Resting energy expenditure and substrate oxidation in human immunodeficiency virus (HIV)–infected asymptomatic men: HIV affects host metabolism in the early asymptomatic stage\textsuperscript{1,2}

Mirjam JT Hommes, Johannes A Romijn, Erik Endert, and Hans P Sauerwein

ABSTRACT To study the effect of persistent human immunodeficiency virus (HIV) infection on host metabolism, we performed indirect calorimetry in 11 asymptomatic HIV-infected patients (Centers for Disease Control group II or III) who were seropositive for ≥ 1 y, but who still had normal numbers of circulating CD4\textsuperscript{+} T cells, and in 11 healthy control subjects of similar age and relative body composition. HIV-infected patients had 8\% (P < 0.05) higher rates of resting energy expenditure than did control subjects. Fat-oxidation rates were significantly higher in the patients (\(\bar{x} \pm SE: 2.90 \pm 0.08\) vs 2.19 \(\pm 0.17\) g \(\cdot\) kg FFM\(^{-1}\) \(\cdot\) d\(^{-1}\)). patients vs control subjects, \(P < 0.01\)) whereas no significant differences in carbohydrate-oxidation rates between patients and control subjects were found. These alterations in metabolism were not associated with increased concentrations of catecholamines, cortisol, or thyroid hormones. Mean concentrations of interleukin 6 in the patients were increased only twofold when compared with healthy control subjects. The results indicate that HIV infection affects host metabolism in the early asymptomatic stage, before CD4\textsuperscript{+} T cell numbers start to decline. Am J Clin Nutr 1991;54:311–5.

KEY WORDS Cachexia, energy metabolism, endocrinology, HIV seropositivity, interleukins

Introduction

Severe weight loss is a common phenomenon in patients with acquired immune deficiency syndrome (AIDS) (1, 2). In addition to malabsorption and inadequate food intake, metabolic alterations resulting in increased energy expenditure are a possible cause for weight loss in AIDS (3). Previously, we reported a 9\% increase of resting energy expenditure (REE) in clinically stable patients with AIDS or AIDS-related complex (ARC) (4). Because the patients in that study were free of clinically active (opportunistic) infections for ≥ 2 mo, it seemed likely that the infection with the human immunodeficiency virus (HIV) was the cause of the hypermetabolism. However, it could not be excluded that the hypermetabolism in those patients was caused by subclinical (opportunistic) infections. If the metabolic alterations resulting in increased REE are caused by the HIV infection itself, they might already be detectable in the early asymptomatic stages. At present, no studies on the influence of asymptomatic HIV infection on host metabolism have been published.

The purpose of the present study was twofold: first, to investigate whether REE is already increased in the early asymptomatic stage of HIV infection and second, to study the relative contributions of carbohydrate and fat oxidation to REE in these patients.

Subjects and methods

Subjects

Eleven consecutive male HIV-infected outpatients (aged 23–50 y) who were clinically asymptomatic, classified as group II or III of the classification of the Centers for Disease Control (CDC) (5), and who had normal absolute CD4\textsuperscript{+} T-cell numbers (normal range: 0.4–1.58 \(\times\) \(10^3\)/L) were studied. Patients with fever (\(>\) 37.8 °C), impaired kidney function (creatinine > 95 \(\mu\)mol/L), or known endocrinologic disease (thyroid disease or diabetes mellitus) were excluded. Eleven healthy, male volunteers (aged 25–51 y) with normal physical examination and medical history served as a control group. These 11 control subjects were also part of another study of REE performed in our laboratory (4). None of the participants used any medication.

The protocol was approved by the Medical Ethical Committee of the Academic Medical Center and participants gave informed consent.

Experimental protocol

Studies were done under strictly standardized conditions in a quiet, temperature-controlled (22 °C) room with participants in the postabsorptive state (10–14 h) confined to bed. After an equilibration period of 2 h, indirect calorimetry was performed between 1000 and 1100.

Afterwards, body composition was measured and blood samples were taken for measurements of concentrations of albumin.

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retinol-binding protein, transferrin, hormones, and interleukin 6 (IL-6). Urine was collected during the test (4 h) and analyzed for nitrogen. Five patients and nine control subjects kept 5-d diet records at home (as part of an ongoing longitudinal study of nutritional habits), which were analyzed by a hospital dietitian.

