Reprod. Nutr. Dev. 43 (2003) 203–214

© INRA, EDP Sciences, 2003
DOI: 10.1051/rnd:2003017

Original article

Effect of medroxyprogesterone acetate on the efficiency of an oral protein-rich nutritional support in HIV-infected patients

Cécile ROCHON[a], Magali PROD'HOMME[a], Henri LAURICHESSE[b], Igor TAUVERON[c], Michèle BALAGE[a]*, Florence GOURDON[b], Olivier BAUD[b], Christine JACOMET[b], Sylvie JOUVENCY[b], Gérard BAYLE[a], Claude CHAMPREDON[a], Philippe THIEBLOT[c], Jean BEYTOUT[b], Jean GRIZARD[a]

[a] Unité de Nutrition et Métabolisme Protéique, INRA, Centre de Clermont-Ferrand-Theix, 63122 Saint-Genès-Champanelle, France
[b] Service des Maladies Infectieuses et Tropicales, Centre Hospitalier Universitaire, 63003 Clermont-Ferrand, France
[c] Service d'Endocrinologie et Maladies Métaboliques, Centre Hospitalier Universitaire, Centre de Recherche en Nutrition Humaine, 63003 Clermont-Ferrand, France

(Received 24 July 2002; accepted 10 April 2003)

Abstract — We have examined the effect of a medroxyprogesterone therapy in HIV-infected patients under appropriate nutrition for anabolism. The experiments were performed on 12 men (mean age 40 y), HIV seropositive but free of any clinically active opportunistic infection for at least one month. The patients underwent a 2-week baseline diet period (1.2 g protein·kg^{-1} body weight (BW)·d^{-1}) and then a 5-week experimental period with again the baseline diet in conjunction with supplements including Tonexis HP (0.7 g protein·kg^{-1} BW)·d^{-1}), L-threonine (0.018 g·kg^{-1} BW·d^{-1}) and L-methionine (0.013 g·kg^{-1} BW·d^{-1}). Indeed HIV-infected patients showed deficiencies in these amino acids. They were randomly divided into groups I and II under double-blinded condition. Group II was given medroxyprogesterone acetate (0.4 g·d^{-1}) during the last 3 weeks whereas group I received a placebo. All the patients significantly increased their body weight ($P < 0.05$) during the experimental periods. Those under medroxyprogesterone tended to show a higher but not significant weight gain (+3.1 ± 1.0 kg in group II and +1.9 ± 0.3 kg in group I). Blood free amino acids were used as rough indicators of amino acid utilization and were analyzed prior and during acute 150 min intravenous infusion of a complete glucose-amino acid mixture. This test was done before and at the end of the experimental periods. Basal essential blood free amino acids were similar in the two groups and did not change during the experimental period. Most essential amino acids increased

* Correspondence and reprints
E-mail: balage@clermont.inra.fr

following glucose-amino acid infusions. The incremental increase was of less magnitude after the experimental period than before when medroxyprogesterone was present ($P < 0.05$ for valine, leucine, lysine, threonine and methionine). This was not the case in the absence of the hormone. We concluded that medroxyprogesterone might improve the efficacy of an oral protein-rich nutritional support in HIV-infected patients.

body weight / amino acid / hormonal therapy / AIDS

1. INTRODUCTION

Infection with the human immunodeficiency virus (HIV) has a devastating effect on nutritional status. Weight loss, often profound in magnitude, is one of the most universal features of HIV infection, and patients may lose 30–50% of their body mass before succumbing to the disease [1, 2]. Acquired immunodeficiency syndrome (AIDS) is characterized by a predominant loss of lean tissue [3]. Multiple factors in different combinations contribute to AIDS related malnutrition and increased host requirements ; these include anorexia, malabsorption, abnormal utilization and excretion of nutrients. This is correlated with the severity of the HIV infection and with secondary infections. Malnutrition has a deleterious effect on immune function and thus may potentially accelerate the progression of immune deficiency in HIV infection [4]. It may also contribute to protein wasting in HIV-infected patients because nutritional support seems to have been beneficial [5–8] but this nutritional support is largely ineffective in terms of repleting lean tissue (see for a discussion Macallan [9]). Because short-term parenteral alimentation enriched with amino acids is capable of reversing the net protein catabolism [10, 11], we hypothesize that some specific amino acid deficiencies may limit the utilization of other nutrients. Accordingly, we previously demonstrated that threonine and possibly methionine may be rate-limiting for whole body protein synthesis in HIV-infected patients [12].

The synthetic progesterone derivatives, e.g. medroxyprogesterone or megestrol acetate (a commonly used American equivalent to medroxyprogesterone) are available for the treatment of anorexia, cachexia or unexplained weight loss in patients with AIDS (see for a review, Corcoran and Grinspoon [13], Mann [14], Miller [15]). Appetite, caloric intake and sense of well-being were all significantly better in AIDS patients under these therapies. Body weight increased. The analysis of body composition revealed that fat was the major component being affected. Triceps skinfold thickness and muscle circumference were also improved suggesting an increase in fat body mass. However, there have been anecdotal reports of development of diabetes, as well as fluid retention. In addition, trials supporting the approval of these compounds in AIDS patients showed a relatively high placebo effect. No specific nutritional support was used in these trials.

