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Depot-specific regulation of glucose uptake and insulin sensitivity in HIV-lipodystrophy

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Abstract

Altered fat distribution is associated with insulin resistance in HIV, but little is known about regional glucose metabolism in fat and muscle depots in this patient population. The aim of the present study was to quantify regional fat, muscle, and whole body glucose disposal in HIV-infected men with lipoatrophy. Whole body glucose disposal was determined by hyperinsulinemic clamp technique ($80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) in 6 HIV-infected men and 5 age/weight-matched healthy volunteers. Regional glucose uptake in muscle and subcutaneous (SAT) and visceral adipose tissue (VAT) was quantified in fasting and insulin-stimulated states using 2-deoxy- ^{18}F fluoro-D-glucose positron emission tomography. HIV-infected subjects with lipoatrophy had significantly increased glucose uptake into SAT (3.8 ± 0.4 vs. $2.3 \pm 0.5 \mu\text{mol} \cdot \text{kg tissue}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$) in the fasted state. Glucose uptake into VAT did not differ between groups. VAT area was inversely related with whole body glucose disposal, insulin sensitivity, and muscle glucose uptake during insulin stimulation. VAT area was highly predictive of whole body glucose disposal ($r^2 = 0.94$, $P < 0.0001$). This may be mediated by adiponectin, which was significantly associated with VAT area ($r = -0.75$, $P = 0.008$), and whole body glucose disposal ($r = 0.80$, $P = 0.003$). This is the first study to directly demonstrate increased glucose uptake in subcutaneous fat of lipoatrophic patients, which may partially compensate for loss of SAT. Furthermore, we demonstrate a clear relationship between VAT and glucose metabolism in multiple fat and muscle depots, suggesting the critical importance of this depot in the regulation of glucose and highlighting the significant potential role of adiponectin in this process.

Keywords

positron emission tomography; adipose tissue; insulin resistance; human immunodeficiency virus-lipodystrophy

Abnormal distribution and metabolism of adipose tissue, as well as insulin resistance, are well-known complications of HIV antiretroviral therapy (3, 9, 21). Although antiretroviral medications such as protease inhibitors may directly impair insulin sensitivity (37),

abnormal adipose distribution may itself play an important role in insulin resistance in this population. For instance, as with congenital lipodystrophy syndromes (18), peripheral lipotrophy is associated with decreased insulin sensitivity in HIV-lipodystrophy (30, 34). Visceral or abdominal adiposity, a known risk factor for insulin resistance, type 2 diabetes, and the metabolic syndrome (10, 11, 51), may also be relatively increased in HIV-lipodystrophy (41).

In the setting of abnormal fat distribution in HIV, insulin resistance may be caused by ectopic lipid accumulation in muscle (17) and liver (44) as well as by abnormalities in adipocytokine physiology (46). Among HIV-infected patients with lipodystrophy, increased visceral adiposity is associated with excess free fatty acids (22, 48), increased intramyocellular fat content (17, 28, 47), and hypoadiponectinemia (1, 33, 45, 46), but little is known regarding the extent to which the visceral and subcutaneous fat depots affect glucose regulation. Understanding the metabolic activity in adipose tissue is central to the pathophysiology of whole body and tissue-specific insulin sensitivity in HIV.

Behrens et al. (4) recently used positron emission tomography (PET) to show that muscle glucose uptake is impaired with HIV antiretroviral therapy. Similar techniques have been used to demonstrate abnormal glucose metabolism in adipose tissue of obese and diabetic subjects, specifically demonstrating relationships between whole body and depot-specific glucose disposal (49, 50). Quantitative measurements of adipose glucose metabolism in relation to body composition and metabolic risk factors for insulin resistance have not been explored previously in HIV-lipodystrophy. In addition to impaired muscle glucose utilization, impaired adipose glucose uptake may play a significant role in insulin resistance in HIV-lipotrophy.

