Altered Myocellular and Abdominal Fat Partitioning Predict Disturbance in Insulin Action in HIV Protease Inhibitor–Related Lipodystrophy

Seng Khee Gan,1 Katherine Samaras,1 Campbell H. Thompson,2 Edward W. Kraegen,1 Andrew Carr,3 David A. Cooper,3,4 and Donald J. Chisholm1

HIV protease inhibitor–related lipodystrophy is characterized by peripheral fat loss, hyperlipidemia, and insulin resistance. Increased availability of lipid to muscle may be one of the mechanisms that induce insulin resistance. Regional fat, intramyocellular lipid (by 1H-magnetic resonance spectroscopy), serum lipids, and insulin-stimulated glucose disposal (by hyperinsulinemic-euglycemic clamp) were quantified in 10 men who had HIV-1 infection with moderate to severe lipodystrophy and a control group of 10 nonlipodystrophic men who had HIV-1 infection and were naïve to protease inhibitors to examine the effects of lipodystrophy on glucose and lipid metabolism. Lipodystrophic subjects showed lower insulin-stimulated glucose disposal than control subjects (P = 0.001) and had increased serum triglycerides (P = 0.03), less limb fat (P = 0.02), increased visceral fat as a proportion of total abdominal fat (P = 0.003), and increased intramyocellular lipid (1.90 ± 0.15 vs. 1.23 ± 0.16% of water resonance peak area; P = 0.007). In both groups combined, visceral fat related strongly to intramyocellular lipid (r = 0.83, P < 0.0001) and intramyocellular lipid related negatively to insulin-stimulated glucose disposal (r = −0.71, P = 0.0005). Fasting serum cholesterol and triglycerides related positively to intramyocellular lipid and visceral fat in lipodystrophic subjects only. The data indicate that lipodystrophy is associated with increased lipid content in muscle accompanying impaired insulin action. The results do not establish causation but emphasize the interrelationships among visceral fat, myocyte lipid, and insulin action. Diabetes 51:3163–3169, 2002

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DEXA, dual-energy X-ray absorptiometry; EMCH, extramyocellular CH; EMCL, extramyocellular lipid; HAART, highly active antiretroviral therapy; 1H-MRS, 1H-magnetic resonance spectroscopy; HOMA-IR, homeostasis model assessment for insulin resistance; IGT, impaired glucose tolerance; IMCH, intramyocellular CH; IMCL, intramyocellular lipid; MRS, magnetic resonance imaging; SAT, subcutaneous abdominal adipose tissue; VAT, visceral adipose tissue

Abdominal obesity is associated with hyperlipidemia and insulin resistance and predicts cardiovascular disease and type 2 diabetes, a cluster termed the “(pluri)metabolic syndrome” or “insulin resistance syndrome” (1). Total adiposity, particularly central abdominal fat, is predictive of insulin resistance (2–4). Increased muscle triglyceride measured biochemically (5) or by computerized tomography (4) also predicts insulin resistance, but these methods do not distinguish extramyocellular from intramyocellular triglyceride. Histological (6) and, more recently, 1H-magnetic resonance spectroscopic (1H-MRS) studies indicate that intramyocellular rather than extramyocellular triglyceride predicts insulin resistance (7–10). Thus, increased lipid availability in myocytes, possibly arising from metabolically active stores such as abdominal fat, may be a major mechanism to induce insulin resistance.

Lipodystrophy is reported in ~50% of patients who receive HIV protease inhibitors as a component of highly active antiretroviral therapy (HAART) (11–14). Prominent changes are loss of subcutaneous fat (lipoatrophy) in the face, buttocks, and limbs. Central abdominal fat does not seem to change greatly. The condition is progressive and is associated with metabolic abnormalities such as hyperlipidemia, insulin resistance, and, in 23% of patients, impaired glucose tolerance (IGT) or type 2 diabetes (11,12). Its cause is unclear despite several hypotheses and in vitro studies (15–22). Components of HAART apart from protease inhibitors may also contribute (23–25).