To better understand the efficiency of these progesterone derivatives, we decided to analyze the effect of a 3-week period of medroxyprogesterone treatment on HIV-infected patients under appropriate nutrition for anabolism (energy balanced diet enriched with amino acids). The response of blood free amino acids to acute intravenous infusion of a glucose-amino acid mixture was used as an indicator of amino acid utilization [12]. We assumed that during infusion, the inhibition of protein synthesis and anabolism due to limiting amino acids would be suppressed; as a result, more amino acids would have been incorporated into body proteins. The concentrations of non-limiting amino acids will be altered, depending on the difference between the supply by perfusion and utilization for body deposition and oxidation. The plasma concentrations of limiting amino acids would stay at a low level if their supply from

infusion compensates only for their utilization or these amino acids will accumulate when in excess. In other words, amino acids that have a low basal level and do not change during infusion give an indication that they are limiting amino acids for protein anabolism.

2. MATERIALS AND METHODS

2.1. Patients

The study protocol was approved by the local Ethical Committee (Comité Consultatif pour la Protection des Personnes en Recherche Biomédicale pour la Région Auvergne). Each subject gave a written informed consent. The experiments were performed from March 1996 – February 1998 with 12 men (mean age: 40 ± 3 years; body weight (BW): 65.7 ± 2.3 kg; body mass index (BMI): 21.4 ± 0.8 $kg \cdot m^{-2}$, means ± SEM, Tab. I) recruited from the Department of Infectious Diseases at the University Hospital in Clermont-Ferrand. All men were HIV seropositive as determined from ELISA and Western blot assay. The known duration of HIV infection was 61 ± 11 months (19 minimum, 129 maximum). A clinical history and a physical examination were performed at the time of the study. Nine patients were classified C3 (T4 lymphocyte count < 200), two C2 (between 200 and 499) and one C1 (above 500) according to the criteria of the Center for Disease Control and Prevention [16]. Only patients who had been free of any clinically active opportunistic infection for a period of at least 1 month before participation were included. Their initial weight loss was –12.7 ± 3.8% (compared to pre-illness body weight). Patients with fever (> 37.8 ºC) or diarrhea (defined as increased frequency or liquidity of stools) were excluded.

The patients were randomly divided into two groups: Group I and Group II under double-blind conditions.

Each group exhibited the same duration of antiretroviral treatments (31.8 ± 4.6 months, minimum 18 months, maximum 46 months in Group I ; 23.3 ± 6.2 months, minimum 14 months, maximum 52 months in Group II). The current antiretroviral therapy (Tab. I) almost always consisted of two nucleosidic reverse transcriptase inhibitors (NRTI) (all Group I, 5/6 Group II). It frequently included an additional protease inhibitor (PI) (5/6 Group I, 2/6 Group II). The patient from Group II who only received one NRTI drug alone was an exception. Patients underwent the current therapy for 5.7 ± 3.1 months in Group I (minimum at inclusion, maximum 19 months) and 1.8 ± 0.9 months in Group II (minimum at inclusion, maximum 5 months).

2.2. Experimental procedure (Fig. 1)

The patients underwent a 2-week baseline and then a 5-week experimental period with nutritional support. Group II was given medroxyprogesterone (0.4 $g \cdot d^{-1}$) on the last three weeks of the experimental period whereas Group I received a placebo. The patients underwent metabolic investigations in a postabsorptive state the last morning of the baseline period and the next morning after the experimental period (after nutritional-hormonal treatments ceased; i.e. after dinner).

The experiment was carried out under the control of a dietician. The patients were asked to complete a report of food consumption every day. They were also advised how to maintain their intake during both the baseline and experimental periods. During the experimental period the subjects had their regular breakfast, lunch and dinner (corresponding to the baseline diet) along with equal amounts of specific amino acid supplements (3 × 400 mg L-threonine, 3 × 300 mg L-methionine daily supplement). In addition, they received Tonexis HP (Nestlé, Clinical Nutrition, Sèvres, France) which provides 700 mg $protein \cdot kg^{-1}$ BW,

Table I. Characteristics of the patients before and after the experimental periods.

