

the glycine group throughout the 7-day period (Fig. 3). During this 3-day period, mean daily nitrogen balance was +0.1 ± 5.3 g in the arginine group compared with −2.8 ± 6.1 g in the glycine group.

Measurement of plasma amino acids of the two groups revealed significant increases in plasma arginine levels, from a preoperative level of 87 μM/l to 213 μM/l by postoperative Day 7. As shown in Figure 4, plasma ornithine levels increased from 57 μM/l to 259 μM/l in the arginine-supplemented group. In the glycine-supplemented group, plasma arginine levels remained similar throughout the postoperative period, whereas plasma ornithine levels seemed to decrease somewhat. Plasma glycine levels increased from 255 μM/l to 586 μM/l in the glycine-supplemented group as expected. As shown in Table 7, plasma concentrations of lysine, leucine, isoleucine, alanine, and glutamate were not significantly different for the arginine- and glycine-supplemented groups.

Immune Studies

As shown in Figure 5, T-lymphocyte activation to con A demonstrated significant decreases from the preoperative levels to the first postoperative day in both arginine and glycine groups. However, there was no significant difference between the arginine and glycine groups on either of these two days. In the arginine-supplemented group, the mean con A stimulation index increased from 45 ± 29 on postoperative Day 1 to 72 ± 47 and 88 ± 50 on postoperative Days 4 and 7, respectively ($p < 0.05$). By contrast, the mean stimulation index in the glycine-stimulated group remained depressed throughout the postoperative period, with levels varying from 27 ± 20 to 34 ± 23 ($p < 0.05$ arginine group *vs.* glycine group). T-lymphocyte activation to phytohemoglutanin decreased in both the arginine- and glycine-supplemented groups from the preoperative value to the first postoperative day. In the arginine group, the mean stimulation index increased significantly ($p < 0.02$) from 94 ± 69 on postoperative Day 1 to 135 ± 93 and 145 ± 121 on the fourth and seventh

TABLE 6. *Nutrient Intake and Nitrogen Balance (Mean ± Standard Deviation)*

	Arginine Group	Glycine Group
Total kcal	1372 ± 549	1389 ± 574
Nonprotein kcal	1059 ± 424	1092 ± 440
Nitrogen Intake (g)	15.3 ± 6.4	15.0 ± 5.6
Nitrogen Balance (g)	−2.3 ± 6.5	−3.9 ± 7.0

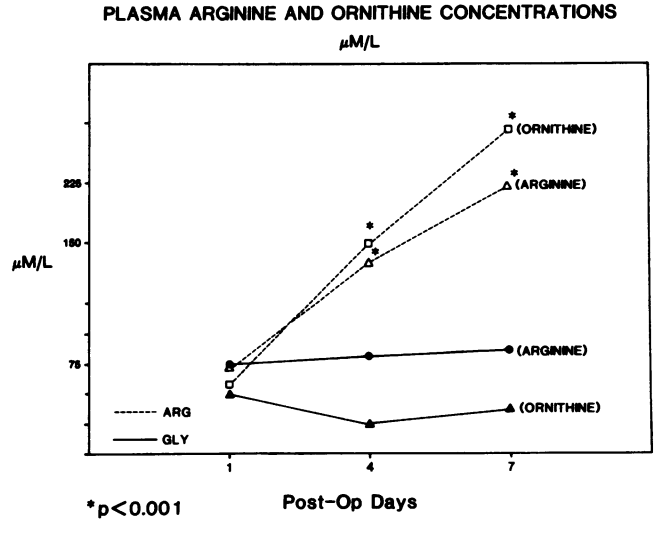

FIG. 4. Mean plasma arginine and ornithine levels increased substantially on Day 4 and Day 7 in the arginine group (dashed lines) compared with the glycine group (solid lines).

