

Original Paper

Low Levels of Linoleic Acid in Plasma Total Lipids of HIV-1 Seropositive Children

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Objective: To assess the plasma fatty acid status of a group of well-nourished children with the human immunodeficiency virus type-1 (HIV-1) and how this relates to the blood total CD4⁺ lymphocyte count.

Subjects: Fourteen HIV-1 seropositive children at various stages of disease and with adequate growth indices were assessed and compared to a control group of 30 healthy children.

Results: The concentrations (mg/dL) of plasma total fatty acids were not different between the two groups. HIV-1 seropositive children presented lower levels of 18-C essential polyunsaturated fatty acids (PUFA: linoleic acid, LA, and alpha-linolenic acid) and higher levels of their 20-C long-chain derivatives (di-homo-γ-linolenic acid, arachidonic acid, AA, and eicosapentaenoic acid) and docosahexaenoic acid in their plasma total lipids. The lowest plasma LA levels were observed in the subgroup of patients with more advanced stages of disease. In bivariate analyses the plasma LA levels related positively (Spearman $r=0.50$, $p=0.06$), while the LA/AA ratio related negatively (Spearman $r=-0.51$, $p=0.06$), to the total CD4⁺ count.

Conclusions: Childhood HIV-1 infection is associated with changes in plasma fatty acid profile suggestive of an increased PUFA turnover. Decreased levels of LA (together with higher plasma AA levels) appear to be associated with more advanced clinical and biochemical stages of disease.

INTRODUCTION

As a 18-C essential polyunsaturated fatty acid (PUFA) deficiency may impair the immune response [1] and longer-chain PUFA derivatives are important mediators in the immune cascade [2], the PUFA pattern in adult human immunodeficiency virus-type 1 (HIV-1) infection has been studied [3–5]. More recently the PUFA status of severely malnourished children infected by HIV-1 has been investigated [6].

The aim of this study was to investigate the PUFA status of well-nourished HIV-1 seropositive children, showing a good development of growth indices, and to investigate its relationship to the blood total CD4⁺ lymphocyte count taken as predictor of progression of disease [7].

PATIENTS AND METHODS

In a study approved by the Departmental Ethical Committee, we considered 14 children who met inclusion criteria from the vertically HIV-1 infected pediatric population followed up at this center. Diagnosis and staging of HIV-1 infection were made in accordance with the CDC Classification, 1994 [8]. Weight and length were recorded for all patients at blood sampling and classified as percentile and z score values according to the National Center for Health Statistics (NCHS) reference charts [9]. Inclusion criteria were: a) weight-for-age and height-for-age values above the 3rd percentile; and b) no decrease in the percentile values of growth parameters during the last 12 months.

Abbreviations: AA=arachidonic acid; DGLA=di-homo-γ-linolenic acid, DHA=docosahexaenoic acid, EPA=eicosapentaenoic acid, FA=fatty acids, HIV-1=human immunodeficiency virus-type 1, LA=linoleic acid, LnA=α-linolenic acid, PUFA=polyunsaturated fatty acids.

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Table 1. Clinical Status, Anthropometric Characteristics and Daily Energy Intakes (Median of Three 24-Hour Interviews) of the 14 HIV-1 Seropositive Children in Decreasing Order of Age per Clinical Stage

Case	Age (year)	Gender	Stage	AVT	L/A z score	W/A z score	W/L z score	BMI	Cal%
1	9.5	M	N1	–	1.74	2.17	1.16	20.9	186
2	2.9	F	N1	–	1.54	0.41	–0.27	15.1	136
3	2.3	F	N1	–	2.29	1.47	0.45	16.4	162
4	1.2	M	N1	–	0.48	0.54	0.11	16.8	105
5	4.4	F	A1	+	1.69	0.83	0.02	15.1	151
6	1.5	M	A1	+	2.37	2.0	1.07	17.5	173
7	5.0	M	A2	+	–0.26	–1.01	–1.08	14.0	158
8	3.6	M	A2	+	0.21	–0.47	–0.66	14.8	172
9	1.5	F	B1	+	2.29	2.09	1.08	17.0	195
10	7.3	F	B2	+	2.26	1.21	–0.66	15.3	175
11	4.1	F	B2	+	0.79	2.04	2.30	18.9	135
12	12.5	F	B3	+	–0.19	0.58	1.74	21.5	124
13	4.7	M	C1	+	0.31	0.35	0.32	16.1	152
14	3.9	M	C3	+	–1.44	–1.60	–0.96	14.6	101

