Psoas muscle attenuation measurement with computed tomography indicates intramuscular fat accumulation in patients with the HIV-lipodystrophy syndrome

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HUMAN IMMUNODEFICIENCY VIRUS (HIV)-infected patients receiving highly active antiretroviral therapy often demonstrate fat redistribution, characterized by subcutaneous fat loss and visceral fat hypertrophy. Changes in fat distribution are often seen in association with insulin resistance and dyslipidemia, and overall lipolysis rates are increased among such patients (17). Excess free fatty acids (FFA) from lipolysis may accumulate in muscle and thereby affect glucose entry and subsequent phosphorylation. Recent studies in this population using proton magnetic resonance (MR) spectroscopy demonstrate increased intramyocellular lipid concentrations (13, 22). Muscle attenuation values obtained with computed tomography (CT) decrease as a function of augmented lipid concentrations and are important independent markers of insulin resistance in non-HIV-infected patients with obesity and Type 2 diabetes (15, 16). However, prior studies using CT scan have not compared muscle attenuation as a surrogate index of intramyocellular fat content in relationship to body composition and metabolic variables in HIV-infected individuals.

MATERIALS AND METHODS

Experimental Subjects and Protocol

Attenuation values of psoas muscles were obtained by CT in 22 HIV-infected men with lipodystrophy syndrome (Lipo), 20 HIV-infected men without lipodystrophy (Nonlipo), and 21 HIV-negative male control subjects (Ctrl) recruited from the multidisciplinary HIV practice at the Massachusetts General Hospital. Subjects were referred for evaluation of observed changes in fat distribution and also were recruited from advertisements seeking HIV-infected patients with and without evidence of fat redistribution.

HIV status was confirmed by enzyme-linked immunosorbent assay and Western blot testing in all subjects. Lipo subjects were selected on the basis of a waist-to-hip ratio of >0.95 and a history of significant change in fat distribution in the trunk, extremities, neck, or face. In all lipodystrophy cases, the presence of changes in fat distribution was confirmed by computed tomography (CT) scan. The presence of changes in fat distribution was solely to indicate this fact.

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firmed by physical examination and scored by a single investigator as severe (1.5 on a scale of 0–2) in one or more areas. Severe lipodystrophy was scored for changes obvious to the casual observer and mild-to-moderate lipodystrophy for changes noticeable to the patient and confirmed by the single investigator. Objective criteria used in the determination of severe lipodystrophy included, but were not limited to, prominent peripheral venocele and a palpable dorsocervical fat pad. In contrast, HIV-positive, Nonlipo subjects were recruited from advertisements seeking HIV-infected men without changes in fat distribution. Nonlipo subjects were selected on the basis of a waist-to-hip ratio of <0.95 and no significant fat redistribution in any area on physical examination. Lipo patients were classified as having significant peripheral lipoatrophy if they demonstrated moderate or severe fat loss in the arms or legs. HIV-infected subjects receiving antiretroviral medications were on a stable regimen for >6 wk. One subject in the Lipo group was receiving stable thyroid hormone replacement. No other subjects were known to have thyroid disease. To prevent enrollment of subjects with primary HIV-related wasting, patients with a body mass index (BMI) of <20 kg/m² were excluded from all groups. Subjects receiving testosterone, growth hormone, anabolic hormones, glucocorticoid, anti diabetic agents, and megestrol acetate were excluded. Exclusion criteria also included known diabetes mellitus, hemoglobin level <9.0 g/dl, and age >60 and <18 yr.

The non-HIV-infected Ctrl subjects were in good health, used no medications, and had a waist-to-hip ratio of <0.95. Written, informed consent was obtained from each subject before testing, in accordance with the Committee on the use of Humans as Experimental Subjects of the Massachusetts Institute of Technology and the Subcommittee on Human Studies at the Massachusetts General Hospital.

Body composition by CT scan and dual-energy X-ray absorptiometry (DEXA), and metabolic indexes such as fasting lipid levels, insulin, glucose, oral glucose tolerance test (OGTT), plasma FFA, CD4⁺ count, and HIV viral load were also assessed. Body composition and other endocrine data have previously been reported in this subpopulation (25, 31).

