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Relationship between plasma L-lysine concentrations and levels of HIV-1 RNA

Evgeny Vlad Butorov

The Municipal Center of HIV/AIDS Prophylaxis; Surgut, Russian Federation

Keywords: HIV, plasma L-lysine, viral load

Introduction

The HIV/AIDS pandemic continues to be a major health problem with an estimated 34 million people living with human immunodeficiency virus, with 2.5 million new infections in 2011 and an estimated 1.7 million deaths from HIV-related illnesses in the same year.[1,2] The development of an effective vaccine, new drugs, methods and approaches in the treatment of HIV-infection remains an urgent and pressing need. Conquering this enormous challenge demands further understanding of the biology of the virus, and its interaction not only with infected cells and the immune system, but also its negative impact on the development of metabolic disturbances.

The significant changes of the host protein and lipid metabolism, catabolic effect and the negative nitrogen equilibrium in HIV progression are widely appreciated,[3-7] but there are few data in the literature about infection-related amino acid imbalance in HIV infected patients.[8-13] At the same time, metabolic changes in patients with various pathological conditions[14-25] lead to alterations in their amino-acid profiles and the concentrations of amino acids in the extracellular medium was found to be critical in the life cycle of some viruses.[26-35] Observational studies indicate that amino acid imbalances are related to increased risk of rapidly progressing virus infections through changes of the protein homeostasis. Thus, amino acid profiles may be useful as early biomarkers of the physiological disturbances and disease progression.[36,37]

Since it is likely that the virus so formed arises from free amino acids of the cell, knowledge of the changes of free amino acids in the host cell and tissue, and its modification by infection, should be of interest. In virus infection, the protein metabolism of the host cell is shifted from the synthesis of cellular protein to the synthesis of virus protein of a different character with significant variations in amino acids concentrations.[38-41] Amino acids are not only useful for protein synthesis but also act as regulators of gene expression via the mechanism of amino acid-regulated transcription factors.[42-44]

The presence or absence of amino acids in the extracellular medium and its concentrations in the cell modulate specific transcription factors so as to promote or repress the synthesis of multiple genes.[45-47] The same mechanism is well known in HIV pathogenesis. The conversion of the single-stranded RNA genome into double-stranded DNA by virus-coded reverse transcriptase (RT) is an essential step in the retrovirus life cycle. Human immunodeficiency virus requires a cellular $tRNA^{Lys}$ as a primer for initiation of reverse transcription and HIV is not infectious unless the cellular $tRNA^{Lys}$ interacts directly on the specific region of the viral RNA genome.[48-50] But only the presence of a sufficient concentration of the essential L-lysine in HIV-infected cells activates the cellular $tRNA^{Lys}$.

To date, there are no direct clinical trial data on the effect of changes in L-lysine concentrations on HIV-1 viral load, and plasma amino acid profiles in HIV-infected patients are not adequately documented. The purpose of this study was to detect a possible relationship between levels of L-lysine and HIV-1 RNA. We hypothesized that the more rapid progression of HIV-infection is associated with the level of excess supply of this essential amino acid. To test these hypotheses, we measured levels of L-lysine, HIV-1 RNA and markers of immunological status, not only in clinical stages of the disease, but in the groups of the elite controllers, slow-progressors, and progressors among HIV-infected individuals. And our current findings do support this hypothesis.

Results

Clinical characteristics of HIV-infected patients. After the screening measurement of plasma L-lysine concentrations, HIV-1 RNA levels and CD4 count lymphocytes, 100 patients had to be excluded from the study: 32 patients had amino acid concentrations above the reference range of 80–470 μmol/l, 38 patients with HIV viral load above 1 000 000 copies/ml and 30 patients with severely immunodepressed (A3, B3, and C3 stages of HIV-infection).

As potential explanatory variables for plasma L-lysine concentration, organ failures (hepatic and renal failure) or biochemical markers of hepatic (AST, ALT, bilirubin, alkaline phosphatase, and lactate dehydrogenase) and renal (creatinine, γ-glutamyltransferase, and urea) function were entered in the analysis.

Clinical data, stages, immunological, and virological status of the HIV-infected patients are summarized in **Table 1**.

Our data revealed the differences between some hematological parameters in HIV-infected patients in CDC stages of HIV infection. The survey shows a significant decrease in the levels of CD4

Correspondence to: Evgeny Vlad Butorov; Email: butorov888@gmail.com
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Table 1. Clinical data, stages, hematological, immunological, and virological characteristics of HIV-infected patients and healthy control subjects

	HIV-infection stage (CDC, 1993)			
	Group I (A1, A2) (*n* = 290)	**Group II (B2, B2) (*n* = 110)**	**Group III (C1, C2) (*n* = 50)**	**Controls (*n* = 120)**
Age (years)	34 ± 5.8	35 ± 5.7	35 ± 5.8	34 ± 4.4
Sex (F/M)	0/290	0/110	0/50	0/120
BMI (kg/m²)	22.75 ± 2.57	22.99 ± 3.32	22.02 ± 2.32	22.62 ± 2.93
Viral charge ± (s.e.) (copies/ml)	107 600 ± 10 100†	188 600 ± 22 050†	327 200 ± 46 990†	-
CD4 (cells/μl)	496.3 ± 236.9*,†	345.6 ± 240.3*,†	245.5 ± 171.1*,†	1253 ± 230.3
CD8 (cells/μl)	1107.0 ± 488.9*	1052.0 ± 544.9*	1092.0 ± 388.6*	770 ± 322.4
CD4/CD8 ratio	0.44 ± 0.19*,†	0.34 ± 0.20*,†	0.29 ± 0.25*,†	1.75 ± 0.18
ALT (IU/ml)	74.3 ± 57.9*	57.0 ± 46.4*	60.6 ± 51.5*	24.2 ± 15.3
AST (IU/ml)	65.0 ± 58.5*	55.6 ± 36.3*,†	74.5 ± 62.4*,†	23.0 ± 14.7
Lactate dehydrogenase (LDH) (IU/l)	412.8 ± 142.3*,†	431.2 ± 75.8*,†	572.3 ± 204.9*,†	280.1 ± 83.5
Gamma-glutamyltransferase (GGT) (IU/l)	88.4 ± 103.6*,†	91.4 ± 78.4*,†	179.1 ± 154.3*,†	27.7 ± 18.7
Alkaline phosphatase (ALP) (IU/l)	111.8 ± 115.2*,†	114.4 ± 86.7*,†	169.6 ± 197.1*,†	54.2 ± 37.4
Creatinine (μmol/l)	89.3 ± 51.1	88.2 ± 18.3	81.2 ± 19.1	91.1 ± 34.5
Bilirubin (μmol/l)	11.7 ± 5.3	11.3 ± 4.9	8.9 ± 5.1	6.2 ± 3.8
Urea (mmol/l)	4.77 ± 3.15	4.56 ± 2.81	4.68 ± 3.12	4.42 ± 3.17

