Branched Chain Metabolic Support

A Prospective, Randomized, Double-Blind Trial in Surgical Stress

FRANK B. CERRA, M.D., JOHN E. MAZUSKI, M.D., EDMUND CHUTE, M.D., NANCY NUWER, R.N., KATHY TEASLEY, R.PH., JOLYNN LYSNE, PHARM.D., EVA P. SHRONTS, R.D., FRANK N. KONSTANTINIDES

A prospective, randomized, double-blind trial of the nutritional effects of branched chain modified amino acid solutions was undertaken in 23 surgical patients within 24 hours of the onset of major general surgery, polytrauma, or sepsis. The effects were evaluated in the absence of abnormalities of oxygen transport and perfusion in an isocaloric/isonitrogenous setting where the major difference between the groups was the amount of branched chain amino acids received. Both groups received balanced parenteral nutrition with 1.5 gm/kg/day of amino acids, 30 calories/kg/day of glucose, and 7 calories/kg/day of fat. At the end of the 7-day study interval, the group receiving the branched chain enriched therapy at 0.7 gm/kg/day of branched chain amino acids had improved nitrogen retention; an elevation of their absolute lymphocyte count from 800 to 1800/mm^3, a reversal of anergy to recall skin test antigens in 60% of the patients, and improved plasma transferrin levels ($p < 0.03$). Nutritional support using the modified amino acid metabolic support solutions has beneficial effects during the stress interval that do not seem as achievable with current commercially available nutritional support regimens.

THE STRESS RESPONSE of major general surgery, polytrauma, and surgical sepsis is associated with negative nitrogen balance, reduced total body protein synthesis, increased proteolysis, and the suppression of immunocompetence.[1-7] Current nutritional support techniques have made a limited impact on this response.[8-13] With the advent of the altered amino acid support solutions that are modified in their amino acid content and enriched with the branched chain amino acids leucine, isoleucine, and valine, there have been claims made of improved nitrogen retention by effects on protein synthesis and proteolysis.[14-19] Effects on immunocompetence and hepatic protein synthesis have not been demonstrated.

The present study was designed as a prospective, randomized, double-blind trial of the modified branched chain enriched amino acid solution's capacity to support stress metabolism and its systemic consequences during the human response to major general surgery and sepsis.

From the Department of Surgery and the Nutrition Support Service, University of Minnesota Hospitals and the Surgical Research Metabolic Laboratories at the St. Paul Ramsey Hospital, Minneapolis, Minnesota

Reprint requests: Frank B. Cerra, M.D., Associate Professor of Surgery, Department of Surgery, Box 42 Mayo Building, 420 Delaware Street S.E., Minneapolis, MN 55455.

Submitted for publication: August 12, 1983.

Materials and Methods

Twenty-three patients were enrolled in this prospective, randomized, double-blind trial in a protocol approved by the Human Investigations Committee of the University of Minnesota Hospitals. All patients were entered with written informed consent.

Patients who entered the study did not have diabetes mellitus, were not receiving steroids or chemotherapy, and were not receiving insulin. All patients were within 24 hours for major polytrauma or elective/emergency general surgery. The group characteristics are summarized in Table 1. Sepsis was defined as an anatomically documented source together with the presence of blood cultures that grew gram-negative organisms. The sources of infection were colon and small bowel anastamotic leaks or perforations, acute cholangitis or gangrenous cholecystitis, or multilobe pneumonias. Systemic findings such as fever, leukocytosis, and tachycardia were also present. Major general surgical procedures included radical cystectomy, renal artery stenosis or aortic aneurysm resections, gastrectomy, total colectomy or panproctocolectomy, choledochojejunostomy, resection of pelvic tumors, and complex small bowel obstructions requiring procedures greater than 5 hours duration with resection of major quantities of small bowel. Most patients who entered the study were moderately malnourished using the criteria of clinical examination: a plasma transferrin of

