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Lipolytic sensitivity to catecholamines in patients with human immunodeficiency virus infection

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ABSTRACT Lipolysis is higher in patients with acquired immunodeficiency syndrome (AIDS) than in healthy control subjects. To evaluate whether this increase in lipolysis is related to increased \( \beta \)-adrenergic sensitivity, we compared the lipolytic response to epinephrine (\( \approx 15 \) ng \( \cdot \) kg\(^{-1} \) \cdot min\(^{-1} \)) of six AIDS patients with that of six matched control subjects. Lipolysis was measured by infusion of \([2H]_{2}\)glycerol and \([2H]_{2}\)palmitate. The baseline rates of appearance of palmitate (2.06 \( \pm \) 0.21 compared with 1.45 \( \pm \) 0.07 \( \mu \)mol \( \cdot \) kg\(^{-1} \) \cdot min\(^{-1} \)) and glycerol (2.35 \( \pm \) 0.16 compared with 1.35 \( \pm \) 0.06 \( \mu \)mol \( \cdot \) kg\(^{-1} \) \cdot min\(^{-1} \)) were higher in AIDS patients (\( P < 0.05 \)). The absolute increase in lipolysis, an indicator of the responsiveness to epinephrine, was higher whereas the lipolytic response to epinephrine was normal in AIDS patients. Increased lipolytic sensitivity to catecholamines is not the cause of increased lipolysis in AIDS.


INTRODUCTION

An increase in whole-body lipolysis has been reported as one of the metabolic consequences of human immunodeficiency virus (HIV) infection (1). The mechanism responsible for this increase in lipolytic activity is not known. Plasma insulin and catecholamine concentrations, the major hormones responsible for regulating lipolysis in humans (2–4), are normal in patients with uncomplicated HIV infection (1, 5–7). Therefore, the increase in lipolytic rates may be related to resistance of adipose tissue to insulin action or increased sensitivity of adipose tissue to \( \beta \)-adrenergic stimulation. It seems unlikely that insulin resistance contributes to increased lipolytic activity in acquired immunodeficiency syndrome (AIDS) because insulin sensitivity, at least with respect to glucose metabolism, is higher not lower in AIDS patients (8). Alternatively, increased sensitivity of adipose tissue to \( \beta \)-adrenergic stimulation may be involved in the pathogenesis of increased lipolysis in AIDS.

The purpose of the present study was to evaluate whole-body lipolytic sensitivity to catecholamines in vivo in patients with HIV infection. We hypothesized that lipolytic sensitivity would be higher in HIV-infected patients and therefore contribute to the increase in lipolytic rates observed previously. The rates of appearance (\( R_{a} \)) in plasma of glycerol (an index of whole-body lipolysis) and palmitate (an index of fatty acid release from adipose tissue) were measured by infusion of stable isotopes of glycerol and palmitate during basal conditions and during epinephrine infusion in patients with HIV infection and in healthy volunteers.

SUBJECTS AND METHODS

Subjects

Six male subjects with HIV infection (CDC-IV stage) [age 46 \( \pm \) 5 y; weight 75 \( \pm \) 2 kg; fat-free mass (FFM) 58 \( \pm \) 2 kg; height 178 \( \pm \) 2 cm; CD4\(^{+}\) lymphocytes: median 50 \( \times \) 10\(^6\)/L, range: 10–190 \( \times \) 10\(^6\)/L] and six healthy male volunteers (age 50 \( \pm \) 4 y, weight 73 \( \pm \) 3 kg, FFM 56 \( \pm \) 2 kg, and height 177 \( \pm \) 2 cm) participated in this study. All subjects were weight-stable and did not have active opportunistic disease. The patients were voluntarily recruited from our outpatient clinic; the healthy control subjects were recruited from hospital staff. Patients who had fever (temperature > 37.5 °C); diarrhea; renal, hepatic, or endocrine disease; malignancies other than Kaposi’s sarcoma of the skin; weight loss; or clinically active opportunistic infection in the 2 mo before the present study were excluded. No patient used any drug other than cotrimoxazole (Roche, Mijdrecht, Netherlands) or zidovudine (Glaxo Wellcome, Zeist, Netherlands). All participants were consuming a weight-maintaining diet containing \( \approx 250 \) g carbohydrate during the 3 d preceding the study. The study protocol was approved by the Institutional Medical Ethical Committee and the Research Committee. Written informed consent was obtained from all participants.