Although alternative mechanisms are possible, we postulate that lipid spill or reduced peripheral adipocyte storage in lipodystrophic subjects may increase lipid availability and storage within myocytes. In this respect, protease inhibitor–related lipodystrophy provides an opportunity to examine how altered body fat partitioning has an impact on myocellular and circulating lipids and insulin sensitivity, before development of advanced metabolic sequelae such as diabetes. This study therefore examined the metabolic characteristics of a group of nondiabetic HIV-positive men, all using protease inhibitors (as a component of HAART), selected on the basis of clinically obvious peripheral lipoatrophy. Controls were men who had HIV-1 infection without lipoatrophy and were naïve to protease inhibitors. The aims of the study were, first, to determine levels of intramyocellular lipid in
lipodystrophy and, second, to examine relationships among muscle lipid, body fat depots, serum lipids, and insulin sensitivity.

RESEARCH DESIGN AND METHODS

**Subject selection.** Male subjects were recruited in an urban hospital HIV outpatient clinic. Subjects with lipodystrophy had moderate to severe lipodystrophy (defined by physician examination using a standardized protocol [11]), and all used HAART, which included protease inhibitors. Control subjects were HIV-1 seropositive but had never received HIV protease inhibitors and had no detectable lipodystrophy. This was preferred to a separate control group of protease inhibitor users without detectable lipodystrophy as peripheral fat loss may already be occurring in subjects who use protease inhibitors before clinical lipodystrophy.

For comparing insulin sensitivity in HIV-1–positive nonlipodystrophic control subjects versus normal HIV-1–negative men, a group of normal HIV-1–negative men of similar mean age and BMI to the HIV-1–positive control subjects, who were undergoing other studies in our unit during the same time period, had fasting blood samples taken for estimation of insulin sensitivity by the homeostasis model assessment (26). Other measurements indicated in the methods were not performed on these HIV-1–negative subjects.

All subjects were in good health. Exclusion criteria were a diabetic response to a screening 75-g oral glucose tolerance test (fasting venous plasma glucose ≥7.0 mmol/l and/or 2-h postload glucose of ≥11.1 mmol/l [27]), a previous or current AIDS-defining condition, use of systemic steroids or hypolipidemic agents within the preceding year, or >2 h/week of strenuous exercise. The protocol was approved by the Human Research Ethics Committee at St. Vincent’s Hospital, Sydney.

**Anthropometric measures and dietary and physical activity assessment.** Measures were weight (nearest 0.1 kg), stadiometer height (barefoot, nearest 0.01 m), waist circumference (nearest 0.5 cm) at the narrowest region between the lower edge of the ribs and iliac crest, and hip circumference (nearest 0.5 cm) at the greater trochanters. BMI was calculated as the [(weight in kg)/(height in m)]^2. Habitual diet composition was assessed by combined food frequency questionnaire and 3-day food record (FoodWorks; Xyris Software, Brisbane, Australia); physical activity was assessed by validated questionnaires (28).

