

Original Research

Nutritional Support in Alcoholic Cirrhotic Patients Improves Host Defenses

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Key words: Nutrition support, cirrhosis, immunity

Background: Malnutrition is usual in patients with alcoholic liver disease and is associated with a poor outcome. Nutritional support decreases nutrition-associated complications.

Aim: To demonstrate that nutritional support in ambulatory alcoholic cirrhotic patients improves host defenses.

Methods: Thirty-one male outpatients with alcoholic cirrhosis CHILD-PUGH B or C were included. Twenty-five subjects completed six months consuming daily a nutritional supplement (Ensure®, 1000 Kcal and 35 g protein), in addition to their regular diet. At entrance and every three months, a clinical assessment, nutritional evaluation and indirect calorimetry were performed. Liver function tests and LPS-induced monocyte production of cytokines, salivary secretory IgA, lactulose/mannitol ratio and breath hydrogen tests were also measured in these intervals. Delayed cutaneous hypersensitivity and IgG and IgM antibody response to endotoxin were assessed at entrance and at the end of the study.

Results: Patients drank 85% of the provided supplement as an average. REE, total body fat and serum albumin increased, basal breath hydrogen decreased and cellular immunity improved significantly during the follow up period ($p \leq 0.03$). All the other parameters remained unchanged throughout the study. Six patients (16.2%) died during the study, five due to upper gastrointestinal bleeding.

Conclusion: Nutritional support in alcoholic cirrhotic patients improves nutritional status and cell mediated immunity.

INTRODUCTION

Malnutrition is invariably present in advanced stages of liver damage [1,2,3,4]. Several factors such as anorexia, alterations in protein and energy metabolism, increased fat oxidation, alcohol ingestion, dietary restrictions or nutrient malabsorption have been implicated in the deterioration of nutritional status [5]. Nitrogen balances are often negative in alcoholic liver disease (ALD) due to low protein ingestion or absorption and increased protein catabolism [6,7]. Protein turnover studies have found increased protein catabolism associated with coexisting events, such as alcohol ingestion, infections and stress [8,9], rather than in stable conditions [10,11]. Concerning energy metabolism, low [12], normal [13,14] or high energy expenditure has been reported in cirrhotic patients [15,16]. In a large study, hypermetabolism was found to be associated with concurrent conditions, so it cannot be considered a constant

feature of cirrhosis [17]. Interestingly, high volume ascites seems to increase resting metabolic rate, since it decreases after ascites removal [18].

Nutrition critically affects the immune system, involving both the antigen-nonspecific and the adaptive or antigen-specific responses. Several investigations have confirmed the adverse effects of protein-energy malnutrition (PEM) over different aspects of host defenses [19] and probably also the gut barrier [20,21,22]. Interestingly, it has been demonstrated that immune alterations recover with nutritional replenishment [23].

As malnutrition adversely affects the outcome of many chronic and acute diseases [24,25,26], numerous trials have attempted to correct nutritional status in ALD, hoping to decrease morbidity and mortality [4,27,28].

We previously demonstrated that long-term nutritional support, using a standard enteral formula, reduced the number of admissions due to infections in Child B and C alcoholic

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cirrhotic patients [4]. The present study was designed to clarify the mechanisms underlying this result. Thus, our aim was to demonstrate that nutritional support in ambulatory alcoholic cirrhotic patients improves host defenses.

PATIENTS AND METHODS

Patients attending an alcoholic liver disease clinic were considered eligible for this study if they fulfilled the following requisites:

- a) Clinical evidence of alcoholic liver disease. Patients were stratified according to CHILD PUGH classification [29] B or C at the time of enrollment.
- b) A history of at least five years of heavy alcohol consumption (daily alcohol intake >150 g).
- c) Absence of hepatitis B surface antigen or hepatitis C antibody.
- d) Absence of significant renal, pulmonary or cardiac disease, clinical diabetes or malignant tumors (including hepatoma).
- e) Residence in the city where the study was performed (Santiago-Chile).

