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Original Communication

Nutritional treatment for acquired immunodeficiency virus infection using an enterotropic peptide-based formula enriched with n-3 fatty acids: a randomized prospective trial

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Objective: Dietary counseling and intervention based on application of conventional criteria have been ineffective in preventing the progressive weight loss associated with HIV infection. The aim of the study was to compare the progression of clinical and nutritional indicators during nutritional supplementation with or without an enterotropic peptide-based formula enriched with n-3 fatty acids.
Design: Randomized trial.
Setting: Tertiary care.
Subjects: Ninety-one patients were screened for the study. Twenty-three did not meet the inclusion criteria, therefore 74 patients were randomized. Of these, 38 were randomized to group I (standard formula) and 36 were randomized to group II supplementation (enterotropic peptide-based formula enriched with n-3 fatty acids).
Interventions: Group I received standard enteral formula and group II received a enterotropic peptide-based enteral formula. The volume was the same (3 cans/day, 236 ml per can). In both groups enteral supplementation were recommended in conjunction with a registered dietitian under a dietary counseling program based on standard nutrition principles. Patients received a prospective serial assessment of nutrition status, nutritional intake with 24 h written food records, GI symptoms, immune function, anthropometric status and intercurrent health events including infections and hospitalization. These determinations were performed at baseline and at 3 months.
Results: Treatment with both supplements resulted in a significant and sustained increase in weight (3.2% in group I and 3.1% in group II); this increase was mostly due to fat mass (12.8% in group I) and (7.5% in group II). Total body water and fat free-mass remained unchanged. CD4 counts remained stable in group I, while a significant increase was detected in group II (576 ± 403 *vs* 642 ± 394 cells/mm^3; $P < 0.05$). After the 3 month period CD4 counts remained higher in group II. Hospitalization events (infections) were also followed during the 3 month period. Group II had fewer hospitalizations than group I, but no statistical differences were found.
Conclusions: Oral nutritional supplements for a 3 month period were well tolerated and resulted in body weight gain in HIV-infected patients. Supplement-enriched formula, with peptides and n-3 fatty acids, increased CD4 count.
Descriptors: HIV; nutrition; randomized trial
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Introduction

The interaction between human immunodeficiency virus (HIV) infection, nutrition and immune function supports a complex relationship. The dominant effect of HIV infection on nutritional state is clear; the impact of nutritional status on immune function and disease pro-

gression is much less clear. Early studies showed the relationship between loss of lean body mass and timing of death in patients with HIV infection (Kotler *et al*, 1989; Greene, 1988; Kotler, 1989; Guenter *et al*, 1993). Surprisingly, studies of dietary intake for patients with HIV infection have demonstrated adequate energy consumption (Chlebowski *et al*, 1991). In addition, dietary counseling and intervention based on application of conventional criteria have been ineffective in preventing the progressive weight loss associated with HIV infection. Some trials of early supplementation of nutrient intake have been beneficial in these patients (Chlebowski *et al*, 1993). These studies showed increased in body weight, body cell mass, and intracellular water; immune status was also improved (Shabert *et al*, 1999), with a significant reduction in the number of hospitalizations (Clark *et al*, 2000).

The aim of our study was to compare the progression of clinical and nutritional indicators in HIV-infected patients during nutritional supplementation with or without an enterotropic peptide-based formula enriched with n-3 fatty acids.

Materials and methods

Patients and procedure

Patients between 18 and 60 y of age with HIV infection with or without AIDS-defining illness (Centers for Disease Control and Prevention, 1992) were elegible for enrollment if they met the following criteria: a confirmed diagnosis of HIV infection, absence of chronic febrile illness, absence of severe GI symptoms (diarrhea for >30 days or >3 times/day); adequate liver function and normal kidney function. All patients gave informed consent before enrollment and remained under routine treatment (highly active antiretroviral therapy-HAART) for HIV infection during the study, a protease inhibitor-containing regimen with three agents. Subjects being of stable weight were chosen to perform the trial to decrease the drop out during the study.