Groups	Age (y)	Height (m)	Weight (kg)		BMI ($kg \cdot m^{-2}$)		$CD_4 \cdot mm^{-3}$		Current treatment	
			Before	After	Before	After	Before	After	NRTI	PI
	61	1.75	85.0	87.2	27.8	28.5	79	68	AZT ddC	
	45	1.78	54.6	57.3	17.2	18.1	6	18	AZT 3TC	IDV
Group I	34	1.80	62.8	63.2	19.4	19.5	50	121	AZT 3TC	IDV
	33	1.75	63.0	65.0	20.6	21.2	43	40	AZT 3TC	IDV
	58	1.73	68.0	69.9	22.7	23.4	221	303	AZT ddI	SQV
	33	1.61	61.0	62.9	23.5	24.3	527	643	AZT 3TC	RTV
Mean	44	1.74	65.7	67.6*	21.9	22.5*	154	199		
SEM	6	0.03	4.2	4.3	1.5	1.5	80	98		
	42	1.72	60.5	60.6	20.4	20.5	28	52	AZT 3TC	–
	46	1.75	64.7	68.9	21.1	22.5	119	131	AZT 3TC	–
Group II	33	1.78	65.0	71.0	20.5	22.4	36	67	ddI	–
	33	1.78	60.7	61.1	19.2	19.3	21	16	AZT 3TC	–
	28	1.79	74.2	77.3	23.2	24.1	176	270	AZT 3TC	IDV
	36	1.80	68.4	73.2	21.1	22.6	51	49	D4T ddI	RTV
Mean	36	1.77	65.6	68.7*	20.9	21.9*	72	97		
SEM	3	0.02	2.1	2.7	0.5	0.7	26	38		

BMI, body mass index; NRTI, nucleosidic reverse transcriptase inhibitor; PI, protease inhibitor; AZT, Zidovudine; ddI, Didanosine; ddC, Zalcitabine; 3TC, Lamivudine; IDV, Indinavir; SQV, Saquinavir; RTV, Ritonavir; D4T, Stavudine. $P < 0.05$; * "after" vs. "before". Group I: + placebo; Group II: + medroxyprogesterone.

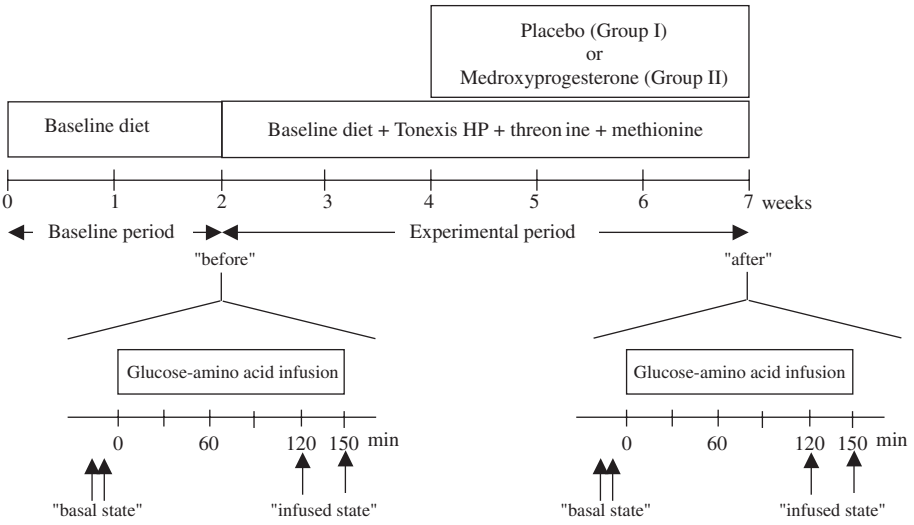

Figure 1. Experimental protocol.

12 kJ·kg^{-1} BW daily in three equal portions, one in the afternoon, two in the evening. As a result, the total dietary essential amino acid supplement (in mg·kg^{-1} BW·day^{-1}) of the patients was as follows: threonine 45.3, valine 43.3, methionine 31.3, cysteine 3.6, isoleucine 32.8, leucine 68.2, tyrosine 29.0, phenylalanine 29.7, lysine 49.7, histidine 18.6, arginine 24.4 and tryptophan 8.2. In fact, the improvement in amino acid intake between our specific nutritional protocol and usual French diets [17] (usually consumed by the patients) was around +25% for threonine, +40% for methionine and +70% for leucine (due to the high level of leucine in Tonexis HP). Dietary intakes were estimated on three occasions by the dietician using the 24-hour dietary recalls over a 3–4 day period (Tab. II). At each time, these estimates did not differ between the two groups suggesting that the medroxyprogesterone therapy was performed (as expected) under constant level of food intake. Moreover the difference in dietary intake between the baseline and the experimental periods correlated with the nutritional value of the Tonexis HP supplement.

Metabolic investigations started at 8 a.m. In order to analyze blood parameters and their response to acute glucose-amino acid provision, a sampling catheter (Venflon 2, 20G, Viggo, Helsingborg, Sweden) was retrogradely inserted into a dorsal vein of the left hand. Another catheter was placed in a contralateral forearm vein and used for infusions. Each experiment consisted of a 150 min period (Fig. 1) of continuous infusion of the Primène 5% amino-acid mixture (1 mL·kg^{-1}·h^{-1}) using a peristaltic pump (Infusomat Secura, Braun Biotrol, Paris). A glucose solution (0.55 mol·L^{-1}, Meram) was concomitantly administrated at a constant rate (1 mL·kg^{-1}·h^{-1}) via the same catheter and by another similar peristaltic pump. The commercially available amino acid solution (Primène 5, Baxter, Maurepas, France) contained the following L-amino acids (in μmol·mL^{-1}): L-leucine 38.17; L-isoleucine 25.57; L-valine 32.48; L-lysine 37.67; L-methionine 8.05; L-phenylalanine 12.73; L-threonine 15.55; L-tryptophan

Table II. Estimates of daily dietary intake during the baseline and on the 2nd and 5th weeks of the experimental periods.