*Patients vs. controls, $P < 0.05$; †Patients in stage A, B vs. patients in clinical stage C (CDC, 1993), $P < 0.05$. Values are given as mean ± SD (range).

count lymphocytes in all clinical stage A (496.3 ± 236.9), B (345.6 ± 240.3), and C (245.5 ± 171.1) patients with HIV compared with healthy controls (1.75 ± 0.18, $P < 0.001$ for all comparison groups; the Student *t* test). At the same time, we found an increase in the levels of HIV-1 RNA ($P < 0.001$), AST ($P < 0.05$), LDH ($P < 0.001$), and ALP ($P < 0.05$) in advanced stages (B, C) of HIV-infection related to the progression of the disease. No significant differences were found between CD8 count lymphocytes, levels of creatinine, urea, bilirubin, cholesterol, ALT, GGT, and alkaline phosphatase in HIV-infected comparison groups. The mean levels of GGT, ALT, AST, LDH, and ALP were significantly higher in HIV-infected subjects compared with our reference values for similar ages ($P < 0.001$ for all comparison groups; the Student *t* test). No relation was observed between BMI, the levels of creatinine, urea, bilirubin in clinical stage (CDC, 1993) HIV-infected patients and healthy controls.

Plasma L-lysine concentrations in HIV-infected patients. The concentrations of L-lysine were evaluated between CDC groups of HIV-infected individuals and with our reference values. No previous studies are available for comparison. The levels of the amino acid in asymptomatic stage A (58%, in the following the percentage denotes the mean of the patients in relation to the mean of healthy controls), stage B (66%), and stage C (67%) were significantly reduced in the HIV patients compared with controls.

Plasma L-lysine concentrations markedly decreased with the progression of HIV-infection. The mean amino acid levels were significantly lower in advanced stage (B and C; 232.3 ± 135.0 and 149.1 ± 110.5) HIV-infected patients compared with healthy subjects (275.8 ± 120.3, $P < 0.005$ and $P < 0.001$, respectively; the Student *t* test) and with the level in asymptomatic stage (A) patients (263.1 ± 144.9, $P < 0.01$ and $P < 0.001$, respectively). No difference was observed between plasma L-lysine concentrations in asymptomatic stage (A1) HIV-infected patients and controls ($P = 0.50$).

At the same time, after initiation of HAART, the levels of L-lysine significantly increased in 80/100 (80%) of our HIV-infected individuals. As regards antiretroviral therapy, plasma amino acid concentrations (322.0 ± 115.9) were significantly higher compared with HIV-infected patients in stage A, B, and C without treatment ($P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively). We examined the difference in plasma L-lysine concentrations between HIV-infected subjects after introduction of HAART and healthy controls ($P < 0.001$).

Among the study patients, low levels of amino acid were found in HIV individuals with recurrent herpes infection (170.1 ± 144.5) which is different from values of controls ($P < 0.01$). The plasma L-lysine concentrations are given in **Table 2**.

Plasma L-lysine concentrations and CD4 count lymphocytes in HIV-infected patients. CD4 count lymphocytes markedly decreased with the progression of HIV-infection. The mean of these immunological parameters in clinical stage B was 345.6 (SD = 240.3) and in stage C, 245.5 (SD = 171.1). We found significant differences in CD4 count lymphocytes between advanced stage (B, C) HIV-infection compared with our reference values (1253 ± 230.3, $P < 0.0001$ and $P < 0.0001$, respectively; the Student *t* test) and levels in asymptomatic stage A patients (496.3 ± 236.9, $P < 0.0001$ and $P < 0.0001$, respectively). A mild positive correlation was observed

Table 2. Plasma L-lysine concentrations in HIV-infected patients and healthy subjects

HIV patients	L-lysine (μmol/l)	Controls (μmol/l) *n* = 120	Student t test
The entire cohort *n* = 450	253.4 ± 152.8	275.8 ± 120.3	*P* < 0.041 (S)
Group I (A1, A2) *n* = 290	263.1 ± 144.9	275.8 ± 120.3	*P* = 0.50 (NS)
Group II (B1, B2) *n* = 110	232.3 ± 135.0	275.8 ± 120.3	*P* < 0.005 (S)
Group III (C1, C3) *n* = 50	149.1 ± 110.5	275.8 ± 120.3	*P* < 0.001 (S)
HIV-infected patients with recurrent herpes infection *n* = 12	170.1 ± 144.5	275.8 ± 120.3	*P* < 0.01 (S)
HIV infected patients on HAART (B1, B2, C1, C2) *n* = 100	322.0 ± 115.9	275.8 ± 120.3	*P* < 0.001 (S)

(S), Differences were considered significant when *P* < 0.05; (NS), not significant. Values are given as mean ± SD (range).

between plasma L-lysine levels and CD4 count lymphocytes in entire cohort HIV-infected patients (r = 0.19, P < 0.008; Pearson correlation coefficient) and in groups I and III (P < 0.04 and P < 0.03, respectively).