Study design

This study was designed to determine the influence, on nutritional and clinical outcomes, of two feed formulas for early supplementation in patients with HIV infection. Seventy-four patients with HIV infection were prospectively randomized (see Table 1). Group I received standard enteral formula and group II received a enterotropic peptide-based enteral formula with n-3 fatty acids, both groups received three cans/day (236 ml per can; Table 2). In both groups, enteral supplementation was recommended in conjunction with a registered dietitian dietary counseling program based on standard nutrition principles.

Patients received prospective serial assessment of nutrition status, nutritional intake with 24 h written food records, GI symptoms, immune function, anthropometric status and intercurrent health events including infections and hospitalizations. These determinations were performed at baseline and at 3 months.

Table 1 Demographic characteristics of patients with HIV at baseline

Characteristics	*Group I*	*Group II*
n	38	36
Age (y)	37.9 ± 10	38.9 ± 8.8
Percentage (male)	77.6%	78.6%
CDC class A-B (%)	57.2%	51.1%
CDC class C (AIDS) (%)	42.8%	48.9%
Intravenous drug use (%)	40.8%	42.9%
Weight (kg)	62.7 ± 10.9	61.1 ± 11.2
Body mass index	22 ± 2.8	21 ± 2.8
CD4 (count/μl)	454 ± 271	561 ± 369
Albumin (g/dl)	4.1 ± 0.8	4.2 ± 0.6

Table 2 Nutrient composition of enteral formulas

	Group I (Standard)[a]	*Group II (Peptide-based)*[b]
Caloric density (kcal/ml)	1.06	1.28
Protein (g/l)	37.2	60
(%VCT)	14	18.7
Fat (g/l)	37.2	22.8
(%VCT)	31.5	15.8
Carbohydrate (g/l)	145	215.8
(%VCT)	54.5	65.5
Dietary fiber (g/l)	0	8.9
n-3 fatty acids (g/ml)	0	9.46

[a]ENSURE®, Abbott Laboratories. Source of proteins: 88% sodium and calcium caseinates, 12% soy protein isolate. Source of carbohydrate: 70% corn syrup and 30% sucrose. Source of fat: 100% corn oil, no beta-carotenes.
[b]ADVERA®, Abbott Laboratories. Source of protein: 78% soy protein hydrosylates, 22% sodium caseinate. Source of carbohydrates: 72% hydrolyzed cornstarch, 23% sucrose, 5% soy polysaccharide. Source of fat: 70% canola oil, 20% medium chain triglyceride, 10% refined deodorized sardine oil, contains beta carotene at 5.1 mg/l.

Weight and anthropometric data

Body weight was measured to an accuracy of 0.5 kg and body mass index (BMI) computed as body weight/(height^2). Bipolar body electrical bioimpedance was used to determine body composition (Pichard *et al*, 1993). An electric current of 0.8 mA and 50 kHz was produced by a calibrated signal generator (Biodynamics Model 310e, Seattle, WA, USA) and applied to the skin using adhesive electrodes placed on right-side limbs. Resistance and reactance were used to calculate total body water, fat and fat-free mass. Regional changes in body mass were estimated by measuring the circumferences and tricep skinfold of the midarm.

Biochemical parameters

Basal blood sampling was performed before and after 3 months of treatment, for determinations of blood chemistry, liver function tests and hematologic parameters (LabCorp, Uniondale, NY). We also measured CD4 counts (LabCorp)

and HIV viral loads (LabCorp); the minimun detection limit was 50 copies per milliliter.

Dietary intake
All patients received instruction in keeping a 24 h written food record, incorporating the use of food scales to enhance portion size accuracy. Records were reviewed by a registered dietitian and analyzed with a computer-based data-evaluation system. Total calorie intake and the contribution of the assigned enteral supplement were used as indicators of nutrient intake and were determined at baseline and at 3 months after entry into the study. Enteral formula consumed was also estimated in relation to the formula dispensed, patient reports and level of consumption.

Intecurrent health events
Unscheduled physician visits and hospitalizations, for any reason, were recorded and evaluated in every patient during the 3 months of the study.

Statistical analysis
The results were expressed as means ± standard deviation. The distribution of variables was analyzed with the Kolmogorov–Smirnov test. Quantitative variables with normal distribution were analyzed with two-factor repeated measures ANOVA including interaction terms. Non-parametric variables were analyzed using the Mann–Whitney *U*-test. Discrete variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. To minimize the potential for introducing bias, all randomized patients were included in the comparisons, irrespective of whether or not and for how long they complied with their allocated regimen (intention-to-treat analysis). A *P*-value under 0.05 was considered statistically significant.