	Group I			Group II		
	Baseline	Experimental		Baseline	Experimental	
Period		2nd week	5th week		2nd week	5th week
Protein	1.22 ± 0.06	1.85 ± 0.08	1.83 ± 0.06	1.12 ± 0.10	1.74 ± 0.09	1.58 ± 0.09
Lipids	1.11 ± 0.22	1.40 ± 0.22	1.36 ± 0.19	1.27 ± 0.06	1.33 ± 0.10	1.26 ± 0.13
Carbohydrate	4.83 ± 0.75	4.53 ± 0.49	4.45 ± 0.53	2.85 ± 0.16	3.79 ± 0.29	3.51 ± 0.35
Energy	143 ± 18	159 ± 16	156 ± 17	115 ± 5	142 ± 9	132 ± 12

The estimates of dietary intake were obtained from the 24-hour dietary recalls on 3–4 days in three occasions: the end of the baseline period, the end of the 2nd week of the experimental period i.e. under Tonexis, the end of the 5th week of the experimental period i.e. under Tonexis along with the placebo (Group I) or medroxyprogesterone (Group II). Values are mean ± SEM and expressed in g· kg $^{-1}$ BW (with protein, lipids and carbohydrates) or kJ· kg $^{-1}$ BW (with energy), $n = 6$.

4.90; L-alanine 44.94; L-arginine 24.14; L-aspartic acid 22.56; L-cysteine 10.16 (chlorydrate); L-glutamic acid 34.01; glycine 26.67; taurine 2.40; L-histidine 12.26; L-proline 13.05; L-serine 19.05; L-tyrosine 2.49; L-ornithine 8.56. Venous blood samples were obtained before (–15 and –5 min; basal state) and during (120 and 150 min; infused state) infusion. Blood samples were collected in heparinized tubes, centrifuged at 4 ºC and the resulting plasma was stored at –20 ºC for subsequent analyses.

In order to evaluate the changes in muscle mass, tricep skinfold thickness (as the mean of three measurements) was recorded on the dominant arm by the same observer with a caliper. Moreover, arm circumference was measured to the nearest 0.5 cm with a flexible, 1-cm-wide tape at midarm. A schematic cross-sectional view of the upper arm tissue resulted in a disk area derived from measurements of arm circumference (C) and tricep skinfold thickness (TS). As $C = 2\pi R$ where C is the arm circumference and R the radius of the disk and disk area = πR^2 then the total upper arm area (TUA) = $C^2/(4\pi)$. The arm muscle area constitutes a disk surrounded by a rim (fat). The unrolled rim is a rectangle whose length is C and width is TS/2. The upper arm fat area estimate is C*(TS/2). Muscle area is obtained by subtracting the fat area from TUA [18].

2.3. Assays

Plasma tryptophan was determined using a fluorometric method [19], using an LS30 Perkin-Elmer spectrofluorometer, Buckinghamshire, England. The concentration of the other amino acids in plasma was measured by ion-exchange chromatography. A special protein precipitation protocol has been developed to avoid sample dilution. An aliquot of 250 µL of a sulfosalicylic acid solution (0.916 mol·L^{-1} dissolved in ethanol with 0.533 mol·L^{-1} thiodiglycol and 1.040 mmol·L^{-1} norleucine) were added to tubes and evaporated to dryness. Plasma (1 mL) was then added to tubes and

incubated on ice for 1 h. Afterwards, the tubes were centrifuged for 1 h (3500 *g*, +4 ºC) and 500 µL of the supernatant was added to 250 µL of 0.1 mol·L^{-1} lithium acetate buffer, pH 2.2. Amino acid concentrations were determined in these extracts using an automated amino acid analyzer (Biotronic LC 3000, Roucaire, Velizy, France with BTC 2410 resin).

Plasma substrates were determined with an automatic analyzer (Chem 1, Bayer diagnostic, Puteaux, France) using enzymes (hexokinase and glucose-6-phosphate dehydrogenase for glucose; urease and glutamate dehydrogenase for urea; cholesterol esterase, cholesterol oxidase and peroxidase for cholesterol; lipase, glycerokinase, pyruvate kinase and lactate dehydrogenase for triglycerides). Plasma insulin was determined by an immunoenzymatic assay (Abbott Diagnostics, Rungis, France). Prealbumin, albumin, retinol binding protein, α1-acid glycoprotein and C-reactive protein were measured by immunonephelometry using routine hospital assays.

2.4. Statistical analysis

All data are expressed as means ± S.E.M. A paired-t-test was used to compare the basal data with data obtained during the infusions. The paired t-test and the two-way analysis of variance test (ANOVA) were also used to analyze the effect of treatments in each group and to compare the groups with $P \leq 0.05$ taken as significant.