The lowest levels of amino acid and CD4 count lymphocytes were found in HIV patients with recurrent herpes infection and patients with AIDS (284.2 ± 173.1 and 245.5 ± 171.1, respectively) (**Table 3**).

Plasma L-lysine concentrations and HIV-1 RNA levels in HIV-infected patients. Our data revealed that HIV-1 RNA levels evidently increased with the HIV-infection stages. The mean viral load concentrations were significantly higher in clinical stage B (188 600 ± 22 050 s.e.) and C (327 200 ± 46 990 s.e.) subjects compared with values in asymptomatic stage (A) HIV-infected patients (107 600 ± 10 100 s.e., P < 0.0001 and P < 0.0001, respectively; the Student t test). We found the same significant differences between these parameters in all clinical stage HIV-infected patients and with subjects after antiretroviral therapy (630.7 ± 95.4 s.e., P < 0.0001 for all comparison groups). The highest HIV-1 RNA levels (1 071 100 ± 647.6) were detected in HIV patients with acute herpes infection.

We observed the mild significant negative correlation between plasma L-lysine levels and viral load in clinical stage A and B HIV-infected patients (r = −0.18, P < 0.006 and r = −0.23, P < 0.03, respectively; Pearson correlation coefficient) and strong negative correlation in stage C (r = −0.48, P < 0.006). The relation between amino acid concentrations and HIV-1 RNA levels was not significant in patients on HAART (P = 0.83) and HIV-infected subjects with acute herpes infection (P = 0.86) (**Table 4**).

The absence of significance in HIV infected patients with recurrent herpes infection and group III may be due to the small number of subjects (n = 12). Our analysis was limited by a cohort size that may have been too small to allow detection of statistical associations when limited to subgroups.

In study, we observed the most significant negative correlation between plasma L-lysine levels and HIV viral load in patients with a range of viremia from 5000 to 50 000 copies/ml (r = −0.42, P < 0.0001; Pearson correlation coefficient). The mild negative correlation was found in subjects with viral load from 5 000 to 75 000 copies/ml and from 5000 to 100 000 copies/ml (r = −0.29, P < 0.002 and r = −0.24, P < 0.007, respectively). The weak negative correlation was also found in HIV-infected patients with viral load from 5000 to 500 000 copies/ml and from 5000 to 1000 000 copies/ml (r = −0.15, P < 0.03 and r = −0.12, P < 0.04, respectively) (**Table 5**; **Fig. 1**).

The results of our study suggest that there is evidence for an association between the triad of plasma L-lysine and HIV-1 RNA levels and CD4 count lymphocytes in clinical A, B, and C stages HIV infected patients (**Fig. 2**).

Plasma L-lysine concentrations and clinical data of HIV-controllers and progressors. From all patients observed in the our center we identified 13 HIV individuals as long-term non-progressors (elite controllers) whose viremia remained controlled for more than 10 years after HIV-infection, with the constant level of HIV-1 RNA less than 500 copies/ml and CD4 count lymphocytes more than 500 cells/μl; and 25 slow-progressors whose viral load remained low for more than 7 years, with the constant level of HIV-1 RNA less than 10 000 copies/ml and CD4 count lymphocytes more than 400 cells/μl. We selected 100 patients infected during the 5 years in comparison groups. The levels of plasma L-lysine, HIV-1 RNA, and CD4 count lymphocytes were evaluated between groups of HIV-1 elite controllers, slow-progressors and progressors (A2, B1, B2 stages of CDC classification). No previous studies are available for comparison.

The mean of viral load in HIV elite controllers was 550.1 (SD = 135.7), in slow-progressors 2631 (SD = 2606), and in HIV progressors 186 500 (SD = 150 100). The mean of CD4 count lymphocytes in HIV elite controllers was 549.6 (SD = 123.8), in slow-progressors 476.7 (SD = 326.3) and in HIV progressors 352.0 (SD = 199.9). Low levels of plasma L-lysine were observed in 9/13 (75%) of HIV elite controllers, 15/25 (60%) slow-progressors and 66/100 (66%) in HIV progressors compared with controls. The mean amino acid levels were significantly reduced in HIV elite controllers and non-progressors compared with HIV progressors (P < 0.0001 and P < 0.007, respectively;

Table 3. Comparison plasma L-lysine concentrations and CD4 count lymphocytes in HIV-infected patients

HIV patients	CD4 lymphocytes (cells/μl)	L-lysine (μmol/l)	Pearson correlation coefficient
The entire cohort *n* = 450	448.5 ± 247.5	253.4 ± 152.8	$r = 0.19$, $P < 0.008$ (S)
Group I (A1, A2) *n* = 290	496.3 ± 236.9	263.1 ± 144.9	$r = 0.14$, $P < 0.049$ (S)
Group II (B1, B2) *n* = 110	345.6 ± 240.3	232.3 ± 135.0	$r = 0.21$, $P = 0.04$ (S)
Group III (C1, C3) *n* = 50	245.5 ± 171.1	149.1 ± 110.5	$r = 0.70$, $P < 0.03$ (S)
HIV infected patients with recurrent herpes infection *n* = 12	284.2 ± 173.1	170.1 ± 144.5	$r = 0.57$, $P = 0.10$ (NS)
HIV-infected patients on HAART (B1, B2, C1, C2) *n* = 100	449.6 ± 214.3	322.0 ± 115.9	$r = 0.02$, $P = 0.90$ (NS)

(S), Differences were considered significant when $P < 0.05$; (NS), not significant. Values are given as mean ± SD (range).