The study was approved by our local ethics committee, and all eligible patients signed a written informed consent. Each subject was instructed to consume, in addition to his regular diet, one liter of a commercial enteral formula (Ensure®, Abbott Laboratories), that provided 35 g protein, 1000 Kcal/day and 800 mg sodium. The total amount of fluid consumed per day was aimed at 2500 mL/d.

Patients were seen twice a month by a nurse practitioner at the liver disease clinic. On each visit, they were invited to void in a small container to measure urine alcohol with reactive strips (Alcohol Dipstick). They were asked about any pharmacological treatment received, alcohol ingestion and compliance with the nutritional support and were given a new supply of Ensure®. The compliance was measured by interrogating the patient about the amount of daily supplement ingested, the Ensure® leftover, the acceptability or adverse effects and the number and kind of regular meals.

Subjects were examined by a physician at entrance to the study and monthly or more frequently, if necessary, during a six month period. Every third month, a complete clinical and nutritional assessment was performed, including anthropometric measurements (weight, midarm circumference, triceps skinfold thickness using a Lange caliper at four standard locations and hand grip muscle strength using a hand grip dynamometer (model No. 0032, Therapeutic Instruments). Subjective Global Assessment (SGA) of nutritional status was performed only at entrance to the study [30]. Additionally, body fat was measured by Dual Energy X-ray absorptiometry (DEXA) in a LUNAR DPX-L densitometer (LUNAR Corp Madison, Wisconsin USA), and resting energy expenditure was assessed by indirect

calorimetry in a canopy system (Sensor Medics 2900). Conventional treatment for encephalopathy (metronidazole and lactulose) and ascites was given if required.

Fasting blood samples were obtained at the beginning of the study and every three months, to measure *in vitro* lipopolysaccharide (LPS)-stimulated peripheral blood monocyte cell (PBMC) production of $\text{IL-1}\beta$, IL-6 and $\text{TNF-}\alpha$, and routine laboratory tests (packed red cell volume, erythrocyte sedimentation rate [ESR], albumin, creatinine, blood urea nitrogen, total bilirubin, alkaline phosphatase, aspartate aminotransferase [AST] and prothrombin time) were performed. At the entrance and at the end of the study, serum IgG and IgM antibody responses to endotoxin were measured. At the same visit, a salivary sample was taken to measure secretory IgA. The lactulose/mannitol urinary excretion and a basal breath hydrogen test were performed to estimate intestinal permeability and microbiological contamination of the small intestine, respectively.

At the beginning and at the end of the study, cell mediated immunity was assessed using a delayed hypersensitivity skin multitest with seven antigens (MULTITEST IMC, Pasteur Merieux). Results were expressed as the sum of the major diameter of all positive reactions (mm).

Analytical Procedures

PBMC production of $\text{IL-1}\beta$, IL-6 and $\text{TNF-}\alpha$ were measured as previously described [31] using a specific commercial enzyme-linked immunoabsorbent assay (R&D System, Minneapolis). IgG and IgM antibody responses to endotoxin were quantified using an enzyme-linked immunoabsorbent assay (EndoCAb), as previously described [32].

Salivary secretory IgA levels were measured by a radial immunodiffusion kit (The Binding Site Limited, Birmingham, England).

The lactulose/mannitol test was performed withdrawing antibiotics and lactulose seven days before the test. After an overnight fast, subjects drank 200 mL of a solution containing seven g lactulose and two g mannitol. Subsequently, urine was collected during the following five hours, where both sugars were measured by gas chromatography.

The breath hydrogen test was performed withdrawing antibiotics and/or lactulose one week before the test. After eight hours' fast, expired air was collected using Milar bags with a two-way valve. Hydrogen was measured the same day by gas chromatography [33].

Results from the four methods just described were compared with values obtained in twelve age and socioeconomic-status paired healthy subjects.