3. RESULTS

The patients exhibited a significant increase in BW at the end of the experimental periods (+1.9 ± 0.3 and +3.1 ± 1.0 kg in groups I and II respectively; $P < 0.05$) and BMI (+0.6 ± 0.1 and 1.0 ± 0.3 kg·m^{-2}; $P < 0.05$; Tab. I). Although not significant, these parameters seemed to be improved when medroxyprogesterone was associated to the nutritional support. In contrast, no significant change could be detected during

Table III. Plasma insulin, glucose and urea before and after the experimental periods.

	Group I		Group II	
	Before	After	Before	After
Insulin (µU·mL^{-1})				
Basal state	11.5 ± 2.5	10.6 ± 4.1	6.3 ± 1.4	13.9 ± 5.1
Infused state	14.3 ± 4.5	18.5 ± 6.1	10.9 ± 2.1*	19.4 ± 4.7
Glucose (mmol·L^{-1})				
Basal state	5.21 ± 0.28	5.12 ± 0.34	5.19 ± 0.12	5.02 ± 0.08
Infused state	5.80 ± 0.40*	5.67 ± 0.46*	5.42 ± 0.21	5.22 ± 0.21
Urea (mmol·L^{-1})				
Basal state	4.87 ± 0.83	5.32 ± 0.92	5.02 ± 0.60	5.96 ± 0.74
Infused state	4.61 ± 0.71	5.04 ± 0.83*	4.85 ± 0.60	5.73 ± 0.65

Group I = + placebo; Group II = + medroxyprogesterone.
Values are mean ± SEM, $n = 6$. $P < 0.05$; * infused vs. basal state.

Table IV. Plasma essential and semi-essential free amino acid concentrations before and after the experimental periods.

	Basal values of plasma amino acids				Absolute changes during glucose-amino acid infusion			
	Group I		Group II		Group I		Group II	
	Before	After	Before	After	Before	After	Before	After
Threonine	118 ± 14	132 ± 13	119 ± 17	114 ± 7	9.3 ± 3.5	3.9 ± 4.1	14.0 ± 5.2	2.6 ± 5.3**
Valine	237 ± 24	236 ± 12	228 ± 22	243 ± 20	51.6 ± 5.1	41.6 ± 15.9	54.9 ± 9.4	37.2 ± 9.2**
Methionine	12 ± 2	12 ± 2	12 ± 1	13 ± 2	3.3 ± 0.4	3.2 ± 0.8	3.4 ± 0.7	1.8 ± 0.8**
Isoleucine	74 ± 9	77 ± 6	70 ± 7	69 ± 9	29.0 ± 3.1	24.5 ± 9.4	30.2 ± 4.0	17.9 ± 4.1
Leucine	127 ± 13	129 ± 9	127 ± 15	125 ± 12	32.9 ± 4.1	41.6 ± 5.0	36.0 ± 4.9	21.5 ± 5.8**
Tyrosine	71 ± 10	73 ± 7	59 ± 4	60 ± 7	–16.5 ± 5.5	–12.7 ± 2.5	–10.9 ± 1.1	–12.3 ± 1.8
Phenylalanine	54 ± 3	53 ± 4	55 ± 4	57 ± 4	7.0 ± 1.7	7.7 ± 3.6	7.2 ± 0.7	9.9 ± 1.2
Lysine	159 ± 16	165 ± 17	180 ± 21	190 ± 17	77.8 ± 3.4	64.8 ± 17.2	89.5 ± 9.0	70.4 ± 10.6**
Histidine	74 ± 6	76 ± 5	78 ± 3	71 ± 4	18.0 ± 1.8	12.4 ± 3.7**	12.0 ± 1.7*	12.6 ± 2.0
Arginine	81 ± 6	68 ± 4	105 ± 13	110 ± 17	27.3 ± 6.8	28.0 ± 9.6	34.7 ± 7.3	30.4 ± 7.2
Tryptophan	59 ± 8	63 ± 6	44 ± 4	48 ± 2	10.0 ± 1.4	7.6 ± 3.1	11.4 ± 2.0	9.6 ± 1.3

Group I = + placebo; Group II = + medroxyprogesterone.
Values are means ± SEM and expressed in $\mu mol \cdot L^{-1}$, $n = 6$. $P < 0.05$; * Group II vs. Group I in the same state; ** after vs. before in the same group.

the experimental period with both arm circumference (from 25.6 ± 1.4 to 26.2 ± 1.5 cm in group I, from 26.0 ± 0.6 to 26.5 ± 0.4 cm in group II) and tricep skinfold thickness (from 0.47 ± 0.04 to 0.46 ± 0.04 cm in group I; from 0.61 ± 0.10 to 0.63 ± 0.10 cm in group II). This was also the case after calculation of the upper arm muscle surface (from 47.0 ± 5.2 to 49.3 ± 5.5 cm^2 in group I, from 46.1 ± 2.8 to 47.7 ± 2.8 cm^2 in group II) and upper arm fat surface (from 6.1 ± 0.7 to 6.2 ± 0.8 cm^2 in group I; from 7.8 ± 1.3 to 8.3 ± 1.2 cm^2 in group II). The CD4 did not change during the treatment.

