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PAPER

Gut Function and Immune and Inflammatory Responses in Patients Perioperatively Fed With Supplemented Enteral Formulas

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Objective: To evaluate if the perioperative administration of a supplemented enteral formula modulates selective inflammatory and immune variables and gut function after surgery.

Design: Prospective, randomized, double-blind, clinical trial.

Setting: Department of surgery, university hospital.

Patients: Forty patients with neoplasm of the colorectum or stomach.

Intervention: Seven days before surgery, the patients drank 1 L/d of a control enteral formula (n=20) or the same formula enriched with arginine, RNA, and ω-3 fatty acids (n=20). Jejunal infusion with the same formulas was started 6 hours after operation and continued until day 7.

Main Outcome Measures: Immune response was determined by phagocytosis ability and respiratory burst of polymorphonuclear cells, and inflammatory response by plasma levels of C-reactive protein. Operative intestinal microperfusion, postoperative intestinal mucosa oxygen metabolism, and plasma intestinal isoenzyme of alkaline phosphatase were used as indicators of gut function. Plasma nitric oxide also was determined.

Results: In the enriched group, phagocytosis ability and respiratory burst after surgery was higher ($P<.01$) and C-reactive protein level was lower ($P<.05$) than in the control group. The enriched group had higher mean (±SD) intestinal microperfusion (180±46 vs 146±59 perfusion units, $P<.05$), intestinal mucosa oxygen metabolism (pHi 7.39±0.2 vs pHi 7.33±0.1, $P<.05$), and 5-fold lower levels of intestinal isoenzyme of alkaline phosphatase ($P<.05$). Postoperative levels of nitric oxide were higher in the enriched group ($P<.05$, analysis of variance).

Conclusion: The perioperative administration of an enriched enteral formula significantly improved gut function and positively modulated postsurgical immunosuppressive and inflammatory responses.

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It is well known that major surgery and other types of trauma are associated with severe alterations of the host defense mechanisms, making the patients highly susceptible to septic and inflammatory complications.[1] More recently, injury has been proved to affect gut function, ie, decreased perfusion and oxygenation, altered mucosal structure, and microflora ecology, all potentially resulting in a loss of barrier function.[2]

Nutritional support after injury may modulate the immune, inflammatory, and metabolic responses; the gut function; and the clinical outcome of critically ill subjects. In different experimental and clinical settings, enteral vs parenteral route,[3-6] early vs delayed enteral feeding,[7-9] and supplemented vs standard diets[10-13] have been associated with better results. Despite early postoperative administration of enriched enteral diets, the recovery of cell-mediated and humoral responses occurred late.[10,14-18] Because the alterations of immune variables were detectable immediately after surgery,[1,10,14-18] the delayed recovery of the host defense mechanisms might be partially effective in improving outcome.[10,11,16,19] Thus, the hypothesis of this phase II trial was that the perioperative administration of an enriched enteral formula could counteract the variations of specific laboratory and biochemical variables observed

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MATERIALS AND METHODS

This was a prospective, randomized, double-blind clinical trial. Forty-four consecutive patients of both genders, aged between 18 and 75 years, with histologically documented adenocarcinoma of the colorectum or stomach and who were candidates for curative surgery, were enrolled. Exclusion criteria were clinically relevant alterations of the pulmonary, cardiovascular, renal, intestinal or hepatic function; history of recent immunosuppressive therapy (including preoperative radiochemotherapy) or immunological diseases; ongoing infection; intestinal obstruction; or emergency surgery. Four patients were intraoperatively withdrawn from the study because of evidence of peritoneal carcinomatosis requiring palliative surgery (n=2) or macroscopic fecal contamination of the peritoneal cavity (n=2). On admission (day −8), the following baseline variables were determined in all patients: plasma levels of arginine (μmol/L), CRP (g/L), α_1-antitrypsin (g/L), fibrinogen (g/L), IIAP, expressed as percentage of total AP (U/L), NO (μmol/L) after nitrate reduction using the method described by Green et al,[20] PMN cell ability to phagocytose zymosan particles as described by Brain and Kavet,[21] and the oxidative metabolism of PMNs by luminometry (mV) according to the technique of Barbour et al.[22] The degree of phagocytosis was expressed as percentage of PMN in which zymosan particles were detected over a hundred PMNs. The PMN oxidative metabolism is closely related to the production of O_2- and H_2O_2 and expresses an indirect measurement of the respiratory burst and intracellular killing. Total number of lymphocytes per cm^3 and T-lymphocytes, B-lymphocytes, natural killer cells, T-lymphocyte subsets (CD4, CD8, and CD4/CD8 ratio) as a percentage of total lymphocytes also were determined.

Patients were randomized to drink 1 L/d for 7 consecutive days of a control or a supplemented liquid diet (Impact, Sandoz Nutrition, Bern, Switzerland). The composition of the diets is given in **Table 1**. In addition to the 2 liquid diets, the patients of both groups were allowed to consume standard hospital food (average, 1200 kcal/d and 100 g protein/d).

