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Nutritional treatment for ambulatory patients with acquired immunodeficiency virus infection and previous weight loss using a formula enriched with n3 fatty acids: a randomized prospective trial

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Abstract. – *Background and Aims:* **Dietary counseling and oral supplementation have unclear results in preventing the progressive weight loss in human deficiency virus (HIV)-infection. The aim of the study was to compare the progression of nutritional indicators with or without a formula enriched with n-3 fatty acids.**

Patients and Methods: **30 HIV patients were enrolled. 15 were randomized to group I (standard formula) and 15 were randomized to group II (formula enriched with n-3 fatty acids). A nutritional evaluation was realized at basal time and at 3 months.**

Results: **An increase in protein and calories intakes was detected in both. There was a significant increase in n3 fatty acid intake from baseline in group II, without statistical changes in group I. Treatment with both supplements resulted in a significant and sustained increase in weight (4.5% in group I and 5.4%, in group II). This increase was mostly due to fat free mass in group I. In group II it was due to an increase in fat free mass and fat mass.**

Conclusions: **Oral nutritional supplements for a 3-months period were well tolerated and resulted in body weight gain in HIV-infected patients with previous weight loss.**

Key Words:

HIV, Randomized trial, n3 fatty acids.

Introduction

The dominant effect of human immunodeficiency virus (HIV) infection on nutritional state is clear, the impact of nutritional status on immune function and disease progression, is much less clear. Early studies showed the relationship between loss of lean body mass and timing of death in patients with HIV infection[1-4]. Surprisingly, studies of dietary intake for patients with HIV infection have demonstrated adequate energy consumption[5]. 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[6]. Some trials of early supplementation of nutrient intake have been beneficial in these patients[7]. These studies showed increased in body weight[8], body cell mass, and intracellular water. Immune status was also improved[9], with a significant reduction in the number of hospitalizations[10], and an increase in CD 4 count[11].

The aim of our study was to compare the influence on nutritional indicators in HIV infected patients with undernutrition during nutritional supplementation with or without a formula enriched with n-3 fatty acids.

Patients and Methods

Patients and Procedure

Patients between 30 and 60 years of age with HIV infection and a previous weight loss (>5% in previous 3 months) were elegible for enrollment if they met the following criteria: a confirmed diagnosis of HIV infection, absence of chronic febrile illness, absence of severe gastrointestinal (GI) symptoms (diarrhea for >30 days or >3 times/day); adequate liver function

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and normal kidney function. All patients gave informed consent before enrollment and remained under treatment (highly active antiretroviral therapy – HAART) for HIV infection during the study and in previous 3 months, a protease inhibitor containing regimen with 3 agents.

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. Thirty patients with HIV infection were prospectively randomized. Group I received a standard enteral formula and group II a formula with n-3 fatty acids; both groups received 2 cans/day (236 ml per can) (Table I). In both groups, enteral supplementation was recommended in conjunction with a dietitian dietary counseling program based on standard nutrition principles.

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

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[12]. 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 mass and fat-free mass (FFM). Regional changes in body mass were stimated 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 (Hitachi, Boehringhen Mannheim, Germany), and hematologic parameters (LabCorp, Uniondale, NY, USA). Plasma fatty acids before study commencement and at 3 months was performed by gas chromatography (Altomsystem detector cid, Boston, MA, USA), as described previously[13].

Dietary Intake

All patients received instruction in keeping a 24 hours written food record, incorporating the use of food scales to enhance portion size accuracy. Records were reviewed by a dietitian and analyzed with a computer-based data-evaluation system. Total calorie intake and the contribution of the assigned enteral supplement were used as an indicator 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.

Table I. Nutrient composition of enteral formulas.

	Group I (standard)[1]	Group II (W3 fatty acid)[2]
Caloric density (Kcal/ml)	1.06	1.2
Protein (g/L)	37.2	66
(%VCT)	14	21.6
Fat (g/L)	37.2	25.6
(%VCT)	31.5	18.8
Carbohydrate (g/L)	145	183
(%VCT)	54.5	59.6
Dietary fiber (g/L)	0	20.4
n-3 fatty acids (mg/L)	0	6.29

[1]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 contein beta-carotenes.
[2]PROSURE®, Abbott laboratories. Source of protein, 47.5% protein hydrosylates, 47.5% sodium caseinate, 5% serum proteins. Source of carbohydrates, 63% syrup of corn, 10% sucrose, 16% maltodextrine, 1% soy polysaccharide. Source of fat, 65% fish oil, 9.3% canola oil, 16.2% medium chain triglycerides, 5.5% soy oil.