Table 4. Comparison plasma L-lysine concentrations and HIV-1 RNA levels in HIV-infected patients

HIV patients	HIV-1 RNA (copies/ml) ± s.e.	L-lysine (μmol/l)	Pearson correlation coefficient
The entire cohort *n* = 450	170 300 ± 15 640	253.4 ± 152.8	$r = -0.14$, $P < 0.023$ (S)
Group I (A1, A2) *n* = 290	107 600 ± 10 100	263.1 ± 144.9	$r = -0.18$, $P < 0.006$ (S)
Group II (B1, B2) *n* = 110	188 600 ± 22 050	232.3 ± 135.0	$r = -0.23$, $P < 0.031$ (S)
Group III (C1, C3) *n* = 50	327 200 ± 46 990	149.1 ± 110.5	$r = -0.48$, $P = 0.006$ (S)
HIV-infected patients with recurrent herpes infection *n* = 12	1 071 100 ± 647.6	170.1 ± 144.5	$r = -0.05$, $P = 0.86$ (NS)
HIV infected patients on HAART (B1, B2, C1, C2) *n* = 100	630.7 ± 95.4	322.0 ± 115.9	$r = -0.02$, $P = 0.83$ (NS)

(S), Differences were considered significant when $P < 0.05$; (NS), not significant. Values are given as mean ± SD (range).

the Student *t* test) and healthy individuals ($P < 0.0001$ and $P < 0.01$, respectively). The mean level plasma L-lysine in HIV progressors is almost the same as in the controls ($P = 0.18$).

Plasma L-lysine concentrations, immunological, and virological status of the HIV-infected patients are summarized in **Table 6**.

Thus, the lowest levels of plasma L-lysine and the highest CD4 count lymphocytes were found in HIV-1 elite controllers and slow-progressors comparison in HIV progressors and controls. Accordingly, the highest viral load was detected in progressors.

Conclusions

In this study, we clearly demonstrated serious changes plasma concentrations of L-lysine in HIV-infected patients. We formerly hypothesized that the amino acid levels were related to increased viral load and to the clinical stages of HIV-infection; and, our current findings support this hypothesis. The mean plasma L-lysine concentrations were significantly lower in advanced CDC stage (B and C) HIV-infected patients than in asymptomatic stage (A) and patients on HAART. We examined that plasma L-lysine concentrations were negatively correlated with HIV-1 RNA levels and inversely with CD4 count lymphocytes. The mean plasma L-lysine concentrations were significantly lower in HIV-1 elite controllers and slow-progressors compared with healthy individuals and HIV-progressors. And the levels of amino acid increased in HIV-infected subjects after introduction of HAART. It sounds paradoxical,

Table 5. Comparison of plasma l-lysine concentrations in HIV-infected patients ranged by HIV-1 RNA levels

HIV-infected patients with HIV-1 RNA levels	HIV-1 RNA (copies/ml)	L-lysine (μmol/l)	Pearson correlation coefficient
5000–20 000 $n = 39$	12 400 ± 4432	313.2 ± 150.4	$r = -0.35$, $P < 0.028$ (S)
5000–30 000 $n = 54$	15 990 ± 7155	296.3 ± 151.5	$r = -0.32$, $P < 0.018$ (S)
5000–40 000 $n = 69$	21 190 ± 11 960	283.1 ± 154.0	$r = -0.35$, $P < 0.003$ (S)
5000–50 000 $n = 74$	24 900 ± 14 870	261.9 ± 134.8	$r = -0.43$, $P < 0.0001$ (S)
5000–75 000 $n = 105$	32 480 ± 23 070	265.9 ± 156.1	$r = -0.29$, $P < 0.002$ (S)
5000–100 000 $n = 120$	41 790 ± 35 000	261.6 ± 154.7	$r = -0.24$, $P < 0.007$ (S)
5000–250 000 $n = 160$	78 490 ± 76 600	256.0 ± 151.2	$r = -0.16$, $P < 0.048$ (S)
5000–500 000 $n = 186$	122 100 ± 133 500	250.6 ± 150.7	$r = -0.15$, $P < 0.035$ (S)
5000–1000 000 $n = 255$	180 900 ± 263 600	247.4 ± 142.4	$r = -0.12$, $P < 0.040$ (S)

(S), Differences were considered significant when $P < 0.05$. Values are given as mean ± SD (range).

but can be explained by the influence of amino acid deficiency on HIV reproduction. Excess of L-lysine concentration leads to active HIV replication and reduce the concentration of plasma amino acid.

We hypothesized that the disease progresses more rapidly among patients with the level of excess supply of this essential amino acid. We now intended that the excess of L-lysine leads to active HIV replication and reduce the concentration of plasma amino acid. To test these hypotheses, we measured levels of this essential amino acid, HIV-1 RNA and markers of immunological status in the groups of the elite controllers, slow-progressors and progressors among HIV-infected individuals. The lowest concentrations of essential amino acid were found in HIV elite controllers and slow-progressors. From these findings it may be assumed that if the infected cells contain a limited amount of L-lysine, synthesis of HIV virions could probably have been prevented or reduced to the level of latent infection. It is possible that this mechanism is used in HIV controllers.

In summary, our study seems to support the hypothesis that changes in plasma L-lysine play a key role in the synthesis of the virus proteins and in transcription initiation of the retrovirus life cycle. It sounds paradoxical, but excessive intake of this essential amino acid may increase the risk of high HIV-1 RNA levels, subsequent acceleration of immunosuppression and HIV progression, and further research is needed to understand the impact of L-lysine amino acid on HIV infection pathogenesis.

Material and Methods

Patients. We included 550 HIV-1-infected males (age range: 20–38 y; mean ± SD; 34 ± 5.9 y), who were periodically monitored in the Municipal Center of HIV/AIDS prophylaxis. We ascertained patients' general attributes (age, sex), medical history, and stage of HIV infection, co-infections and the simultaneous survey period, without antiretroviral therapy. Blood samples for this study were collected during routine clinical control and analytical monitoring, which included hematological, immunological and virological evaluation. Clinical stages of the disease were based on the 1993 revised guidelines for HIV-1 infection (Centers for Disease Control and Prevention). All patients, 550 HIV-1-infected males without highly active antiretroviral therapy (HAART), were classified into groups I (asymptomatic; A1, A2), II (symptomatic, non AIDS; B1, B2) and III (AIDS; C1, C2). Patients who were severely immunodepressed (A3, B3, and C3) were excluded from the study.