Baseline variables were reassessed 1 day before surgery (day −1) and 1 (day +1), 4 (day +4), and 8 (day +8) days after surgery.

At laparotomy, before any manipulation of the gut, the intestinal microperfusion was evaluated by laser Doppler flowmetry system (Periflux 4001 Master, Perimed, Stockholm, Sweden), which has been used for calibration of the flowmeter signal in absolute flow units in the human colon.[23] Recordings were made with a probe with a measuring depth of about 6 mm in the bowel wall, which is transmural in the human intestine. The probe had 1 emitting and 2 receiving fibers with a diameter of 0.7 mm and separation of 0.7 mm. The instrument setup has been used in clinical studies of intestinal microcirculation.[24,25] The measurement was repeated for 3 different segments of the ileum and colon and the mean of the 3 values was calculated. Just before suturing the abdominal wall, intestinal microperfusion was remeasured and an enteral feeding tube (Kangaroo 2.6 mm outer diameter, Sherwood Medical, Tullamore, Ireland) and an intestinal tonometer (Trip Sigmoid Catheter, Tonometrics Inc, Hopkinton, Mass), previously tied together, were inserted through the nose and advanced by the surgeon to reach the ligament of Treitz in the patients undergoing colorectal surgery. In the subjects undergoing gastric surgery (all with Roux-en-Y reconstruction), the tonometer and the feeding tube were placed about 30 cm below the esophagojejunal or gastrojejunal anastomosis and above the jejunoileal anastomosis.

Postoperative enteral feeding with supplemented or control diet (Table 1) was started 6 hours after surgery with an infusion rate of 10 mL/h, which was progressively increased to reach the full nutritional regimen (25 kcal/kg per day). In the first 3 postoperative days, intravenous saline and electrolytes were administered according to clinical requirement as volume integration to the enteral diet. Oral food intake was allowed on day +8. None of the patients received parenteral nutrition before or after surgery. All patients had food and water restricted from the night before surgery.

Postoperative intestinal tonometry was performed on days +1, +4, and +8. Fifteen minutes before measurement, the enteral infusion was stopped and the feeding tube was flushed with 20 mL of saline. Intestinal mucosa oxygen metabolism was calculated according to the formulas suggested by the manufacturer.

Intestinal washout with an iso-osmotic solution (3 L) was carried out the day before operation in patients undergoing colorectal surgery. The evening before and the morning of the operation, patients also were given an enema. These patients were given antibiotic prophylaxis with a single intravenous dose (2 g) of cefotetan disodium 30 minutes before surgery. Patients undergoing gastric surgery were treated with intestinal washout (1 L) the day before operation and antibiotic prophylaxis with a single intravenous dose (2 g) of cefazolin sodium 30 minutes before surgery. The administration of the antibiotics was repeated if the surgical procedure lasted more than 4 hours. All patients, except those who underwent total gastrectomy, were treated with ranitidine (150 mg/d) for 10 days after surgery. None of the patients was treated with epidural anesthesia.

Enteral feeding side effects such as abdominal discomfort and cramps, bloating, diarrhea (defined as more than 3 liquid stools per day), and emesis were recorded. If any side effect appeared, the jejunal infusion was reduced or discontinued for 4 hours and then resumed at a lower infusion rate.

The sample size was determined to detect a 20% variation of the variables studied based on results of previous trials. Treatment group balance was assessed by exact contingency table for discrete variables such as sex, diagnosis, type of surgery, blood transfusion, and weight loss. The data of continuous variables were analyzed by using the 2-factor analysis of variance with post hoc testing. Probability (P value) of less than .05 was accepted as significant. Results are reported as mean ±SD.

shortly after surgery in patients with cancer. The end points of the study were the evaluation of the immune response (by polymorphonuclear [PMN] cell respiratory burst and phagocytosis ability, lymphocyte subsets, and nitric oxide [NO]), the inflammatory response (by C-reactive protein [CRP], α_1-antitrypsin, and fibrinogen levels), and the gut function (by intestinal microperfusion, intestinal mucosa oxygen

Table 1. Composition of the Diets

Variable	Composition of Formula, per 100 mL			
	Preoperative Period		Postoperative Period	
	Supplemented	Control	Supplemented	Control
Total proteins, g	5.6	4.35	5.6	5.6
Free L-arginine	1.25	...	1.25	...
L-Serine	...	...	...	0.93
L-Glycine	...	...	...	0.77
L-Alanine	...	...	...	0.51
L-Proline	...	...	...	0.45
RNA, g	0.12	...	0.12	...
Total lipids, g				
Fatty acids, %	2.8	2.8	2.8	2.8
ω-3	10.5	...	10.5	...
ω-6	8.3	35.7	8.3	24.1
Carbohydrates, g	13.3	14.55	13.4	13.4
Total energy, kcal	101	101	101	101
Osmolarity, mOsm/L	...	...	293	486

metabolism [pHi], and intestinal isoenzyme of alkaline phosphatase [IIAP]).