**Insulin-stimulated glucose disposal (hyperinsulinenic-euglycemic clamp), homeostasis model assessment of insulin resistance, and substrate oxidation (indirect calorimetry).** Subjects were studied after an overnight fast and magnetic resonance imaging (MRI; see below). Intravenous cannulae were placed in each forearm, one for glucose and insulin infusions and the other retrogradely in a warmed forearm to collect arterialized venous blood. After 20 min of rest, indirect calorimetry (Deltatrac; Datex, Helsinki, Finland) was performed for 25 min (repeated during final 25 min of the clamp). Measurements of oxygen consumption and carbon dioxide production allowed calculation of respiratory quotient, energy expenditure, and oxidation rates for fat and carbohydrate (29). After basal calorimetry, insulin was infused at 2 μU·min·kg^−1·h^−1 for 150 min, achieving serum insulin levels of 1,350 ± 111 pmol/l (187.5 ± 15.4 μU/l) in lipodystrophic subjects and 1,390 ± 208 pmol/l (193.2 ± 28.9 μU/l) in control subjects. This insulin dose was used to suppress hepatic glucose production and allow the steady-state glucose infusion rate to reflect predominantly muscle insulin-stimulated glucose disposal. Plasma glucose was measured every 10 min (30), and the glucose infusion rate was adjusted to maintain plasma glucose close to 5.0 mmol/l (YSI 2300 StatPlus; YSI, Yellow Springs, OH). Steady-state glucose infusion rate, a measure of whole-body insulin sensitivity, was calculated from the last 40 min of the clamp, adjusted for fat-free mass from dual-energy X-ray absorptiometry (DEXA) measurement. Differences in insulin sensitivity between the HIV-1–positive and the normal HIV-1–negative subjects were assessed from fasting plasma glucose and insulin levels using the homeostasis model assessment of insulin resistance (HOMA-IR) index ([fasting plasma glucose × fasting plasma insulin]/22.5) (26).

**Body composition.** DEXA (Lunar DPX-L, Madison, WI) was usually performed within 2 weeks of metabolic studies. Regional fat compartments included central (total BMI [limb fat = sum of arms and legs], and central abdomen). Central abdominal fat was defined as fat mass in a window 0.8 cm in vertical dimension with the lower border at superior iliac crest level; lateral dimensions were adjusted to the lateral borders of the costal margin (3). Visceral adipose tissue and subcutaneous abdominal adipose tissue. Twelve T1-weighted axial MRI scats (5-mm thickness, 5-mm intervals) were performed between the levels of L4/5 and L1/2 intervertebral discs. Planimetric analysis (NIH Image 1.62; National Institutes of Health, Bethesda, MD) was used to quantify areas of visceral adipose tissue (VAT); within inner margin of abdominal wall and subcutaneous abdominal adipose tissue (SAT; between skin and outer margin of abdominal wall). Volumes of VAT and SAT were estimated from interpolation between scanned slices. Total abdominal adipose tissue (TAT) was the sum of VAT and SAT.

**H-MRS.** Subjects were studied using a 1.5Tesla medical magnetic resonance scanner (General Electric, Milwaukee, WI) after an 8-h overnight fast, avoiding strenuous physical exertion for 5 days. The right lower leg of each subject was scanned within an extremity coil. A voxel (2.10 × 2.00 × 2.0 cm) was positioned within the soleus muscle. Spectra were acquired by PRESS (point resolved spectroscopy) sequence with echo time 1.35 ms and repetition time 1,500 ms. Proton resonance time-domain quantitation was performed with AMARES (MRUI version 99.2; European Union) using constraints to improve fitting reliability similar to recent approaches (30). Line shapes were gaussian apart from a lorentzian line shape for the water peak. Peaks of pairs assigned equal line widths were creatine and carnitine, intramyocellular CH2 (IMCH2) and extramyocellular CH3 (EMCH3) and extramyocellular CH2 (EMCH2). A line-width ratio of 0.93 was assigned for IMCH3:EMCH3 and EMCH3:EMCH2. Peak areas were corrected for T1 and T2 times. Intramyocellular lipid (IMCL) and extramyocellular lipid (EMCL) were quantified as areas of IMCH2 and EMCH2 peaks, respectively, as a percentage of water peak area.

**Biochemical analysis.** All serum samples were collected after a 10-h overnight fast. Radioimmunoassays were performed for insulin and leptin (Linco Research, St. Charles, MO). Total and HDL cholesterol, triglycerides (all by enzymatic colorimetry; Roche, Indianapolis, IN), free fatty acids (enzymatic colorimetry; Wako, Osaka, Japan), and apolipoprotein B (immunoturbidimetric method; Roche) were measured.

