Increased intramyocellular lipid accumulation in HIV-infected women with fat redistribution

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chronic diseases, and using no medications. Data were obtained during early follicular phase of the menstrual cycle. HIV-infected and control subjects were characterized as eumenorrheic (normal menstrual function), oligomenorrheic (less than three menstrual periods in the 3 mo before study), and amenorrheic (zero menstrual periods in the 3 mo before the study) to determine clinically significant menstrual dysfunction. Among the HIV-infected subjects, 72% were eumenorrheic, 8% were oligomenorrheic, and 20% were amenorrheic. Among the controls, 86% were eumenorrheic, 5% were oligomenorrheic, and 9% were amenorrheic. One subject in the HIV-infected group was on low-dose estrogen therapy. Written, informed consent was obtained from subjects before testing. This protocol was approved by the Committee on the Use of Humans as Experimental Subjects of the Massachusetts Institute of Technology and the Human Research Committee at the Massachusetts General Hospital.

1H-MRS. All scans were performed using a 1.5-T system (Signa LX, version 8.3; GE Medical Systems, Milwaukee, WI). 1H-MRS of tibialis anterior (n = 46; 21 Ctrl, 25 HIV-infected) and soleus (n = 24; 15 Ctrl, 9 HIV-infected) muscles was performed between 0700 and 0800 after 8-h overnight fasting. Subjects were positioned feet first in the magnet bore, and the right calf was placed in an extremity coil. A triplane gradient echo localizer pulse sequence with echo time (TE) of 1.6 ms and repetition time (TR) of 49.0 ms and axial T1-weighted images (TR, 600 ms; TE, 14 ms; slice thickness, 4 mm; interslice gap, 1 mm) of the calf were performed for voxel placement. Single-voxel MRS data was acquired using point-resolved spatially localized spectroscopy pulse sequence with TE of 25 ms, TR of 3,000 ms, 32 acquisitions, and 8 number of excitations. In all cases, a 3.4 ml voxel was placed on the largest cross-sectional area of the muscle, avoiding visible interstitial tissue, fat, or vessels. Fitting of all 1H-MRS data (Fig. 1) was performed using LCModel software (version 6.0–2, running on a Linux workstation. The signal corresponding to IMCL (1.3 ppm) methylene protons was automatically scaled to unsuppressed water peak, with values being expressed in institutional units (IU).

**Body composition analysis.** Weight was determined after an overnight fast, and waist-to-hip ratio was determined from the circumferential 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. A single cross-sectional CT image at L4 was utilized to assess distribution of subcutaneous and visceral abdominal fat. All scans were performed with a LightSpeed CT scanner (GE Medical Systems, Milwaukee, WI) using standardized parameters (144-cm table height, 80 kV, 70 mA, 2 s, 1-cm slice thickness, 48-cm field of view). Fat attenuation values were set between −50 and −250 Hounsfield units, and intra-abdominal visceral (VAT) and subcutaneous (SAT) fat areas were determined on the basis of tracings obtained utilizing commercial software (Alice, Parexel, Waltham, MA). Tracings for determination of right calf subcutaneous fat area were obtained from a single axial magnetic resonance (MR) T1-weighted image located 8.0 cm distal to the proximal fibular tip. Fat and fat-free mass were determined by dual-energy X-ray absorptiometry (DEXA) using a Hologic 4500 densitometer (Hologic, Waltham, MA), providing measures of regional trunk fat and extremity fat (combined fat content of upper and lower extremities).