Experimental Methods

CT attenuation of psoas muscle. All scans were performed with a LightSpeed CT scanner (General Electric, Milwaukee, WI). A lateral scout image of the abdomen was obtained to identify the L₄ pedicle, which served as a landmark for a single-slice image at this level. Scan parameters for each selected on the basis of a waist-to-hip ratio of <0.95 and no significant fat redistribution in any area on physical examination. Lipo patients were classified as having significant peripheral lipoatrophy if they demonstrated moderate or severe fat loss in the arms or legs. HIV-infected subjects receiving antiretroviral medications were on a stable regimen for >6 wk. One subject in the Lipo group was receiving stable thyroid hormone replacement. No other subjects were known to have thyroid disease. To prevent enrollment of subjects with primary HIV-related wasting, patients with a body mass index (BMI) of <20 kg/m² were excluded from all groups. Subjects receiving testosterone, growth hormone, anabolic hormones, glucocorticoid, anti diabetic agents, and megestrol acetate were excluded. Exclusion criteria also included known diabetes mellitus, hemoglobin level <9.0 g/dl, and age >60 and <18 yr.

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Body composition analysis. The single cross-sectional CT image at L₄ was utilized to assess distribution of subcutaneous and visceral abdominal fat. Fat attenuation values were set at −50 to −250 HU as described by Borkan et al. (5), and intra-abdominal visceral and subcutaneous fat areas were determined on the basis of tracings obtained utilizing commercial software (Alice, Parexel, Waltham, MA). Abdominal visceral and subcutaneous fat and the ratio of abdominal visceral fat to total abdominal cross-sectional area were determined. Fat and fat-free mass were determined by DEXA with a Hologic 4500 densitometer (Hologic, Waltham, MA). The technique has a precision error of 3% for fat and 1.5% for fat-free mass (23). Baseline weight was determined after an overnight fast, and percent ideal body weight was calculated on the basis of standard height and weight tables (26). Waist-to-hip ratio was determined from the circunferential measurements of the waist at the level of the umbilicus and the hips at the level of the iliac crest taken with the patient in an upright standing position.

Hormonal assessment and laboratory methods. Serological assessment included fasting plasma FFA, triglycerides, and low-density lipoprotein (LDL), high-density lipoprotein, and total cholesterol. A standard 75-g OGTT with insulin and glucose levels, CD4⁺, and HIV viral load were also performed. All parameters were determined by using previously published methods (20, 31).

Statistical analysis. Comparisons were made between the groups (Lipo vs. Nonlipo, Lipo vs. Ctrl, and Nonlipo vs. Ctrl) by the Wilcoxon rank-sum test. χ² analysis was used to assess group differences for categorical variables. Univariate regression analyses were performed, comparing CT attenuation values and indexes of body fat and composition among all HIV-infected patients. Forward stepwise regression analysis, P = 0.1 to enter the model, was performed to determine relevant factors contributing to muscle attenuation and hyperinsulinemia. Statistical significance was defined as P ≤ 0.05. Results are median plus interquartile range. Statistical analyses were made by using JMP Statistical Database Software (SAS Institute, Cary, NC).

RESULTS

Age and BMI were not significantly different between the groups (Table 1). As previously reported, no significant difference in whole body fat mass measured by DEXA was detected between groups (31). Regional trunk fat was increased, and extremity fat decreased in Lipo compared with Nonlipo and Ctrl subjects. The Nonlipo and Ctrl groups showed no significant difference in either truncal or extremity fat. Lipo subjects demonstrated significantly increased visceral fat area and visceral fat to total abdominal cross-sectional area determined by CT, compared with Nonlipo and Ctrl.

![Fig. 1. Axial computed tomography of the abdomen obtained at level of L₄ showing subcutaneous fat (SC) and intra-abdominal (visceral) fat (IA) interspersed between bowel loops. Placement of regions of interest used to measure psoas (P) attenuation values is shown.](http://jap.physiology.org/10.1152/japplphysiol.00989.2003)
No significant differences in visceral fat area were observed between Nonlipo and Ctrl subjects. CD4 cell count and HIV viral load were not significantly different between HIV-infected groups, although duration of HIV, protease inhibitor (PI) use, and nucleoside reverse transcriptase inhibitor (NRTI) use were tested as independent variables utilizing a forward stepwise regression analysis was performed with psoas muscle attenuation value as the dependent variable (Table 3). Visceral fat, BMI, plasma FFA, subcutaneous fat area, and PI and NRTI use were tested as independent variables utilizing a forward stepwise regression analysis.