TABLE 1. *Patient Characteristics*

	Control Group		Branched Chain Amino Acid Group	
	At Entrance			
Per cent male	70		65	
Age (years)	56 ± 13		54 ± 14	
Height (cm)	170 ± 13		167 ± 9	
Weight (kg)	66 ± 10		70 ± 10	
Per cent ideal body weight	100 ± 18		111 ± 14	
Number patients	11		12	
Per cent sepsis	50		60	
Per cent elective	50		40	
	Day		Day	
	0	7	0	7
Allbumin (gm/dl)	3 ± 0.5	2.4 ± 0.6	3 ± 0.6	2.9 ± 0.7
Glucose (mg/dl)	169 ± 40	170 ± 70	160 ± 50	130 ± 50
Blood urea nitrogen (mg/dl)	30 ± 20	29 ± 16	20 ± 16	23 ± 19
Creatinine (mg/dl)	1.4 ± 0.7	1.2 ± 0.6	1.2 ± 0.6	0.9 ± 0.4
Lactate (mm/l)	1.9 ± 1	1.2 ± 1	1.5 ± 0.5	0.9 ± 0.4
Pyruvate (mm/l)	0.1 ± 0.05	0.07 ± 0.04	0.08 ± 0.06	0.05 ± 0.05
Lactate/pyruvate ratio	17	18	18	18
Bilirubin (mg/dl)	3 ± 4	4 ± 6	3 ± 3	5 ± 6
SGOT (U/l)	40 ± 25	49 ± 44	52 ± 36	46 ± 33
LDH (U/l)	429 ± 190	410 ± 190	480 ± 200	350 ± 100
AST (U/l)	250 ± 190	470 ± 250	237 ± 160	393 ± 186

Values $\bar{X}$ ± SD.

less than 200 mg/dl, albumin of less than 3 gm/dl, and an absolute lymphocyte count of less than 1000/mm^3.

All patients were in the Surgical Intensive Care Unit. As part of their postoperative support regimen, they had optimized cardiac outputs to maintain an oxygen consumption index of greater than 140 ml/m^2 and had normal lactate to pyruvate ratios. The absolute level of lactate on entrance into the study was approximately 1700 μmol/l. Thus, great care was taken to assure that there was no perfusion deficit present such that the metabolic study might proceed in the absence of oxygen deprivation effects.[1,8,9,14]

The patient's care was given in accordance with sound clinical practice. Nutritional support was begun as soon as oxygen transport was stabilized in the postoperative period. An isocaloric/isonitrogenous study was implemented where the major nutritional difference between the control and the branched chain amino acid group was the quantity of branched chain amino acids administered. The patients were randomized in central pharmacy using a list that was randomly derived by the computer. The treatment group was unknown to patients or to anyone involved in the patient's care. Beginning on Day 0, the solutions were started and rapidly increased to provide 30 glucose calories/kg/day; 7 fat calories/kg/day; and 1.5 gm/kg/day of protein as amino acids. The study continued for a full 7 days of support. The control group received a commercial amino acid solution that was approximately 24% branched chain amino acids. The branched chain amino acid group (BCAA) received the same commercial amino acid solution except that it was enriched with branched chain amino acids such that the branched chain concentration was now 45% and the ratio of leucine, isoleucine, and valine was 1:4:7. Thus, the total protein that the patients received was the same but the BCAA group received approximately 0.7 gm/kg/day of branched chain amino acids while the control group received 0.36 gm/kg/day of branched chain amino acids.

Twenty-four-hour urines were collected daily for the determination of total nitrogen excretion and for the determination of 3-methylhistidine excretion. The presence of 3-methylhistidine in the urine was used as an index of the degree of muscle proteolysis that was occurring.[14,16] All patients had a glomerular filtration rate at entrance into the study of greater than 25 ml/minute as determined by 24-hour urinary creatinine clearance.