Results

Baseline evaluation and adherence
Ninety-one patients were screened for the study. Twenty-three did not meet the inclusion criteria, therefore 74 patients were randomized to one of the two study groups. Of these, 38 were randomized to group I and 36 were randomized to group II. Patient characteristics are outlined in Table 1; there were no significant differences between the two groups in the demographic characteristics assessed.

To assure adherence to study supplementation program, we dispensed enough formula to our patients to provide 3 cans/day. The volumetric consumption rates of the formula were identical for the two groups. Although the caloric density of supplement was greater in formula II, total calorie and protein consumption, based on both formula and dietary intake with 24 h food records, were similar in both groups (group I 2171 ± 519 *vs* group II 2087 ± 461 cal/day and group I 99.5 ± 30 *vs* group II 103 ± 28 g/day; $P > 0.05$).

Anthropometric measurements
Table 3 shows the results of anthropometric parameters in both groups. During the 3 months' supplementation period, an increase in caloric intake of 18.6% and protein intake of 18.4% above baseline was achieved in group I and 17.4 and 19.8%, respectively, in group II, without differences between groups. Treatment with both supplements resulted in a significant and sustained increase in weight (3.2% in group I and 3.1%, in group II; $P < 0.05$); this increase was mostly due to fat mass (12.8% in group I and 7.5% in group II; $P < 0.05$). There was a statistically significant increase in both groups in tricipital skinfold. Changes in fat-free mass, midarm circumference and total body water did not change significantly in either group.

Biochemical parameters
Table 4 shows the results of several biochemical indices of nutrition status. CD4 counts remained stable in group I, while in group II a significant increase was detected (576 ± 403 *vs* 642 ± 394 cells/mm^3; $P < 0.05$) at 3 months. No statistically significant changes were detected in albumin, prealbumin, transferrin or viral load, in both groups.

Safety and intercurrent illness
Hospitalization events (infections) were recorded during the 3 month period. Group II had fewer hospitalizations than group I, without statistical differences (group I 10.5% events *vs* group II 5.5% events; NS), the mean stay did not reach statistical differences (22.7 ± 8.7 *vs* 12.5 ± 3.5 days). No side effects to the supplements were reported. All patients followed the whole oral supplementation treatment period.

Table 3 Anthropometric parameters

	Group I		*Group II*	
Characteristics	*Baseline*	*3 months*	*Baseline*	*3 months*
Weight (kg)	62.7 ± 10.9	64.7 ± 11.6*	61.1 ± 11.2	63 ± 11.3*
Body mass index	22.04 ± 2.8	22.7 ± 3*	21 ± 2.8	21.9 ± 2.6*
Fat-free mass (kg)	52.3 ± 9.9	52.8 ± 10	52.9 ± 10.5	52.2 ± 12.9
Fat mass (kg)	10.2 ± 4.6	11.7 ± 4.9*	8.6 ± 3.8	9.3 ± 3.6*
Total water (kg)	38.3 ± 7.3	38.2 ± 7.1	38.6 ± 8	38.4 ± 7.5
Tricipital skinfold (mm)	17.9 ± 4.3	20.2 ± 4.8*	18.02 ± 4.7	19.5 ± 4.5*
Circumference arm (cm)	26.7 ± 2.9	27.1 ± 2.7	26.9 ± 3.7	27.2 ± 3.2

*$P < 0.05$, differences between time 0 and at 3 months in each group.

Table 4 Biochemical parameters

	Group I		*Group II*	
Characteristics	*Baseline*	*3 months*	*Baseline*	*3 months*
Albumin (g/l)	4.05 ± 0.9	4.4 ± 2	4.17 ± 0.6	4.23 ± 0.66
Prealbumin (mg/dl)	23.2 ± 7.5	23.7 ± 7.8	21 ± 2.8	21.9 ± 2.6
Transferrin (mg/dl)	248.7 ± 63.3	239 ± 48	252.2 ± 58	249 ± 56
CD4 (cells/mm^3)	479 ± 261	515 ± 243	576 ± 403	642 ± 394*
Viral load (copies/ml)	9352 ± 26633	3925 ± 9162	13733 ± 40109	7989 ± 22589

*$P < 0.05$, differences between time 0 and at 3 months in CD4 count in group II *vs* group I, and in group II at basal time *vs* 3 months.