The aim of the present study was to quantitatively assess regional glucose metabolism in muscle and adipose tissues and to characterize the relationship between measurements of whole body insulin sensitivity, lipolysis, endogenous glucose production, and adipocytokines in lipotrophic HIV-infected patients receiving highly active antiretroviral therapy (HAART). Our results demonstrate for the first time that glucose uptake into a well-defined subcutaneous fat depot is significantly increased, which may help to compensate for the overall lack of this storage depot and reduced glucose uptake into muscle among lipotrophic patients. Furthermore, we demonstrate that visceral fat area largely determines overall insulin sensitivity, and is tightly related to glucose uptake into fat and muscle compartments, under conditions of hyperinsulinemia.

MATERIALS AND METHODS

Subjects

Subjects were recruited through advertisements in newspapers as well as postings in community and HIV resource centers. Seven HIV-infected and six healthy men without a history of HIV infection were recruited to participate in the study. Written, informed consent was obtained from each subject in accordance with the Massachusetts General Hospital and the Massachusetts Institute of Technology institutional review boards. The study was approved by the Institutional Review Board of the Massachusetts General Hospital and by the Committee on the Use of Humans as Experimental Subjects of the Massachusetts Institute of Technology.

The inclusion criteria for HIV-infected subjects were lipotrophy, defined as body fat $\leq 20\%$ by bioelectrical impedance (BIA) analysis (model BIA 101; RJL Systems, Clinton Township, MI), and evidence of fat atrophy, rated by the investigator as present in one or more body regions, such as the face, arms, or legs, and stable antiretroviral regimen

containing a protease inhibitor (PI) for ≥ 3 mo. Control subjects were required to be healthy, on no medications, and of comparable age and weight. Exclusion criteria for all subjects were hemoglobin < 11.0 g/dl, history of diabetes mellitus, use of antidiabetic medication, glucocorticoids, growth hormone, or other anabolic agents currently or within the previous 3 mo (except for physiological testosterone replacement), and current substance abuse. One control subject was excluded on the basis of abnormally low subcutaneous fat [the subject's abdominal subcutaneous adipose tissue (SAT) area was more than one standard deviation below the mean of the HIV-lipoatrophy subjects]. In addition, one HIV-infected subject was excluded due to insufficient SAT for adequate analysis with PET imaging.

Experimental Design

After a screening visit to determine eligibility, subjects completed metabolic testing on 2 days separated by ≥ 2 wk.

Test day 1—Subjects were asked to report at 7 AM after an overnight fast and to refrain from heavy exercise for 1 day before the visit. HIV antiretroviral medications were taken after test procedures were completed. Each subject completed proton magnetic resonance spectroscopy (^1H MRS) of the calf, cross-sectional magnetic resonance imaging (MRI) of the abdomen, and PET of the right thigh and abdomen with 2-deoxy- ^{18}F fluoro-D-glucose (FDG) infusion.

^1H MRS of the Tibialis Anterior Muscle and Mri of the Midabdomen: ^1H -MRS of the tibialis muscle was performed to quantify lipid concentration in skeletal muscle. Conventional MR images were acquired for anatomic reference of ^1H -MRS overlays. The subjects were scanned using a 1.5-Tesla system (Signa LX, software version 8.3; General Electric Medical Systems, Milwaukee, WI). ^1H -MRS of tibialis anterior muscle was performed by positioning the feet first in the magnet, and the right calf was placed in a standard radio frequency transmit-and-signal-receive 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 was obtained. Axial T1-weighted images (TR, 600 ms; TE, 14 ms; slice thickness, 4 mm; interslice gap, 1 mm; matrix, 128^2 ; NEX, 1; FOV 16 cm) of the proximal two-thirds of the calf were prescribed using the proximal tip of the fibula as an osseous landmark. The first slice was always placed at the level of proximal fibular tip, and the prescription stack was propagated distally with the above-mentioned thickness and spacing parameters. Single-voxel MRS data were acquired using point-resolved spatially localized spectroscopy (PRESS) pulse sequence with TE of 25 ms, repetition time TR of 3,000 ms, 32 acquisitions, and 8 NEX. In all cases, a voxel measuring $15 \times 15 \times 15$ mm (3.4 ml) was placed on the axial T1-weighted slice with largest muscle crosssectional area, avoiding visible interstitial tissue, fat, or vessels. Automated optimization of gradient shimming, water suppression, and transmit-and-receive gain was followed by manual adjustment of central frequency and performed for each voxel placement, obtaining water line widths of 10-12 Hz. Water presaturation was used for metabolite spectral acquisition, and unsuppressed water spectra of the same voxel were obtained for each scan. Neither metabolite levels nor unsuppressed water levels were corrected for T1 and T2 relaxation times. An axial T1-weighted slice (fast spin-echo pulse sequence, 5-mm slice thickness, 48-cm field of view, TR 300 ms, TE 12 ms, echo train of 4, 256×256 matrix, 1 NEX) of the midabdomen at the L4 pedicle was obtained to localize fat depots on PET scans of the same region.