If necessary, patients were admitted to the hospital and followed by the staff in charge of the protocol. Criteria for admission were the following:

- a) Upper gastrointestinal bleeding evidenced by hematemesis, melena or rectal bleeding.

b) Progressive ascites despite the use of diuretics and salt restriction.

c) Progressive encephalopathy despite adequate ambulatory management (lactose and/or neomycin).

d) Clinical evidences of severe infections such as pneumonia or spontaneous bacterial peritonitis.

e) Any other life threatening condition.

In case of admission to another hospital (usually due to emergencies), relatives were instructed to provide information about such events and the outcome data (death or discharge diagnoses). All hospitalizations were registered.

If a patient died, the cause was recorded, and if he failed to attend two or more follow up visits, efforts were made to find him. Those who were not located after one month were considered definitively lost from control and not readmitted to the protocol even if they returned, although their clinical care was maintained.

Statistical Analysis

Results are expressed as median and range or mean ± standard deviation. ANOVA for repeated measures was used for comparison of parametric variables, and the Scheffé *post hoc* comparison was done to find where the significant differences were in the data that had significant p value for ANOVA. A Wilcoxon matched pair test was used for comparison of non-parametric variables.

RESULTS

Fifty-one alcoholic cirrhotic patients were initially considered eligible to participate in the study. Nine of these were excluded, five due to positive hepatitis C virus antibody, two were diabetic, one was classified as Child A, one died in a traffic accident, and five did not attend the first control. Thirty-seven patients (Child B or C) were thus admitted to the study. The initial nutritional features and laboratory tests are shown in Tables 1, 2 and 3. According to SGA, 34 patients were classified as malnourished. LPS-stimulated PBMC production of IL-6

Table 1. Initial Nutritional Features of All Patients Considered Eligible for the Study (n=37)

	Mean±STD	Range
Age (years)	46.3±7.4	31–65
Weight (kg)	64.6±11.7	48.2–100
Height (cm)	163.6±5.9	155–174
Triceps skinfold (mm)	10.7±6.4	5–31
Mid-arm circumference (cm)	26.1±4.2	21–38
Muscle strength (kg)	28.8±5.9	19–44
Resting energy expenditure (Kcal/day)	1683.9±265.3	1347–2309
RQ (VCO ₂ /VO ₂)	0.75±0.1	0.64–0.95
Total body fat by DEXA (%)	18.8±5.9	10.1–30.6

Table 2. Initial Routine Laboratory Features of all Patients Considered Eligible for the Study (n=37)

	Mean±STD	Range
Serum total protein (g/L)	76±10	51–89
Serum albumin (g/L)	34±7	21–47
Serum creatinine (μmol/L)	97.4±17.7	70.7–114.9
Blood urea nitrogen (mmol/L)	4.6±1.9	2.9–8.2
Total bilirubin (μmol/L)	49.6±37.6	13.7–150.4
AST (μkat/L)	0.55±0.007	0.22–1.37
Alkaline phosphatase (μkat/L)	4.1±1.2	1.9–7.0
Packed red cell volume (l)	0.35±0.06	0.27–0.46
Leukocyte count (10 ⁶ /L)	8.273±5.208	2.800–21.800
ESR (mm/h)	59.1±41.2	7–126
Prothrombin time (%)	58.4±20.4	19–100
Blood glucose (mmol/L)	4.9±0.73	4.2–6.77

Table 3. Initial Immunological and Intestinal Function Tests of all Patients Considered Eligible for the Study (n=37)

	Study Group	Healthy Controls
Skin multitest (mm)	16.2±11.3	15.0±7.7
LPS stimulated PBMC of:		
IL-1β (ng/mL)	8.4±4.7	9.0±2.3
IL-6 (ng/mL)*	26.0±12.8	16.4±2.3
TNF-α (ng/mL)	5.4±3.3	3.8±2.2
Salivary IgAs (mg sIgA/mg albumin)	15.0±11.9	14.5±7.8
Lactulose/mannitol ratio	0.029±0.014	0.028±0.016
Breath hydrogen test (ppm)**	13.4±12.0	4.3±2.8