Plasma cholesterol (4.52 ± 0.54 $mmol \cdot L^{-1}$), prealbumin (0.23 ± 0.02 $g \cdot L^{-1}$), albumin (37.7 ± 2.2 $g \cdot L^{-1}$), retinol binding protein (43.2 ± 2.7 $mg \cdot L^{-1}$), α1-acid glycoprotein (1.02 ± 0.11 $g \cdot L^{-1}$) and C-reactive protein (3.44 ± 1.73 $mg \cdot L^{-1}$) were at normal levels and did not differ from those previously found in AIDS patients without opportunistic infection [12]. By contrast, all patients in the present study had high triglyceride levels (2.67 ± 0.89 $mmol \cdot L^{-1}$). The normal value of C-reactive protein was used as a marker to establish the absence of opportunistic infection at the time of the study. All these biochemical variables were similar in the two groups and did not show any changes during the experimental periods (not shown).

Because insulin resistance has been sometimes demonstrated in HIV-infected patients [20], especially under progesterone derivative therapy [15], the responses of insulin, glucose and urea were also analyzed before and after experimental periods during basal and infused states (Tab. III). In a previous experiment, we showed that combined glucose-amino acid infusion significantly increased plasma insulin and glucose but decreased plasma urea [12]. The same changes were observed in the present exper iment but the statistical significance ($P < 0.05$) was only occasionally obtained. Whatever the conditions (basal or infused states) the values neither changed in each group during the experimental period nor differed between the groups.

The basal concentrations of plasma free essential amino acids did not significantly change during the experimental periods ("after" compared to "before" treatment) whatever the group (Tab. IV). Before the experimental period, glucose-amino acid infusion resulted in a significant increase in most of the essential amino acids in each group ($P < 0.05$). These increases were similar in the two groups except for histidine which increased to a lesser extent in group II than in group I ($P < 0.05$) (Tab. IV). The increases following the infusions were not maintained after the experimental period for threonine in the two groups, for tryptophan and phenylalanine in group I and for methionine in group II. They had a lower magnitude than before the treatment for histidine in group I and threonine, valine, methionine, leucine and lysine in group II ($P < 0.05$). In contrast, tyrosine always decreased under infusions in each condition.

4. DISCUSSION

In the present study; we showed that a 5-week period of nutritional support (protein-rich oral supply supplemented with threonine and methionine) was able to significantly increase BW and BMI in HIV-infected patients. Moreover, additional hormonal treatment (medroxyprogesterone) for the last 3 weeks tended to improve weight gain (+3.1 vs. +1.9 kg in Group II and Group I respectively; i.e. +4.7% vs. +2.9% of the initial BW) and BMI (+4.8% vs. +2.7% $kg \cdot m^{-2}$). Note that more than 30 subjects would be needed to obtain the statistical significance $P < 0.05$ of the difference between the two groups (with a test power of 0.8). A beneficial effect of medroxyprogesterone should not be surprising because this hormone turns the nitrogen balance in tumor-bearing animals from negative to positive [21]. It is also able to stimulate appetite and to reverse fat loss

in patients with non-hormone-sensitive cancer [22]. In AIDS patients, medroxyprogesterone has been shown to increase body weight and appetite [23, 24]. The effect of medroxy- progesterone in our experiment was obtained under an appropriate nutritional status for protein deposition. This beneficial effect of nutrition could be related to the high protein oral supplement enriched with specific amino acids including the limiting amino acids for protein anabolism (e.g. threonine and methionine) and leucine which have been shown to stimulate skeletal muscle protein synthesis [25]. In other words, the nutritional treatment met the total protein requirements for HIV-infected patients (i.e. around 2 g protein·kg^{-1} BW·d^{-1}, [26]) and allowed high threonine, methionine and leucine intakes. A beneficial effect of the increased energy intake per se could not be ruled out. An unexpected increase in energy and protein intakes through the baseline diet did not seem likely based on the estimates of dietary intake throughout the experiment.

The response of blood free amino acids to an acute infusion of glucose-amino acids before and after the experimental periods was investigated as an indicator of amino acid utilization. As expected, before the experimental periods, most of the essential amino acids increased during glucose-amino acid infusion in both groups. After the experimental period, the incremental increases in essential amino acids were maintained in patients with the placebo (Group I) whereas they were lower than before in patients with medroxyprogesterone (Group II). Indeed, the increase in valine, leucine and lysine was less important after the experimental period than before in group II. This was also the case for threonine in both groups. Even, the increase of this amino acid after the experimental period never reached statistical significance ($P < 0.05$) as it did before. Moreover the increase in methionine after the experimental period was abolished in group II but not in group I. The incremental increase in the sum of essential amino acids was altered by about 50% after the experimental period compared to before in patients from group II suggesting a better utilization of amino acids in this group. The increase in essential blood free amino acids after glucose-amino acid infusion are a reflection of the enlargement in the difference between amino acid appearance (mainly from infusion, proteolysis and endogenous synthesis) and disappearance rates (for protein synthesis and oxidation). Our results suggest that this difference following glucose-amino acid infusions could be lower under the medroxyprogesterone therapy. With the amino acid infusion rates being constant, we could speculate that either proteolysis was more inhibited or oxidation and protein synthesis more stimulated.