**Statistical analysis.** Data were analyzed using StatView5 (SAS Institute, Cary, NC). Results were expressed as mean ± SE. Two-tailed Student’s t tests were used for comparisons between groups. Simple regression analysis was performed with the regression coefficient (r) expressing magnitude of relationships. Logarithmic transformation of serum triglycerides was applied in analyses because of a skewed distribution. ANCOVA tested for homogeneity of relationships between subject groups; where there was no significant difference, a single regression analysis was presented.

**RESULTS**

**Subject characteristics (Table 1).** The 10 lipodystrophic subjects and 10 control subjects studied were similar in age, duration of known HIV seropositivity, CD4 lymphocyte counts, and plasma HIV RNA (Table 1). Duration of recognized lipodystrophy in lipodystrophic subjects was 16.4 ± 5.3 months. Physical activity (215 ± 7 vs. 213 ± 55 mets/week; P = 0.86), dietary energy intake (11.6 ± 1.1 vs. 9.9 ± 0.6 MJ/day; P = 0.21), and dietary fat (35 ± 3 vs. 31 ± 2% of energy intake; P = 0.30) did not differ. Plasma glucose after 75 g oral glucose was significantly higher in lipodystrophic subjects than in control subjects (P < 0.04). Five lipodystrophy subjects and two control subjects had IGT (2 h after 75 g glucose ≥7.8 mmol/l and <11.1 mmol/l [26]). Lipodystrophy subjects used protease inhibitors for a mean duration of 33 ± 4 months. Three control subjects did not use any antiretroviral therapy (their exclusion from the control group did not change group differences for DEXA and abdominal MRI). The 27 normal HIV–negative males used for comparison of HOMA-IR only had similar mean age (39.9 ± 2.0 years) and BMI (26.2 ± 0.7 kg/m^2).

**Body composition, serum lipids, and leptin.** Lipodystrophic subjects were similar to control subjects in fat-free mass and trunk fat (Table 2). As expected, limb fat was lower in lipodystrophic subjects compared with control subjects as mass (P = 0.02) or as a proportion of total body fat (P < 0.0001). Although central abdominal fat (by DEXA) or TAT (by MRI) did not differ between groups, central abdominal fat composed a greater proportion of total body fat in lipodystrophic subjects (P < 0.0001). Within the abdominal region, VAT as a percentage of TAT...
TABLE 1
Characteristics of 10 men with HIV protease inhibitor–related lipodystrophy compared with 10 HIV–1–infected men without lipodystrophy and naïve to protease inhibitors (controls)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Lipodystrophy (n = 10)</th>
<th>Control (n = 10)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.4 ± 3.2 (26–58)</td>
<td>42.1 ± 2.7 (28–55)</td>
<td>0.59</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 1.3 (18.9–30.7)</td>
<td>25.4 ± 1.6 (21.9–38.9)</td>
<td>0.47</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>91.5 ± 3.0 (78–105)</td>
<td>90.5 ± 4.0 (77–122)</td>
<td>0.88</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.98 ± 0.01 (0.93–1.05)</td>
<td>0.95 ± 0.02 (0.87–1.02)</td>
<td>0.16</td>
</tr>
<tr>
<td>Duration of known HIV-1 infection (years)</td>
<td>9.9 ± 1.0 (5.0–15.5)</td>
<td>7.7 ± 1.5 (3.0–15.0)</td>
<td>0.24</td>
</tr>
<tr>
<td>CD4+ lymphocyte count (× 10⁹/l)</td>
<td>490 ± 88 (117–1100)</td>
<td>392 ± 102 (40–1160)</td>
<td>0.46</td>
</tr>
<tr>
<td>HIV-1 RNA (log copies/ml plasma)</td>
<td>3.3 ± 0.3 (2.6–4.9)</td>
<td>3.2 ± 0.3 (2.6–5.6)</td>
<td>0.94</td>
</tr>
<tr>
<td>Fasting plasma insulin (mU/l)</td>
<td>14.7 ± 1.8 (5.0–19.2)</td>
<td>10.9 ± 3.5 (4.1–42.0)</td>
<td>0.36</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.5 ± 0.2 (4.6–6.3)</td>
<td>5.2 ± 0.2 (4.5–6.7)</td>
<td>0.39</td>
</tr>
<tr>
<td>Plasma glucose 2 h after 75-g glucose load (mmol/l)</td>
<td>7.6 ± 0.4 (5.9–9.9)</td>
<td>6.1 ± 0.6 (4.0–9.5)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Protease inhibitors used at time of study (no. of subjects)
- Indinavir 5
- Ritonavir 5†
- Nelfinavir 1