ANOVA * p=0.007, ** p=0.02.

and basal breath hydrogen was significantly higher than controls' values (p=0.007 and p=0.002 respectively). Five subjects that continued to drink and one that moved to another city had to be withdrawn from the study due to lack of compliance in the first two months. Thus, the final sample comprised 31 patients. Subjects consumed 85% of the nutritional supplement. Alcohol ingestion was detected in 13 patients throughout the study. Metronidazole and or lactulose were required in 15 subjects. In the same period, 13 patients were admitted to the hospital on 19 occasions, due to upper gastrointestinal bleeding (12 episodes in eight subjects), spontaneous bacterial peritonitis in two subjects (one during the first month and the second during the second month of nutritional support), prostatitis in one patient, liver failure in three cases and one case as the result of an emergency surgical procedure (complicated umbilical hernia). Six of these patients died (16.2%) during the hospitalization, five in the first three months and one during the fifth month of follow-up. Causes of death were upper gastrointestinal bleeding in five subjects and spontaneous bacterial peritonitis in one patient; these events were associated with alcohol intake in four patients. Consequently, 25 patients completed the six-month follow up. Initial nutritional and laboratory parameters between hospitalized, deceased and surviving patients were not different.

Resting energy expenditure, body fat (measured both anthropometrically and by DEXA), increased significantly ($p \leq 0.05$) during the first three months and serum albumin at the end of the study compared with the basal assessment (Table 4). Liver function tests and salivary secretory IgA did not change during the study. Packed red cell volume increased, and ESR decreased significantly at the end of the study period compared with the basal assessment (Table 5).

Basal breath hydrogen decreased, regardless of metronidazole utilization or alcohol ingestion during the study (Fig. 1), and cellular immunity improved significantly (Fig. 2). No variations in LPS-stimulated PBMC production of $IL-1\beta$, $IL-6$ and $TNF-\alpha$, or lactulose/mannitol ratio were observed during 6-month (Table 6).

Metronidazole, lactulose prescription (nine patients) or intermittent alcohol ingestion (nine patients) did not have an effect on the evolution of nutritional, liver function or immunological parameters during the study period.

Serum IgG and IgM endotoxin antibodies levels did not change significantly during the study. However, those patients with persisting alcohol intake had higher initial and final levels of IgG endotoxin antibodies $p < 0.006$ (1217 ± 747 initial, 1380 ± 626 MU/mL final) compared with abstinent patients (588 ± 400 initial, 641 ± 574 MU/mL final) (Fig. 3).

DISCUSSION

In this study we were able to demonstrate that six-month's nutritional support with a standard enteral formula improves cellular immunity and nutritional status in ambulatory alcoholic cirrhotic patients, Child B and C. We previously demonstrated the benefits of nutritional support in a group of totally comparable patients; therefore, on this occasion, we did not include a control group for ethical reasons [4].

Throughout the study, the rate of hospitalizations due to infections was comparable to that of the supplemented group of our previous trial (three cases out of the 31 patients included in

Table 4. Nutritional Features during the Study Period (n=25)

	Initial	3 Months	6 Months
Weight (kg)	63.2±12.2	70.4±13.6	71.2±13.1
Triceps skinfold (mm)	12.1±7.6 α	14.5±7.6 β	14.4±7.4 β^*
Mid-arm circumference (cm)	27.0±4.7	29.7±5.1	30.2±5.0
Muscle strength (kg)	29.0±6.3	29.5±7.0	30.2±5.0
Resting energy expenditure (Kcal/day)	1711±260 α	1848±255 β	1883±218 β^*
RQ	0.75±0.06	0.77±0.06	0.73±0.06
Total body fat by DEXA (%)	19.8±1.6 α	21.5±5.5 β	22.3±5.7 β^*
Serum albumin (g/L)	35±6 α	38±6	39±6 β^*

* ANOVA for repeated measures $p < 0.03$.