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Study design

Subjects were admitted to the metabolic research unit the evening before the isotope-infusion study was performed. After consuming an evening meal at 1800, subjects fasted until the end of the isotope-infusion study. Body composition was measured with a bioelectrical impedance analyzer (BIA 109; Akern, Florence, Italy) (9) in the morning before the start of the isotope-infusion study. At 0745 a 16-gauge catheter was inserted in an antecubital vein of the left lower arm to infuse isotope tracers and epinephrine. Another catheter, inserted retrogradely in a right dorsal wrist vein, was kept in a thermostatted (65 °C) box and was used to sample arterialized venous blood (10). Both catheters were kept patent by infusion of 0.65% NaCl (30 mL/h). After blood samples were taken for determination of background isotope enrichment, intravenous infusions of [2H2]palmitate bound to albumin (∼0.04 μmol·kg⁻¹·min⁻¹) and [2H2]glycerol (∼1.6 μmol/kg priming dose and ∼0.11 μmol·kg⁻¹·min⁻¹ continuous infusion) were started and continued for 120 min. Exact infusion rates were determined for each subject by measuring tracer concentrations in the infuses.

Epinephrine (∼15 ng·kg⁻¹·min⁻¹, or ∼85 pmol·kg⁻¹·min⁻¹) was infused during the last 60 min of isotope infusion. This specific dose of epinephrine was chosen because it results in adequate stimulation of lipolysis at physiologic plasma concentrations of epinephrine (11). Blood samples were obtained at 45, 50, 55, and 60 min of isotope infusion to determine basal lipid kinetics and every 5 min during epinephrine infusion to determine the lipolytic response to epinephrine. Blood samples for plasma ketone body, triacylglycerol, and hormone concentrations were obtained every 15 min during epinephrine infusion. Blood pressure and heart rate were monitored every 10 min.

All samples were put immediately in an ice bath. Plasma was separated by centrifugation at 1500 × g for 10 min at 4 °C within 10 min and stored at −20 °C until analyzed.

Analysis

Samples for catecholamine analysis were collected in 5-mL glass tubes containing reduced glutathione and ethyleneglycol-bis-(2-aminoethyl) tetraacetic acid. Plasma epinephrine concentrations, infused epinephrine concentrations, and plasma norepinephrine concentrations were determined by HPLC and electrochemical detection. Plasma triacylglycerol, glycerol, acetoacetate, and butyrate concentrations were measured by enzymatic methods (Boehringer Mannheim, Almere, Netherlands) on a Cobas Bio centrifugal analyzer (Roche). Plasma palmitic acid and total fatty acid concentrations in plasma were quantified by gas chromatography (12). Radioimmunoassays were used to measure plasma concentrations of insulin (Pharmacia Diagnostics, Uppsala, Sweden), C-peptide (RIA-mat C-Peptid; Mallinckrodt Diagnostica, Dietzenbach, Germany), thyroxine, and triiodothyronine (in-house assay). Plasma cortisol was determined by fluorescence polarization immunoassay on a Technical Device X (Abbott Laboratories, North Chicago).

Blood for analysis of palmitate and glycerol enrichment was collected in prechilled tubes with heparin. Isotope enrichment of palmitic acid and glycerol in plasma was determined by gas chromatography–mass spectrometry using an MSD 5971 system (Hewlett-Packard, Palo Alto, CA) as described previously (11). A trimethylsilyl derivative of glycerol was generated and glycerol enrichment was determined by monitoring ions at mass-to-charge ratios (m/z) of 205.1, 206.1, 207.1, and 208.1. A methyl ester of palmitate was formed and palmitate enrichment was determined by selectively monitoring ions at m/z 270.2, 271.2, and 272.2.

Calculations

Steele’s equation for steady state conditions adjusted for stable isotopes (13) was used to calculate baseline R5, for glycerol and palmitate. During epinephrine infusion, glycerol and palmitate R5 were calculated by using the Steele equation for nonsteady state kinetics. The effective volume of distribution of palmitate was assumed to be 40 mL/kg body wt and that of glycerol to be 235 mL/kg. Enrichment and concentration data obtained during epinephrine infusion were smoothed by spline fitting (14) and substrate kinetics were calculated by using these smoothed data.

