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Abnormal Free Fatty Acids and Cortisol Concentrations in the Serum of AIDS Patients

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Abstract—*The serum free fatty acid (FFA), cortisol and urinary creatinine, 17-hydzoxycorticosteroid and 17-oxosteroid concentrations of acquired immunedeficiency syndrome (AIDS-I: beginning and AIDS-II: end phase) and AIDS-related complex (ARC) patients were determined. Both groups were compared to a control group (healthy men).*

ARC and AIDS-I patients. *The ratios of stearic* (C18:0) *to oleic* (C18:1) *acid were* 75%, $P < 0.01$ *(ARC) and* 45%, $P < 0.05$ *(AIDS-I) greater than normal, due to a decrease in the relative percentage of monounsaturated fatty acids by* 25%, $P < 0.001$ *(ARC) and* 20%, $P < 0.01$ *(AIDS-I). In contrast, the relative percentage of polyunsaturated fatty acids was* 85% *greater than normal* ($P < 0.001$) *in ARC and* 100% *greater than normal* ($P < 0.001$) *in AIDS-I patients. Total FFA levels did not differ from controls. Serum cortisol levels were* 35% ($P < 0.01$) *above normal in ARC and* 60% ($P < 0.001$) *above normal in AIDS-I patients. Urinary 17-hydroxycorticosteroids and 17-oxosteroids were very low (2–3-fold lower than normal values,* $P < 0.001$) *in both groups of patients. Urinary creatinine did not differ from controls.*

In AIDS-II patients *the total FFA concentration was below normal* 35% ($P < 0.01$) *and the stearic/oleic acid ratio was* 50% *above normal* ($P < 0.05$). *The relative percentages of monounsaturated and polyunsaturated fatty acids in this group were similar to those of controls. Serum cortisol concentrations were significantly higher,* 50% ($P < 0.001$), *but he urinary 17-hydroxycorticosteroids and 17-oxosteroids were 2-fold lower* ($P < 0.001$) *than those of controls. Urinary creatinine did not differ from controls.*

These significant differences from normal may be implicated in the pathophysiology of AIDS and could represent not only a good index of diagnosis and prognosis, but also indicate new therapeutic approach to the disease.

INTRODUCTION

RECENT studies have clearly shown that the fatty acid contents of red blood cells and white blood cells are abnormal in acquired immunodeficiency syndrome (AIDS) patients [1]. The oleic acid content of the lipids extracted from blood cells is higher than those of normal subjects or symptom-free anti-human immunodeficiency virus (HIV) positive adult men. This abnormality is particularly clear when the results are expressed as the ratio between the stearic and oleic acid concentrations (saturation index [1]). The ratio is significantly lower than normal in AIDS patients but not in anti-HIV positive patients.

It is also known that ethanolic extracts of plasma from AIDS sufferers can cause considerable modification of various immune responses, especially lymphocyte viability and the T-CD4+/T-CD8+ ratio [2]. This action is mimicked *in vitro* by free unsaturated fatty acids (UFA) such as linoleic acid. The action of the UFA is dose-dependent, as is that of cortisol which when added together with linoleic acid, greatly enhances its negative effect on lymphocyte viability [3].

The free fatty acid (FFA) and cortisol contents of the serum of AIDS patients may, therefore, be of particular significance. This study was designed to measure the serum levels of FFA, both the total and those of each class and cortisol from patients at various stages of the disease. Urinary creatinine, 17-hydroxycorticosteroids and 17-oxosteroids were also determined.

MATERIALS AND METHODS

Patients

Sixty HIV seropositive patients were studied as in-patients or out-patients at the Claude Bernard Hospital, Paris, between March and September 1987: 16 patients with AIDS-related complex

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(ARC), 25 patients with early AIDS (diagnosis since less than 3 months—AIDS-I) and 19 patients with advanced AIDS (diagnosis since more than 5 months—AIDS-II).

The criteria or the diagnosis of AIDS was opportunistic infections (OI) in 29 patients, Kaposi's sarcoma (KS) in four and both OI and KS in 11.

All were adult male, mean age 35 years (range 23–57), 50 European patients and 10 immigrants from Africa (9 patients) or Brazil (1 patient) had at least one risk factor (49 homo- or bisexual men, 4 drug addicts, 4 blood-recipients, 3 heterosexuals).