RESULTS

Table 2 gives the characteristics of the 2 groups of patients. The group receiving the supplemented diet was slightly older than the control group but without reaching significant difference ($P=.09$). For baseline performance status, rate of weight loss, hemoglobin level, cancer site, surgical variables, and blood transfusions, the 2 groups were similar.

Table 3 gives the variations of arginine, NO, and IIAP plasma levels, and pHi. Baseline (day −8) arginine plasma level was similar in the 2 groups. After a week of oral feeding, the arginine level increased from 70.4 to 105.6 μmol/L ($P<.05$) in the patients receiving the supplemented formula, but, in the control group, no variation was observed (67.0-64.9 μmol/L). Moreover, the level of arginine remained significantly higher in the supplemented group than in the control group 8 days after surgery. The circulating levels of NO did not change after 7 days of oral feeding with either formula, but the group treated with the supplemented diet had significantly higher NO levels on days +1 and +4 ($P<.05$). Tonometry showed that the control group had a significantly lower pHi on days +1 and +4 ($P<.05$). The IIAP peaked 1 day after surgery in the control group, but in the patients receiving the supplemented formula, the levels remained similar to presurgical values. On days +4 and +8, the IIAP levels were still significantly higher in the control group compared with the supplemented group.

Operative gut microperfusion was significantly different in the 2 groups (**Figure 1**). The patients receiving the supplemented diet had a higher small-bowel and colon perfusion at the beginning of operation than did the control patients. In the control group, a significant reduction of microperfusion occurred at the end of surgery. The patients receiving the supplemented formula had values after the operation that were higher than the baseline values of the control patients.

Table 2. Characteristics of the 2 Groups

Characteristic	Group	
	Supplemented (n=20)	Control (n=20)
Mean (±SD) age, y	64.1±12.5	58.1±9.3
M/F	11/9	14/6
Mean (±SD) performance status*	85.5±10.5	88.5±9.9
Weight loss >10%, No. (%) of patients	4 (20)	2 (10)
Mean (±SD) hemoglobin level, g/L	121±38	128±31
Cancer, No. of patients		
Site		
Stomach	8	8
Colon	7	5
Rectum	5	7
Stage		
Stomach		
II	3	2
III	5	6
Colorectum		
B	7	6
C	5	6
Mean (±SD) time of surgery, min	213±52	246±75
Mean (±SD) operative blood loss, mL	391±385	434±444
Transfused patients, No. (%) of patients	6 (30)	7 (35)

**Performance status was measured using the Karnofsky scale, with 0 indicating nonfunctional and 100, normal function.*

The ex vivo ability of PMNs to phagocytose zymosan particles is shown in **Figure 2**. The 2 groups had a similar baseline phagocytosis ability that did not change throughout the week of presurgical feeding. A significant decrease in phagocytosis was observed on days +1 and +4 in the control group. Conversely, in patients receiving the supplemented diet, phagocytosis ability did not decrease after surgery, remaining similar to the preoperative values. A significant difference between the 2 groups was found on days +1 and +4.

The PMN oxidative metabolism is shown in **Figure 3**. Baseline (day −8) degree of respiratory burst was significantly lower in the supplemented group ($P<.05$). The week of prefeeding did not significantly change the response in either group, but, on day −1, the 2 groups were similar. A sharp postoperative increase of the oxidative metabolism compared with the presurgical values was observed only in the supplemented group ($P<.01$). On day +8, a significant difference existed between the 2 groups ($P<.05$).

Table 4 gives the perioperative variations of total lymphocyte count and the percentage of lymphocyte subsets. One week of feeding before surgery did not affect any of the cell lines studied. B-lymphocytes did not change after operation in both groups. A significant postoperative decrease in natural killer cells was observed in both groups, but they recovered on days +4 and +8 only in the supplemented group. The percentage of the CD8 subset increased on day +1 in the control group and decreased in the supplemented group. Also, on days +1,

Table 3. Variations of Arginine, Nitric Oxide, and Intestinal Isoenzyme of Alkaline Phosphatase Plasma Levels and Intestinal Mucosa Oxygen Metabolism*

Variable	Group	Days −8	−1	+1	+4	+8
Arginine, μmol/L	Supplemented	70.4±10.3	105.6±45.8†	ND	ND	109.6±51.2†
	Control	67.0±8.1	64.9±20.0	ND	ND	71.4±34.0
Nitric oxide, μmol/L	Supplemented	20.30±9.82	25.79±7.66	30.65±10.50†‡	33.82±14.98†‡	22.46±13.52
	Control	21.55±10.70	23.50±10.80	26.70±9.94	23.70±14.12	22.27±18.41
IIAP, %	Supplemented	2.25±2.13	3.32±3.70	2.89±3.52†	6.77±7.51†	4.55±3.13†
	Control	2.32±2.43	3.75±4.21	19.28±11.68‡	15.85±8.59‡	12.25±6.32‡
pHi	Supplemented	ND	ND	7.39±0.23†	7.41±0.16†	7.40±0.12
	Control	ND	ND	7.33±0.18	7.36±0.21	7.38±0.10

*Values are given as mean±SD. ND indicates not determined; IIAP, intestinal isoenzyme of alkaline phosphatase; pHi, intestinal mucosa oxygen metabolism.
†P<.05 vs control.
‡P<.05 vs days −8 and −1.