Group I: 290 patients were asymptomatic or had slight symptoms, non- and moderately immunodepressed (A1, A2).

Group II: 110 patients were moderately symptomatic and immunodepressed (B1, B2).

Group III: 50 patients were severely symptomatic and moderately immunodepressed (C1, C2).

Comparison groups: 100 subjects after introduction of HAART for more than two years, 25% HIV patients on nucleoside analog reverse-transcriptase inhibitors (NARTIs) and non-nucleoside reverse-transcriptase inhibitors (NNRTIs) therapy, 5% only on NARTIs and 70% HIV patients on NNRTIs and protease inhibitors (PIs) therapy (age range: 25–45 y, mean ± SD: 36 ± 5.1 y).

Reference values were established in healthy regional donors (age range: 23–45 y, mean ± SD: 34 ± 4.4 y) by measurement of plasma L-lysine concentrations ($n = 120$). Blood samples were obtained after informed consent from the patients.

Laboratory studies. Fasting venous blood samples for measurement of hematological and immunological parameters, HIV-1 RNA levels and amino-acid

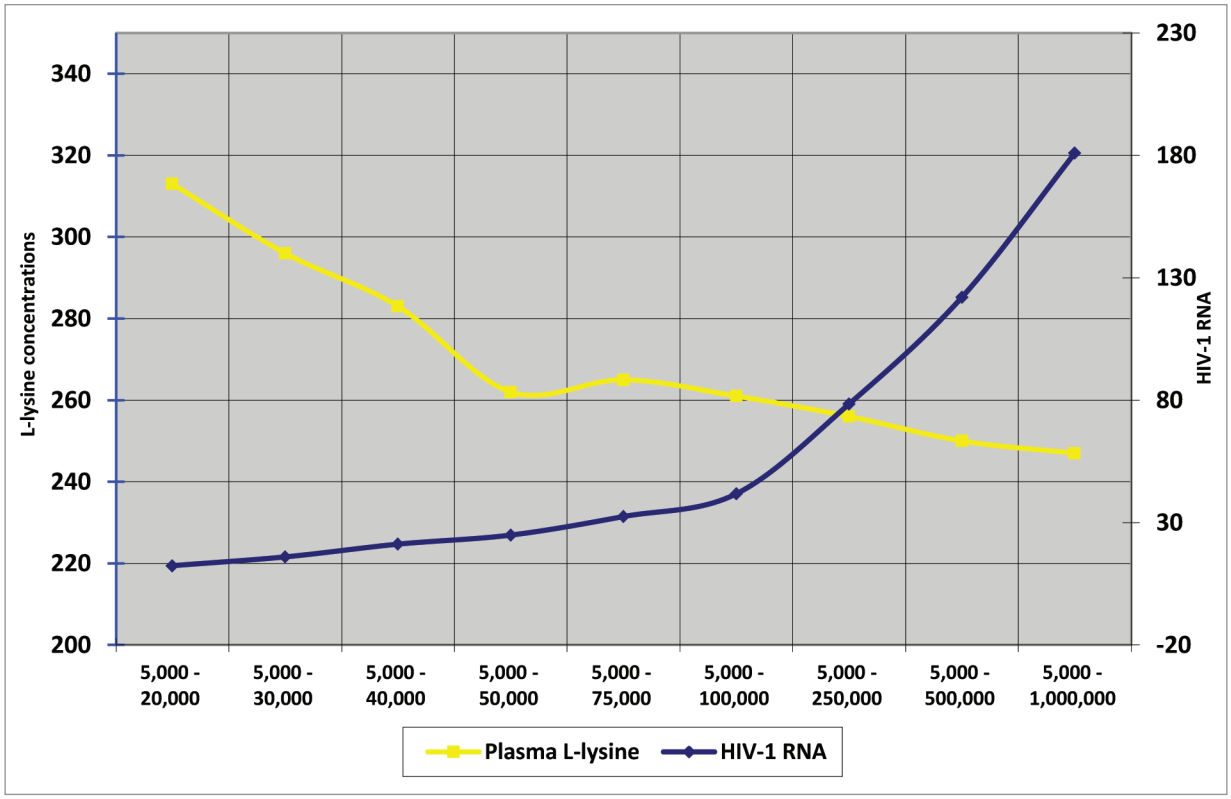

Figure 1. Plasma L-lysine in HIV-infected patients ranged by HIV-1 RNA levels.

profiles were collected in 5 ml tubes containing 1.6 mg/ml K^2 EDTA (BD Vacutainer®) and were centrifuged (3500 rpm; 10 min). For the analysis of L-lysine concentrations, venous blood was deproteinized with 3% sulphosalicylic acid, carefully mixed and immediately centrifuged (3500 rpm; 10 min) to remove plasma proteins. Aliquots, the supernatant of 100 µl, were pipetted into Eppendorf tubes, stored at −40 °C and analyzed within the following fortnight in the same laboratory.

Blood samples for measurement of HIV-1 RNA levels were immediately centrifuged (3500 rpm; 10 min), aliquots plasma of 1.0 ml were pipetted into Eppendorf tubes and stored at −40 °C until ready for use. For other hematological and immunological parameters, whole venous blood samples were immediately analyzed at the center laboratory.

Quantification HIV-1 RNA assay was performed by quantitative competitive (RT-PCR) reverse-transcriptase polymerase chain reaction using the commercially available Amplisens® HIV-monitor-FRT kit (Amplisens®) with a sensitivity limit of 500 HIV-1 RNA copies/ml and real-time PCR cycler (Rotor-Gene Q, QIAGEN).

A laboratory survey of the immune function was conducted by use of a flow cytometer according to the method, defining CD3, CD4, and CD8 lymphocytes count (Coulter Epics XL, Beckman Coulter).