Scheffé post-hoc comparison of means, α significantly different to β .

Table 5. Laboratory Features during the Study Period (n=25)

	Initial	3 Months	6 Months
Serum total protein (g/L)	76±10	76±06	75±07
Serum creatinine ($\mu\text{mol/L}$)	97.4±17.7	88.4±17.7	97.2±16.8
Blood urea nitrogen ($\mu\text{mol/L}$)	4.3±1.5	4.6±1.3	4.5±1.5
Total bilirubin ($\mu\text{mol/L}$)	44.6±35.9	35.9±39.3	30.8±20.5
AST ($\mu\text{kat/L}$)	0.47±0.26	0.39±0.16	0.52±0.33
Alkaline phosphatase ($\mu\text{kat/L}$)	3.94±1.38	3.75±1.32	3.95±1.56
Packed red cell volume (l)	0.35±0.06 α	0.37±0.07	0.39±0.05 β
Leukocyte count ($10^9/L$)	6.633±3.915	5.800±3.222	4.850±1.298
ESR (mm/h)	55.8±39.4 α	40.2±39.4	34.8±33.9 β
Prothrombin time (%)	61.6±19.7	56.3±20.0	59.0±17.7

* ANOVA for repeated measures $p < 0.02$.

Scheffé post-hoc comparison of means, α significantly different to β .

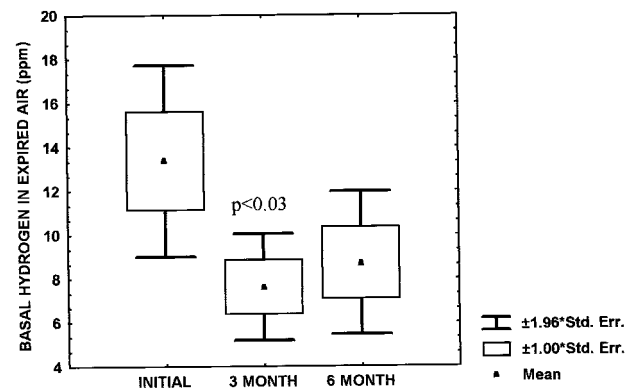


Fig. 1. Effect of a nutritional support during six months on basal hydrogen breath test. ANOVA for repeated measures $p < 0.03$.

this study, *versus* two out of 26 in the former trial). Mortality was also similar to that of previous studies [4,34] and was determined mostly by upper gastrointestinal bleeding associated with alcohol ingestion, a factor we could not directly control.

Resting energy expenditure increased during the period of nutritional supplementation. This change probably reflects an increase in food intake or lean body mass accretion during this period [35]. Unfortunately, we were not able to demonstrate directly an increase in lean body mass, since body composition was assessed using DEXA, that estimates lean tissues incorrectly in patients with cirrhosis and renal failure, as it depends on the hydration status of soft tissues. Therefore, under the given conditions, this method is only reliable for the estimation of body fat mass, which contains a low proportion of water [36]. In fact, we observed an increase in this in our patients, as assessed by DEXA and skinfold thickness. Thus, we did not

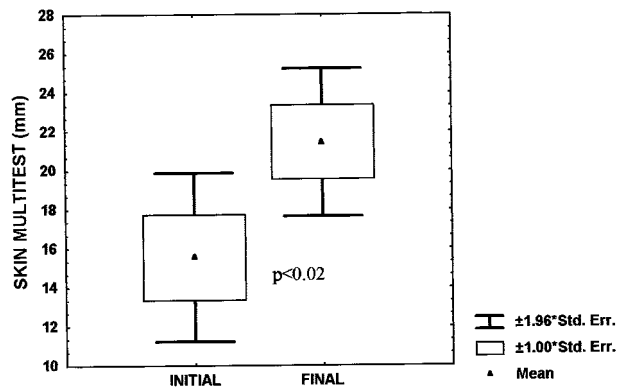


Fig. 2. Effect of nutritional support during six months on delayed coetaneous hypersensitivity, measured by skin multitest with seven antigens. Results are expressed as the sum of the major diameter of all the positive reactions. Paired *t* test $p < 0.02$.