Further experiments in 1998 in HIV patients under NRTI for 0.5 – 3 years showed that similar glucose – amino acid infusion inhibited whole body proteolysis and stimulated leucine oxidation without any effect on protein synthesis (Grizard, unpublished). Similar effects were recorded in normal volunteers and dysthyroid patients when glucose and amino acids were given along with insulin [27, 28]. Indeed insulin is known to mediate the decrease in proteolysis. In the present experiment it does not seem likely that proteolysis might be more inhibited in patients under medroxyprogesterone therapy because the rise in plasma insulin following glucose amino acid infusion (although only occasionally significant) was of the same magnitude in every case. Alternatively the medroxyprogesterone therapy might result in an insulin resistant state. If so, proteolysis should be less inhibited by infusions rather than more inhibited. More presumably amino acid utilization was more stimulated by glucose – amino acid infusion in patients under the medroxyprogesterone therapy. However our experiment did not allow to determine amino acid partitioning between protein synthesis and oxidation.

Assuming that the response to glucose-amino acid infusion would be a mirror of the effect of absorbed nutrients, we may speculate that the medroxyprogesterone therapy increased amino acid utilisation for protein deposition. Indeed megestrol increases both fat and lean body mass in AIDS patients [15, 29, 30]. However, an increase in specific amino acid catabolism is also likely. The latter might limit the growth promoting effect of the hormone.

Note that patients from each group did not undergo exactly the same current drug therapy. The NRTI were almost always given along with a PI in group I (with 5 subjects) but not in group II (with only 2 subjects). This may represent a confounding effect because there is growing evidence that PI drugs result in a number of metabolic side effects, including peripheral lipoatrophy or lipodystrophy with central obesity, hyperlipidemia and insulin resistance [31, 32]. The potential action of PI on protein metabolism is not known. In fact our data from patients under PI did not differ from those not under PI (not shown). Indeed the period under PI therapy was of short duration (only some months).

Although the eradication of HIV infection should represent the only curative issue for patients, our study suggested that a medroxyprogesterone therapy could be considered as a potential efficient choice to improve the effect of an appropriate oral protein-rich nutritional support.

ACKNOWLEDGEMENTS

This study was supported by grants from SIDACTION and the Région Auvergne, France. We would like to thank D. Bonin and H. Lafarge for literature requisitions, Dr G. Dutot and Baxter France for providing the amino acid mixture.

REFERENCES

[1] Gorbach SL, Knox TA, Roubenoff R. Interactions between nutrition and infection with human immunodeficiency virus. Nutr Rev 1993, 51: 226–234.

[2] Sauerwein HP. Nutrition and AIDS. Clin Nutr 1993, 12: 64–68.

[3] Kotler DP, Wang J, Pierson RN. Body composition studies in patients with the acquired immunodeficiency syndrome. Am J Clin Nutr 1985, 42: 1255–1265.

[4] Chandra RK. 1990 McCollum Award lecture. Nutrition and immunity: lessons from the past and new insights into the future. Am J Clin Nutr 1991, 53: 1087–1101.

[5] Melchior JC, Chastang C, Gelas P, Carbonnel F, Zazzo JF, Boulier A, Cosnes J, Boulétreau P, Messing B. Efficacy of 2-month total parenteral nutrition in AIDS patients: A controlled randomized prospective trial. AIDS 1996, 10: 379–384.

[6] Burger B, Ollenschlager G, Schrappe M, Stute A, Fischer M, Wessel D, Schwenk A, Diehl V. Nutrition behavior of malnourished HIV-infected patients and intensified oral nutritional intervention. Nutrition 1993, 9: 43–44.

[7] Pichard C, Karsegard V, Kruseman M. Syndrome d'immunodéficience acquise. In: Leverve X, Cosnes J, Erny P, Hasselmann M (Eds), Traité de nutrition artificielle de l'adulte. Editions Mariette Guéna, 1998. p 853–869.

[8] Kotler DP, Tierney AR, Wang J, Pierson RN. Magnitude of body cell mass depletion and the timing of death from wasting in AIDS. Am J Clin Nutr 1989, 50: 444–447.

[9] Macallan DC. Wasting in HIV infection and AIDS. J Nutr 1999, 129: 238S–242S.

[10] Macallan DC, McNurlan MA, Milne E, Calder AG, Garlick PJ, Griffin GE. Whole-body protein turnover from leucine kinetics and the response to nutrition in human immunodeficiency virus infection. Am J Clin Nutr 1995, 61: 818–826.

[11] Selberg O, Süttmann U, Melzer A, Deicher H, Müller MJ, Henkel E, McMillan DC. Effect of increased protein intake and nutritional status on whole-body protein metabolism of AIDS patients with weight loss. Metabolism 1995, 44: 1159–1165.