Methods

Indirect calorimetry to determine whole-body substrate oxidation rates was performed as described elsewhere (6), by use of a ventilated hood with a mass flow meter, a zirconium oxygen sensor, and an infrared absorption carbon dioxide analyzer (model 2900, Computerized Energy Measurement System, Sensor Medics, Anaheim, CA) (7). Oxygen consumption and carbon dioxide production were measured continuously for >30 min. To allow for adaptation to the hood, the results of the first 10 min were discarded. During these measurements the room was darkened and subjects were asked to close their eyes.

Body composition was measured with a body-electrical impedance analyzer (BIA 109, Akern, Florence, Italy). The principle of this method is that the conductivity of an electric current is proportional to the amount of fat-free mass (FFM), i.e., the amount of water and electrolytes in the body (8). Studies have shown very good correlations of bioelectric-impedance measurements with total body water and FFM determined from body density in healthy subjects, and the BIA is being used for determination of body fat and FFM (9).

Analyzes

Isolation of leukocytes, cell separations, and lymphocyte-subpopulation determinations were performed as described elsewhere (10). Plasma albumin was quantified by a SMAC-II Technicon AutoAnalyzer (Tarrytown, NY). A radial immunodiffusion technique was used to measure concentrations of transferrin and retinol-binding protein (Behring Diagnostics, La Jolla, CA). Plasma concentrations of thyroxine and triiodothyronine were measured by in-house radioimmunoassays (RIAs) (11), catecholamines by in-house radioenzymatic method (12), and cortisol by fluorescence polarization immunoassay on TDx (Abbott Laboratories, North Chicago, IL). The LA Aarden's B9-cell assay (13) was used to determine concentrations of IL-6 in heat-inactivated serum samples. Total urinary nitrogen was measured by the microKjeldahl technique.

Calculations and statistics

REE was calculated from oxygen consumption, carbon dioxide production, and urinary nitrogen excretion, according to published methods (14, 15). To correct for the differences in REE attributable to differences in FFM (16), rates of REE were compared by analysis of covariance.

Caloric intake was calculated from the food table with the use of a computer program (VOEDING, Bazis, Leiden, The Netherlands) (17).

All data are expressed as mean ± SE. Student’s unpaired t test was used to compare mean group values where appropriate.

Results

The clinical and laboratory findings of the subjects studied are summarized in Table 1. Average age and relative body composition were not significantly different between patients and control subjects. Average height and body weight were significantly lower in the patients than in control subjects (height: 176 ± 2 vs 182 ± 1 cm, respectively, P < 0.05; weight: 66.7 ± 2.0 vs 78.1 ± 2.1 kg, respectively, P < 0.001). The difference in average body weight was also reflected in absolute FFM (55.8 ± 1.2 and 62.9 ± 1.9 kg, respectively, P < 0.01). However, when expressed as percentages of total body weight, no differences between patients and control subjects in body fat (15.9 ± 1.5% and 19.3 ± 1.6%, respectively) and FFM (84.1 ± 1.5% and 80.7 ± 1.6%, respectively) were found.