Table 6. Cytokines, Intestinal Permeability and Secretory Immunoglobulin during the Study Period (n=25)

	Initial	3 Months	6 Months
LPS stimulated PBMC of:			
IL-1 β (ng/mL)	7.7 \pm 3.8	7.1 \pm 3.8	6.2 \pm 2.7
IL-6 (ng/mL)*	28.2 \pm 11.2	29.8 \pm 11.7	27.9 \pm 8.7
TNF- α (ng/mL)	4.7 \pm 3.1	5.2 \pm 3.3	4.9 \pm 3.0
Salivary IgAs (mg sIgA/ mg albumin)	12.5 \pm 10.9	12.0 \pm 8.9	10.4 \pm 10.2
Lactulose/mannitol ratio	0.029 \pm 0.014	0.030 \pm 0.018	0.022 \pm 0.012

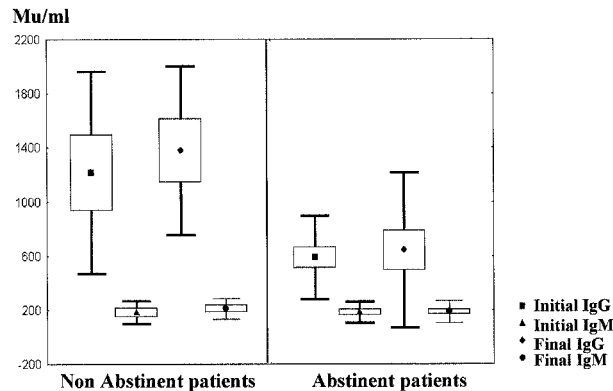


Fig. 3. Effect of a nutritional support during six months on serum IgG and IgM endotoxin antibodies levels. ANOVA for repeated measured=ns. Serum IgG and IgM endotoxin antibodies levels between patients with persisting alcohol intake compared with abstinent patients, ANOVA $p < 0.006$ for IgG.

attempt to draw conclusions regarding the effects of nutrition support on lean body mass. Other causes of increased REE, such as infections and stress, were ruled out. Our patients were free of these complications at the entry to the study, and resting

energy expenditure was always performed when no acute event was present.

In cirrhosis, both the hepatic and the whole body respiratory quotient are markedly reduced. In overnight fasted cirrhotic patients, lipid oxidation increases and glucose oxidation, decreases, compared with normal subjects. These changes in endogenous substrate use is similar to those of subjects adapted to prolonged starvation and persisted even after variable periods of nutritional support [12,13,37]. The behavior of our patients did not differ from that of those in other series.

Although no differences in delayed cutaneous hypersensitivity were observed between normal subjects and our patients in the basal period, an improvement in this parameter was observed after the nutritional support. A boosting effect of repeated skin test measurements is highly unlikely after six months, since it has only been observed when the tests are repeated after a short period, of three weeks or less [38]. Cirrhosis and alcoholism are both associated with immunological changes. Alcohol consumption appears to attenuate the production and migration of polymorphonuclear leukocytes and inhibits cell-mediated immunity [39,40]. Cirrhosis is associated with a lower T lymphocyte count, cutaneous anergy, chemotactic activity disturbances, decreased complement factors and depressed macrophage phagocytic activity [5]. These changes are very similar to those caused by protein energy malnutrition [41,42]. As discussed previously, we had no objective indicator of lean body mass depletion in our patients. However the low SGA of nutritional status at baseline likely reflects the nutritional derangement caused by alcoholism and cirrhosis. The later method has been extensively validated by us and other authors [30,43].