Statistical Analysis

Sample size was calculated to detect differences over 4% in weight gain with 90% power and 5% significance (n=15, in each group). The results were expressed as means±standard deviation. The distribution of variables was analyzed with 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

Thirty three patients were screened for the study. Three did not meet the inclusion criteria. Therefore, 30 patients were randomized to one of the two study groups. Of these, 15 were randomized to group I and 15 were randomized to group II. Patient characteristics are outlined in Table II. 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 2 cans per day. The volumetric consumption rates of the formula were similar 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-hours food records, were similar in both groups at 3 months with a significant increase in each group (group I 1800±523 vs 2330±575 cal/day: $p<0.05$) and (group II 1774±332 vs 2500±851 cal/day: $p<0.05$). Protein intake showed a significant increase in both groups after supplementation, without differences between groups (group I 74.5±27.5 vs 106±26.8 g/day: $p<0.05$) vs (group II 81.8±23.9 vs 108±29.3 g/day: $p>0.05$).

The recommended supplement dose was 2 cans per day. However, intake of the supplements (group I and II) averaged 1.5 cans per day. There was a significant increase in total n3 fatty acid intake (meals plus supplements) from baseline in group II; as we showed; (EPA: 0.01±0.05 vs 1.8±0.5 g/day; $p<0.05$) and (DHA: 0.04±0.2 vs 0.9±0.2 g/day; $p<0.05$). Ratio of w6/w3 decreased in group II (7±1.2 vs 1.7±1.2: $p<0.05$). In group II, a no significant differences was detected in w6 ratio intake from meals (4.4±1.8 vs 5±3.1 g/day: ns) and linolenic acid intake (w3) (linolenic acid: 0.65±0.22 vs 1.4±0.6 g/day; $p<0.05$), too. Nevertheless, no significant differences were detected in group I in EPA (0.05±0.18 vs 0.1±0.2 g/day: ns), DHA (0.05±0.18 vs 0.1±0.3 g/day: ns), w6 fatty acid (4.4±1.5 vs 6.1±2 g/day: ns) and ratio w6/w3 (6.8±3.5 vs 6.4±1.9: ns) intakes.

Anthropometric Measurements

Table III shows the results of anthropometric parameters in both groups. During the 3 months supplementation period, an increase in caloric intake of 18.8% and protein intake of 18.5% above baseline was achieved in group I and 19% and 18.4 % in group II, without differences between groups. Treatment with both supplements resulted in a significant and sustained increase in weight (4.5% in group I and 5.4 %, in group II; $p<0.05$). This increase was mostly due to FFM by bioimpedance in group I. In group II it was

Table II. Characteristics of patients with hiv at baseline.

Characteristics N	Group I 15	Group II 15	*p*
Age (yr)	41.6 ± 8.4	42.1 ± 4.8	ns
CDC class A-B (%)	54.7%	55.7%	ns
CDC class C (AIDS) (%)	53.3%	60%	ns
Intravenous drug use (%)	46.7%	40%	ns
Weight (kg)	57.1 ± 6.8	51.3 ± 8.6	ns
BMI (kg/h^2)	19.2 ± 0.9	18.8 ± 2.2	ns
Albumin (g/dl)	3.9 ± 0.9	4.1 ± 0.2	ns

Table III. Anthropometric parameters.

Characteristics	Group I		Group II	
	Baseline	3 month	Baseline	3 month
Weight (kg)	57.1 ± 6.8	59.6 ± 6.8*	51.3 ± 8.6	54.2 ± 6.6*
BMI (kg/h²)	19.2 ± 0.9	22.6 ± 0.9	18.8 ± 2.2	19.7 ± 1.5
Fat free mass (kg)	48.2 ± 8.1	50.9 ± 7.3*	45.7 ± 8.2	47.9 ± 8.2*
Fat mass (kg)	9.8 ± 5.2	8.4 ± 3.2	6.1 ± 3.5	7.7 ± 4.3*
Tricipital skinfold (mm)	6.4 ± 1.9	8.1 ± 2.9*	5 ± 1.6	5.2 ± 2.4
Circumference arm (cm)	21.9 ± 1.7	22.6 ± 2.2	21.1 ± 3.8	22.4 ± 2.6

*p <0.05, differences between time 0 and at 3 months in each group. No statistical differences between groups.

due to an increase in FFM and fat mass by bioimpedance, too. There was a statistically significant increase in group I in tricipital skinfold.

Biochemical Parameters

Table IV shows the results of several biochemical indices of nutrition status. No statistically significant changes were detected in albumin, prealbumin or transferrin, in both groups. Table V shows the eicosapentaenoic(EPA), idocosahexaenoic (DHA), linolenic acid, linoleic acid and w6/w3 ratio in plasma phospholipids by gas chromatography. EPA, DHA and ratio w6/w3 improved in group II without differences in group I.

Safety and Intercurrent Illness

Hospitalization events were recorded during the 3 months period. No hospitalization events were recorded in both groups. No side effects to the supplements were reported nor observed. All patients followed the whole oral supplementation treatment period.

Discussion

Weight loss has become a defining characteristic of the progression of HIV infection[14,15]. Wasting and weight loss have been associated with malnutrition, increased susceptibility to infection, reduced gastrointestinal function and increased mortality[16].