TABLE 7. *Plasma Amino Acids (μM/L)*

	Arginine				Glycine			
	Preoperative	Day 1	Day 4	Day 7	Preoperative	Day 1	Day 4	Day 7
Arginine	87 ± 15	76 ± 8	120 ± 73	213 ± 72	103 ± 29	75 ± 16	72 ± 21	80 ± 43
Ornithine	57 ± 21	62 ± 36	181 ± 94	259 ± 76	67 ± 11	60 ± 6	49 ± 14	57 ± 21
Glycine	262 ± 46	169 ± 60	129 ± 24	136 ± 31	255 ± 71	144 ± 67	650 ± 430	586 ± 416
Lysine	184 ± 41	85 ± 24	139 ± 38	141 ± 35	167 ± 35	111 ± 38	177 ± 63	171 ± 46
Leucine	108 ± 23	76 ± 24	139 ± 30	125 ± 50	123 ± 35	83 ± 19	123 ± 29	131 ± 41
Isoleucine	66 ± 28	33 ± 12	69 ± 13	75 ± 26	54 ± 17	27 ± 15	62 ± 22	65 ± 29
Alanine	435 ± 65	243 ± 72	249 ± 76	296 ± 80	364 ± 90	356 ± 367	273 ± 102	374 ± 153
Glutamate	80 ± 24	86 ± 21	60 ± 19	90 ± 48	105 ± 44	64 ± 23	70 ± 46	133 ± 115

postoperative days, respectively. In the glycine-supplemented group, there was minimal elevation of the mean stimulation index, from 44 ± 23 to 57 ± 41 by the seventh postoperative day. The changes in T-lymphocyte activation by PHA were significantly different at the $p < 0.05$ level for the arginine- and glycine-supplemented groups. Measurement of total T-lymphocytes and lymphocyte subsets, using isofluorescence and dual color markers with FACS analysis, demonstrated an increase in the CD4 (T helper cell) expression from 33 ± 13 on the first postoperative day to 43 ± 14 on the seventh postoperative day, in comparison with the glycine group, in which the CD4 expression varied from 33 ± 13 to 29 ± 13 during the same period ($p < 0.05$). As shown in Table 8, other phenotype subsets, including total T-cells, CD8, CD4/CD8 ratio, CD4DR, CD8DR, CD4DR/CD8DR and M3DR showed no significant differences between the arginine- and glycine-supplemented groups during the perioperative period.

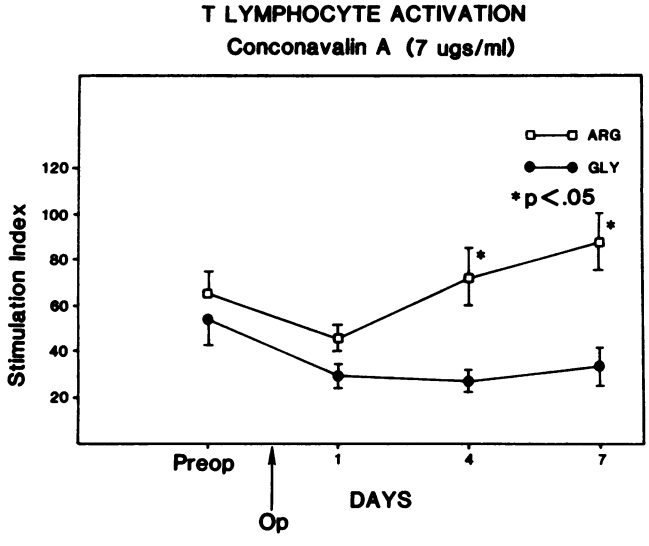

FIG. 5. In both groups T-lymphocyte activation to con A decreased significantly from the preoperative period to postoperative Day 1. However, in the arginine group there was a rapid rise back to normal levels on Days 4 and 7, whereas mean values remained low in the glycine group. (Mean ± SEM).