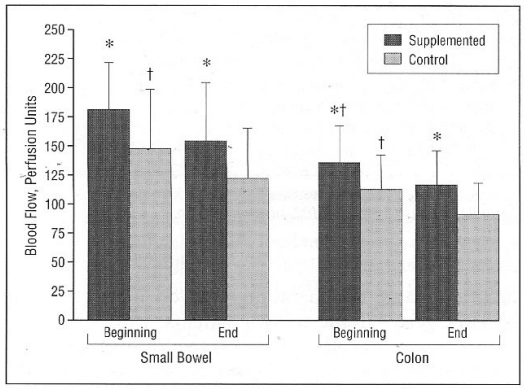

Figure 1. *Small-bowel and colon microperfusion at the beginning and end of surgery, as measured by laser Doppler flowmetry. Asterisk indicates P<.05 vs control; dagger, P<.05 vs end of surgery.*

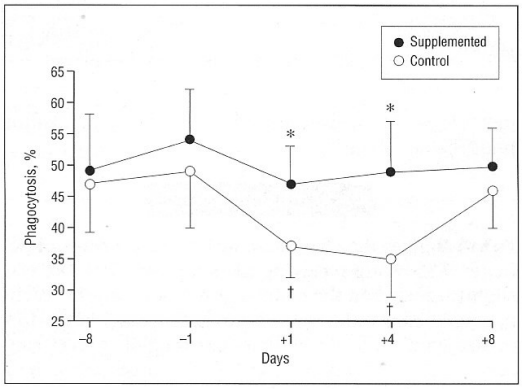

Figure 2. *Perioperative variations of the ex vivo phagocytosis ability. Asterisk indicates P<.01 vs control; dagger, P<.05 vs days −1 and −8.*

+4, and +8, the patients fed with the supplemented diet had a higher CD4/CD8 ratio than did the control group.

As expected, CRP levels significantly increased 1 day after operation in both groups, but, in patients receiving the supplemented formula, this increase was significantly lower than in the control group (**Figure 4**). The α_1-antitrypsin and fibrinogen levels slightly increased after surgery, and no difference between the 2 groups was observed.

None of the patients complained of adverse effects during presurgical oral intake of both formulas. Regardless of the composition, postoperative enteral feeding was well tolerated. Abdominal distention and cramps occurred in 3 patients (2 supplemented and 1 control) (7.5%), diarrhea in 2 (1 supplemented and 1 control) (5%), and emesis in 1 (control) (2.5%) for intestinal obstruction. Temporary discontinuation or lowering of the infusion rate allowed cramps and diarrhea to regress, so the nutritional goal was achieved in all patients but 1, who had persisting abdominal cramps. Displacement or clogging of the nasojejunal feeding tube occurred in 5 patients (3 supplemented and 2 control) (12.5%), always after the third postoperative day. In all of these cases but 1, enteral formulas were given orally until postop-

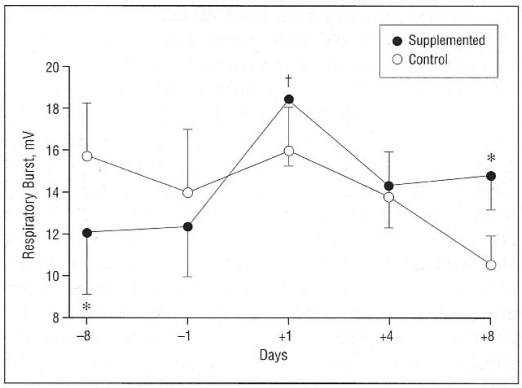

Figure 3. *Perioperative variations of the respiratory burst of polymorphonuclear cells as measured by luminometry (mV). Asterisk indicates P<.05 vs control; dagger, P<.01 vs days −1 and −8.*

erative day 7. Overall, permanent discontinuation of the enteral infusion was necessary in 2 patients (1 intestinal obstruction and 1 persisting cramps).