**Hormonal assessment and laboratory methods.** Serum estradiol was measured by radioimmunoassay kit (Diagnostic Systems Laboratories, Webster, TX) with an intra-assay coefficient of variation of 6.5–8.9%. Insulin levels were measured in serum using a radioimmunoassay (Diagnostic Products, Los Angeles, CA), with intra- and interassay coefficients of variation ranged from 3.1 to 9.3 to and from 4.9 to 10.0%, respectively. Low-density lipoprotein was measured directly (Genzyme Diagnostics, Cambridge, MA). Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, fasting glucose, and 75-g oral glucose tolerance test (OGTT) were performed using standard techniques. CD4+ T-cell count was determined by flow cytometry (Becton Dickinson Biosciences, San Jose, CA), and HIV viral load was determined by ultrasensitive assay (Amphloc HIV-1 Monitor Assay, Roche Molecular Systems, Branchburg, NJ) with limits of detection of 50–75,000 RNA copies/ml. Insulin sensitivity index (ISI) (12) was calculated from the values of insulin obtained during the 2-h OGTT.

**Statistical analysis.** Comparisons were made between the groups by t-test and analysis of variance. Univariate and multivariate regression analyses were performed, comparing IMCL values and metabolic and body composition indexes in each group separately. Statistical significance was defined as P ≤ 0.05. Results are expressed as means ± SE. Statistical analyses were made using JMP Statistical...
Database Software (SAS Institute, Cary, NC). Potential outliers in the data were identified as extreme values using the Mahalanobis distance procedure in JMP. Outlier data points were identified and eliminated in IMCL (2 values in Ctrl group and 1 in HIV-infected group).

RESULTS

There were no notable differences between the two groups with respect to mean age, race, BMI, and whole body fat measured by DEXA (Table 1). HIV-infected patients had mean disease duration of 113 ± 10 mo, with 56% reporting use of protease inhibitor, 72% of nucleoside reverse transcriptase inhibitor, and 36% of nonnucleoside reverse transcriptase inhibitor. CD4+ T-cell counts were significantly different (P = 0.0004) between HIV-infected (526.4 ± 255.8 cells/mm3) and control subjects (881.8 ± 66.9 cells/mm3). HIV-infected subjects demonstrated reduced extremity fat by DEXA and subcutaneous fat of the calf by MR and increased VAT compared with control subjects (Table 1). Insulin sensitivity, assessed by ISI and fasting insulin levels, was reduced in HIV-infected subjects compared with control subjects (Table 1). Estradiol data were available in a limited subset of patients (n = 33, 9 Ctrl and 24 lipodystrophy subjects), with no significant difference between groups (75.1 ± 28.2 vs. 85.2 ± 13.1 pg/ml, Ctrl vs. lipodystrophy, P = 0.72).

The mean IMCL concentration of tibialis anterior and soleus muscles measured by 1H-MRS was significantly higher in HIV lipodystrophy subjects (Table 1). Tibialis anterior IMCL showed an inverse correlation with extremity fat as percentage of whole body fat by DEXA (r = −0.51, P = 0.01) and an positive correlation with serum triglycerides (r = 0.39, P = 0.05) in the HIV-infected group. Absolute measures of SAT and VAT by CT, whole body fat by DEXA, BMI, waist-to-hip ratio, and insulin sensitivity measures were not significantly associated with tibialis anterior IMCL among HIV-infected subjects, but significant correlations between ISI (r = −0.36, P = 0.04), insulin area under the curve (AUC; r = 0.46, P = 0.007) and fasting glucose (r = 0.33, P = 0.03) with tibialis anterior IMCL were seen in an analysis of the combined groups. In the subset of HIV-infected subjects in whom soleus IMCL was obtained (n = 9), no significant correlations were established with metabolic and body composition parameters. No relationship between IMCL and either extremity fat or VAT was seen in the control subjects. Duration in months of antiretroviral therapy with protease inhibitor (r = 0.16, P = 0.48), nucleoside reverse transcriptase inhibitor (r = 0.12, P = 0.65), and nonnucleoside reverse transcriptase inhibitor (r = −0.25, P = 0.31) did not correlate with tibialis anterior IMCL.