The hematological levels of leukocytes, erythrocytes, platelets, hemoglobin, total protein, albumin, globulin, ALT, AST, creatinine, bilirubin, cholesterol, and glucose, were determined using standard techniques (ABX Micros 60, ABX Diagnostics), (SABA 18, AMS).

The amino acids were separated by the thin layer chromatography (TLC) method and L-lysine concentrations were detected spectrophotometrically after reaction with ninhydrin reagent.

Reference values of plasma L-lysine were established in apparently healthy donors. Low amino acid concentration was considered to be present when plasma L-lysine values were below and above one and a half standard deviations (SD) of our mean reference values (80 µmol/l < plasma L-lysine < 470 µmol/l). No significant differences in plasma amino acid concentrations were observed in repeat samples obtained from any given patient. The overall reproducibility of results was consistent to within ± 5%.

Body mass index (BMI) at baseline was calculated using the formula weight (kg)/(height[m])^2 and categorized as underweight (< 18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2).

Statistical analysis. Plasma L-lysine values follow a Gaussian distribution (Kolmogorov–Smirnov), so the Student *t* test was applied to compare patients' concentrations to reference values, as well as those found in patients in comparison groups, with a 95% confidence interval. The correlation between HIV-1 RNA and amino-acid concentrations was estimated by a Pearson correlation coefficient. Data are reported as mean ± SD (standard deviation), and *P* values below 0.05 were considered to indicate statistical significance. Statistical analyses were performed using the statistical software package Biostat®.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

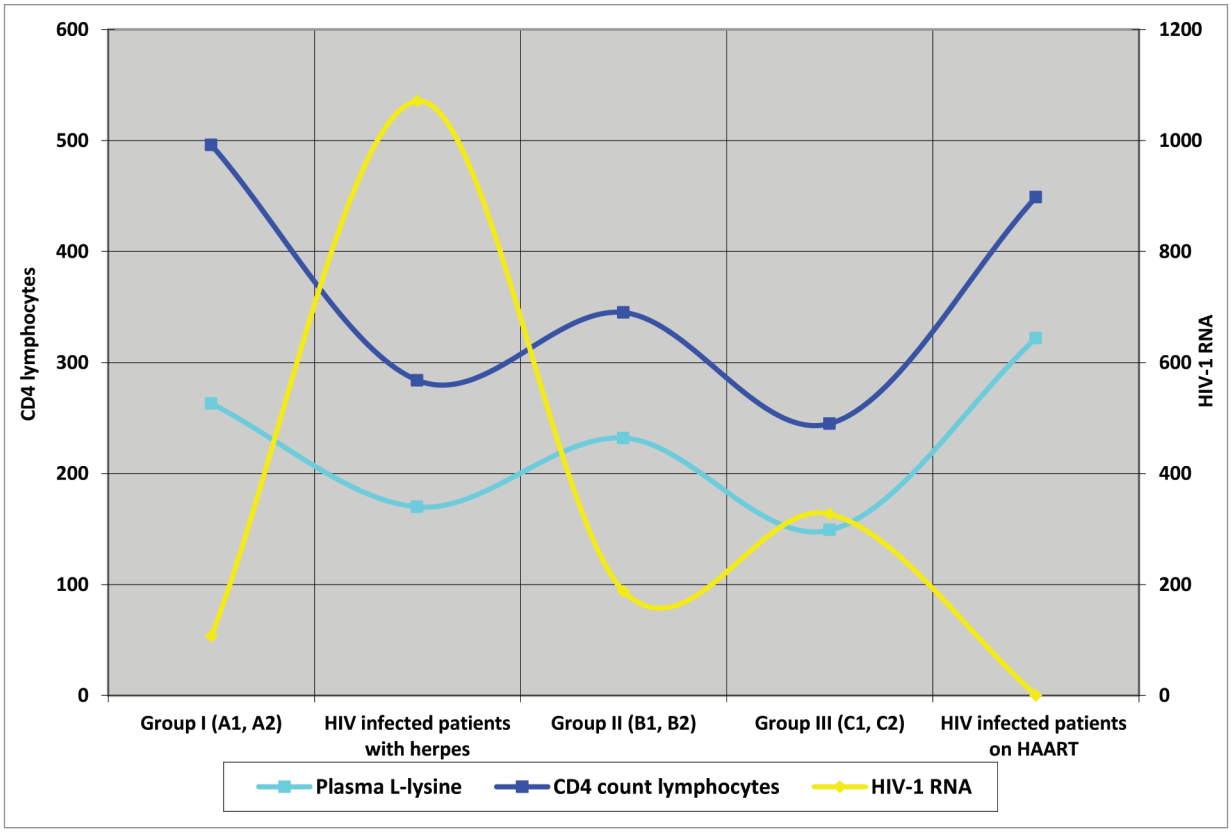

Figure 2. Correlation between the plasma L-lysine, HIV-1 RNA levels, and CD4 count lymphocytes in clinical A, B, and C stages HIV-infected patients.

Table 6. Compared plasma L-lysine concentrations, HIV-1 RNA levels and CD4 count lymphocytes in HIV-1 elite controllers, slow-progressors, and progressors

	HIV-1 elite controllers	HIV-1 slow-progressors	HIV progressors	Controls
L-lysine (μmol/l)	162.5 ± 116.4*,†	221.1 ± 130.8*,†	239.9 ± 148.9	275.8 ± 120.3
HIV-1 RNA levels (copies/ml)	550.1 ± 135.7†	2631.0 ± 2606†	186 500.0 ± 150 100.0	-
CD4 lymphocytes (cells/mm³)	549.6 ± 123.8*,†	476.7 ± 326.3*,†	352.0 ± 199.9*	1253.0 ± 230.3

*Patients vs. controls, $P < 0.01$; †Patients vs. HIV progressors, $P < 0.01$. Values are given as mean ± SD (range).