In the postoperative period, the mean caloric in-

Table 4. Variations of Lymphocytes*

Variable	Group	Days −8	−1	+1	+4	+8
Total lymphocytes, No. cells $\times 10^6/cm^3$	Supplemented	1586±400	1545±680	1122±386	1239±278	1353±302
	Control	1733±620	1662±393	984±487†	900±429†‡	1019±475†‡
T-lymphocytes, %	Supplemented	70.9±7.1	70.8±10.3	70.1±8.9	70.5±6.2	75.2±4.6
	Control	71.7±10.0	70.3±8.2	61.4±3.9†‡	65.7±1.6	64.2±2.3‡
CD4, %	Supplemented	43.0±9.6	43.8±8.7	46.9±8.1	51.3±10.9‡	54.0±7.0†‡
	Control	44.4±9.4	43.2±6.1	39.8±11.1	39.4±10.7	41.1±8.9
CD8, %	Supplemented	24.9±10.4	19.7±6.5	17.5±7.2†‡	17.2±5.7†	18.7±6.3
	Control	23.4±8.6	21.9±8.3	22.1±6.3	19.3±7.3	19.6±8.4
CD4/CD8	Supplemented	1.7±0.8	2.2±1.6	2.7±1.3†‡	3.0±1.9†‡	2.9±1.8†‡
	Control	1.7±0.7	2.0±1.2	1.7±1.4	2.0±1.4	2.1±1.6
Natural killer cells, %	Supplemented	17.4±6.8	18.3±7.2	12.3±9.7†	14.1±5.1	16.2±5.8‡
	Control	16.5±7.6	15.9±7.6	11.1±8.2†	12.6±4.6	10.2±3.8†
B-lymphocytes, %	Supplemented	9.9±3.6	11.8±6.0	15.5±5.6	13.4±3.5	12.8±1.8
	Control	11.7±6.2	10.9±7.2	12.9±8.4	12.1±4.1	11.9±2.6

*Values are given as mean±SD.
†P<.05 vs days −8 and −1.
‡P<.05 vs control.

take per day was 1085±314 kcal in the control group vs 1043±418 kcal in the supplemented group (P=.30). The mean protein intake per day was 69.2±20.2 g in the control group vs 66.8±23.7 g in the supplemented group (P=.40). The full nutritional regimen (25 kcal/kg per day) was reached after 4.2±0.5 and 4.4±0.3 days in the control and supplemented groups, respectively (P=.30).

COMMENT

Early enteral vs parenteral feeding in traumatized and surgical patients is gaining wide consensus after the promising results showing good tolerance and notable reduction of septic morbidity.[3-5,15,16] Our data confirm that early postoperative enteral feeding may be carried out safely in patients undergoing major abdominal surgery for gastric or colorectal cancer. Gastrointestinal side effects occurred in 15% of patients, but this rarely compromised the achievement of the nutritional goal. Moreover, preoperative supplementation allowed a good substrate bioavailability as confirmed by the significant preoperative increase in the arginine blood level.

In different clinical trials, the effect of dietary supplementation with arginine, ω-3 fatty acids, and nucleotides on host immune response after injury or surgery has been evaluated. The administration of supplemented diets improved the host defense mechanisms and helped to overcome postsurgical immune depression more rapidly than standard diet. However, this enhancement occurred with some delay. In fact, in the first days after surgery, a similar impairment of phagocytosis ability, alteration of cytokine profiles, reduction of immunoglobulin levels and number of activated T and B cells, and lymphocyte mitogenesis were found by comparing patients fed with supplemented or standard diets.[10,14-18] The delayed recovery in immune response might explain why supplemented diets given solely in the postoperative course led to variable improvements in outcome.[10,11,15,19] In our previous experience, the early postoperative administration of the supplemented diet

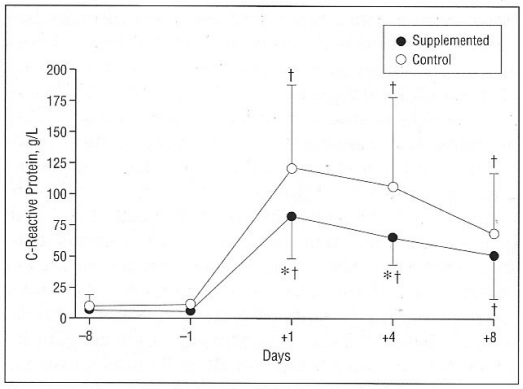

Figure 4. *Perioperative variations of the C-reactive protein. Asterisk indicates P<.05 vs control; dagger, P<.01 vs days −1 and −8.*

significantly reduced severity of infections and postoperative stay, but the infection rate was not significantly lower than in the group fed with the standard diet.[16] A suitable hypothesis to explain these findings is that, however early postoperative feeding is started, the amount of substrates given in the first days and the time required to reach adequate plasma and tissue substrate concentrations might be insufficient to allow a prompt burst of the immune system and an effective bacterial clearance shortly after surgery.

Experimental studies strongly suggest the usefulness of immunonutrition given before and after injury.[26-28] In burned animals, a significant reduction of overall mortality rate was found only when the administration of immunonutrients began 5 days before injury and continued afterward.[29,30] This observation stimulated us to evaluate possible benefits of perioperative administration of supplemented diets in patients with gastrointestinal cancer undergoing major surgery.

Preoperative supplementation with immunonutrients prevented the early postoperative decrease in phago-

cytosis and number of circulating lymphocytes, which play a key role in the control of postoperative infections. Moreover, the group receiving the supplemented diet had a significantly more effective activation of the intracellular respiratory burst early after surgery. The prompt and effective immune response observed in the supplemented group might be due to adequate plasma and tissue concentrations of specific nutrients such as arginine, ω-3 fatty acids, and RNA already available at the time of surgical stress.