Other antiretrovirals used at time of study (no. of subjects)
- Nucleoside RTI lamivudine 8
- stavudine 5
- zidovudine 3
- didanosine 1
- abacavir 1

Other medications used (no. of subjects)
- Bactrim 5
- acyclovir 2
- Histamine-2
- Antagonist 2
- Fosinopril 1
- Nefazodone 1

Values are mean ± SE. Ranges are presented in brackets. *P values were calculated by a two-tailed t test. †Ritonavir was used by four subjects at a pharmacokinetic (not virologic) dose of 100 mg twice daily in combination with another protease inhibitor. RTI, reverse transcriptase inhibitors.

was greater in lipodystrophic subjects (P = 0.002), as was the VAT:SAT ratio (P = 0.01; Table 2, Fig. 1A and B).

Fasting total cholesterol and leptin (per kilogram of total fat) were similar in both groups (Table 2). Serum triglycerides were higher (P = 0.03) and HDL cholesterol was lower in lipodystrophic subjects (P = 0.03). Apolipoprotein B was higher in lipodystrophic subjects (P = 0.03). Serum free fatty acids did not show a significant difference (P = 0.24).

Insulin-stimulated glucose disposal, HOMA-IR, and substrate oxidation. In the resting, postabsorptive state, there were no significant differences in energy expenditure, respiratory quotient, or fat and glucose oxidation rates. During hyperinsulinemia, the steady-state glucose infusion rate was 45% lower in lipodystrophic subjects than in control subjects (P = 0.001), indicating insulin resistance in lipodystrophic subjects. Percentage reduction of fat oxidation during hyperinsulinemia was lower in lipodystrophic subjects (P = 0.003), consistent with reduced insulin sensitivity (Table 3).

HOMA-IR analysis reflected these differences between lipodystrophic HIV–1–positive subjects and HIV–1–positive control subjects (3.8 ± 0.5 vs. 2.8 ± 1.1, respectively). HOMA-IR in normal HIV–1–negative men (2.4 ± 0.3) was not significantly different from nonlipodystrophic HIV–1–positive control subjects (P = 0.61) but was substantially lower than lipodystrophic HIV–1–positive subjects (P = 0.013).

Intramyocellular lipid. Soleus IMCL was 50% higher in lipodystrophic subjects versus control subjects (P = 0.007), whereas EMCL was similar, resulting in a higher ratio of IMCL:EMCL (P = 0.026; Table 4, Fig. 1). Significant differences persisted even with exclusion of subjects with IGT. Representative spectra are shown in Fig. 1A’ and B’.

Relationships among IMCL, serum lipids, and other metabolic parameters. In both groups combined, there was a strong negative relationship between IMCL and insulin-stimulated glucose disposal (r = −0.71, P = 0.0005; Fig. 2A). IMCL related strongly to VAT (r = 0.83, P < 0.0001; Fig. 2B) but not to SAT (r = 0.25, P = 0.21; relationships not significantly different between groups by ANCOVA).

In lipodystrophic subjects, IMCL related strongly to total cholesterol (r = 0.91, P = 0.0002) and log serum triglycerides (r = 0.77, P = 0.009) but not in control subjects (cholesterol: r = −0.18, P = 0.61; log triglycerides: r = 0.49, P = 0.16). In lipodystrophic subjects, VAT related positively to total cholesterol (r = 0.78, P = 0.008) and log serum triglycerides (r = 0.63, P = 0.049) but again not in control subjects (cholesterol: r = −0.18, P = 0.61; log triglycerides: r = 0.25, P = 0.49). Serum free fatty acids

DIABETES, VOL. 51, NOVEMBER 2002 3165
did not relate to VAT or IMCL in either group or in both combined (data not shown).