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References

1. Joint United Nations Programme on HIV/AIDS (UNAIDS). Report on the Global AIDS Epidemic (2012).
2. Joint United Nations Programme on HIV/AIDS (UNAIDS). World AIDS Day Report - Results (2012).
3. Grunfeld C, Feingold KR. Metabolic disturbances and wasting in the acquired immunodeficiency syndrome. N Engl J Med 1992; 327:329-37; PMID:1620172; http://dx.doi.org/10.1056/NEJM199207303270506
4. Kotler DP, Tierney AR, Brenner SK, Couture S, Wang J, Pierson RN Jr. Preservation of short-term energy balance in clinically stable patients with AIDS. Am J Clin Nutr 1990; 51:7-13; PMID:2296930
5. Macallan DC. Wasting in HIV infection and AIDS. J Nutr 1999; 129(Suppl):238S-42S; PMID:9915906
6. Kotler DP, Tierney AR, Wang J, Pierson RN Jr. Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. Am J Clin Nutr 1989; 50:444-7; PMID:2773823
7. Wheeler DA, Gibert CL, Launer CA, Muurahainen N, Elion RA, Abrams DI, Bartsch GE; Terry Beirn Community Programs for Clinical Research on AIDS. Weight loss as a predictor of survival and disease progression in HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol 1998; 18:80-5; PMID:9593462; http://dx.doi.org/10.1097/00042560-199805010-00012
8. Eck HP, Gmünder H, Hartmann M, Petzoldt D, Daniel V, Dröge W. Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. Biol Chem Hoppe Seyler 1989; 370:101-8; PMID:2784973; http://dx.doi.org/10.1515/bchm3.1989.370.1.101
9. de Quay B, Malinverni R, Lauterburg BH. Glutathione depletion in HIV-infected patients: role of cysteine deficiency and effect of oral N-acetylcysteine. AIDS 1992; 6:815-9; PMID:1418777; http://dx.doi.org/10.1097/00002030-199208000-00008
10. Fuchs D, Möller AA, Reibnegger G, Stöckle E, Werner ER, Wachter H. Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. J Acquir Immune Defic Syndr 1990; 3:873-6; PMID:2166783
11. Laurichesse H, Tauveron I, Gourdon F, Cormerais L, Champredon C, Charrier S, Rochon C, Lamain S, Bayle G, Laveran H, et al. Threonine and methionine are limiting amino acids for protein synthesis in patients with AIDS. J Nutr 1998; 128:1342-8; PMID:9687554
12. Abdollahi A, Shoar TS. Hyperhomocysteinemia in HIV-Infected Individuals: Correlation of a frequent prothrombotic factor with CD4+ cell count. Oman Med J 2012; 27:224-7; PMID:22811772; http://dx.doi.org/10.5001/omj.2012.50
13. Hortin GL, Landt M, Powderly WG. Changes in plasma amino acid concentrations in response to HIV-1 infection. Clin Chem 1994; 40:785-9; PMID:7909726