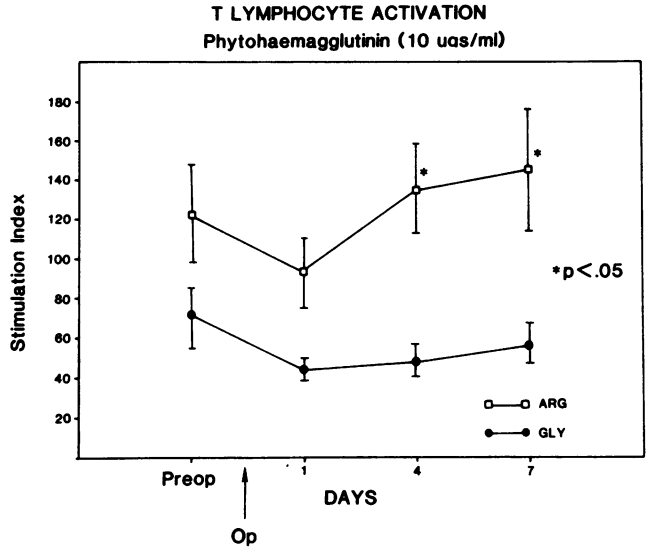

FIG. 6. T-lymphocyte activation to PHA decreased significantly from the preoperative period to postoperative Day 1 in both groups, but rose back to normal levels only in the arginine group. Due to high values in three patients, preoperative mean values were higher in the arginine group than in the glycine group. If matching preoperative values are used, the same findings are noted. (Mean ± SEM).

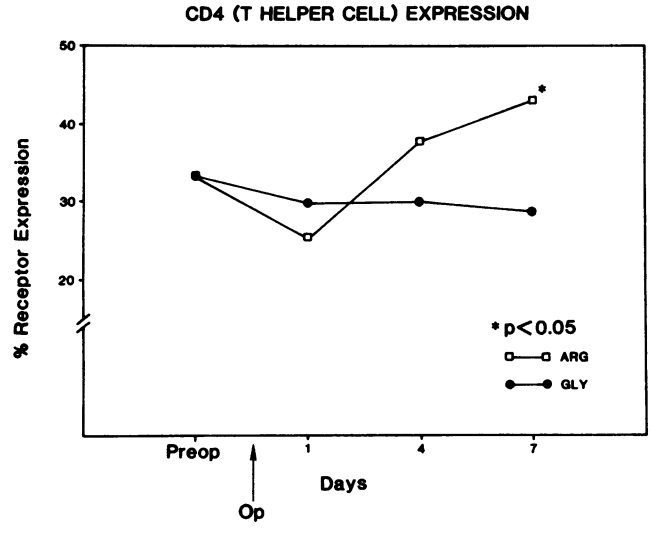

FIG. 7. On Day 7, mean CD4 (T-helper cell) expression increased significantly in the arginine group compared with the glycine group.

TABLE 8. *Lymphocyte/Macrophage Phenotype Subsets**

	Arginine (Days)				Glycine (Days)			
	1§	1¶	4¶	7¶	1§	1¶	4¶	7¶
Total T	49 ± 15	47 ± 20	60 ± 12	61 ± 20	54 ± 14	50 ± 16	46 ± 17	46 ± 13
CD4	33 ± 13	25 ± 9	38 ± 11	43 ± 14†	33 ± 13	30 ± 14	30 ± 12	29 ± 13
CD8	17 ± 9	19 ± 13	18 ± 11	16 ± 10	24 ± 10	21 ± 10	17 ± 11	17 ± 11
CD4/CD8	2.4 ± 1.1	1.8 ± 0.8	2.6 ± 1.5	3.9 ± 2.8	1.7 ± 1.0	1.6 ± 1.1	1.5 ± 1.6	2.3 ± 1.7
CD4-DR	5.1 ± 4.3	7.0 ± 5	5.6 ± 2.8	5.3 ± 3.8	9.1 ± 6.0	7.5 ± 5.0	7.8 ± 4.4	9.2 ± 4.3
CD8-DR	8.0 ± 5.7	11.6 ± 4.7	9.4 ± 3.7	7.0 ± 3.3	11.5 ± 6.4	14.8 ± 11.7	15 ± 14	16.7 ± 9
CD4-DR/CD8-DR	0.62 ± 0.29	0.57 ± 0.3	0.71 ± 0.6	0.74 ± 0.4	0.97 ± 0.6	0.71 ± 4.0	0.8 ± 0.4	0.6 ± 0.3
M3-DR†	59 ± 24	44 ± 22	42.6 ± 22	48 ± 19	50 ± 18	46 ± 23	46 ± 26	49 ± 24