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>As percentage</th>
<th>Absolute</th>
<th>Lymphocytes *</th>
<th>CD4+ cells †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y</td>
<td>cm</td>
<td>kg</td>
<td>%</td>
<td>kg</td>
<td>×10⁹/L</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>34</td>
<td>177</td>
<td>81.6</td>
<td>72.0</td>
<td>59.0</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>161</td>
<td>61.0</td>
<td>81.7</td>
<td>49.8</td>
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</tr>
<tr>
<td>3</td>
<td>33</td>
<td>182</td>
<td>68.0</td>
<td>85.3</td>
<td>58.0</td>
<td>2.9</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>179</td>
<td>71.0</td>
<td>87.7</td>
<td>62.2</td>
<td>2.1</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>178</td>
<td>66.5</td>
<td>83.4</td>
<td>55.4</td>
<td>2.2</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>182</td>
<td>63.8</td>
<td>85.9</td>
<td>54.8</td>
<td>2.4</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>177</td>
<td>67.5</td>
<td>80.4</td>
<td>54.2</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>178</td>
<td>60.5</td>
<td>88.0</td>
<td>53.2</td>
<td>2.3</td>
<td>0.7</td>
</tr>
<tr>
<td>9</td>
<td>41</td>
<td>179</td>
<td>72.5</td>
<td>86.1</td>
<td>62.4</td>
<td>1.9</td>
<td>0.8</td>
</tr>
<tr>
<td>10</td>
<td>32</td>
<td>181</td>
<td>61.5</td>
<td>83.5</td>
<td>51.3</td>
<td>1.8</td>
<td>0.7</td>
</tr>
<tr>
<td>11</td>
<td>31</td>
<td>166</td>
<td>59.5</td>
<td>90.8</td>
<td>54.0</td>
<td>2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Control subjects (n = 11)</td>
<td>32 ± 3</td>
<td>182 ± 1</td>
<td>78.1 ± 2.1</td>
<td>80.7 ± 1.6</td>
<td>62.9 ± 1.9</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Normal range 1.2–3.6 × 10⁹/L.
† Normal range 0.4–1.6 × 10⁹/L.
‡‡‡ Significantly different from control subjects: §P < 0.05, §§P < 0.001, §§§P < 0.01.
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TABLE 2
Fat- and carbohydrate-oxidation rates and REE in HIV-infected patients and control subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fat oxidation</th>
<th>Carbohydrate oxidation</th>
<th>Nitrogen-excretion</th>
<th>REE</th>
<th>Caloric intake</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Per day</td>
<td>Per FFM per day</td>
<td>g/d</td>
<td>g · kg FFM⁻¹ · d⁻¹</td>
<td>Per day</td>
</tr>
<tr>
<td>1</td>
<td>172</td>
<td>2.92</td>
<td>0</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>2</td>
<td>145</td>
<td>2.91</td>
<td>0</td>
<td>0</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>158</td>
<td>2.73</td>
<td>11</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>4</td>
<td>181</td>
<td>2.91</td>
<td>34</td>
<td>0.54</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>157</td>
<td>2.83</td>
<td>0</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>6</td>
<td>191</td>
<td>3.49</td>
<td>0</td>
<td>0</td>
<td>0.12</td>
</tr>
<tr>
<td>7</td>
<td>171</td>
<td>3.16</td>
<td>0</td>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td>8</td>
<td>131</td>
<td>2.47</td>
<td>9</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>9</td>
<td>177</td>
<td>2.84</td>
<td>0</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>10</td>
<td>157</td>
<td>3.06</td>
<td>0</td>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td>11</td>
<td>141</td>
<td>2.62</td>
<td>47</td>
<td>0.87</td>
<td>0.13</td>
</tr>
<tr>
<td>Control subjects</td>
<td>136 ± 10</td>
<td>2.19 ± 0.17</td>
<td>45 ± 21</td>
<td>0.70 ± 0.33</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(80-189)</td>
<td>(1.28-3.02)</td>
<td>(0-203)</td>
<td>(0-3.26)</td>
<td>(0.07-0.25)</td>
</tr>
</tbody>
</table>

* Not available.
†† Significantly different from control subjects: *P < 0.05, †P < 0.01.
§ Range in parentheses.

Despite significantly lower absolute FFM in the patients, total REE did not differ between patients and control subjects (7116 ± 173 and 7058 ± 205 kJ/d, respectively) (Table 2), indicating relative hypermetabolism in the patients. Analysis of covariance, taking into account the differences in absolute FFM between patients and control subjects, showed that patients had 8% (565 kJ/d) higher rates of REE (P < 0.05). Total caloric intake, calculated from diet records in five patients and nine control subjects, did not differ between patients and control subjects. There were no differences in clinical or laboratory data of the five patients who had kept diet records when compared with the data of the six patients for whom no diet records were available (Table 2).

No differences were observed in the respiratory quotients between patients and control subjects (0.71 ± 0.01 vs 0.74 ± 0.02, respectively) or in urinary nitrogen excretion. However, whole-body fat-oxidation rates in the patients were higher when compared with control subjects (Table 2). When expressed as g · kg FFM⁻¹ · d⁻¹, the difference in fat-oxidation rates between patients and control subjects was even more pronounced. Carbohydrate-oxidation rates were slightly but not significantly lower in the patients than in the control subjects (Table 2).

TABLE 3
Catabolic hormone and IL-6 concentrations in patients and control subjects

<table>
<thead>
<tr>
<th>Patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxine (nmol/L)</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>Triiodothyronine (nmol/L)</td>
<td>1.67 ± 0.10</td>
</tr>
<tr>
<td>Epinephrine (pmol/L)</td>
<td>339 ± 66</td>
</tr>
<tr>
<td>Norepinephrine (nmol/L)</td>
<td>1.36 ± 0.18</td>
</tr>
<tr>
<td>Cortisol (μmol/L)</td>
<td>0.27 ± 0.04</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>5.8 ± 1.8</td>
</tr>
</tbody>
</table>

*† Significantly different from patients: *P < 0.01, †P < 0.001.