Eleven patients were randomized to the control group and 12 patients were randomized in the BCAA group. Upon entrance into the study and on Day 7, the following laboratory tests were done: electrolytes, blood urea nitrogen and creatinine, prothrombin time, partial thromboplastin time, platelet count and fibrinogen, SGOT, LDH, alkaline phosphatase, bilirubin, complete blood count with differential, plasma albumin and transferrin, and a plasma amino acid profile on heparinized arterial blood. Skin tests were applied upon entrance to the study and on Day 7. The skin test battery included intradermal injections of mumps, candida, and second strength pu-

TABLE 2. *Nutritional Input Data*

	Control Group			Branched Chain Amino Acid Group		
	Day			Day		
	0–1	3–4	6–7	0–1	3–4	6–7
Source amino acids total						
Gm/kg/day	1.0 ± 0.5	1.5 ± 0.3	1.5 ± 0.5	0.9 ± 0.4	1.4 ± 0.3	1.3 ± 0.4
Nitrogen in gm/day	14 ± 6	15 ± 4	14 ± 5	11 ± 5	14 ± 4	15 ± 4
Glucose						
Cal/Kg/Day	24 ± 8	30 ± 8	34 ± 5	22 ± 7	30 ± 8	30 ± 11
Fat						
Cal/kg/day	7	7	7	7	7	7
Number patients	11	11	11	12	12	12

Values $\bar{X}$ ± SD.

rified protein derivative (PPD). A positive response was the presence of 5 mm of induration at 48 to 72 hours after application of this skin test.

Statistical analysis was performed with a t-test, using appropriate corrections for sample size and variance where indicated. Inter- and intragroup differences were analyzed. The nutritional input, urinary output, and plasma amino acid data were combined for data analysis purposes into Days 0 and 1, Days 3 and 4, and Days 6 and 7.

Results

All patients successfully completed the 7-day study interval. As is summarized in Table 1, there were no differences between the groups in age, sex, nutrition indices, laboratory tests, incidence of sepsis, or types of surgical procedures. The nutritional input data are summarized in Table 2. The patients received the same amount of calories and fat irrespective of their group: 30 calories/kg glucose and 7 calories/kg fat. There were also no differences in the amino acid load with approximately 1.5 gm/kg/day being administered to each patient irrespective of the group. Thus, the major nutritional difference between the groups is the amount of branched chain amino acid that the groups received. The BCAA group received approximately twice the branched chain amino acid load as the control group. There were no significant differences between the groups in the caloric or nitrogen inputs.

There were no significant changes during the 7-day study interval in patient weight, coagulation factors, electrolytes, blood urea nitrogen (BUN), creatinine, and creatinine clearance. The urinary excretion data are summarized in Table 3. Because of the severity of the patients' illnesses, and the immediate postoperative period of the evaluations, bowel movements were a rare occurrence. Therefore, a factor of 1.5 gm/day was added to the urinary nitrogen excretion in the calculation of the nitrogen balance. There was no statistical difference in the urinary nitrogen excretion on the first day of the study. However, by Day 7, the urinary nitrogen output in the BCAA group was less than the control group at the $p = 0.05$ level. As the study progressed, 3-methylhistidine excretion declined in both groups, but there were no differences between the groups. Both groups started in negative nitrogen balance. The BCAA group, however, showed an increased nitrogen retention and a progressively positive nitrogen balance on Days 3 and 7 that was greater than the control group on Day 7 at the $p < 0.03$ level. Cumulative nitrogen balance was also greater in the BCAA group (9 ± 3 *vs.* −2.5 ± 3.5, $p < 0.03$).

TABLE 3. *Urinary Excretion*

	Control Group			Branched Chain Amino Acid Group		
	Day			Day		
	0–1	3–4	6–7	0–1	3–4	6–7
Total nitrogen (gm/day)	16 ± 8	15 ± 7	14 ± 4	12 ± 3	13 ± 4	11 ± 3*
3-Methylhistidine (μm/day)	335 ± 180	233 ± 150	142 ± 86	256 ± 88	261 ± 168	188 ± 100
Nitrogen balance (gm/day)	−2.0 ± 4	0.2 ± 4	−0,9 ± 6	0.6 ± 5	1.4 ± 6	5 ± 3†
Cumulative total‡			−2.5 ± 3.5			9 ± 3†
Number of patients	11	11	11	12	12	12

Values $\bar{X}$ ± SD.
* Significant at $p = 0.05$.
† Significant at $p < 0.03$.
‡ Also includes Day 5 balance.