* Percent lymphocytes.
† Percent macrophage.
‡ $p < 0.05$ (Arginine *vs.* Glycine).
§ Preoperative.
¶ Postoperative.

Measurement of serum concentrations of creatinine, glucose, and blood urea nitrogen showed no significant differences in the results in the arginine and glycine groups. As shown in Table 9, mean plasma insulin levels rose during the perioperative period, but there was no significant difference between arginine and glycine groups. Changes in mean plasma glucagon levels were variable and not significantly different between the groups. Mean plasma cortisol levels increased, as expected, from the preoperative to postoperative time periods, but increased similarly in both arginine- and glycine-supplemented groups. Mean plasma growth hormone levels increased in both groups from the preoperative to the first postoperative day. Thereafter, there was no significant difference in mean serum growth hormone levels of the groups. However, mean serum somatamedin C (IGF1) levels were significantly increased in the arginine-supplemented group on postoperative Day 7, in comparison with results of the glycine-supplemented group, as shown in Figure 8.

G.I. complications of the jejunostomy feeding were not common. In both groups, nausea occurred in two patients. Also in both groups, diarrhea occurred in four patients. Abdominal cramping and bloating occurred in four patients who received arginine and six patients who received glycine. Feeding required interruption in two of the patients who received arginine and three of the patients who received glycine. There were no mechanical or infectious complications with the jejunostomy catheters in any patient.

Respiratory, infectious, and G.I. complications occurred in ten of 16 patients of the arginine group, compared with nine of 14 patients of the glycine group. Respiratory complications were most common and occurred predominantly in the patients undergoing esophagogastrectomy. Five respiratory complications occurred in patients of both groups who had atelectasis (five), pulmonary infiltrates with fever (two), and pulmonary embolus (one), pneumothorax (one), and respiratory insufficiency (one). Postoperative intestinal ileus (lasting for more than 7 days) occurred in four instances; pancreatitis occurred in two instances, and a gastric bezoar formed in one patient after the study period had completed. Postoperative oral temperature elevation greater than 38 C by the fifth postoperative day occurred in 25% of the patients of the arginine group and 43% of those of the glycine group. None of the patients had positive blood cultures or hypotension associated with fever. A superficial perineal wound infection occurred in one patient. Three months after surgery, one

TABLE 9. *Cortisol and Circulating Hormone Levels*

Hormone	Group	Preoperative	Postoperative Day 1	Postoperative Day 4	Postoperative Day 7
Cortisol (mg/dl)	Arginine	29.7 ± 18.6	37.2 ± 11.6	30.9 ± 17.6	40.8 ± 28.8
	Glycine	23.4 ± 14.6	42.5 ± 24.9	40.8 ± 25.4	38.9 ± 28.4
Insulin (ng/ml)	Arginine	1.04 ± 0.5	1.36 ± 0.5	1.7 ± 1.1	2.5 ± 1.2
	Glycine	1.24 ± 0.5	1.96 ± 1.5	2.1 ± 1.6	2.5 ± 1.4
Glucagon (pgs/ml)	Arginine	192 ± 82	244 ± 112	162 ± 37	182 ± 59.8
	Glycine	261 ± 130	227 ± 64	290 ± 119	210 ± 73.0
Growth Hormone (ng/ml)	Arginine	2.3 ± 3.0	7.2 ± 5.7	3.6 ± 2.3	5.4 ± 3.3
	Glycine	4.2 ± 3.7	7.1 ± 8.0	4.9 ± 3.4	3.5 ± 4.5
Somatomedin c (U/ml)	Arginine	0.71 ± 0.3	0.33 ± 0.09	0.47 ± 0.2	0.59 ± 0.18*
	Glycine	0.70 ± 0.3	0.435 ± 0.2	0.36 ± 0.2	0.39 ± 0.12