M = Male, F = Female, AVT = antiviral therapy (Azidothymidine and/or Dideoxynosine), L/A = length for age, W/A = weight for age, W/L = weight for length, BMI = body mass index, Cal % = caloric percentage of the Italian Recommended Dietary Allowances for age and gender.

The control group was made up of 30 healthy children of both sexes born to HIV-seronegative mothers and aged 1 to 13 years (median age: 6). It has been shown that data from this age range may be used as reference values for healthy children since no change in the fatty acid (FA) composition occurs in lipid subclasses in this bracket [10]. The controls were in the same age bracket as subjects with HIV-1 infection, with at least two subjects per year of age and 16 subjects in the 1 to 6 year range which included 11/14 HIV-1 seropositive children.

Dietary habits of both groups were measured by three 24-hour dietary recalls administered to mothers in consecutive weeks. The first recall was administered on the day the blood sample was taken, and then by home interview on two other days within the next 2 weeks. The conversion of food intake into energy and nutrients was based on the food composition tables issued by the Italian National Institute of Nutrition, completed by data from other sources for any food item not listed. Medians from the three interviews were considered for

each subject and compared with the Italian Recommended Dietary Allowances [11].

The FA composition of plasma total lipids was measured by high-resolution capillary gas-chromatography (MEGA 2 GC, Fisons, Rodano, Italy) of FA methyl esters obtained by acid-catalyzed transmethylation after extraction of lipids by chloroform-methanol [12] from fasting blood samples. A 17:0 fatty acid was used as an internal standard for FA quantification. Individual fatty acids (expressed as weight percentage) were identified by comparisons with pure reference compounds and by means of mass spectrometry (Trio 1000, Fisons Instruments, Rodano, Italy). The plasma total FA concentrations (mg/dL) represent the sums of the amounts of single FA molecules identified in the chromatograms.

The blood total CD4⁺ count in HIV-1 seropositive subjects was determined by flow cytometry and expressed as absolute number of cells per cubic millimeter. The arachidonic acid (AA)/linoleic acid (LA) and the eicosapentaenoic (EPA)/ α -linolenic acid (LnA) ratios were taken as indices of metabolic balance (expression of dietary intake, metabolic processes, fat

Table 2. Fatty Acid Composition of Plasma Total Lipids (Weight%, Mean \pm SD, 95% Confidence Interval for Difference) of HIV-1 Seropositive Children vs. the Control Group

Fatty acid	HIV-1 (n=14)	Controls (n=30)	95% CI for difference	P
Linoleic acid	22.5 \pm 3.2	30.0 \pm 2.2	–9.17, –5.83	<0.001
α -Linolenic acid	0.22 \pm 0.04	0.31 \pm 0.11	–0.15, –0.03	0.005
Di-homo- γ -linolenic acid	2.2 \pm 1.6	1.6 \pm 0.2	0.01, 1.19	<0.05
Arachidonic acid	8.5 \pm 1.8	6.5 \pm 0.9	1.18, 2.82	<0.001
Eicosapentaenoic acid	0.42 \pm 0.09	0.33 \pm 0.11	0.02, 0.16	0.01
Docosahexaenoic acid	1.9 \pm 0.7	1.3 \pm 0.2	0.32, 0.88	<0.001
Eicosatrienoic acid	0.24 \pm 0.19	0.15 \pm 0.10	0.00, 0.18	0.05
Saturated FA	36.3 \pm 2.6	34.3 \pm 1.6	0.72, 3.28	0.003
Monounsaturated FA	26.2 \pm 3.1	24.2 \pm 2.2	0.36, 3.64	0.01
Polyunsaturated FA	37.3 \pm 4.0	42.5 \pm 2.5	–7.19, –3.28	<0.001