TABLE 4. *Plasma Amino Acid Data*

	Control Group			Branched Chain Amino Acid Group		
	Day			Day		
	0–1	3–4	6–7	0–1	3–4	6–7
Alanine	30 ± 16	38 ± 18	37 ± 17	29 ± 13	39 ± 17	38 ± 19
Isoleucine	5 ± 3	8 ± 4	10 ± 5	4 ± 2	21 ± 10	18 ± 12*
Leucine	9 ± 3	12 ± 4	9 ± 4	9 ± 2	10 ± 3	11 ± 2
Valine	19 ± 6	32 ± 13	30 ± 9	18 ± 5	56 ± 40	107 ± 80*
Phenylalanine	11 ± 3	10 ± 3	11 ± 3	9 ± 5	8 ± 2	10 ± 4
Tyrosine	5 ± 3	10 ± 3	5 ± 2	6 ± 3	5 ± 2	6 ± 2
Methionine	4 ± 3	5 ± 2	5 ± 2	3 ± 2	4 ± 2	4 ± 2
Glutamine	34 ± 11	38 ± 12	36 ± 11	39 ± 9	38 ± 11	40 ± 14
Proline	15 ± 6	26 ± 8	30 ± 14	14 ± 5	24 ± 9	23 ± 11
Taurine	3 ± 1	5 ± 3	9 ± 15	4 ± 2	4 ± 2	5 ± 3

Values are μm/100 ml plasma from arterial blood.

* Signifies $p < 0.03$ relative to control.

The plasma amino acid data are summarized in Table 4 and in Figure 1. The BCAA group evolved a profile pattern that was significantly increased in total branched chain amino acid and in the isoleucine and valine concentrations by Day 7 of the study. The remainder of the amino acid profiles were not different between the groups.

The liver function tests are summarized in Table 1. There were no differences between the groups either at entrance into the study or at any point during the study. The nutritional effect data are also summarized in Table 5. The plasma transferrin levels were higher on Day 7 in the BCAA group ($p < 0.05$) and there was a greater rise