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The HIV-lipodystrophy syndrome is characterized by increased visceral adiposity, loss of subcutaneous extremity fat, insulin resistance, and dyslipidemia (9, 10, 27, 36). The mechanisms of the syndrome are unknown and may be related to PI or NRTI inhibitor effects (8, 21) or an interaction of drug and nondrug factors. In addition, it is not known whether the HIV-lipodystrophy syndrome represents a single pathophysiological entity or several distinct subsyndromes. Hypertriglyceridemia is associated with increased concentrations of muscle lipid and insulin resistance in animal models (11), healthy subjects (3), and obese and Type 2 diabetic subjects (15, 29). Intramuscular lipid may contribute to skeletal muscle insulin resistance through inhibition of insulin signaling at phosphatidylinositol 3-kinase kinase and related inhibition of glucose transport (30). The extent and metabolic consequences of intramuscular lipid accumulation in HIV-infected patients with fat redistribution remains unknown. In non-HIV-infected patients, data obtained with CT suggest skeletal muscle attenuation decreases as a function of augmented lipid concentrations (14) and is an important independent marker of insulin resistance in obesity and Type 2 diabetes (15, 16). Goodpaster et al. (14) have validated CT attenuation values of psoas, thigh, and calf muscles as reliable indicators of lipid content. CT attenuation values of skeletal muscle have not previously been assessed in HIV lipodystrophy, and the relationship between muscle attenuation value, body composition, plasma FFA, and glucose homeostasis remains unknown in this syndrome. Our study demonstrates low CT attenuation values of the psoas muscle in Lipo patients. Furthermore, CT attenuation values correlate significantly with insulin response to OGTT and plasma FFA. The Lipo subjects in our study had a mixed lipodystrophy pattern with predominant visceral hypertrophy. Visceral fat was increased to nearly twice the level of controls, whereas subcutaneous fat area was decreased by only 23% compared with BMI-matched control subjects. Visceral fat area was a significant predictor of muscle attenuation values in HIV-infected individuals, whereas subcutaneous fat area was not significantly associated with psoas muscle attenuation in either univariate or multivariate regression analysis.

Increased basal rates of lipolysis and plasma FFA have been demonstrated in patients with HIV infection and fat redistribution receiving highly active antiretroviral therapy (17, 24, 32, 33, 36). Increased lipolysis was recently shown to be a strong predictor of insulin resistance in HIV-infected men (17), and improved insulin sensitivity was demonstrated after acute inhibition of lipolysis with acipimox in this population (18). Results from the present study extend these prior observations and demonstrate increased intramuscular lipid in HIV-infected patients, in association with fat distribution and FFA. Furthermore, our data are in agreement with the results of Behrens et al. (1) demonstrating hyperinsulinemia and reduced insulin-mediated glucose uptake of skeletal muscle in HIV-lipodystrophic patients with increased levels of FFA.

### Table 2. Univariate correlations of body composition, WHR, and metabolic status with psoas muscle density in HIV-infected subjects

<table>
<thead>
<tr>
<th>Body composition and anthropometrics</th>
<th>Psos Muscle Density, HU</th>
<th>r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.43</td>
<td>-</td>
<td>0.008</td>
</tr>
<tr>
<td>WHR</td>
<td>0.54</td>
<td>-</td>
<td>0.0006</td>
</tr>
<tr>
<td>Visceral fat by CT</td>
<td>0.49</td>
<td>-</td>
<td>0.002</td>
</tr>
<tr>
<td>Subcutaneous fat by CT</td>
<td>-0.23</td>
<td>-</td>
<td>0.17</td>
</tr>
<tr>
<td>Whole body fat by DEXA</td>
<td>-0.32</td>
<td>-</td>
<td>0.05</td>
</tr>
<tr>
<td>Trunk fat by DEXA</td>
<td>-0.50</td>
<td>-</td>
<td>0.002</td>
</tr>
<tr>
<td>Metabolic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFA</td>
<td>0.38</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-0.42</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.30</td>
<td>-</td>
<td>0.07</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-0.47</td>
<td>-</td>
<td>0.004</td>
</tr>
<tr>
<td>Glucose response to OGTT (AUC)</td>
<td>0.38</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>-0.32</td>
<td>-</td>
<td>0.06</td>
</tr>
<tr>
<td>Insulin response to OGTT (AUC)</td>
<td>-0.60</td>
<td>-</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