Table 3. Fatty Acid Composition of Plasma Total Lipids: Weight%, Median and Range in HIV-1 Seropositive Children Subdivided According to Stage of Disease

Fatty acid	N+A (8)	B+C (6)
Linoleic acid	23.2 (21.0–28.2)	20.8 (17.5–24.1)*
α -Linolenic acid	0.20 (0.18–0.29)	0.22 (0.18–0.29)
Di-homo- γ -linolenic acid	1.89 (1.51–2.36)	1.74 (1.25–2.94)
Arachidonic acid	7.9 (7.0–10.9)	8.4 (5.4–11.5)
Eicosapentaenoic acid	0.44 (0.37–0.56)	0.36 (0.29–0.60)
Eicosatrienoic acid	0.19 (0.10–0.59)	0.16 (0.06–0.69)
Docosahexaenoic acid	1.7 (1.0–2.7)	2.1 (0.9–3.2)
Saturated FA	35.7 (34.4–40.2)	35.1 (31.5–39.9)
Monounsaturated FA	25.3 (20.4–28.1)	28.1 (25.9–30.7)*
Polyunsaturated FA	37.1 (33.6–44.2)	36.2 (29.6–39.6)

* $p < 0.05$.

mobilization and storage) between the precursor n-6 PUFA and their most functionally relevant 20-C derivatives. Results in the text are expressed as mean \pm SD. Comparisons between the study population and the control group were analysed by Student's *t* test and fatty acid levels in the HIV-1 seropositive subgroups were compared by the Mann-Whitney's *U* test considering the small number of subjects in each subgroup. The limit of significance was set at 0.05. Correlation tests (Spearman's *r*) were performed to investigate the relationships between PUFAs with their product/precursor ratios and total CD4⁺ count.

RESULTS

The anthropometric, clinical data and energy intakes of the 14 HIV-1 seropositive children who met inclusion criteria are reported on Table 1.

Protein and micronutrient (including iron and zinc) intakes were within the recommended limits for all subjects. By comparing the daily macronutrient intakes (expressed as percentage of total energy) of HIV-1 seropositive patients with those in the control group we did not find significantly different total fat

(34 \pm 5% vs. 33 \pm 6% in controls) and polyunsaturated lipid (4.2 \pm 2.4% vs. 5.1 \pm 2.9% in controls) intakes.

Concentrations of plasma total fatty acids did not significantly vary between the two groups (180 \pm 88 vs. 204 \pm 41 mg/dL in controls). HIV-1 seropositive children had lower levels of the two 18-C essential PUFA and higher levels of their 20-C derivatives and docosahexaenoic acid (DHA, C22:6 n-3) in plasma total lipids (Table 2). Plasma levels of eicosatrienoic acid (C20:3 n-9) derived from the non-essential oleic acid were higher in the HIV-1 group. The eight subjects in stages N and A showed a greater decrease in LA levels compared to the six subjects in stages B and C, with a concomitant increase in the monounsaturated FA fraction (Table 3). At bivariate analyses, LA levels correlated positively with CD4⁺ count (Fig. 1). There was a negative association for the AA/LA ratio and CD4⁺ count (Spearman $r=-0.51$, $p=0.06$) (Fig. 2). LnA, 20-C PUFA, DHA and the EPA/LnA ratio did not show any significant association with CD4⁺ count.

DISCUSSION

In the early phase of HIV-1 infection, PUFA levels of free fatty acids were found to be higher than in controls [3]. Among