**DISCUSSION**

This study showed that lipodystrophic subjects had 50% more IMCL compared with control subjects and were insulin resistant with increased serum triglycerides and relatively increased visceral fat. IMCL related negatively to insulin-stimulated glucose disposal but positively to VAT and, in the lipodystrophic subjects only, to serum cholesterol and triglycerides. These data advance characterization of metabolic changes in HIV protease inhibitor–related lipodystrophy and highlight aspects of fat metabolism pertaining to insulin action. The results of the hyperinsulinemic-euglycemic clamp show that lipodystrophic subjects are insulin resistant, confirming previous indirect estimates from fasting glucose and insulin levels (11,12) and complementing recent limited clamp data (31). This study did not attempt to examine in detail whether HIV-1 infection is also associated with reduced insulin sensitivity, but the HOMA-IR comparison with normal HIV−1−negative men indicates, only a small difference in insulin sensitivity, if any. A larger study would be necessary to pursue this issue and determine the relationship of any change in insulin sensitivity to HIV-1 infection itself or antiretroviral therapy (in the absence of lipodystrophy).

Abdominal MRI shows that VAT composed 53% of VAT in lipodystrophic subjects compared with 37% in control subjects. A previous study had shown increases in VAT: TAT ratios on single-cut abdominal computed tomographic scans in subjects selected for abdominal enlargement, without the necessary presence of peripheral lipoatrophy (32), leaving some doubt as to whether lipoatrophy was consistently associated with visceral fat accumulation. Using 12 MRI slices and case selection on the basis of peripheral lipoatrophy, the current study more firmly establishes increased VAT in HIV protease inhibitor–related lipodystrophy.

IMCL was increased 50% in lipodystrophic subjects compared with control subjects, and IMCL related negatively to insulin-stimulated glucose disposal. This negative association has been previously reported in healthy subjects (7,8) or those genetically predisposed to type 2 diabetes (5,9,10). IMCL was also examined in four subjects with congenital generalized lipodystrophy where soleus IMCL (by 1H-MRS) was approximately double that in healthy control subjects (33). In contrast to the current study, a number of those subjects had diabetes, which may explain the increased magnitude of IMCL compared with our findings. Five lipodystrophic subjects and two control subjects in our study had IGT, but even with exclusion of these subjects, a significant increase in soleus IMCL persisted in lipodystrophic subjects. Together with a recent abstract reporting that IMCL was raised in 11 subjects with lipodystrophy associated with HIV protease inhibitors compared with healthy HIV-negative control subjects (34), the current study represents one of the first major spectroscopic demonstrations that IMCL is elevated in nondiabetic subjects with an acquired lipodystrophy of relatively recent onset. This result is in accordance with other previously studied human situations of insulin resistance (5,9,10) and further supports the hypothesis that increased lipid availability in myocytes is a cause of impairment of insulin action.

It is possible, however, that this is not the only mechanism for insulin resistance in the protease inhibitor–related lipodystrophy syndrome. Protease inhibitors can reduce glucose transport in isolated skeletal muscle di-

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**TABLE 2**

Body composition as measured by DEXA and abdominal MRI, with fasting serum lipids and leptin