Discusion

Weight loss and general cachexia have become a defining characteristic of the progression of HIV infection (Macallan *et al*, 1993; Teng *et al*, 1991). Wasting and weight loss have been associated with malnutrition, increased susceptibility to infection, reduced GI function, increased work absenteeism and premature mortality (Guenter *et al*, 1992).

We attempted to resolve this problem by increasing energy and protein intake, via enteral supplementation, in HIV-infected patients on both treatment models of our study. The use of standard and peptide-based formulae was associated with a significant increase in energy and protein intake. Body weight gain was associated with an increase in fat mass (Table 3), with no differences in fat-free mass. Our data differ from other studies; Pichard *et al* (1998) found an increase in fat mass and fat-free mass, but nitrogen/energy ratio intake was greater than in our trial and in one group they gave a supplementation with arginine, a known anabolic amino acid. Another study showed an increase in fat-free mass but not in fat mass (Clark *et al*, 2000); the intervention group in this study used acid-L-glutamine and antioxidants. Glutamine plays a major metabolic role in the maintenance of visceral and muscle tissues; during stress and inflamation, consumption of glutamine exceeds the ability of skeletal muscle to supply this amino acid (Shabert & Wilmore, 1996). The increase in fat mass with oral supplementation in the present study is supported by the results of a previous randomized controlled trial of oral supplementation which also showed an increase in fat mass with no significant change in fat-free mass (Alvarez *et al*, 2000). Perhaps the differences of our results could be explained by the duration of the trial, which was shorter in our study, or the better nutritional status at the begining of the study of our HIV population on HAART therapy with a mean BMI over 20. As protein and calorie intake during the 3 months of the study were substantially above the recommended energy (30 kcal/kg body weight per day) and protein (1.2 g/kg body weight per day) requirements, we suggest that energy and protein needs for HIV-infected patients are markedly higher than those of healthy subjects and international recommendations for HIV-infected patients. This observation could be related to a reduced metabolic efficiency (Melchior *et al*, 1993), an increased in diet-induced thermogenesis in relation to food intake (Macallan *et al*, 1995) or due to a malabsortive process (Kambwa *et al*, 1990).

Patient compliance with the supplementation was remarkable with all patients randomized completing the 3 month trial. This might be explained by the selection of patients who were receiving HAART therapy, by a continuous nutritional evaluation during the trial or by the low degree of side effects during the study. This study confirms that prolonged oral nutrition supplementation is feasible in HIV-infected patients (Süttmann *et al*, 1996).

The increase in CD4 cells in peptide-based group suggest an improvement in immune status; this was also observed in an other study (Shabert *et al*, 1999), but it is not known whether this effect is due to some nutrient or is secondary to the increase in energy and protein intake. Perhaps in our study the increase in CD4 is related to the enterotropic peptide and fish oil supplemented formula. The n-3 fatty acids of the fish oil are incorporated into cellular membranes and replace arachidonic acid, which is derived from n-6 fatty acids. This substitution results in a less inflammatory state producing fewer cytokines (Endres *et al*, 1989). In addition, the presence of n-3 fatty acids in the membrane reduces the production of inflammatory and immunosuppressive eicosanoids (eg prostaglandins of the 2 series, leukotrienes of the 4 series, thromboxanes of the 2 series). On the other hand, less inflammatory and less immunosuppressive eicosanoids are produced.

This change in immune status did not decrease hospitalizations events; in the study of Chlebowski *et al* (1993), where patients with peptide-based supplementation showed a decrease in hospitalizations events, supplementation duration of the study was 6 months. Perhaps 3 months is too short a time to detect differences.

In conclusion, oral nutritional supplements for a 3 month period were well tolerated and resulted in body weight gain in HIV-infected patients. Supplement enriched with peptides and n-3 fatty acids increases CD4 count.

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