Statistical analysis

Data are presented as means ± SEs. Baseline values were compared by using Student’s t test for unpaired observations. Changes related to time and differences in these changes between groups were tested by repeated-measurements analysis of variance (two-way ANOVA) and the Newman-Keuls test. Statistical analysis was performed by using the Number Cruncher Statistical System (NCSS, Kaysville, UT). A P value < 0.05 was considered statistically significant.

RESULTS

Basal data

Plasma concentrations of epinephrine, norepinephrine, insulin, C-peptide, cortisol, and thyroid hormones were not significantly different between groups (Table 1). Plasma concentrations of palmitate were 35% higher in AIDS patients than in control subjects although the plasma concentrations of glycerol were not significantly different between groups. Plasma concentrations of ketone bodies (given as the sum of β-hydroxybutyrate and acetoacetate) were 125% higher in AIDS patients than in control subjects. Plasma concentrations of triacylglycerol were not significantly different between groups at baseline (Table 2).

Palmitate R5 expressed per kg body weight in patients with AIDS compared with the control group (2.06 ± 0.21 and 1.45 ± 0.07 μmol·kg⁻¹·min⁻¹, respectively) and per kg fat mass (8.6 ± 0.9 and 6.8 ± 0.4 μmol·kg fat⁻¹·min⁻¹, respectively) were 42% and 26% higher in patients with AIDS than in the control group (P < 0.05). Glycerol R5 expressed per kg body weight in AIDS patients and control subjects (2.35 ± 0.16 and 1.35 ± 0.06 μmol·kg⁻¹·min⁻¹, respectively) and per kg fat mass (9.5 ± 1.0 and 6.4 ± 0.5 μmol·kg fat⁻¹·min⁻¹, respectively) were 74% and 48% higher, respectively, in patients with AIDS than in control subjects (P < 0.05).

Epinephrine infusion data

The infusion rate of epinephrine was not different between patients with AIDS (61.4 ± 5.1 pmol·kg⁻¹·min⁻¹) and the
control group (65.6 ± 2.1 pmol·kg⁻¹·min⁻¹). Plasma epinephrine concentrations increased significantly during the infusion of epinephrine in both AIDS patients and control subjects (Figure 1) but was not significantly different between the two study groups at any time point during epinephrine infusion.

Pulse rate increased by ~10% in both groups during epinephrine infusion. None of the subjects noticed palpitations or tremor. Plasma concentrations of other regulatory hormones did not change significantly during epinephrine infusion (Table 1).

The relative contribution of palmitate to total fatty acids in plasma was not significantly different between patients with AIDS and the control group and remained constant throughout the administration of epinephrine (at t = 0: 26.3 ± 0.9% for AIDS patients and 28.6 ± 0.8% for control subjects; at t = 60: 26.3 ± 0.9% for AIDS patients and 28.6 ± 0.8% for control subjects). Therefore, palmitate Rₚ was directly related to fatty acid Rₚ throughout the study and all our data are presented as palmitate Rₚ.

Plasma palmitate and glycerol concentrations increased transiently in both patients with AIDS and in control subjects during epinephrine infusion (Figures 2 and 3). Although the absolute plasma palmitate and glycerol values were higher in patients with AIDS than in the control group, the increase above baseline was not significantly different between both groups. Epinephrine caused a 120% increase in plasma ketone body concentrations in both groups (Table 2). Plasma triacylglycerol concentrations did not change during the infusion of epinephrine (Table 2).