These patients had received no parenteral or enteral artificial nutrition. The percentage of weight loss was carefully estimated for each group of patients.

ARC patients: no loss (45%), loss <10% (50%), loss >10% (5%).

AIDS-I patients: no loss (20%), loss <10% (50%), loss >10% (30%).

AIDS-II patients: no loss (15%), loss <10% (10%), loss >10% (75%).

All patients receiving azydothymidine or interferon were excluded. Anti-infection drugs (such as cotrimoxazole, sulfadiazine, pyrimethionine, ketoconazol) had been prescribed for almost all AIDS-I or -II patients before the blood samples were taken. In contrast very few of the ARC patients received drugs. Anxiolytic drugs, particularly the benzodiazepines, were very often administered to all three groups.

The data from both groups of HIV seropositive patients were compared to those obtained from a control group ($n = 38$) of healthy adult males (mean age 40 years, extremes 25–50 years) receiving no medication.

It was not possible to perform all the tests on all the patients and controls, their number is indicated for each type of determination in the tables.

Blood and urine samples

All blood samples were obtained at 8 a.m. and allowed to coagulate before separation of serum by centrifugation (3000 rpm/10 min at 4°C).

Serum samples were treated 30 min at 56°C and stored at −20°C until assayed.

Twenty-four hours urine, checked by creatinine determination [4], was collected.

Fatty acids standards

Myristic, palmitic, stearic, heptadecanoic, palmitoleic (*n*-7), oleic (*n*-9), linoleic (*n*-6), linolenic (*n*-3), arachidonic (*n*-6), docosatetraenoic (*n*-6) and docosahexaenoic (*n*-3) acids were purchased from Sigma Chemical Company.

Serum free fatty acid (FFA) extraction and gas chromatographic analysis

Heptadecanoic acid was added to serum samples as an internal standard. Each sample (0.5 ml) was extracted three times with 5 ml organic solvent (ethyl acetate/cyclohexane v/v) and the aqueous phase was removed by freezing (−20°C). The organic extracts were pooled, evaporated to dryness, taken up in 0.5 ml of chromatography solvent (benzene–ethanol, 95:5) and placed on Sephadex LH20 microcolumns (0.5 × 6 cm). FFA were eluted with 3 ml of solvent. Extracts were evaporated to dryness and methylated in boron trifluoride–methanol (Merck). The methylated fatty acids were chromatographed on a Packard Chromatograph, model 419 (Packard, Beker, U.S.A.) using a capillary column (WCOT Fused Silica CP-WAX-52 CB, 25 m × 0.32 mm). The column temperature was 175°C for the first 5 min and increasing thereafter by 4°C/min to 230°C. The injector temperature was 240°C and the detector temperature was 260°C.

Peak area ratios and internal standard values were compared to standard ratios. Concentrations of fatty acids were determined in a Packard 604 (United Technologies).

Cortisol determination

The serum cortisol and urinary free cortisol levels were determined by fluorescence polarization immunoassay, an immunofluorimetric method using TDX System cortisol from Abbott Diagnostics Division (U.S.A.).

Determination of urinary 17-hydroxycorticosteroids and 17-oxosteroids

Urinary 17-hydroxycorticosteroids and 17-oxosteroids were estimated respectively by the methods of Silber and Porter [5] and Zimmermann [6].

Statistical analysis

The data are reported as means ± S.E.M. Student's *t* test was used to compare mean. Results were considered significant when the probabilities were: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

RESULTS

Serum concentration of free fatty acids (FFA) in different groups of ARC and AIDS patients

The serum levels of total FFA, the relative percentages of saturated, mono- or polyunsaturated fatty acids and the ratios of stearic (C18:0) to oleic acid (C18:1) in each group of ARC and AIDS patients are shown in Table 1.

The serum levels of total FFA were significantly lower than normal (35%, $P < 0.01$) only in the AIDS-II patients. Total FFA in the ARC and AIDS-I were not different from normal controls.