INFLAMMATION has been long known to be an essential part of the healing and immune processes and successful recovery after injury. Recently, an exuberant systemic and uncontrolled inflammatory response has been recognized to lead to organ dysfunction and adverse outcome.[31] It has been proposed that injury promotes the switching of protein synthesis from constitutive to acute-phase proteins through the release of proinflammatory cytokines such as tumor necrosis factor α and interleukin-6. This may be associated with a rapid decrease of the nitrogen balance, loss of lean body mass, and catabolism. ω-3 fatty acids exert anti-inflammatory, vasodilatatory, and immunomodulatory properties through their ability to modulate the synthesis of different eicosanoids.[32,33] Preoperative and postoperative[11,34,35] administration of diets enriched in ω-3 fatty acids showed reduced plasma and tissue levels of specific leukotrienes, thromboxanes, and prostaglandins (or their metabolites) with proinflammatory, immunosuppressive, and vasoconstrictive effects. Other clinical studies documented lower plasma levels of interleukin-6 and tumor necrosis factor α when surgical patients were fed with similar supplemented diets.[15-18] These mechanisms might account for lower postoperative levels of CRP and could constitute an important cofactor in improving intestinal blood flow and oxygen metabolism in the splanchnic district.

Monitoring of the gastrointestinal mucosa perfusion by tonometry during major surgery and soon after trauma seems to be a sensitive method to predict the development of organ failure and poor outcome.[36] It has been shown repeatedly that patients with low postinjury pHi have a high risk of morbidity and mortality.[37,38] Yet, the variations of pHi in critically ill subjects do not seem to correlate with other standard monitoring systems, such as cardiac rate and output, arterial pH, blood pressure, and global assessment of oxygen delivery and consumption,[39] suggesting that the gut has an oxygen metabolism independent from other tissues and compartments. Boyd et al[40] showed that it was possible to improve outcome after major surgery by the prophylactic increase of oxygen delivery with the aim of avoiding intestinal mucosa hypoxia. It is reasonable to think that the maintenance of an adequate gut function was involved. We achieved a better postoperative intestinal oxygenation by giving specific nutrients before surgery. Although an adequate blood flow does not guarantee good tissue oxygen tension, delivery, and utilization, our present data showed that a higher intestinal microperfusion, as directly measured by laser Doppler flowmetry technique, paralleled a better intestinal pHi. It also should be stressed that a deficient blood flow and oxygenation of the gut could be a detrimental event, especially during abdominal surgery, in which intestinal anastomoses are often performed. Moreover, the detrimental sequelae observed after splanchnic hypoperfusion in critically ill patients may be mediated by translocation of endotoxin and bacteria, which has been proved in several experimental models to activate proinflammatory and catabolic responses, organ failure, and septic morbidity and mortality.[41-43]

An additional indirect demonstration of the impaired gut mucosa perfusion during major abdominal operations and of the significant amelioration of intestinal oxygen delivery and consumption by immunonutrients is the variation of plasma levels of the IIAP. In fact, this isoenzyme is released in the peritoneal fluid and plasma after intestinal hypoxic insults.

Another important component of this supplemented diet is arginine, which exerts different functions, most of them mediated by the L–arginine-NO pathway. Nitric oxide is synthesized from L-arginine by 2 types of enzyme: constitutive and inducible synthases. The constitutive synthesis of NO by vascular endothelium is responsible for the vasodilator tone that is essential for the regulation of blood pressure. A widespread network of adrenergic and cholinergic nerves ensures, through an NO-dependent mechanism, the regulation of neurologic vasodilation.[44] During bacteremia, severe intestinal microvascular vasoconstriction and hypoperfusion were reported after administration of NO synthase inhibitors.[45] It is reasonable to hypothesize that the increased splanchnic microperfusion observed at laparotomy in the supplemented group is due to the constitutive component of NO because time was too short to allow inducible NO synthesis. Nevertheless, the role of the inducible NO pathway cannot be excluded to explain later findings. The increased NO levels observed 1 and 4 days after surgery in the supplemented group may be a mechanism involved in the enhanced immune response early after surgery as shown by the improved phagocytosis ability and respiratory burst of PMNs. In fact, the administration of arginine before and after trauma improved host survival by enhancing bacterial clearance, and this protective effect was reversed when NO inhibitors were administered.[26,46] The NO inducible pathway is also involved in the cytokine-mediated inflammatory response. This is especially true when large quantities of NO are produced and released.[44] These observations seem to be in contrast with our data showing lower levels of CRP and higher levels of NO in the supplemented group. Nevertheless, in the present study, the patients receiving the supplemented diet had moderate absolute values of NO. Thus, it is suitable to speculate that in this case, the inducible NO pathway is not the primary mechanism involved in the modulation of the inflammatory response and that the anti-inflammatory properties of ω-3 fatty acids play a more specific role.