After the start of epinephrine infusion, palmitate Rₚ reached a peak value at 20 min in both groups (Figure 2). The peak value was significantly higher in patients with AIDS than in control subjects (4.28 ± 0.45 compared with 3.35 ± 0.18 μmol·kg⁻¹·min⁻¹, P < 0.05). After 15 min of epinephrine infusion, peak glycerol Rₚ values were reached in both groups (Figure 3). Peak glycerol Rₚ values were significantly higher in patients with AIDS than in control subjects (4.75 ± 0.54 compared with 3.28 ± 0.19 μmol·kg⁻¹·min⁻¹, P < 0.05). Both palmitate and glycerol Rₚ values were reached in both groups (Figures 2 and 3). Total areas under the curve from t = 0 to t = 60 of palmitate Rₚ (211 ± 18 μmol·min/L for AIDS patients and 162 ± 6 μmol·min/L for control subjects, P < 0.05) and glycerol Rₚ (219 ± 18 μmol·min/L for AIDS patients and 139 ± 5 μmol·min/L for control subjects, P < 0.05) were significantly larger in AIDS patients than in control subjects. However, the total increase in palmitate Rₚ and glycerol Rₚ above baseline was not significantly different between patients with AIDS (86 ± 14 and 79 ± 13 μmol·L⁻¹·min⁻¹, respectively) and control subjects (75 ± 7 and 59 ± 6 μmol·L⁻¹·min⁻¹, respectively).

**DISCUSSION**

The results of the present study indicate that basal lipolytic rates were higher in patients with AIDS although plasma concentrations of the major hormones that affect lipolysis, epinephrine and insulin, were not significantly different between

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**TABLE 1**

<table>
<thead>
<tr>
<th>Hormone concentration</th>
<th>AIDS patients (n = 6)</th>
<th>Control subjects (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t = 0</td>
<td>t = 60</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>43 ± 6</td>
<td>63 ± 10</td>
</tr>
<tr>
<td>C-peptide (nmol/L)</td>
<td>0.68 ± 0.12</td>
<td>0.84 ± 0.15</td>
</tr>
<tr>
<td>Cortisol (μmol/L)</td>
<td>0.30 ± 0.04</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>Thyroxine (nmol/L)</td>
<td>102 ± 8</td>
<td>ND</td>
</tr>
<tr>
<td>Triiodothyronine (nmol/L)</td>
<td>1.56 ± 0.15</td>
<td>ND</td>
</tr>
<tr>
<td>Norepinephrine (nmol/L)</td>
<td>0.81 ± 0.10</td>
<td>1.15 ± 0.15</td>
</tr>
<tr>
<td>Epinephrine (nmol/L)</td>
<td>0.18 ± 0.05</td>
<td>1.27 ± 0.15</td>
</tr>
</tbody>
</table>

^1 t ± SE. AIDS, acquired immunodeficiency syndrome. 
^2 Significantly different from control subjects, P < 0.05.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Baseline substrate concentrations^1</th>
<th>AIDS patients (n = 6)</th>
<th>Control subjects (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate (mmol/L)</td>
<td>0.19 ± 0.02^2</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>Fatty acids (mmol/L)</td>
<td>0.69 ± 0.06^2</td>
<td>0.50 ± 0.03</td>
</tr>
<tr>
<td>Glycerol (μmol/L)</td>
<td>50 ± 5</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Ketone bodies (mmol/L)</td>
<td>0.31 ± 0.04^2</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/L)</td>
<td>1.34 ± 0.27</td>
<td>1.17 ± 0.20</td>
</tr>
</tbody>
</table>

^1 t ± SE. AIDS, acquired immunodeficiency syndrome. 
^2 Significantly different from control subjects, P < 0.05.
The mechanism responsible for the increase in basal lipolysis in patients with AIDS is not clear. The major hormonal regulators of lipolysis are catecholamines and insulin. Although differences in insulin's antilipolytic effect on adipose tissue could explain the differences in lipolysis observed in our two study groups, we believe this explanation is unlikely. Plasma insulin and C-peptide concentrations in our AIDS patients were not different from those in our healthy control subjects. Further investigation is needed to understand the underlying mechanisms.

AIDS patients and control subjects. In addition, our data show that increased adipose tissue sensitivity to β-adrenergic stimulation was not responsible for the increase in basal lipolytic activity. During epinephrine infusion, absolute lipolytic rates were higher in patients with AIDS than in normal subjects but the increase above baseline was not significantly different between groups.

**FIGURE 2.** Plasma concentrations of palmitate, palmitate enrichment, and palmitate rate of appearance. ○, acquired immunodeficiency syndrome (AIDS) patients; □, control subjects; ■ and ● are significantly different from baseline values in AIDS patients and control subjects, respectively. *Baseline value for AIDS patients significantly different from control subjects, \( P < 0.05. \) \( P \) values of the response from \( t = 5 \) to \( t = 60 \) are given at the top of each panel.