Measurements of concentrations of thyroxine, triiodothyronine, catecholamines, cortisol, and IL-6 revealed significantly lower concentrations of norepinephrine in the patients than in the control subjects (226 ± 30 vs 367 ± 38 ng/L, respectively, P < 0.01) and significantly higher concentrations of IL-6 (5.8 ± 1.8 vs 2.9 ± 0.6 ng/L, P < 0.001) (Table 3).

No differences were observed in plasma albumin concentrations (41.4 ± 0.9 vs 43.6 ± 0.7 g/L, patients vs control subjects), serum concentrations of retinol-binding protein (50.7 ± 4.0 vs 52.2 ± 4.3 mg/L, patients vs control subjects), or transferrin (2.22 ± 0.11 vs 2.44 ± 0.13 g/L, patients vs control subjects).

Discussion

These data demonstrate increased REE with increased rates of fat oxidation in healthy men infected with HIV. Although the men had been antiHIV antibody positive for ≥ 1 y, circulating CD4⁺ T-cell numbers were normal and the patients were clinically asymptomatic, classified as CDC group II or III.

Whole-body rates of REE and substrate oxidation depend on body size, or, more accurately, on the metabolically active body mass (16). Therefore, when REE or substrate oxidation are compared between different groups of subjects, it is necessary to normalize the data to some index of body size. One of the best available estimates of the metabolically active body mass is the FFM (16), which can be accurately measured with the body-impedance analyzer (9). However, in addition to the metabolically active body mass, FFM also includes extracellular fluid and mineral mass. The FFM, therefore, can only be used as a reliable estimate of the metabolically active body mass in the absence of changes in the state of hydration. Kotler et al (18) reported an increase in extracellular water volume with decreased serum concentrations of albumin and retinol-binding protein in underweight patients with symptomatic (AIDS or ARC) HIV infection, most of whom were suffering from opportunistic infections at the time of study. In contrast, our patients were clinically healthy (asymptomatic HIV infection), weight-stable men,
with no signs of overhydration on physical examination and with completely normal plasma albumin concentrations. Therefore, the FFM, derived from bioelectric-impedance measurement, seems to be a valid estimate of the metabolically active body mass in our patients.

Whole-body fat-oxidation rates were significantly higher in the patients (19%) whereas carbohydrate-oxidation rates were only slightly but not significantly lower in the patients. As a result, total REE did not differ between patients and control subjects, even though patients had a lower absolute FFM, indicating relative hypermetabolism in the patients. Comparing the resting metabolic rate of groups of individuals who differ considerably in their absolute value of FFM by simply dividing REE by FFM would overestimate the metabolic rate in the individuals with a lower FFM, as was recently discussed (16). A more appropriate way of comparing REE is by analysis of covariance, which takes into account the differences in absolute FFM. This showed that our patients had 8% (135 kcal/d) higher rates of REE when compared with healthy control subjects. This degree of hypermetabolism closely approximates our previously reported 9% increase of REE in clinically stable patients with AIDS or ARC (4).

It could be argued that hypermetabolism in clinically stable patients with AIDS or ARC is caused by subclinical opportunistic infections, even when there is no evidence for active opportunistic infections in the 2 mo preceding the metabolic study. The present study provides evidence that similar increases of REE as observed in these later stages of HIV-induced disease already exist in asymptomatic HIV-infected men, even before CD4+ cell depletion is detectable. These findings support the hypothesis that infection with HIV itself, directly or indirectly, results in metabolic alterations characterized by increased rates of fat oxidation and REE.

Hypermetabolism can be mediated by elevated concentrations of thyroid hormones, catecholamines, or cortisol (19). Thyroid hormone concentrations in our patients were not significantly different from those in the control subjects. These results agree with a previously reported study in which serum triiodothyronine and thyroxine concentrations in 10 asymptomatic, HIV-positive outpatients did not differ from those in healthy control subjects (20). As in our study of REE in AIDS and ARC patients, measurements of catabolic hormone concentrations only showed slightly lower concentrations of norepinephrine in the patients. The possible causes and consequences of lower concentrations of norepinephrine in HIV-infected patients, which were still within the normal range (150-650 ng/L in our laboratory), are not clear from this study.

The hypermetabolism in our patients could not be explained by higher concentrations of catabolic hormones, suggesting HIV infection causes metabolic alterations through another mechanism, at present unknown, independent of elevated catabolic hormones. Cytokines, like tumor necrosis factor (TNF), interleukin 1 (IL-1), and IL-6 might be involved in this alternative mechanism. TNF and IL-1 appear to have metabolic effects related to tissue wasting (21); IL-6 induces the hepatic acute phase response to inflammation or tissue injury (22).