BMI, body mass index; WHR, waist-to-hip ratio
Visceral fat area, but not subcutaneous fat area, was highly related to CT attenuation. In stepwise regression modeling, visceral fat remained strongly associated with muscle attenuation in a model including PI and NRTI use as well as BMI. These data are consistent with and extend those of Gan et al. (13), demonstrating a relationship between visceral, but not subcutaneous, fat and intramyocellular lipid by proton MR spectroscopy in this population. Our results support the hypothesis that the lipodystrophy syndrome represents several distinct subsyndromes in which lipoatrophy per se is not associated with intramuscular lipid accumulation and is related to use and duration of NRTI therapy (2, 12). In contrast, our data suggest that visceral adiposity and a marked metabolic syndrome is associated with intramuscular lipid accumulation. Although the mechanism of visceral hypertrophy is not known, preliminary data suggest that use of PIs may contribute to visceral fat accumulation and to related insulin resistance and dyslipidemia (10). Of note, the Nonlipo group also demonstrated decreased psoas muscle attenuation, although not to the same degree as the Lipo patients. This suggests a spectrum of metabolic derangement among HIV-infected patients that is not entirely defined by the presence of fat redistribution.

We demonstrate that muscle attenuation is a strong predictor of hyperinsulinemia, suggesting significant metabolic consequences of intramuscular lipid accumulation in this population. Our data suggest an overall schema whereby visceral fat hypertrophy may contribute to intramuscular fat accumulation and hyperinsulinemia. Increased lipolysis in association with visceral adiposity may result in increased FFA levels that accumulate in the muscle, particularly if there is relatively less subcutaneous or extremity fat to serve as a depot for fat substrate. In turn, excess intramyocellular lipid may reduce glucose uptake into the muscle through effects on phosphatidylinositol 3-kinase and thereby contribute to insulin resistance.

Because of the cross-sectional nature of this study, we cannot determine causality, and it is also plausible that insulin resistance as a primary event impairs insulin-mediated inhibition of lipolysis, resulting in excess FFA and lipid accumulation in the muscle. Furthermore, Luzi et al. (22) demonstrated decreased lipid oxidation rates in association with increased intramyocellular lipid accumulation among HIV-infected patients receiving antiretroviral therapy, but regional and whole body lipolytic rates were not quantified.

Although skeletal muscle fat content can be estimated on the basis of X-ray attenuation value by CT (6, 7, 14, 28), this methodology is incapable of directly measuring muscle lipid content, and distinction of intramyocellular and extramyocellular fat is not possible. Proton MR spectroscopy has been recently validated as an effective and specific method to directly quantify skeletal muscle lipids (4, 35), with reliable results correlating intramyocellular lipids with biopsy samples (19) and insulin sensitivity (34). This technique has been successfully employed by Gan et al. (13), who found increased intramuscular lipid in association with insulin resistance in HIV-lipodystrophy patients. Nevertheless, single-slice CT scanning, as performed in this study, appears to be effective for assessment of body composition and overall psoas muscle adiposity overcoming anatomic limitations of MR spectroscopy that is limited to two muscles of the lower leg. The capability of quantifying attenuation in a variety of muscles as well as fat distribution around muscle represent a significant advantage of CT methodology. The widespread availability of CT, relative low cost, and prompt acquisition of data also make it well suited for large-scale clinical investigation.

In summary, this study demonstrates decreased psoas muscle attenuation in HIV-infected patients with evidence of the lipodystrophy syndrome. Diminished psoas attenuation is associated with abnormal body composition and lipid and glucose metabolism in this population. Our data support the hypothesis that overaccumulation of intramuscular lipids contributes to decreased insulin sensitivity and hyperinsulinemia in this population. Further studies investigating the pathogenesis of intramuscular lipid accumulation and effects on glucose homeostasis are necessary to determine optimal treatment strategies for dyslipidemia and insulin resistance in HIV-infected patients.

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DISCLOSURES

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