We attempted to resolve this problem by increasing energy and protein intake, via enteral supplementation, as other Authors[11,17]. The use of standard and omega 3 fatty acid enriched formula was associated with a significant increase in energy and protein intake. Body weight gain was associated with an increase in fat and FFM in group II (omega 3 fatty acid enriched formula) and FFM in group I. Our data differ from other investigation[18], where the Authors found an increase in fat mass and FFM only with enriched formula. However, nitrogen/energy ratio intake was greater than in our trial and in enriched formula group they gave them a supplementation with arginine, a known anabolic aminoacid.

In other study design during one year[19], within the context and limitations of this long study, standard and immune-enhancing oral formulas consumed daily had no differential effects on weight gain, as shown our data.

Another research showed an increase in FFM but not in fat mass[10]; 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,

Table IV. Biochemical parameters.

Characteristics	Group I		Group II	
	Baseline	3 month	Baseline	3 month
Albumin (g/L)	3.9 ± 0.9	4.1 ± 0.9	4.17 ± 0.6	4.23 ± 0.66
Prealbumin (mg/dl)	20.6 ± 10.9	18.6 ± 13	21 ± 2.8	21.9 ± 2.6
Transferrin (mg/dl)	231 ± 85	232 ± 73	252.2 ± 58	249 ± 56

No statistical differences.

Table IV. Percentage of EPA, DHA, linolenic acid, linoleic acid and ratio w6/w3 in plasma phospholipid profile.

Plasma levels	Group I		Group II	
	Baseline	3 month	Baseline	3 month
EPA (%)	0.97 ± 0.9	0.58 ± 0.5	0.57 ± 0.4	0.8 ± 0.6*
DHA (%)	2.8 ± 0.9	2.1 ± 0.6	2.1 ± 0.5	3.7 ± 1.1*
Linolenic acid (%)	0.22 ± 0.4	0.15 ± 0.5	0.3 ± 0.2	0.26 ± 0.1
Linoleic acid (%)	24.3 ± 4.2	26.8 ± 6.9	26.1 ± 5.7	28.7 ± 5.2
W6/W3 ratio	4.01 ± 1.8	2.86 ± 1.1	4.8 ± 1.2	2.9 ± 0.8*

EPA (20:5n-3): eicosapentaenoic acid; DHA (22:6n-3): idocosahexaenoic acid; linolenic acid (18:3n-3); linoleic acid (18:2n-6). *p <0.05, differences between time 0 and at 3 months.

during stress and inflammation, consumption of glutamine exceeds the ability of skeletal muscle to supply this aminoacid[9].

The increase of weight after enteral supplementation in HIV patients is a controversial area, for example in a previous randomized controlled trial of oral supplementation which also showed an increase in fat mass with no significant change in fat free mass[20]. Perhaps, the differences of our data could be explained by the duration of the trial, that was shorter in us. A second reason could be the better nutritional status at the beginning of the study of our HIV population on HAART therapy with a mean BMI over 19. A third reason could be the protein and calorie intake during the 3 months of our design, which 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.

Patients compliance with the supplementation was remarkable with all patients randomized completing the 3-month trial, despite the presence and lower palatability of fish oil in some immune-enhancing formulas. This might be explained by the selection of patients who were received a HAART therapy, by a continuous nutritional evaluation during the trial or by the low degree of side effects during the study. This research confirms that prolonged oral nutrition supplementation is feasible in HIV-infected patients[6,21].

The experimental supplement used in this investigation was designed to deliver a phamacological dose of n-3 fatty acids within a nutrient matrix to provide extra protein and energy and allows net deposition of FFM. However, standard supplementation improved fat free mass, too. Firstly, the increase in energy and protein intake was similar in both groups, with a theorical anabolic effect. In previous studies, nutritional supplement with polymeric diets has been well tolerated and permitted to achieve a significant weight and fat free mass gain in HIV infected patients[8]. For example, standard and immune-enhancing oral formulas consumed daily for 1 year had no differential effects on nutrition or immune parametes in asymptomatic HIV-infected patients[18,19]. Secondly, an average intake of EPA less than 2 g per day in both groups was achieved. Previous researches in cachectic cancer patients suggested that EPA alone at a dose of 2 g/day was associated with weigth stability[22]. Average can consumption in the present trial was 1.5 cans per day (containing 1.8 g EPA), showing a modest improvement in plasma profile of fatty acids. Thirdly, an increased turnover of polyunsaturated fatty acids has been described in HIV infected children[22]. This fact could explain a higher dietary recommendations in HIV infected patients than controls.

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[23]. In addition, the presence of n-3 fatty acids in the membrane reduces the production of inflammatory and immunosuppressive eicosanoids (e.g., 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 theorical change in immune status did not decrease hospitalizations events in our design. In the study of Chlebowski et al[7], patients with peptide-based supplementation showed a decrease in hospitalizations events without changes in death

rate. These different results must be investigated with new interventional trials and with new enhanced-formulas.

In conclusion, oral nutritional supplements for a 3-months period were well tolerated and resulted in body weight gain in HIV-infected patients. Fat mass and fat free mass increased in patients with formula enriched with n-3 fatty acids and fat free mass increased with a control formula. Further studies are necessary to investigate this association.

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