PET of Right Thigh and Abdomen at L4: Subjects were placed in a supine position and rested for ~ 50 min, during which resting energy expenditure (REE) was determined (see REE), followed by placement of a venous catheter in one arm for infusion and an arterial catheter in the opposite arm for blood sampling. The midthigh region was centered in the

camera's field of view after a reference using the scanner-positioning laser to the midpatella was made. Transmission images of 10 min duration were acquired with a rotating ^{68}Ge pin source. Approximately 10 mCi of FDG were then injected over 1.5 min through the venous catheter, and sequential PET images were acquired. Arterial blood samples were obtained every 15 s for 3 min, every 30 s for 2 min, every 1 min for 5 min, every 5 min for 55 min, and at 75 and 90 min. PET images were acquired for 30 s/frame for 3 min, 60 s/frame for 4 min, 120 s/frame for 32 min, and 300 s/frame for 50 min. Arterial FDG blood plasma radioactivity was measured using a calibrated Baird Atomic well counter. At 90 min, the abdominal acquisition was started, and the camera was centered on a localizing mark made on the skin corresponding to the level of the L4 pedicle. A 15-min image was acquired followed by a 10-min transmission image.

REE: REE was measured for 20 min by indirect calorimetry (Deltatrac; Sensormedics, Anaheim, CA) after subjects had been resting in the PET gantry for 30 min and before placement of the intravenous and arterial catheters.

Dual-Energy X-Ray Absorptiometry: Whole body dual-energy X-ray absorptiometry (DEXA) was performed using a Hologic QDR-4500 densitometer (Hologic, Bedford, MA) to determine total body and regional percent fat and lean body mass. The technique has a precision error (1 SD) of 3% for fat and 1.5% for lean body mass.

Test Day 2—As in *day 1*, subjects reported at 7 AM after a 12-h overnight fast. Weight did not vary more than 2 kg in any individual subject between the two visits. This test day consisted of a crosssection computed tomography (CT) scan of the midthigh and abdomen, a hyperinsulinemic euglycemic clamp with stable isotope tracers of glycerol and glucose, and FDG infusion and PET imaging during hyperinsulinemic steady state.

CT of Right Thigh and Abdomen at L4: CT 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 image were standardized (144-cm table height, 80 kV, 70 mA, 2 s, 1-cm slice thickness, and 48-cm field of view). The single cross-sectional CT image at L4 was used to assess distribution of subcutaneous and visceral abdominal adipose tissue (SAT and VAT, respectively). Fat attenuation values were set at -50 to -250 Hounsfield units, as described by Borkan et al. (7), and intra-abdominal VAT and abdominal SAT areas were determined on the basis of tracings obtained using graphic analysis software (Alice; Parexel, Waltham, MA).