Despite conflicting reports on serum concentrations of TNF and IL-1 in HIV-infected patients (4, 23, 24), evidence of increased in vitro production of these cytokines by purified blood monocytes of both asymptomatic HIV-positive patients (CDC group II or III) and patients with ARC or AIDS (CDC group IV) is accumulating (25–27). However, at present the significance of increased in vitro production of cytokines by purified blood monocytes for energy metabolism in HIV-infected patients is purely speculative.

Mean serum concentrations of IL-6 in our patients, though statistically significant, were increased only twofold when compared with the control subjects and were all still within the normal range in our laboratory (< 15 ng/L). The IL-6 concentrations in our patients also remained far below the reported concentrations in patients with severe bacterial infections such as acute bacterial meningitis and/or sepsis (28). However, it should be realized that the increase in REE in our patients was also relatively small when compared with the degree of hypermetabolism in severe bacterial infections (29). Therefore, although it seems unlikely, it cannot be excluded that the slightly higher concentrations of IL-6, possibly in conjunction with other cytokines, that have not been measured in this study, were involved in the pathophysiological mechanism responsible for the hypermetabolism in our HIV-infected patients.

Recently, two studies suggested increased production of IL-6 by blood monocytes in HIV-infected patients (30, 31). In one of these studies Breen et al (31) reported a 16-fold increase in mean plasma concentration of IL-6 in a heterogeneous group of 28 HIV-infected patients (asymptomatic HIV seropositive patients with AIDS or ARC, all with or without other known bacterial or viral infections) when compared with healthy control subjects. This apparent discrepancy between the study of Breen et al and our present study is unlikely to be caused by the use of different cell lines for the IL-6 assay because both the assay with the MH60. BSF-2 cell line used by Breen et al and the LA Aarden’s B9 cell assay used in this study are well-established assays for the determination of IL-6. A more reasonable explanation for the difference in IL-6 concentrations in the two studies could be that our study included only HIV-infected patients who were strictly clinically asymptomatic and who still had normal CD4+ T-cell numbers, whereas only 6 of the 28 patients studied by Breen et al were truly asymptomatic. Unfortunately, the IL-6 concentration of these six patients is not given separately in their article, making a correct comparison impossible.

None of our patients had experienced weight loss (defined as a loss of ≥3% of prediagnosis body weight), indicating a balance between energy expenditure (comprising REE, thermic effect of exercise, and dietary induced thermogenesis) and energy intake (dependent on food intake and nutrient absorption). Our findings of comparable concentrations of albumin, retinol-binding protein, and transferrin in HIV-positive patients and control subjects also argues against catabolism in our patients. The increased REE in the HIV-positive subjects may have been compensated for by an increase in total caloric intake (although analysis of diet records in five patients did not support this possibility) and/or a reduction in physical activity (not measured in this study). However, because of the increased REE, the balance between energy expenditure and energy intake in asymptomatic HIV infection might be more difficult to maintain during, and to restore after, periods of even minor (common) illness and so places the asymptomatic HIV-positive individual at risk of catabolism. Although an uncompensated increase in REE of 565 kJ/d, if derived from body-fat stores (caloric equivalent of fat: 9 kcal/g), would cause a weight loss of only 15 g/d after 1 mo, the weight loss would have accumulated to 450 g. The importance of maintaining body mass in AIDS patients has been stressed in a recent
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study in which death from wasting in AIDS was directly related to the magnitude of body-cell-mass depletion (32). Our observation of increased REE in the early asymptomatic stage of HIV infection has to be kept in mind when dietary advice to prevent weight loss is given to HIV-infected patients.

Our patients were clinically asymptomatic, were classified as CDC group II or III, and had normal absolute CD4+ T-cell numbers. Nevertheless, they were hypermetabolic. This symptom adds to the growing puzzling panoply of subtle aberrations [eg, of the endocrine (33) and the immune (10) system] present in asymptomatic HIV-infected patients. These hormonal, immunological, and metabolic changes reflect that various aspects of host physiology are already affected by HIV in the early asymptomatic phase in the course of HIV infection, before CD4+ T-cell numbers start to decline. Therefore, consideration of antiretroviral therapy may be appropriate even in asymptomatic, HIV-seropositive subjects with normal numbers of CD4+ T-cells.

We thank RJM Ten Berge and FNJ Van Diepen for performing the IL-6 assay.

References