Bacterial translocation and intestinal permeability are increased in animal models of cirrhosis [44,45]. Other causes of elevated intestinal permeability are malnutrition and the metabolic response to injury [22,46]. Bacterial translocation is associated with a higher risk of developing gut derived infections such as spontaneous bacterial peritonitis and bacteraemia. It also activates phagocytic cells to produce lymphokines, whose adverse effects can perpetuate liver damage or induce wasting. Although endotoxemia is common in cirrhotic patients with portal hypertension [47,48], intestinal permeability has been reported to be normal [49], as in our patients. The lack of effect of nutritional support on intestinal permeability is consistent with other studies, performed in acutely ill patients, in whom this parameter did not improve after a successful nutritional replenishment [50]. Other factors such as acute infectious events or burns also influence intestinal permeability [51,52]. A normal intestinal permeability, assessed through the lactulose/mannitol ratio, does not exclude an increased bacterial translocation or a reduced clearance of intestinal bacteria in cirrhosis [43]. The effect of alcohol ingestion on endotoxin antibody levels probably indicates that ethanol increases bacterial translocation, although it was not reflected by changes in cytokine

production or lactulose/mannitol ratio. We have not previously found modifications in lactulose mannitol ratio in alcoholics [53].

In this study, the reduction in breath hydrogen presumably reflects less intestinal contamination [54]. This effect could be due to an improvement in nutritional status or a more efficient management of intestinal microflora with oral antibiotics or lactulose. The lack of alcohol effect on breath hydrogen is in contradiction to the reports showing a higher intestinal contamination in recently drinking alcoholics [55]. Noteworthy, as mentioned before, our patients were receiving treatment for encephalopathy.

PBMC production of IL-1 β and TNF- α has been reported normal or elevated in cirrhotic patients [56]. An enhanced production of these cytokines is associated with acute events such as infections and acute alcoholic hepatitis [30]. IL-6 is elevated in patients with chronic liver disease, associated with ascites and end stage cirrhosis [57,58,59]. As expected in this study, performed in stable Child B or C cirrhotic patients PBMC production of IL1- β and TNF- α at baseline were not different from normal controls, and IL-6 was higher. On the other hand, we did not observe changes in PBMC production of cytokines during the nutritional supplementation, suggesting that the initial low rate of endotoxemia did not worsen throughout the study.

Salivary secretory IgA levels were high in our healthy subjects and in cirrhotic patients, when compared with other reference populations. These differences are possibly explained by a high oral contamination that is common in low socioeconomic levels. Secretory IgA is lower in animals models of stress or starvation and in malnourished children [60,61]. The lack of changes with nutritional supplementation in our patients indicates that the regulation of salivary secretory IgA depends on multiple factors, besides nutrition.

Serum albumin levels increased during the nutritional intervention. However, this parameter is not a sensitive indicator of visceral protein storage in cirrhotic patients. Albumin concentrations levels are a function of its rate of synthesis, volume of distribution and catabolism. The causes of hypoalbuminemia in cirrhotic subjects are an enlarged volume of distribution and increased catabolic rate, without a compensatory increase in albumin synthesis, due to inadequate synthetic reserve, inadequate protein intake and frequent superimposed infections [28]. The observed improvement in serum albumin levels probably reflect an increase in protein intake and a low incidence of infections in these patients, since other liver function tests did not change. Volume depletion due to a more efficient ascites management could also play a role.

In summary, nutritional support in alcoholic cirrhotic patients improves nutritional status and host defenses due to better cellular immunity and less intestinal bacterial overgrowth. In addition, alcohol abstinence in these patients probably exerts an additive effect.

ACKNOWLEDGMENTS

Marcela Bonnefoy for her devotion to the patients and Sergio Latorre for his technical assistance. Financing from Fondecyt, Chile, Grant # 1950-386.

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Received February 1999; revision accepted July 1999.