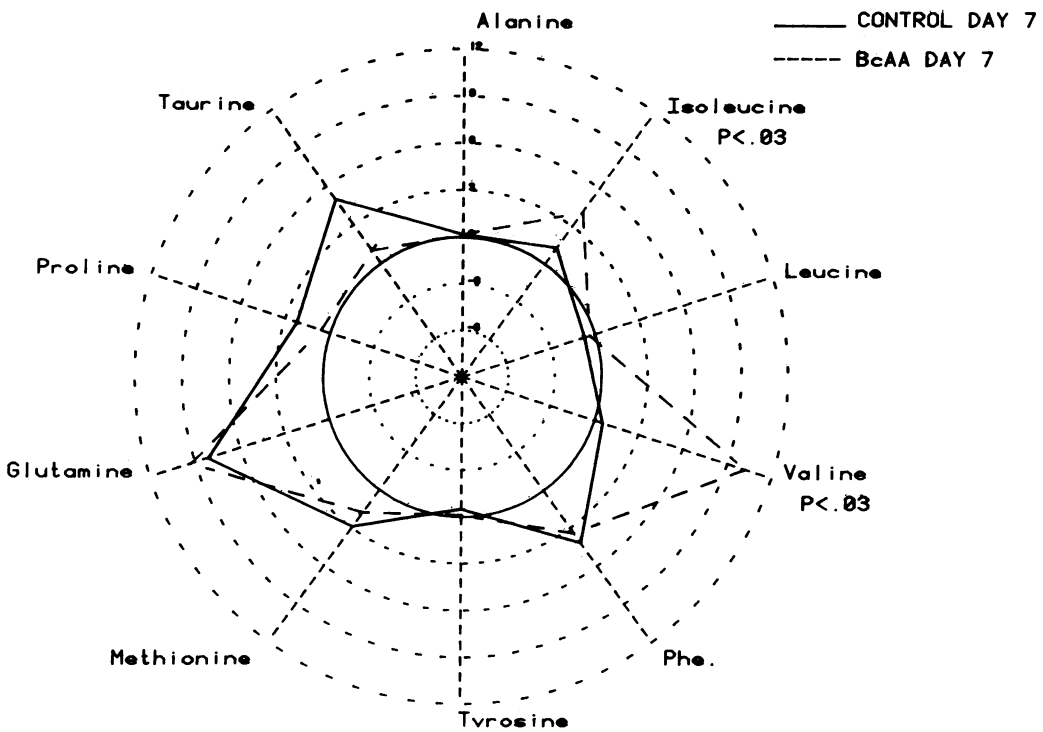

FIG. 1. Depicted are the plasma amino acids of the control and branched chain amino acid enriched groups relative to overnight nonstressed fasting man. The dark inner circle represents the mean value for each variable (radians) for nonstressed, overnight fasting man. Each of the dotted circles then represents the standard deviational differences from this fasting nonstressed control. Plotted against this are the values on Day 7 from the control group and from the branched chain enriched modified amino acid Group. This latter group has an elevation of the total branched chain content and of the concentrations of isoleucine and valine. Otherwise, the amino acid patterns throughout the course were typical of those of stressed surgical, polytrauma, and septic patients.

TABLE 5. *Branched Chain Amino Acid Effects*

	Control Group		Branched Chain Amino Acid Group	
	Day		Day	
	0	7	0	7
Absolute lymphocyte count (No./mm³)	900 ± 38	1200 ± 600	800 ± 500	1800 ± 250*
Per cent recall skin test positive	0	10	0	60†
Plasma transferrin level (mg/dl)	182 ± 32	186 ± 21	173 ± 39	203 ± 46*
Change in plasma transferrin from day 0 (mg/dl)		4.6 ± 28		31 ± 46*
Number of patients	11		12	

* = $p < 0.05$.
† = $p < 0.01$.

from baseline in the BCAA group ($p < 0.05$). The absolute lymphocyte counts demonstrated a rise in the BCAA group at the $p < 0.05$ level. The control group had some rise in the absolute lymphocyte count, but not of statistical significance. All patients were anergic at the entrance to the study. After Day 7 of the study, 60% of the BCAA group were now reactive to at least one recall antigen skin test; only one patient in the control group was now positive ($p < 0.01$).

Discussion

This prospective, randomized, double-blind study of the effects of branched chain enriched amino acid therapy in a setting of isonitrogenous, isocaloric nutritional support demonstrated several beneficial effects in the BCAA group. This group achieved better nitrogen retention and positive nitrogen balance, improved plasma transferrin levels, and demonstrated significant improvements in both absolute lymphocyte count and skin test reactivity after 7 days of infusion.

The metabolic response to surgical stress and sepsis runs a predictable course following the onset of that stress. The effects on the metabolic machinery by the neurohumeral modulating system produce a predictable sequence of measurable events.[3,5,9,10,12,13] Using a classification that describes and characterizes these events, the admission values would indicate that the current group under study was a level 2 of 4 stress (a moderate level).[12,14] Thus, a substantial quantity of stress was present in this group of moderately malnourished patients at the time of entrance into the study.

The endpoints of nutritional support regimens during the stress response are to provide effective support of the lean body mass, effective support of hepatic protein synthesis, and, hopefully, to support immunocompetence. Under these conditions, the use of standard nutritional support to provide 150 nonprotein calories/kg/day and 1 gm of protein/kg/day has had disappointing results and an increased incidence of pulmonary and liver complications.[1,4,5,8,10,13,14] Adjusting the nutritional support during the stress phase such that it is more appropriate to the stress response has minimized the complication rate but has not improved the response rate substantially.[10–12,18–21] This was achieved by providing approximately 2 gm/kg/day of amino acids and 35 nonprotein calories/kg/day with approximately 30% to 40% of those nonprotein calories as exogenous fat.

The inability of standard amino acid solutions and nutritional support regimens to achieve these goals, and a better understanding of the amino acid requirements of stress both on an organ level and organism level has led to the development of modified amino acid support solutions.[8,12,16,17,22] The principle modifications have been the enrichment of the basic balanced amino acid solutions with branched chain amino acids. This rationale was the result of research demonstrating the increased oxidation of branched chain amino acids under stress along with an increased demand to meet hepatic protein synthesis.[1,2,8,10] Thus, in the present study, the branched chain amino acid levels do not rise until the last 2 days, probably reflecting the change in stress level and reduction in the "branched chain amino acid-sink" effect.