**FIGURE 3.** Plasma concentrations of glycerol, glycerol enrichment, and glycerol rate of appearance. ○, acquired immunodeficiency syndrome (AIDS) patients; □, control subjects; ■ and ● are significantly different from baseline values in AIDS patients and control subjects, respectively. *Baseline value for AIDS patients significantly different from control subjects, \( P < 0.05. \) \( P \) values of the response from \( t = 5 \) to \( t = 60 \) are given at the top of each panel.
thermore, we have found that insulin sensitivity, at least with respect to glucose metabolism, is actually increased in patients with AIDS (8). Therefore, it seems unlikely that lipolytic activity in our patients was resistant to insulin action.

Lipolysis is also stimulated by cortisol (15). In the present study, plasma concentrations of cortisol were not different between AIDS patients and control subjects. Although the plasma concentration of cortisol in HIV infection has been reported to be slightly higher in some reports (16, 17), other studies showed normal plasma concentrations of cortisol in HIV infection (18). Although we cannot exclude the possibility of a small difference in cortisol concentrations between our study groups, it seems unlikely that the considerable increase in basal lipolytic rates in AIDS patients is merely explained by the effects of cortisol.

Locally secreted substances, such as adenosine and cytokines like tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), interferon α (IFN-α), and interferon γ (IFN-γ) are well-known modulators of lipolysis (19–23). Adenosine is an inhibitor of lipolysis, so decreased secretion of adenosine would increase lipolytic rates (24, 25). However, there are no reported studies that have evaluated adenosine secretion in HIV. TNF-α, IL-1, IFN-α, and IFN-γ are cytokines with potent lipolysis-stimulating properties (19–21, 24). Increased plasma concentrations of TNF-α, increased ex vivo production of TNF-α by isolated HIV-infected monocytes, and increased concentrations of soluble TNF receptors have been reported in patients with AIDS (26–28). In addition, the production of IFN-α and IFN-γ is higher in patients with AIDS and plasma concentrations of IFN-α are correlated with alterations in triacylglycerol concentrations in AIDS (29–31). Therefore, increased local production of TNF-α, IL-1, IFN-α, or IFN-γ provides a possible explanation for the higher lipolysis in AIDS.

In previous studies, it was shown that plasma concentrations of triacylglycerols were significantly higher in AIDS patients, with the highest values found in patients with advanced disease or active opportunistic infections (32–34). In the present study, the fasting plasma concentrations of triacylglycerols were not significantly different between AIDS patients and control subjects, probably because of the relatively small number of patients and exclusion of patients with active opportunistic disease. In the fasted state, triacylglycerols in plasma originate from very-low-density-lipoprotein synthesis in the liver. These triacylglycerols are formed by reesterification of fatty acids derived from plasma and, to a small extent, from de novo synthesis using other precursors. Although de novo lipid synthesis is increased in AIDS patients (35), reesterification still accounted for > 95% of lipogenesis in these patients. Therefore, increased reesterification of fatty acids derived from lipolysis is probably the major mechanism for increased concentrations of triacylglycerols in AIDS.

Our study underscores the importance of using isotope tracers rather than relying on plasma substrate concentrations alone when evaluating substrate metabolism. Plasma glycerol and palmitate concentrations were not significantly different in patients with AIDS compared with normal subjects during epinephrine infusion. However, analysis of the kinetic data showed significant differences between our two study groups.

It may be argued that an increase in lipolytic sensitivity to basal catecholamine concentrations occurs despite an increase in lipolytic sensitivity to infused catecholamines. Although we cannot exclude this possibility in our study, it seems to be unlikely. In vitro studies do not provide evidence for the existence of increased sensitivity to catecholamines at basal concentrations but normal sensitivity at slightly higher catecholamine concentrations. Rather, lipolytic sensitivity is higher at a wide range of catecholamine concentrations (36–38).

In summary, we found that patients with AIDS have high basal lipolytic rates despite the presence of normal plasma catecholamine and insulin concentrations and normal adipose tissue sensitivity to β-adrenergic stimulation. Therefore, increased basal lipolysis in patients with AIDS is not caused by changes in adrenergic system function.

REFERENCES