[12] Laurichesse H, Tauveron I, Gourdon F, Cormerais L, Champredon C, Charrier S, Rochon C, Lamain S, Bayle G, Laveran H, Thieblot P, Beytout J, Grizard J. Threonine and methionine are limiting amino acids for protein synthesis in patients with AIDS. J Nutr 1998, 128: 1342–1348.

[13] Corcoran C, Grinspoon S. Treatments for wasting in patients with the acquired immunodeficiency syndrome. N Engl J Med 1999, 340: 1740–1750.

[14] Mann M. Approved pharmacologic interventions for wasting: an overview and lessons learned. J Nutr 1999, 129: 303S–305S.

[15] Miller TL. Nutrition in paediatric human immunodeficiency virus infection. Proc Nutr Soc 2000, 59: 155–162.

[16] Centers for Disease Control. Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Morb Mortl Wkly Rep 1993, 41: 1–19.

[17] Patureau Mirand P, Beaufrère B, Grizard J, Obled C, Arnal M. Les protéines et les acides aminés. In: Martin A (Ed), Les apports nutritionnels conseillés à la population française, AFSSA-CNERNA. Tech. et Doc, Lavoisier, 2001. p 37–62.

[18] Rolland-Cachera MF, Brambilla P, Manzoni P, Akrout M, Sironi S, Delmaschio A, Chiumello G. Body composition assessed on the basis of arm circumference and triceps skinfold thickness: A new index validated in children by magnetic resonance imaging. Am J Clin Nutr 1997, 65: 1709–1713.

[19] Bloxam DL, Warren WH. Error in the determination of tryptophan by the method of Denkla and Dewey. A revised procedure. Anal Biochem 1974, 60: 621–625.

[20] Hardin DS, LeBlanc A, Young D, Johnson P. Increased leucine turnover and insulin resistance in men with advanced HIV infection. J Investig Med 1999, 47: 405–413.

[21] Pannuti F, Martoni A, Camaggi CM. Medroxyprogesterone acetate high-dose in advanced breast cancer: Italian experience. German Italian Oncological Symposium, Milan. 1992.

[22] Simons JP, Schols AM, Hoefnagels JM, Westerterp KR, ten Velde GP, Wouters EF. Effects of medroxyprogesterone acetate on food intake, body composition, and resting energy expenditure in patients with advanced, nonhormone-sensitive cancer: a randomized, placebo-controlled trial. Cancer 1998, 82: 553–560.

[23] Saint-Marc T, Livrozet JM, Fournier F, Rambeaud B, Touraine JL. Intérêt de la médroxyprogesterone acétate dans le traitement de la cachexie du SIDA. Presse Med 1993, 22: 597.

[24] Scevola D, Zambelli A, Bottari G. Appetite stimulation and body weight gain with medroxyprogesterone acetate in AIDS anorexia and cachexia. Int J Drugs Ther 1991, 3: 77–83.

[25] Dardevet D, Sornet C, Balage M, Grizard J. Stimulation of in vitro rat muscle protein synthesis by leucine decreases with age. J Nutr 2000, 130: 2630–2635.

[26] Pichard C, Sudre P, Karsegard V, Yerly S, Slosman DO, Delley V, Perrin L, Hirschel B. A randomized double-blind controlled study of 6 months of oral nutritional supplementation with arginine and omega-3 fatty acids in HIV-infected patients. AIDS 1998, 12: 53–63.

[27] Tauveron I, Charrier S, Champredon C, Bonnet Y, Berry C, Bayle G, Prugnaud J, Obled C, Grizard J, Thiéblot P. Response of leucine metabolism to hyperinsulinemia under amino acid replacement in experimental hyperthyroidism. Am J Physiol Endocrinol Metab 1995, 269: E499–E507.

[28] Rochon C, Tauveron I, Dejax C, Benoit P, Capitan P, Bayle G, Prugnaud J, Fabricio A, Berry C, Champredon C, Thieblot P, Grizard J. Response of leucine metabolism to hyperinsulinemia in hypothyroid patients before and after thyroxine replacement. J Clin Endocrinol Metab 2000, 85: 697–706.

[29] Oster MH, Enders SR, Samuels SJ, Cone LA, Hooton TM, Browder HP, Flynn NM. Megestrol acetate in patients with AIDS and cachexia. Ann Intern Med 1994, 121: 400–408.

[30] Von Roenn JH, Armstrong D, Kotler DP, Cohn DL, Klimas NG, Tchekmedyian NS, Cone L, Brennan PJ, Weitzman SA. Megestrol acetate in patients with AIDS-related cachexia. Ann Intern Med 1994, 121: 393–399.

[31] Penzak SR, Chuck SK. Hyperlipidemia associated with HIV protease inhibitor use: pathophysiology, prevalence, risk factors and treatment. Scand J Infect Dis 2000, 32: 111–123.

[32] Mulligan K, Grunfeld C, Tai VW, Algren H, Pang MY, Chernoff DN, Lo JC, Schambelan M. Hyperlipidemia and insulin resistance are induced by protease inhibitors independent of changes in body composition in patients with HIV infection. J Acquir Immune Defic Syndr 2000, 23: 35–43.