In experimental studies, the increased loads of leucine, isoleucine, and valine have been shown to have a metabolic regulatory function with a promotion of protein synthesis and a reduction of protein degradation.[16,17,22] In randomized, prospective, blinded trials in humans, the nitrogen retention effect has been demonstrated.[14] With a failure to reduce the excretion rate of 3-methylhistidine in the urine, this effect on nitrogen retention was felt to be from an increase in protein synthesis. The BCAA formula used in this study was a 50% BCAA-enriched balanced commercial amino acid solution with an equimolar ratio of the BCAA. The nitrogen effect was probably better than with the present solution as the patients in that study were in better nitrogen retention by Day 3. As the amino acid loads were comparable, this discrepancy may reflect different levels of stress or an effect of a different ratio of the BCAA.

To date, an effect on immunocompetence has not been demonstrated,[14,15] although there is experimental evidence that deficiencies of isoleucine and valine do affect immune function.[23,24] The depression of immune function following the onset of major general surgery and sepsis is a well-described phenomenon.[1,7,8,18,25,26] Indeed, a reduction in absolute lymphocyte count and the presence of skin test anergy are not only an integral part of this response but are also associated with moderate levels of protein calorie

malnutrition.[6,19–21,27,28] In the present study, whether the initial reduction in absolute lymphocyte counts and the presence of skin test anergy was due to the preexisting malnutrition or the onset of stress is nonsolvable with the current data. Since the patient type was identical in both groups, the degree of metabolic stress was the same at entrance into the study, and the major difference between the groups was the amount of branched chain amino acids that one group received, the anergy reversal and increase in absolute lymphocyte count would appear to have been significantly impacted by the branched chain amino acids.

In the studies of the nitrogen retention effect of the branched chain amino acids, the dose required to achieve this effect was at least 0.5 gm/kg/day. The dose that our present group received of 0.7 gm/kg/day is well within this therapeutic effect range.[15,16]

Branched chain modified amino acid solutions are thus capable of favorably impacting the patient's metabolic response to stress. The solutions seem to induce nitrogen retention, apparently resulting through a rebalancing of protein kinetics in favor of protein synthesis. Perhaps the improved plasma transferrin levels are also reflecting this improved synthetic milieu.

A possible, previously unobserved effect of the solution used in this study is on the support of immune function. The precise origin of these effects is currently unknown. How the branched chain amino acids would help to restore lymphocyte count and skin test reactivity is also a matter of conjecture. Possibilities would include: a local cellular effect on protein synthesis or adenosine triphosphate (ATP) production, a milieu effect following a "normalization" of the plasma amino acid pattern, or a secondary phenomenon following the systemic effects of the branched chain. More detailed studies of the effects of the modified branched chain enriched amino acid solutions on the specifics of leukocyte cell and lymphocyte function need to be undertaken.

Thus, nutritional support solutions will be available that are specifically designed for use during the stress response. Utilization of these solutions in nonstressed patients seems to have no increased effect over current commercial solutions and has an adverse-effect potential. The need to monitor the level of stress and adjust the kind of support solution becomes an integral part of nutritional support during the stress interval. Whether these metabolic support tools will impact on morbidity and mortality and the subsequent occurrence of multiple system organ failure in these groups of patients is currently unknown, but under study.