Mean ± SD.
* $p < 0.05$.

patient in the glycine group died of complications that developed after the study had completed.

Discussion

Immunosuppression is a major factor in the cancer patient. Tumor burden, malnutrition, and surgery, with its attendant anesthesia and blood transfusions, have all been found to depress the immune system.[2] Patients with cancer show depression of both cellular and humoral immune functions. Classically, T-cell proliferative responses to both mitogen and alloantigen are reduced with increasing tumor burden. Patients with advanced disease often lack a delayed type hypersensitivity reaction to skin recall antigens (*i.e.*, anergy), the absence of this response being predictive of increased sepsis and mortality. In analyzing anergy, recent studies have found defects in T-cell activation to be the primary abnormality in the delayed type hypersensitivity response.[14] When removed from the "anergic environment" and cultured *in vitro* with recall antigen, however, T-cells proliferate normally. These activated T-cells are capable of eliciting the delayed type hypersensitivity response when reinjected into the patient. Cytotoxic T-lymphocytes isolated from patients with breast, lung, and colon carcinomas, as well as from sarcomas, demonstrate decreased cytotoxicity to autologous tumor.[15] Natural killer (NK) cells, the body's putative first line against cancer, are also less active when isolated from both tumor-bearing animals and patients.

In addition to the immunosuppression that occurs in the presence of malignancy, operative therapy intended to eradicate tumor occasionally promotes metastatic growth. Patients undergoing diverting colostomy followed by colectomy for cancer have been shown to do less well than those treated by one operation.[16] Controlled experiments in animal models corroborate enhancement of tumor growth, but mechanisms remain obscure.[17] Functionally, T-cells show depressed responses to mitogen or alloantigen.[18-20] Eggermont et al., for example, noted that laparotomy abrogated the antitumor effect of lymphocyte-activated killer cells (LAK) in a murine sarcoma model.[21]

Although blood transfusions are often unavoidable in oncologic surgery, they may adversely affect prognosis in the cancer patient.[22-24] Regardless of stage, perioperative blood transfusion has been associated with significantly poorer prognosis in breast carcinoma, colon cancer, non-small cell lung cancer, and sarcoma.[25-28] Although the mechanisms involved are complex and multifactorial, multiple histocompatible blood transfusions depress the immune response that is manifest *in vitro* by poor T-cell proliferative indices and decreased effector cell response to various lymphokines. These defects are implicated in contributing to accelerated tumor growth. For this reason, patients in our current study were stratified for perioperative blood transfusions. Generic nutritional support with protein and calories has not been found to dramatically improve immune function. But certain nutrient substrates such as arginine may play a role in regulating the immune system.