14. Beisel WR, Sawyer WD, Ryll ED, Crozier D. Metabolic effects of intracellular infections in man. Ann Intern Med 1967; 67:744-79; PMID:4964043; http://dx.doi.org/10.7326/0003-4819-67-4-744
15. Sherman IW. Amino acid metabolism and protein synthesis in malarial parasites. Bull World Health Organ 1977; 55:265-76; PMID:338183
16. Truswell AS, Hansen JD, Wannenburg P. Plasma tryptophan and other amino acids in pellagra. Am J Clin Nutr 1968; 21:1314-20; PMID:4235335
17. Hofford JM, Milakofsky L, Vogel WH, Sacher RS, Savage GJ, Pell S. The nutritional status in advanced emphysema associated with chronic bronchitis. A study of amino acid and catecholamine levels. Am Rev Respir Dis 1990; 141:902-8; PMID:2327654; http://dx.doi.org/10.1164/ajrccm/141.4_Pt_1.902
18. Jeevanandam M, Young DH, Ramias L, Schiller WR. Effect of major trauma on plasma free amino acid concentrations in geriatric patients. Am J Clin Nutr 1990; 51:1040-5; PMID:2349917
19. Pisters PW, Brennan MF. Amino acid metabolism in human cancer cachexia. [Review]. Annu Rev Nutr 1990; 10:107-32; PMID:2200459; http://dx.doi.org/10.1146/annurev.nu.10.070190.000543
20. Shiota T, Nakatsukasa H, Fujiwara M, Takei N, Yamauchi Y, Kobayashi M, Watanabe A, Nagashima H. Plasma amino acid imbalance in alcoholic liver cirrhosis. Biochem Med 1984; 32:181-8; PMID:6391476; http://dx.doi.org/10.1016/0006-2944(84)90071-1
21. Penttilä KE. Role of cysteine and taurine in regulating glutathione synthesis by periportal and perivenous hepatocytes. Biochem J 1990; 269:659-64; PMID:1975168
22. Wannemacher RW Jr., Pekarek RW, Bartelloni PJ, Vollmer RT, Beisel WR. Changes in individual plasma amino acids following experimentally induced and fly fever virus infection. Metabolism 1972; 21:67-76; PMID:4621526; http://dx.doi.org/10.1016/0026-0495(72)90021-2
23. Wigand R, Kümel G. Amino acid requirement of adenovirus multiplication. J Gen Virol 1978; 39:281-92; PMID:650177; http://dx.doi.org/10.1099/0022-1317-39-2-281
24. Tankersley RW Jr. Amino acid requirements of herpes simplex virus in human cells. J Bacteriol 1964; 87:609-13; PMID:14127578
25. Goldblum N, Ravid Z, Becker Y. Effect of withdrawal of arginine and other amino acids on the synthesis of tumour and viral antigens of SV 40 virus. J Gen Virol 1968; 3:143-6; PMID:4300565; http://dx.doi.org/10.1099/0022-1317-3-1-143
26. Everitt E, Sundquist B, Philipson L. Mechanism of the arginine requirement for adenovirus synthesis. I. Synthesis of structural proteins. J Virol 1971; 8:742-53; PMID:5002382
27. Inglis VBM. Requirement of arginine for the replication of herpes virus. J Gen Virol 1968; 3:9-17; PMID:4300568; http://dx.doi.org/10.1099/0022-1317-3-1-9
28. Loh PC, Oie HK. Role of lysine in the replication of reovirus: I. Synthesis of complete and empty virions. J Virol 1969; 4:890-5; PMID:16789122
29. Naito T, Irie H, Tsujimoto K, Ikeda K, Arakawa T, Koyama AH. Antiviral effect of arginine against herpes simplex virus type 1. Int J Mol Med 2009; 23:495-9; PMID:19288025; http://dx.doi.org/10.3892/ijmm_00000156
30. Yamasaki H, Tsujimoto K, Koyama AH, Ejima D, Arakawa T. Arginine facilitates inactivation of enveloped viruses. J Pharm Sci 2008; 97:3067-73; http://dx.doi.org/10.1002/jps.21224; PMID:18186461
31. Ikeda K, Yamasaki H, Suzuki Y, Koyama AH, Arakawa T. Novel strategy with acidic arginine solution for the treatment of influenza A virus infection. [Review]. Exp Ther Med 2010; 1:251-6; PMID:22993536
32. Steigmann F, Szanto PB, Poulos A, Lim PE, Dubin A. Significance of serum aminograms in diagnosis and prognosis of liver diseases. J Clin Gastroenterol 1984; 6:453-60; PMID:6501833; http://dx.doi.org/10.1097/00004836-198410000-00012
33. Brosnan JT. Interorgan amino acid transport and its regulation. J Nutr 2003; 133(Suppl 1):2068S-72S; PMID:12771367
34. Okamoto N, Miyagi Y, Chiba A, Akaike M, Shiozawa M, Imaizumi A, Yamamoto H, Ando T, Yamakado M, Tochikubo O. Diagnostic modeling with differences in plasma amino acid profiles between non-cachectic colorectal/breast cancer patients and healthy individuals. Int J Med Med Sci 2009; 1:1-8
35. Holm E, Sedlaczek O, Grips E. Amino acid metabolism in liver disease. Curr Opin Clin Nutr Metab Care 1999; 2:47-53; PMID:10453330; http://dx.doi.org/10.1097/00075197-199901000-00009
36. Maeda J, Higashiyama M, Imaizumi A, Nakayama T, Yamamoto H, Daimon T, Yamakado M, Imamura F, Kodama K. Possibility of multivariate function composed of plasma amino acid profiles as a novel screening index for non-small cell lung cancer: a case control study. BMC Cancer 2010; 10:690; PMID:21176209; http://dx.doi.org/10.1186/1471-2407-10-690
37. Zhang PC, Pang CP. Plasma amino acid patterns in cancer. Clin Chem 1992; 38:1198-9; PMID:1597002
38. Wannemacher RW Jr. Key role of various individual amino acids in host response to infection. Am J Clin Nutr 1977; 30:1269-80; PMID:407785
39. Schneider RJ, Shenk T. Impact of virus infection on host cell protein synthesis. Annu Rev Biochem 1987; 56:317-32; PMID:3113326; http://dx.doi.org/10.1146/annurev.bi.56.070187.001533
40. Willems M, Penman S. The mechanism of host cell protein synthesis inhibition by poliovirus. Virology 1966; 30:355-67; PMID:4288415; http://dx.doi.org/10.1016/0042-6822(66)90114-0
41. Kääriäinen L, Ranki M. Inhibition of cell functions by RNA-virus infections. Annu Rev Microbiol 1984; 38:91-109; PMID:6093688; http://dx.doi.org/10.1146/annurev.mi.38.100184.000515
42. Avin-Wittenberg T, Galili G. Metabolism: Amino acid regulatory wisdom. Nat Chem Biol 2012; 8:23-4; http://dx.doi.org/10.1038/nchembio.743; PMID:22173355
43. Fafournoux P, Bruhat A, Jousse C. Amino acid regulation of gene expression. Biochem J 2000; 351:1-12; PMID:10998343; http://dx.doi.org/10.1042/0264-6021:3510001
44. Kilberg MS, Hutson RG, Laine RO. Amino acid-regulated gene expression in eukaryotic cells. FASEB J 1994; 8:13-9; PMID:8299885
45. Caldara M, Minh PN, Bostoen S, Massant J, Charlier D. ArgR-dependent repression of arginine and histidine transport genes in Escherichia coli K-12. J Mol Biol 2007; 373:251-67; PMID:17850814; http://dx.doi.org/10.1016/j.jmb.2007.08.013
46. Calvo JM, Matthews RG. The leucine-responsive regulatory protein, a global regulator of metabolism in Escherichia coli. Microbiol Rev 1994; 58:466-90; PMID:7968922
47. Cho BK, Federowicz S, Park YS, Zengler K, Palsson BØ. Deciphering the transcriptional regulatory logic of amino acid metabolism. Nat Chem Biol 2012; 8:65-71; http://dx.doi.org/10.1038/nchembio.710; PMID:22082910
48. Tisné C, Roques BP, Dardel F. The annealing mechanism of HIV-1 reverse transcription primer onto the viral genome. J Biol Chem 2004; 279:3588-95; PMID:14602716; http://dx.doi.org/10.1074/jbc.M310368200
49. Yu Q, Morrow CD. Essential regions of the tRNA primer required for HIV-1 infectivity. Nucleic Acids Res 2000; 28:4783-9; PMID:11095691; http://dx.doi.org/10.1093/nar/28.23.4783
50. Guo F, Cen S, Niu M, Javanbakht H, Kleiman L. Specific inhibition of the synthesis of human lysyl-tRNA synthetase results in decreases in tRNA(Lys) incorporation, tRNA(3)(Lys) annealing to viral RNA, and viral infectivity in human immunodeficiency virus type 1. J Virol 2003; 77:9817-22; PMID:12941890; http://dx.doi.org/10.1128/JVI.77.18.9817-9822.2003