It is appealing that the maintenance of adequate gut microperfusion and intestinal pHi and immune and inflammatory responses would leave the host better

equipped to handle the insult of the surgical event. This type of intervention should be an important part of a wider strategy to reduce preoperatively surgical risk factors.[47-50]

Larger trials should follow to confirm that the control of postoperative immunosuppression and inflammatory response and the amelioration of the intestinal perfusion by means of immunonutrition will result in improved outcome.

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DISCUSSION

J. Wesley Alexander, MD, Cincinnati, Ohio: There are now 7 prospective randomized controlled clinical trials in which the effect of immunonutrition has been studied against a controlled diet. Six of these have been randomized so that they receive the same amount of calories, and 3 of them have been randomized so that they get isonitrogenous diets. The important findings in these studies have been that in the aggregate, there has been a significant reduction in infection and wound complications of about 50% to 75%, and hospitalization has been reduced by approximately 20%. Two of the studies have also done cost analysis and there is a significant reduction in the cost for patients receiving immunoenhancing diets.

Dr Braga and his colleagues have presented a very important study to expand these, for 3 reasons. First, it looks at the mechanisms of the diet, which has never been really accomplished in humans; second, it is the first study in which feeding before the time of the injury or operative procedure has been done; and third, it looks at the effect of the immunonutrients on the bowel function, which I think has never been done before. The results of this study have shown that there is improved bowel function, a decreased acute-phase protein response, improved phagocytic function, dampened responses for CRP, and a diminished effect on lymphocyte numbers and subsets in patients receiving the immunoenhancing diets.

There are basically 4 questions that I would like to ask of this important study. First, did the immunoenhancing diets have an effect on infectious complications as they have in the preceding 7 studies? As I indicated, 6 of these are published and 1 was presented by Dr Kudsk recently at the American Surgical Association. Perhaps in this very small study, which was not designed to look at the result of infection but rather the response, the numbers are not big enough yet.

Second, do you have any information on the cytokine response? There are some animal studies with individual nutrients, particularly the omega-3 fatty acids, that suggest that they alter cytokine response, but there has been very little done in humans with the complete diets which have used multiple immunoenhancing components.

Third, would you comment on the mechanism for the improvement of PMN function? I think this is a very important aspect, and, again, I know of little evidence in the literature suggesting which of these components may be important. Do you think it is perhaps the arginine or the omega-3 fatty acids? Dr Ogle has done some work in the laboratory that suggests that glutamine will improve phagocytic function, and we do know that with this particular diet you are using, glutamine levels are increased because of shunting from arginine to glutamine.

Finally, would you speculate on the beneficial effect of the improved oxygenation of the gut and the stabilization of the mucosal pH in your studies? This may be an extremely important aspect.

Dr Braga: At present, the sample size is too small to draw any conclusion on clinical outcome, but we observed an interesting trend with the reduction of postoperative infections in the supplemented group. Another interesting finding is that the only 2 anastomotic dehiscences of our series were in the control group. This might be meaningful because a better microperfusion and oxygenation of the gut could be a key factor to ameliorate the healing of the surgical anastomosis. Patient enrollment is continuing to reach a suitable number of patients to evaluate the clinical outcome.

About the second question, we studied the effect of the same supplemented diet given postoperatively on cytokines. A delayed improvement in the IL-2 receptors and a delayed reduction in IL-6 was observed. We collected plasma samples in all patients to determine some cytokine profiles. The data will be available in the future; in particular, we plan to determine IL-2 receptors, IL-6, and IL-8 to better study the function of polymorphonuclear cells, which play a key role in the immune response after surgery. The evaluation of both phagocytosis ability and intracellular killing demonstrated that PMN function was upgraded by the supplemented diet.

I agree with Dr Alexander that the addition of glutamine to such enteral formulas could further ameliorate both PMNs and lymphocyte function. Moreover, the addition of glutamine could improve gut barrier structure and function with subsequent reduction of microbial translocation.

Kenneth A. Kudsk, MD, Memphis, Tenn: The operations you talked about, colectomies and the gastric resections, are in people who are probably fairly well-nourished; preoperative nutrition may not play an important role. You didn't stratify your patients by degree of malnutrition. You had several people that had lost 10% of their body weight, which we haven't found to be a valuable predictor of length of stay or complications. Have you stratified either group of patients by degree of malnutrition through albumin, prealbumin, or other factors, and is there a difference in the response of people who are malnourished compared with those who are well nourished both within groups and between groups?

Dr Braga: The nutritional status of patients is an important issue, and I showed during my presentation that the severity of weight loss was comparable in the 2 groups. In the present study, the number of malnourished patients in both groups was small and this makes the evaluation of the subgroup of malnourished patients not reliable. Yet, the main goal of this study was not to replace energy and proteins but to ameliorate both immune response and gut function which are impaired after major operations also in well-nourished patients.