Table 1. *Serum FFA levels, relative percentage of the various classes of FFA and ratio of stearic* (C18:0) *to oleic* (C18:1) *acids in ARC and AIDS patients*

	FEA (mM/l)	FFA(%)				C18:0/C18:1
		Saturated	Monounsaturated	Diunsaturated	Polyunsaturated	
Controls $n = 21$	0.87 ± 0.1	30 ± 3	38 ± 1.4	26.2 ± 1.5	5.8 ± 0.5	0.2 ± 0.02
ARC $n = 13$	0.75 ± 0.1	34 ± 1.2	30 ± 1.4***	25 ± 1.3	10.8 ± 0.9***	0.35 ± 0.02**
AIDS-I $n = 11$	0.85 ± 0.1	34.6 ± 1.6	31.2 ± 1.7**	22.5 ± 1.7	11.7 ± 1***	0.29 ± 0.01*
AIDS-II $n = 12$	0.58 ± 0.08**	34 ± 1.6	34.6 ± 2.1 (N.S.)	24.2 ± 1.3	7.2 ± 0.7 (N.S.)	0.30 ± 0.05*

Gas chromatographic quantification of FFA serum levels.
Means of determinations ± S.E.M.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The relative percentages of monounsaturated FA (C18:1 and C16:1) were 25% lower than normal ($P < 0.001$) in ARC patients and 20% lower than normal ($P < 0.01$) in AIDS-I patients. This difference was particularly marked for oleic acid (*C18:1*). By contrast, the relative percentages of polyunsaturated fatty acids (C20:4, C22:4 and C22:6) in these same two groups were almost double those of controls (5.8%), being 10.8% in ARC patients ($P < 0.001$) and 11.7% ($P < 0.001$) in AIDS-I patients. The relative percentages of saturated and diunsaturated FA were not significantly different from controls in any of the groups. The ratio of stearic acid to oleic acid was significantly higher in ARC (75%, $P < 0.01$) and AIDS-I and -II patients (50%, $P < 0.05$) than in controls.

There were no significant differences in the relative percentages of the various classes of FFA in the AIDS-II patients.

Serum concentrations of cortisol and urinary creatinine, 17-hydroxycorticosteroids and 17-oxosteroids in different groups of ARC and AIDS patients

The serum cortisol and urinary creatinine 17-hydroxycorticosteroid and 17-oxosteroid levels of controls, ARC and AIDS-I and -II patients are shown in Table 2.

The serum cortisol concentrations were significantly higher than control values in the ARC (35%, $P < 0.01$), AIDS-I (60%, $P < 0.001$) and AIDS-II (50%, <0.001) patients. In contrast, the urinary levels of 17-hydroxycorticosteroids and 17-oxosteroids were very significantly lower than normal (2–3-fold, $P < 0.001$) in the ARC and all the AIDS patients.

The creatinine levels in ARC and AIDS patients were not different from normal controls.

DISCUSSION

The data clearly show that, although the total FFA concentrations are not different from the control values, the serum monounsaturated FA content is decreased and the polyunsaturated fatty acid (PUFA) content is increased in the AIDS-related complex (ARC) and AIDS-I patients. These abnormalities are highly significant, particularly for C18:1. The C18:0/C18:1 ratio is also clearly increased, particularly in the ARC patients. These results can be compared with those obtained in a recent study [1] where this ratio was found to be significantly decreased in the red and white blood cells of AIDS patients. The existence of such a reciprocity between the blood cells and the surrounding serum may be the result of a transfer of oleic acid from the serum to the cells and/or strong inhibition of the release of monounsaturated fatty acids from the cells. It is difficult, at present, to explain why the concentration of PUFAs is enhanced in the serum of these patients. Determination of the cellular PUFA concentration and activities of the various intra- or extracellular lipases may provide the data required to interpret this result.

The situation is quite different in the serum of highly critical AIDS-II patients, which have a significantly lower than normal concentration of total free fatty acids. This may result from major metabolic perturbations, with an increase in the use of lipids for energy, as is usually observed in starved and severely stressed patients in the terminal phase of the disease. The other lipid perturbations

Table 2. Serum cortisol and urinary 17-hydroxycorticosteroid, 17-oxosteroid and creatinine concentrations in ARC and AIDS patients