A coronal scout image was used to prescribe an axial CT image of the thigh. The image was obtained at the midpoint between the articular surface of the femoral head and medial femoral condyle by using 120 kV, 170 mA, 2 s, 1-cm slice thickness, and 36-cm field of view. The subcutaneous tissue and muscle areas were measured using graphic analysis software (Alice, Parexel). Manual tracings separated subcutaneous fat and muscle compartments. Subfascial adipose tissue interspersed between muscle was excluded from the determination of muscle area and included in the determination of the subcutaneous fat compartment.

Isotope Infusion and Hyperinsulinemic Euglycemic Clamp: Two hours before insulin infusion, subjects were positioned supine on the PET scanner gantry, and intravenous infusions of $[6, 6 - ^2\text{H}_2]$ glucose (22 $\mu\text{mol/kg}$ prime followed by $0.22 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ continuous infusion) and $[^2\text{H}_5]$ glycerol (1.6 $\mu\text{mol/kg}$ prime followed by $0.11 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ continuous infusion) were commenced (Fig. 1). Thirty minutes before the

start of the hyperinsulinemic clamp, blood samples were drawn every 10 min for determination of isotopic enrichment in the fasting state. Samples were subsequently obtained every 10 min from 90 through 150 min for assessment of enrichment during hyperinsulinemia. Two hours after the start of the isotope infusions, and after placement of an arterial catheter for blood sampling in the contralateral arm, a primed continuous infusion of insulin ($80 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) was maintained for ~205 min, until the end of the PET imaging period (Fig. 1). Blood samples were obtained every 5 min for determination of blood glucose concentration (B-Glucose Analyzer; Hemocue, Lake Forest, CA). A variable amount of 20% dextrose solution was infused to maintain the blood glucose at 90 mg/dl.

This was enriched with $[6, 6 - ^2\text{H}_2]$ glucose ($15.2 \mu\text{mol} [6, 6 - ^2\text{H}_2]$ glucose/ml 20% dextrose) to minimize the influence of exogenous glucose administration on plasma isotopic enrichment (16). Insulin and free fatty acid (FFA) concentration were determined every 20 min during the clamp test. All blood samples were placed on ice, spun, separated, and maintained on ice until frozen at -80°C and stored until analysis.

PET Scan of Right Thigh and Abdomen at L4: PET FDG imaging commenced at ~90 min of the hyperinsulinemic clamp, when the variable glucose infusion rate required minimal adjustments for ≥ 20 min. The PET technique used was identical to the procedures performed on *test day 1*. Subjects were already appropriately positioned on the PET scanner gantry for the infusion study and, hence, could be shifted into position automatically.

REE: We measured REE during two 20-min intervals: once before the start of the hyperinsulinemic clamp (~60-90 min after the start of the isotope infusions) and once at steady state during the hyperinsulinemic clamp (~90-120 min into the clamp and 30 min into the PET scan; Fig. 1).

Calculations

Intramyocellular lipid content— ^1H -MRS raw data were analyzed employing LCModel (version 6.0-2). Data were transferred from the scanner to a Linux workstation, and metabolite quantification was performed using Eddy current correction and water scaling. The fitting algorithm was customized for muscle analysis, providing estimates for lipid peaks (0.9, 1.1, 1.3, 1.5, 2.1, and 2.3 ppm), creatine (2.8 and ~3.0 ppm), choline (3.2 ppm), and putative taurine signal (~3.5 ppm). Only data for intramyocellular lipid (IMCL) CH₂-protons at 1.3 ppm were used for statistical analysis. The lipid estimates were scaled to the unsuppressed water peak (IMCL_W) and expressed in institutional units (IU).

Glucose and glycerol turnover rates—Fasting glucose and glycerol rates of appearance (R_a) were derived using the Steele equation, relating tracer infusion to plasma enrichment (53): $R_a = F/\text{MFE} - F$, where F is tracer infusion rate, and MFE is mole fraction excess enrichment over baseline. MFE in the fasting state was the average of samples between 90 and 120 min of the isotope infusion. This has been determined in prior studies to be adequate time to allow for equilibration (27, 52). Glycerol turnover between 90 and 150 min of the hyperinsulinemic clamp was also determined using the Steele equation, during which time enrichment was relatively constant. Glucose turnover during this interval was determined using the steady-state assumption but taking into account the added source of labeled glucose and the variable-rate exogenous glucose administration (31). The R_a of glucose during the hyperinsulinemic clamp was suppressed to zero for all subjects.