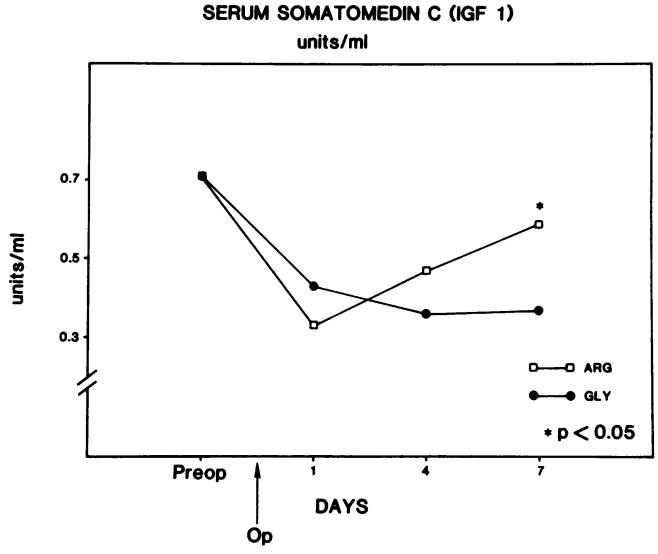

FIG. 8. Mean serum somatomedin C (IGF1) levels decreased on the first postoperative day in both groups. It then rose towards normal levels in patients of the arginine group, but remained depressed in patients in the glycine group.

Arginine is a dibasic amino acid considered semi-essential for times of metabolic stress.[29,30] In such a situation (*e.g.*, after operation or malnutrition), in animal studies, exogenous arginine supplementation consistently improves nitrogen retention, protein turnover, and wound-healing.[5,31] Elsair et al. showed that, compared with control patients, parenteral infusion of arginine (15 g/day) in patients who had undergone cholecystectomy reduced nitrogen excretion by 60%.[9] In our study, as nitrogen and caloric intake improved, the nitrogen balance of both groups improved daily. However, positive mean nitrogen balance was achieved only in the arginine group. Our goal of 25 calories/kg/day was not achieved because of a conservative initial approach to advancement of flow rate and concentration. Abdominal cramping, bloating, and diarrhea occurred in our early patients. Removal of vitamins and electrolytes from the formula reduced the osmolality and abrogated these adverse G.I. symptoms.

The relationship of metabolic effects to the secretagogue properties of arginine is unclear. Arginine infusion induces the secretion of growth hormone, insulin, glucagon, prolactin, and somatostatin.[32-35] Barbul et al. concluded that an intact hypothalamo-pituitary axis was essential in order for arginine to mediate its effects.[36] In our study, there were no significant differences between dietary groups in mean insulin, glucagon, cortisol, and growth hormone

levels. Because of the kinetics of growth hormone secretion, measurement of plasma somatomedin C levels is a better monitor of the overall effects of arginine on growth hormone secretion. Mean somotomedin C levels were significantly higher in the arginine group on Day 7 compared with the glycine group, suggesting a stimulatory effect of arginine on the pituitary.

Supplemental dietary arginine has thymotrophic properties and enhances the responsiveness of thymic lymphocytes to mitogens in normal and traumatized animals.[37] Arginine augments cellular immunity, as evidenced by enhanced skin allograft rejection in normal mice, and improves delayed hypersensitivity responses as well as survival in a 30% burn animal model.[7] In our study, compared with preoperative levels, the mean peripheral lymphocyte responses to the mitogens con A and PHA were significantly depressed in both arginine and glycine groups on the first postoperative day. The mean stimulation index to con A and PHA was 79% and 90% in the arginine group, and 70% and 87% in the glycine group. The arginine group demonstrated a return to baseline levels by Day 4, as shown in Figures 5 and 6, with a continued increase at Day 7 to levels above baseline. By contrast, mean stimulation indexes in the glycine group remained depressed and did not return to baseline levels by the seventh postoperative day. This increase in lymphocyte responsiveness to mitogens is similar to the results of normal volunteers reported by Barbul et al.[10] As shown in Figure 7, the per cent receptor expression for CD4 decreased in both arginine and glycine groups on the first postoperative day. However, in the arginine group, mean CD4 levels increased above baseline on Day 4 and continued to increase on Day 7, whereas mean CD4 levels remained depressed in the glycine group. Other lymphocyte subsets did not demonstrate any significant differences between arginine and glycine groups at any time period measured. Mean monocyte-DR, which measures the functional capacity of the monocyte to present antigen to the host T-lymphocytes, was chosen for measurement, based on the work of Polk et al. suggesting that decreased levels with a slow rise or failure to rise after operative were predictive of sepsis.[38] Mean M-3 DR levels were below the normal range (80–100%) before the operation, and were perhaps associated with the study population, age, or malignancy. Mean M3-DR either decreased further after surgery or remained low throughout the postoperative period. There were no significant differences in mean levels between the arginine and glycine groups. The intriguing possibility exists, therefore, that arginine may have "tissue-specific" properties that influence components of the immune system, and thus may be of value in clinical situations where the immune system is compromised.