In a forward stepwise multivariate regression analysis, we assessed the relationship of VAT, SAT, and extremity fat as percentage of whole body fat by DEXA to IMCL, controlling for antiretroviral use in the HIV-infected group (Table 2). Extremity fat as percentage of whole body fat by DEXA (β = −633.53, P = 0.03) but not VAT and SAT areas, was significantly associated with tibialis anterior IMCL (r2 = 0.29 for model). This model indicated that a decrease of 0.1 in extrem-
ity fat as percentage of whole body fat by DEXA correlated with a 63-1U increase in tibialis anterior IMCL. In contrast, this same regression analysis showed no association of extremity fat as percentage of whole body fat by DEXA, VAT, or SAT to IMCL in controls.

**DISCUSSION**

For the present study, we investigated HIV-infected women similar in age and BMI to healthy controls, but with reduced subcutaneous fat, characterizing a model of fat redistribution and mixed lipodystrophy, rather than pure visceral obesity. Antiretroviral treatment-related alterations of adipose compartments are well known to occur in HIV-infected women, with a particular pattern characterized by breast and visceral fat accumulation, wasting of the glutei and lower limbs (7), as well as marked peripheral lipoatrophy (19). However, it is unknown whether this population exhibits differences in fat accumulation within muscle that could potentially contribute to insulin resistance and other metabolic abnormalities. Although there is evidence that IMCL levels are elevated and correlate with body composition and metabolic parameters in HIV-lipodystrophic men (9), prior studies have not assessed IMCL levels exclusively in HIV-infected women. In our study, examination of female-only HIV-lipodystrophic and control groups allowed us to control for gender variations in fat redistribution that may cause differential effects in metabolic dysregulation.

Evaluation of peripheral lipoatrophy in HIV-lipodystrophic subjects was an important end point of our study. Extremity fat mass by DEXA was analyzed as percentage of whole body fat to minimize variations introduced by differences in body habitus of our cohort. Our data demonstrated that reduced extremity fat as percentage of whole body fat by DEXA was strongly associated with tibialis anterior IMCL in HIV-infected subjects, e.g., lower extremity fat was associated with increased IMCL, and this association remained significant in forward stepwise multivariate analysis controlling for antiretroviral regimen and VAT and SAT. This finding may be related to differences in fat redistribution occurring in HIV-infected women. In a mixed-genre study by Luzzi et al. (11), the fat content measured by DEXA in the legs was significantly lower in HIV-infected subjects compared with healthy controls. In a prior study by Gan et al. (9), relationships of soleus IMCL with VAT but not abdominal SAT were identified in a study examining HIV-lipodystrophic men. Although significant extremity lipodystrophy was present in both studies, the authors did not examine its association with IMCL levels. Our findings extend these observations, suggesting that loss of extremity fat is associated with increased IMCL in HIV-infected women (13, 14). Causality cannot be determined from our cross-sectional data, and further studies are necessary to investigate the potential mechanisms of increased IMCL in the HIV population and whether peripheral fat atrophy is mechanistically linked to IMCL, examining whether fat loss and increased lipolysis may contribute to increased triglyceride and lipid deposition, or whether excess fat accumulation occurs by other mechanisms, such as reduced mitochondrial oxidation of fat in muscle. At a minimum, our data argue against a simple relationship with excess VAT, as this variable was not related to IMCL in either univariate or multivariate regression analyses among HIV-infected women.

In our study, lipodystrophic subjects were hypertriglyceridemic and showed significantly lower levels of HDL cholesterol compared with age- and BMI-matched female control subjects. Serum triglyceride concentrations were strongly positively associated with tibialis anterior IMCL among the HIV-infected subjects. These data are consistent with prior observations demonstrating that increased serum triglyceride concentrations in male patients with HIV-lipodystrophy correlate with IMCL (9) and with data demonstrating that increased free fatty acid concentrations correlate with IMCL in healthy subjects (1). Fat loss may contribute to increased triglyceride levels via increased lipolysis and associated hepatic conversion to triglyceride, as suggested by Sekhar et al. (17). It is unknown whether abnormalities in muscle lipoprotein lipase further contribute to excess lipid accumulation in the muscle.