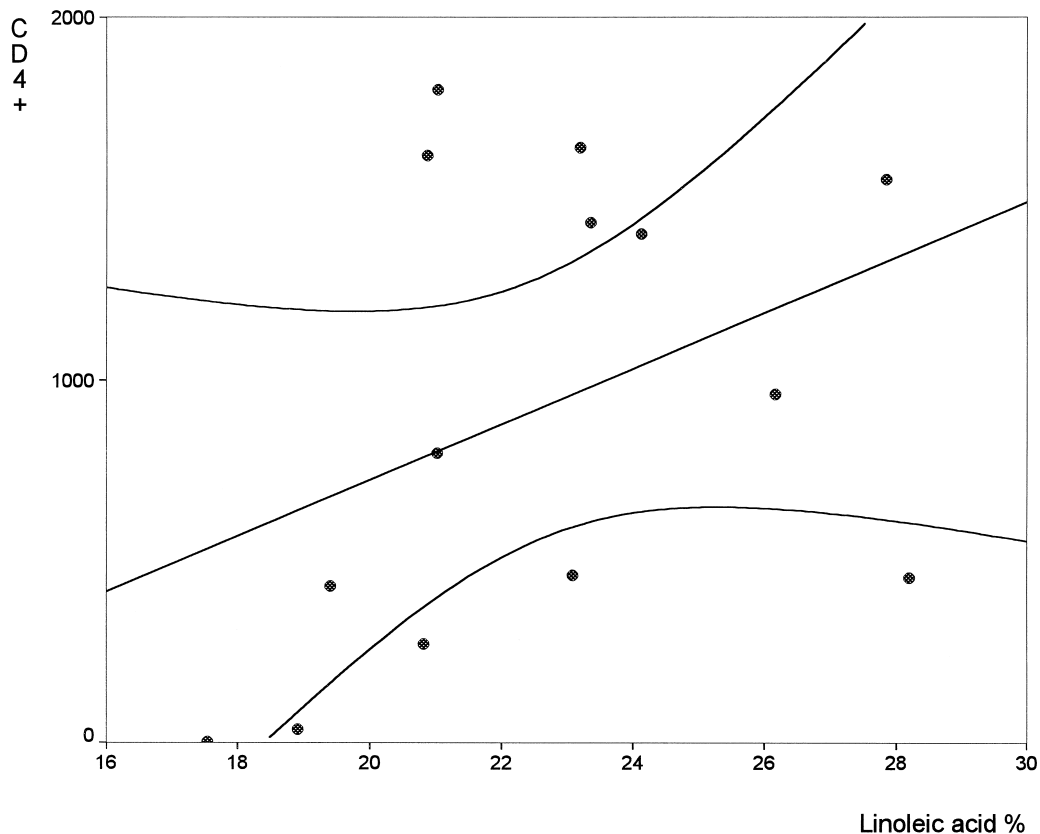


Fig. 1. Correlation between LA levels in plasma total lipids and total CD4⁺ lymphocyte count/mm³ in 14 HIV-1 seropositive children. Outer lines represent 95% CI.