Ornithine shares the thymotrophic and immunostimulatory effects of arginine. It is therefore likely that they either share the same cellular mechanism of action or that arginine acts via increasing the concentration of available ornithine.[4] Hacker-Shakin and Droge demonstrated that L-ornithine augments *in vivo* immunization against minor histocompatibility antigens and against tumor cells with a peak effect at a concentration of 0.8 mM.[39] Droge also reported that ornithine supplementation augments interleukin-2 (IL-2) production, postulating that the effect may be mediated by augmenting ornithine decarboxylase (ODC) activity in activated lymphocytes.[40] Thus, ornithine may have a strong influence on T-lymphocyte processes.[41] In our study, mean plasma amino acid levels demonstrated a nearly equimolar increase in arginine and ornithine concentrations in the group of patients who received supplemental dietary arginine. Maximum plasma levels obtained were 0.2–0.3 mM/L. In our laboratory, we have noted a direct correlation between arginine and ornithine concentrations and splenocyte activation by con A, IL-2 production, IL-2 receptor kinetics, cytotoxic T-cell activity, NK cell activity, and LAK cell generation. Recently, Hibbs et al. showed that arginine is the only amino acid "essential" for activated macrophage effector mechanisms against tumor targets.[8] Regulation of lymphocyte function may relate to changes in intracellular ornithine decarboxylase activity and levels of polyamines.

These daily pharmacologic doses of arginine that were administered as a dietary supplement (7% of maximum caloric intake) were nontoxic. G.I. side effects were similar for the two groups. In both the arginine and glycine groups, mean postoperative serum urea nitrogen concentrations rose in a similar fashion. Finally, additional mean plasma amino acid levels that were measured showed no significant differences between groups. Our level of supplementation was chosen empirically, based on the study of normal volunteers by Barbul et al.[10] It is possible that, because these may be drug effects rather than nutrient effects, lower doses may produce similar results.

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Discussion

DR. STANLEY J. DUDRICK (Houston, Texas): I would like to congratulate Dr. Daly and his co-authors on a study which has been elegant in its conception, execution, and presentation. I think that this work is a highly important extension of that also undertaken by many of us here in this room, as well as by others who have worked in the area of specific amino acid substrates in overall nutrition while measuring such gross indices as weight gain, serum albumin levels, and nitrogen balance. The excitement for now, and in the future, is that individual amino acids are rightfully being perceived as potent substrates having individual pharmacologic type effects that could be beneficial when applied to various physiologic functions or as modulators of pathophysiologic malfunctions.

Last year at this meeting, we presented work showing reductions in serum cholesterol levels and reversal of atherosclerosis related to individual plasma amino acid levels.[1] Arginine was the key amino acid in the intravenous mixture. Although at that time we reported data from four patients only, we have now completed studies in 24 patients, and arginine still remains the most important single amino acid with which we can correlate specific anti-cholesterol, anti-atherosclerosis effects.

In this study, Dr. Daly has shown favorable effects of amino acids on some of the immunologic indices that he measured, and I hope that he will continue to study these effects even more specifically. He is actually giving "industrial strength" doses of arginine, and he observed no toxicity.

It would be interesting to know whether outputs of arginine and other amino acids were measured in the urine. Plasma levels of the various