Strong negative relationships between IMCL measured with 1H-MRS and insulin sensitivity were demonstrated in previous studies in men (9) and a mixed-genre cohort (11) with HIV-lipodystrophy. Gan et al. (9) compared HIV-infected men with and without lipodystrophy and demonstrated a strong correlation between soleus IMCL and stimulated glucose disposal using the hyperinsulinemic-euglycemic clamp technique.
in both groups combined. Luzi et al. (11) found increased IMCL in tibialis anterior and soleus muscles and significant association of IMCL with insulin sensitivity combining 12 HIV-infected lipodystrophic patients (8 male, 4 female) with matched healthy controls. In our study, lipodystrophic subjects showed significantly higher glucose AUC, fasting insulin, and insulin AUC levels, as well as decreased insulin sensitivity by ISI compared with the control group. Furthermore, fasting glucose, insulin AUC, and ISI showed significant correlations with IMCL concentrations in both groups combined, but this relationship was not significant among the HIV-infected women. In a prior study using CT, we demonstrated that increased adiposity of psoas muscle and VAT were strongly associated with insulin response to glucose challenge in HIV-lipodystrophic men (20). Accumulation of fatty acid metabolites (triglycerides, diacylglycerol, fatty acyl CoAs) at the intramyocellular level may contribute to insulin resistance by decreased glucose transport through deficient activation of the phosphatidylinositol 3-kinase cascade (15). Measures of fasting insulin and glucose and related indexes may better represent hepatic insulin sensitivity, rather than glucose uptake into muscle. We did assess dynamic insulin response to OGTT, but further tests to investigate glucose uptake in the muscle in relationship to IMCL among HIV-infected women are needed.

In this study, 1H-MRS of tibialis anterior and soleus muscle was employed for quantification of IMCL. This technique was developed by Boesch et al. (2) and subsequently validated as a reliable noninvasive technique for measurement of IMCL concentrations in muscle (18). Our methodology included calculation of IMCL concentrations relative to the unsuppressed muscle water signal, which is easily implemented and widely used in the literature. Nevertheless, it is unknown whether there are changes in muscle water concentrations of HIV-lipodystrophy subjects due to effects of chronic antiretroviral therapy. Calculation of absolute concentrations or use of an external standard may help minimize potential differences in concentrations of internal references such as water and creatine in IMCL estimation.

Our data showed IMCL was increased ~60% in tibialis anterior and ~45% in soleus muscle compared with healthy controls. The average IMCL concentration in soleus muscle of HIV-infected subjects was 200 IU higher than controls, whereas in the tibialis anterior muscle this difference was 50 IU. The lack of significant correlations of soleus IMCL with body composition parameters and metabolic indexes in our study is likely due to the limited number of subjects in which soleus data was obtained. However, the marked difference of soleus IMCL concentrations between HIV-infected and control subjects may reflect its higher sensitivity to changes in insulin homeostasis. A few prior reports on nonlipodystrophic subjects have shown soleus IMCL correlating more consistently with insulin sensitivity compared with tibialis anterior muscle (16; 8). These findings may be explained by observations in animal models that muscles with predominance of type I fibers (e.g., soleus) are more insulin sensitive than muscles with larger fractions of type II fibers (e.g., tibialis anterior) (16, 6). On the other hand, the tibialis anterior muscle contains a considerable fraction of type I fibers ranging from 65% to above 90% (10), with an architecture allowing optimal peak separation and good repeatability indexes (21), which may be advantageous in populations with altered partitioning of fat compartments, such as the HIV-infected subjects in our study. Further studies on specific clinical populations may be necessary to determine the most appropriate muscle for evaluation of conditions affecting IMCL concentrations.

In conclusion, this study demonstrates increased IMCL concentrations in HIV-infected women in association with lipodystrophic changes in fat, specifically decreased extremity fat. IMCL is associated with serum triglyceride concentrations and in turn may contribute to increased insulin resistance. Further studies are needed to determine the mechanisms and consequences of intramuscular lipid accumulation in HIV-infected women.

GRANTS

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