<table>
<thead>
<tr>
<th></th>
<th>Lipodystrophy</th>
<th>Control</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-body DEXA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>58.62 ± 2.54</td>
<td>57.54 ± 1.23</td>
<td>0.71</td>
</tr>
<tr>
<td>Total body fat (kg)</td>
<td>14.27 ± 2.54</td>
<td>19.83 ± 3.64</td>
<td>0.23</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>18.8 ± 2.7</td>
<td>24.3 ± 9.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Trunk fat (kg)</td>
<td>9.23 ± 1.75</td>
<td>9.97 ± 2.04</td>
<td>0.79</td>
</tr>
<tr>
<td>Central abdominal fat (kg)</td>
<td>1.41 ± 0.24</td>
<td>1.34 ± 0.26</td>
<td>0.85</td>
</tr>
<tr>
<td>Central abdominal fat (% of total body fat)</td>
<td>9.9 ± 0.5</td>
<td>6.7 ± 0.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Limb fat (kg)</td>
<td>4.24 ± 0.75</td>
<td>9.01 ± 1.63</td>
<td>0.02</td>
</tr>
<tr>
<td>Limb fat (% of total body fat)</td>
<td>29.8 ± 1.9</td>
<td>45.9 ± 2.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Abdominal MRI scans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT (l)</td>
<td>2.75 ± 0.46</td>
<td>2.77 ± 0.59</td>
<td>0.98</td>
</tr>
<tr>
<td>VAT (l)</td>
<td>1.49 ± 0.29</td>
<td>1.00 ± 0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>SAT (l)</td>
<td>1.26 ± 0.22</td>
<td>1.77 ± 0.43</td>
<td>0.31</td>
</tr>
<tr>
<td>VAT as % of TAT</td>
<td>52.7 ± 3.6</td>
<td>37.0 ± 2.6</td>
<td>0.002</td>
</tr>
<tr>
<td>VAT:SAT ratio</td>
<td>1.25 ± 0.21</td>
<td>0.61 ± 0.08</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum lipids and leptin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.8 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>0.32</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.86 ± 0.05</td>
<td>1.07 ± 0.07</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>2.9 ± 0.5</td>
<td>1.6 ± 0.5</td>
<td>0.03†</td>
</tr>
<tr>
<td>Free fatty acids (mmol/l)</td>
<td>0.44 ± 0.05</td>
<td>0.35 ± 0.05</td>
<td>0.24</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>1.11 ± 0.08</td>
<td>0.89 ± 0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>2.8 ± 0.7</td>
<td>4.5 ± 1.6</td>
<td>0.30</td>
</tr>
<tr>
<td>Leptin (ng · ml⁻¹ · kg⁻¹ total body fat)</td>
<td>0.21 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *P values were calculated by a two-tailed t test. †Logarithmic transformation applied to serum triglycerides for statistical analyses.
rectly in vitro (19), and treatment of healthy subjects with indinavir for 4 weeks produces insulin resistance in the absence of change in serum lipids or body fat (35). Although this direct effect by protease inhibitors has been suggested as the primary mechanism for the appearance of the protease inhibitor–related lipodystrophy syndrome and the resulting hyperinsulinemia could also result in accumulation of muscle lipid, the appearance of lipoatrophy is less easy to explain if this were the sole mechanism. Data from the current study cannot differentiate between the importance of these direct and indirect mechanisms. The evidence, however, that insulin resistance is present in virtually all known human and rodent lipodystrophy syndromes and that disturbed lipid partitioning with raised muscle triglyceride is a likely cause of insulin resistance in a transgenic lipodystrophic rodent model (36) is persuasive that the effects of disturbed lipid metabolism from lipoatrophy is an important contributor to insulin resistance in the HIV protease inhibitor–related lipodystrophy syndrome.

Recent in vitro studies have also shown that protease inhibitors adversely affect adipocyte differentiation (18,21,22) and even promote apoptosis (20), providing a possible mechanism for the lipoatrophy. In lipodystrophic subjects, our results show that VAT, IMCL, serum cholesterol, and triglycerides all are positively related to each other. Despite the limited numbers in this study, it is interesting that the strong relationships between VAT or IMCL with serum cholesterol and triglycerides seen in lipodystrophic subjects were not present in control subjects. One reason could be that reduction in peripheral fat stores in lipodystrophy resulted in circulating lipids becoming more dominantly related to the quantities of both VAT and IMCL. Although other mechanisms are not ex-