References

1. Border JT, Chenier R, McMenamy RH. Multiple systems organ failure: muscle fuel deficit with visceral protein malnutrition. Surg Clin North Am 1976; 56:1147–1150.
2. Long CL, Jeevanandan M, Kinney JM. Whole body protein synthesis and catabolism in septic man. Am J Clin Nutr 1977; 30:1340–1345.
3. Cuthbertson D, Tilstone W. Metabolism during the post injury period. Adv Clin Chem 1969; 12:1–55.
4. Beisel WR. Malnutrition as a consequence of stress. *In* Suskind RM, ed. Malnutrition and the Immune Response. New York: Raven Press, 1977; 330–345.
5. Waterlow JC, Golden M, Picou D. The measurement of rates of protein turnover, synthesis, and breakdown in man and the effects of nutritional status and surgical injury. Am J Clin Nutr 1977; 30:1333–1339.
6. Law DK, Dudrick SJ, Abdow NI. Immunocompetence of patients with protein-calorie malnutrition. Ann Intern Med 1973; 79:545–550.
7. Kahan BD. Nutrition and host defense mechanisms. Surg Clin North Am 1981; 61:557–570.
8. Cerra FB, Siegel JH, Coleman B. Septic autocannibalism: a failure of exogenous nutritional support. Ann Surg 1980; 192:570–574.
9. Cerra FB, Siegel JH, Border JR. Correlation between metabolic and cardiopulmonary measurement in patients after trauma, general surgery and sepsis. J Trauma 1979; 19:621–626.
10. Clowes GHA, Heidman M, Lindberg B. Effects of parenteral alimentation on amino acid metabolism in septic patients. Surgery 1980; 88:531–535.
11. Tweedle DE. Metabolism of amino acids after trauma. JPEN 1980; 4:165–172.
12. Cerra FB. Profiles in nutritional management: the trauma patient. Chicago: Monograph-Medical Directions, 1982.
13. Wolfe BM. Substrate-endocrine interactions and protein metabolism. JPEN 1980; 4:188–194.
14. Cerra FB, Upson D, Angelico R, et al. Branched chains support postoperative protein synthesis. Surgery 1982; 92:192–199.
15. Cerra FB, Mazuski J, Teasley T, et al. Nitrogen retention is proportionate to branched chain load: a randomized, double blind prospective study. Crit Care Med, in press.
16. Blackburn GL, Moldawer LL, Usui S, et al. Branched chain amino acid administration and metabolism during starvation, injury, and infection. Surgery 1979; 86:307–315.
17. Freund H, Yoshimura N, Fischer JE. The effect of branched chain amino acids and hypertonic glucose infusions on postinjury catabolism in the rat. Surgery 1980; 87:401–408.
18. Willicuts HD, Linderme D, Chlastawa D, et al. Anergy: is nutrition reversal possible/outcome significant. JPEN 1979; 3:292.
19. Sakamota J, Momoi T, Imaizumi M, et al. The effect of intravenous hyperalimentation on cell mediated immunity. Jpn J Surg 1979; 9:89–94.
20. Copeland EM, Daly JM, Ota DM, et al. Nutrition, cancer, and intravenous hyperalimentation. Cancer 1979; 43:2108–2116.
21. Harvey KB, Bothe A, Blackburn GL. Nutritional assessment and patient outcome during oncological therapy. Cancer 1979; 43:2065–2069.
22. Freund H, Fischer JE. Nitrogen-conserving quality of the branched-chain amino acids: possible regulator effect of valine in postinjury muscle catabolism. Surg Forum 1978; 29:69–72.
23. Chevalier J, Aschkenasy A. Hematological and immunological effects of excess dietary leucine in the young rat. Am J Clin Nutr 1977; 30:1645–1654.
24. Aschkenasy A. Dietary proteins and amino acids in leucopoiesis: recent hematological and immunological data. World Rev Nutr Diet 1975; 21:151–197.
25. Meakins JL, Pietsch JB, Bubenick O, et al. Delayed hypersensitivity: indicator of acquired failure of host defenses in sepsis and trauma. Ann Surg 1977; 186:241–250.
26. Meakins JL, Christow NV, Forse A, et al. Malnutrition and anergy in the surgical setting. Third Ross Conference on Medical Research, Captiva Island, 1982; 96–100.
27. Copeland EM, MacFadyen BV, Dudrick SJ. Effects of intravenous hyperalimentation on established delayed hypersensitivity in the cancer patient. Ann Surg 1976; 184;60–64.
28. Chandra RK. Rosette-forming T lymphocytes and cell-mediated immunity in malnutrition. Br Med J 1974; 3:608–609.