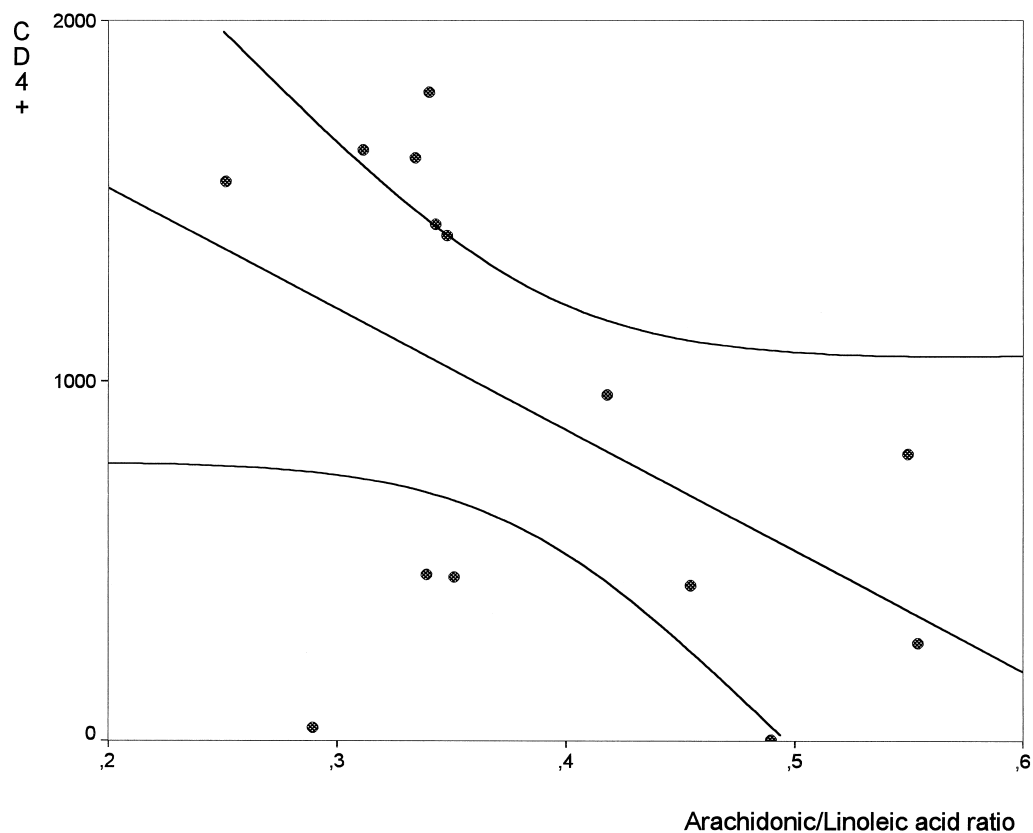


Fig. 2. Correlation between AA/LA ratio in plasma total lipids and total CD4⁺ lymphocyte count/mm³ in 14 HIV-1 seropositive children. Outer lines represent 95% CI.

young adults, early-stage HIV-1 infection was associated with decreased levels of plasma n-6 PUFA (above all AA), but not n-3 long-chain PUFA [5]. Among adults with full-blown disease, absolute plasma LA levels were significantly lower than in controls, although proportions of AA did not differ among the two groups [4]. The only study carried out on pediatric patients was of severely malnourished children in various stages of disease, and showed a depletion of LA, AA and DHA (but not EPA) when compared to reference values [6].

Our data suggest that HIV-1 infection in children at various stages of disease, but with adequate dietary intake and stable growth indices, are associated with lower levels in the two essential 18-C PUFA and higher levels in their 20-C derivatives and DHA of plasma total lipids. Lower LA levels also lower total PUFA, just as the saturated and the monounsaturated fractions increase. These findings are reminiscent of the changes in FA status of patients with cystic fibrosis [13] in whom an increased AA turnover has been implicated in increased synthesis of inflammatory metabolites with varying degrees of fat malabsorption [14]. The observed decrease in LA with LnA and the increase in 20-C PUFA (DGLA and AA derived from LA, and EPA from LnA) with DHA, therefore, may be consistent with a faster turnover of both n-6 and n-3 PUFA classes. Thus the requirements for essential precursors,

are increased with a possible defect in fat and LA absorption which may be secondary to early gastrointestinal mucosa alterations typical of HIV-1 infection [15]. Accordingly, higher levels of the non-essential eicosatrienoic acid, of the n-9 series, could be a result of LA deficiency or nonspecific enzymatic activity of increased desaturation and elongation [16]. Both symptomatic adult patients with HIV and severe weight loss as well as asymptomatic seropositive adults with normal T-cell count have been demonstrated to undergo increased *de novo* lipogenesis [17].

Protein-energy malnutrition in children leads to a depletion of long-chain PUFA in plasma lipids [18]. This explains the difference between our results and those of the population studied by Decsi et al [6] which was severely malnourished, since the inclusion criterion of that study was body weight below or in the third centile of growth charts. The authors found also associations between body weight and AA with DHA in plasma phospholipids [6].

We found correlations between the PUFA profile and the total CD4⁺ lymphocyte count. *In vitro* observations have shown a decline of LA and AA in the CD4⁺ membrane phospholipid composition of the lymphocytes of HIV-1 symptomatic subjects, maybe as a consequence of an activation of the cyclo-oxygenase pathway [19]. A higher LA turnover with

more advanced stages of disease is supported in our study by the negative association found between AA/LA ratio and CD4⁺ count. LA is essential for both growth and immune defense mechanisms, and study designs involving radiolabeling of fatty acids should be mounted to clarify the absorption and metabolic pathways of LA in HIV-seropositive children.

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