Donald E. Fry, MD, Albuquerque, NM: It seems that the feeding frenzy of immunonutrition is certainly on and I see no likelihood that that is going to settle down any time soon. The number of different variables in these nutrition formulas is actually fairly staggering, and yet we seem to focus only on 1 or 2 elements as having particular significance. So I would be a little bit cautious in everybody assuming that arginine or omega-3 fatty acids are somehow going to close the antibiotic pharmacy in the hospital. I would like to ask a couple of questions about, first, statistical methods that were used. Sitting from the back here, it looked like analysis of variance had been used for many of those observations. I would be a little bit skeptical about whether there were genuine differences between the 2 groups in many of the observations that were made.

Second, we don't know much about what was in the control formula, which preparation was used for the control nutrition. Were these fiber-containing nutritional preparations or not? I always end up coming to the fiber issue, since fermentable fiber and the consequences of having short-chain fatty acids is of some considerable significance relative to overall gut function, so was there fermentable fiber in the control or in the

supplemented diet presentations? And finally, antibiotics become fairly significant issues in terms of effectiveness of nutrition. Was there a standardized antibiotic strategy in the patients? There is a tendency in the United States, perhaps not in Italy, for surgeons to get carried away with systemic preventive antibiotics, which may actually have an adverse effect on gut colonization and may adversely affect, potentially, the nutritional support strategy.

Dr Braga: Antibiotic prophylaxis was given in all patients by a single dose of a first- or second-generation cephalosporin. A second dose was administered only when the operation lasted more than 4 hours. The 2 formulas were similar. The amount of arginine added in the supplemented formula was substituted with glycine, proline, alanine, and serine in the control formula. Neither diet contains fiber.

In regard to statistics, we used the Fisher exact test to compare discrete variables, and the Student *t* test to compare continuous variables. The Student *t* test was chosen because the variables were normally distributed.

William S. Helton, MD, Seattle, Wash: As Dr Alexander said, most of the studies previously have all been isonitrogenous and isocaloric: Were your two diets isocaloric, isonitrogenous and did they have the same amount of fat?

Dr Braga: The first study included surgical patients who were given supplemented formula before surgery. And the difference among the results in previous studies in which enteral nutrition was started after surgery or after trauma could be linked to this problem, because in the first days after surgery the risk is to administer it at not sufficient amount of substrates. I think it is very important that in the first hours after surgery, the challenge between the immune system and the bacteria is a key point.

Dr Josef E. Fischer, MD, Cincinnati, Ohio: Dr Helton is asking, are the diets isonitrogenous and isocaloric and equal in fat content on each given day.

Dr Braga: Yes, the 2 diets are isonitrogenous and isocaloric.

Dr Helton: The comment I would like to make has to do with Dr Alexander's mentioning that there are now 6 prospective randomized clinical trials reporting beneficial effects from enterally administered, immune-enhancing diets. I rise to inform some members of the audience and remind others that there will be an upcoming publication in *Shock* of a study presented by Cynthia Mendez of our department at the last AAST meeting. This study was a prospective, randomized, double-blind trial where patients receiving an immune-enhancing enteral diet, rich in fish oil and arginine, had a quicker return of normal monocyte function compared with patients administered a control diet. This improved monocyte function was associated with a significantly increased incidence of ARDS, time spent on the ventilator and time in hospital. Hence, the immune-enhancing effect of the experimental diet was associated with adverse clinical outcome. This study illustrates that we must pay attention not only to the cellular effects of these immunomodulating diets but more importantly to their clinical effects.

Dr Braga: Yes, I am unable to report to you data about clinical outcome.

Hebert R. Freund, MD, Jerusalem, Israel: Dr Braga, this is a very extensive and nicely carried out study for which I congratulate you; however, most of us I believe are sending colon and gastric cancer patients home within 5 to 6 or 7 days nowadays, so I fail to see the relevance of this kind of a study to the current practice of colon and gastric surgery. My other comment is, it would be very beneficial if you could see both formulas and judge for ourselves what is in either one of them.

James P. Fidler, MD, Cincinnati, Ohio: Did you have any concern about the volume of fluid administered in front of the anastomosis for the colon? Were you limited at all in what you could give?

Dr Braga: In our experience, the early infusion of fluids through the colonic anastomosis had no detrimental consequences. I would also emphasize that in patients operated on for gastric cancer, the tip of the nasojejunal feeding tube was placed above the jejunoileal anastomosis, and we never observed any anastomotic dehiscence. I believe that the better the perfusion of the gut, the better chance for anastomotic healing.

R. Neal Garrison, MD, Louisville, Ky: Just one quick methodology type question. When you used laser Doppler, how did you baseline the reading? Because laser Doppler basically is a relative flow change and if you don't have a baseline you can't use absolute numbers to measure absolute blood flow. So how did you use the laser Doppler method?

Dr Braga: Do you want any details about the laser Doppler technique?

Dr Fischer: No. What he is asking is, the laser Doppler, which Dr Garrison has significant experience with, is a relative change, and you have absolute numbers and he is asking how you did that.

Dr Garrison: That's correct.

Dr Braga: The measurement by laser Doppler was expressed in perfusion units. This method to quantitate perfusion units was validated in earlier studies. The method was the same for both groups, thus the relative differences between the 2 groups remain valid.

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