**FIG. 1.** Transverse abdominal MRI scans (L4 vertebral level; fat appears white with T1 weighting) and 1H-MRS soleus muscle spectra from a control subject (A and A’) and a lipodystrophic subject (B and B’). Both subjects have equivalent amounts of central abdominal fat (by DEXA) and TAT (by MRI). There is less subcutaneous abdominal fat and more visceral abdominal fat in the lipodystrophic subject (B) compared with the control subject (A). Muscle lipid partitioning also differs substantially with increased IMCH2 and reduced EMCH2 in the lipodystrophic subject (B’) compared with the control subject (A’). (Chemical shift indicated in parts per million.) Cn, carnitine; Cr, creatine.

### TABLE 3
Whole-body insulin-stimulated glucose disposal as measured by the hyperinsulinemic-euglycemic clamp and substrate oxidation measurements by indirect calorimetry

<table>
<thead>
<tr>
<th></th>
<th>Lipodystrophy</th>
<th>Control</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clamp glucose infusion</td>
<td>36.7 ± 4.2</td>
<td>66.8 ± 6.5</td>
<td>0.001</td>
</tr>
<tr>
<td>rate (µmol · min⁻¹ · kg⁻¹ fat-free mass)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting energy expenditure (kcal · day⁻¹ · kg⁻¹ fat-free mass)</td>
<td>30.6 ± 0.8</td>
<td>30.1 ± 1.0</td>
<td>0.72</td>
</tr>
<tr>
<td>Resting respiratory quotient</td>
<td>0.87 ± 0.02</td>
<td>0.87 ± 0.01</td>
<td>0.82</td>
</tr>
<tr>
<td>Resting glucose oxidation rate (µmol · min⁻¹ · kg⁻¹ fat-free mass)</td>
<td>16.1 ± 1.9</td>
<td>16.5 ± 1.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Resting fat oxidation rate (g fat · day⁻¹ · kg⁻¹ fat-free mass)</td>
<td>1.08 ± 0.21</td>
<td>0.96 ± 0.11</td>
<td>0.61</td>
</tr>
<tr>
<td>% Reduction in fat oxidation during clamp</td>
<td>40.0 ± 8.3</td>
<td>95.7 ± 14.1</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *P values were calculated by a two-tailed t test.
cluded and cause and effect cannot be proved, these relationships in lipodystrophic subjects and the differences compared with control subjects are consistent with the hypothesis that reduction in peripheral adipose storage capacity from lipodystrophy leads to increased circulation of “displaced” lipids and contributes to lipid accumulation in unaffected depots such as myocytes and VAT. This function of peripheral adipocytes in maintaining normal lipid disposal/storage with protection of normal insulin action is also supported by other observations, e.g., improvement in insulin action when subcutaneous fat is transplanted into a lipoatrophic rodent (37). Thiazolidinediones, agonists of peroxisome proliferator–activated receptor-γ nuclear receptors (which promote adipocyte differentiation), also improve insulin sensitivity in humans while increasing subcutaneous abdominal but decreasing visceral fat (38,39).

In summary, this study shows that subjects with HIV protease inhibitor–related peripheral lipodystrophy have increased IMCL, are insulin resistant, and have relatively greater VAT. IMCL is associated positively with VAT and negatively with insulin-stimulated glucose disposal. Although protease inhibitors may have direct effects on muscle insulin action and caution in interpreting these correlations is necessary, the relationships seen in this human lipodystrophy model are consistent with the hypothesis that disruption of peripheral adipocyte function leads to disturbances in myocellular and abdominal lipid partitioning and insulin sensitivity. These results support but by no means prove that this mechanism is significantly involved in the generation of insulin resistance in this syndrome. These results also support current concepts about both the significance of myocyte lipid in determining muscle insulin sensitivity and the important role of peripheral adipocytes in maintaining normal lipid and glucose metabolism.

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