	Cortisol (nM/l)	17-Hydroxy-corticosteroids (μmol/24 hr)	17-Oxosteroids (μmol/24 hr)	Creatinine (mmol/24 hr)
Controls	341 ± 28 $n = 17$	11.6 ± 0.6 $n = 18$	44.6 ± 2 $n = 18$	13 ± 0.7 $n = 18$
ARC	471 ± 34** $n = 16$	6.7 ± 1*** $n = 6$	15.6 ± 6.7*** $n = 6$	13 ± 2.7 $n = 6$
AIDS-I	537.5 ± 32*** $n = 25$	5.5 ± 3.6*** $n = 13$	21.5 ± 4*** $n = 13$	10.7 ± 1.2 $n = 13$
AIDS-II	516.6 ± 44*** $n = 19$	5.5 ± 1.4*** $n = 7$	20.6 ± 7.7*** $n = 7$	12.8 ± 1.4 $n = 7$

Serum cortisol levels were determined by immunofluorimetric method using TDX system cortisol (Abbott Diagnostics).
Urinary creatinine levels were determined by the Jaffé reaction, urinary 17-hydroxysteroids and 17-oxosteroids respectively by the methods of Silber and Porter and Zimmermann.
Values are means ± S.E.M.; $^{**}P < 0.01$; $^{***}P < 0.001$.

observed in the ARC and AIDS-I are not found in this AIDS-II group, except that of the C18:0/C18:1 ratio which is still slightly increased.

The combination of high concentrations of serum cortisol and low urinary concentration of 17-hydroxycorticosteroids in ARC and AIDS-I and -II patients, without any modification of the urinary creatinine, can be compared with the *in vitro* results showing that ethanol extracts of sera from ARC and AIDS patients, cancer and cirrhosis patients and the serum of newborns all inhibit the metabolism of glucocorticoids by lymphocytes [7,8].

Indirect evidence suggests that one of the ethanol soluble compounds responsible for this effect on cortisol metabolism is probably an UFA like linoleic acid [3]. The existence of a very low concentration of 17-hydroxycorticosteroids in the urine of AIDS patients can be explained by impaired metabolism of the steroid due to the high concentration of UFAs.

Thus, it is clear from the same *in vitro* studies that lymphocyte viability, although affected by cortisol and linoleic acid when used alone, is more influenced when these two compounds are associated [3].

The present results provide evidence that the pathophysiological circumstances produced experimentally *in vitro* may correspond to a real *in vivo* situation.

One of the differences between *in vitro* and *in vivo* studies is the fact that the immunosuppressive effect of cortisol *in vitro* is potentiated by the free fatty acid, linoleic acid [3], but the concentration of this UFA is unchanged in ARC and AIDS patients. PUFAs such as arachidonic, docosatetraenoic and docosahexaenoic acids may be involved in this phenomenon *in vivo* instead of linoleic acid, as their concentrations in the sera of ARC and AIDS-I patients are high.

More investigations are needed to define the hormonal status of these patients:

—Dynamic stimulation by ACTH exploring the various steroids produced by adrenals (gluco-, mineralocorticoids, androgens)

—Determination of the concentration of the corticosteroid binding globulin which control the cortisol catabolism.

—Determination of the urinary free cortisol.

Preliminary results of the urinary free cortisol obtained in AIDS patients (264 ± 70 nM, $n = 7$) show that this compound is significantly enhanced by comparison with controls (98.3 ± 12.5 nM, $n = 9$). Furthermore, the observation that the urinary 17-oxosteroids are also significantly decreased during the various stages of the HIV infection suggested that an exploration of the androgenic function (adrenal and testicular) could also provide relevant information.

More work is also needed to explain how this high concentration of cortisol (hypersecretion and/or hypocatabolism), contrasting with clinical and biological symptoms of adrenal insufficiency observed in AIDS [9], can act in association with high concentrations of PUFAs and/or some of their metabolites, amplyifying each other's immunosuppressive activity and hence aggravate the immunosuppressed state observed in AIDS.

In addition to the pathophysiological interest of these results, knowledge of the variation in the concentrations of glucocorticoids and lipids in the sera of ARC and AIDS patients could also be useful as indicators of the stage of the disease, providing a good index for diagnosis and prognosis.

The implication of fatty acids and cortisol in the pathophysiology of AIDS raises the possibility of a new therapeutical approach to the disease, which

may involve induced modifications of the lipidic structure of the immune cells and a reduction in the deleterious effects induced on these cells by the simultaneous presence of high concentrations of glucocorticoids and PUFAs.

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