Regional FDG glucose uptake—A General Electric/Scanditronix PC4096 15-slice whole body tomograph was used to produce 15 contiguous slices 6 mm thick, 6.5 mm center to center with 6 mm in-plane and axial resolution full width half-maximum. Images were reconstructed using a filtered back-projection algorithm, and all projection data were

corrected for nonuniformity of detector response, dead time, random coincidences, and scattered radiation.

Thigh Muscle Glucose Metabolism: Sequential images over the thigh muscle were obtained over a period of 90 min. Four to six irregular-shaped regions of interest (ROIs), each containing $\sim 6 \text{ cm}^3$ of tissue, were placed on selected slices, taking particular caution to exclude bone and major vascular structures. A three-compartment kinetic model was used to estimate glucose kinetics (25). A nonlinear least squares fitting procedure was used to determine the rate constants (k_1, k_2, k_3, k_4) using the arterial input function and the composite tissue time-activity curves. FDG metabolic rate (K) was calculated by relating the rate constants according to the equation $K = (k_1 k_3) / (k_2 + k_3)$ (25). Muscle glucose uptake was obtained by multiplying FDG uptake (K) by the plasma glucose concentration and then dividing by the lump constant value of 1 (38).

Abdominal Adipose Tissue Glucose Metabolism: After the dynamic data acquisition of thigh muscle was completed, a static 15-min emission image was acquired over the abdomen followed by a 10-min transmission image for attenuation correction. We used the Sokoloff autoradiographic method to determine glucose uptake in SAT and VAT depots (43). ROIs were drawn as described above, and cross-sectional images from CT and MRI were used to guide placement of the ROIs (Fig. 2). Tissue glucose utilization rates were calculated as follows:

$$(\text{Glc}) \cdot C(T) / \left\{ \text{LC} \cdot \left[\int_0^T (\text{arterial plasma FDG}) dt \right] \right\} \quad (1)$$

where [Glc] denotes plasma glucose, $C(T)$ radioactive concentration in tissue at time T , and LC the lump constant, which was set at 1 (23, 43).

Insulin-stimulated glucose disposal—We employed the method of DeFronzo et al. (13) for determination of glucose metabolism during hyperinsulinemia. Insulin-stimulated glucose disposal (M) was determined for three 20-min intervals: immediately before FDG injection, following the injection of the FDG, and following reposition of the PET camera from leg to abdomen. These values were tightly correlated, and therefore the three determinations were averaged for a mean overall M value for each subject. M values ($\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$) were indexed to DEXA-derived fat-free mass (FFM). Homeostatic model of assessment of insulin resistance (HOMA-IR) was calculated using fasting insulin and glucose levels (40).

Bioassays and Isotope Derivatization—Fasting adiponectin, leptin, and resistin were measured using radioimmunoassays (adiponectin and leptin: Linco Research, St. Charles, MO; resistin: ALPCO Diagnostics, Windham, NH). Intra- and interassay coefficients of variation were, respectively, 1.78-6.21 and 6.90-9.25% for adiponectin, 3.4 and 3.0-6.2% for leptin, and 2.86-5.17 and 4.2-7.2% for resistin. FFA levels were measured with an in vitro enzymatic colorimetric assay kit (Wako Chemicals, Richmond, VA). The intra-assay coefficients of variation ranged from 1.1 to 2.7%. Total cholesterol, high-density lipoprotein cholesterol, triglyceride, and glucose concentrations were measured using standard techniques.

[$^2\text{H}_5$] glycerol—Plasma samples were deproteinized with acetonitrile prior to derivitization and were prepared as the trimethylsilyl derivative according to the method of Beylot et al. (5). A Hewlett-Packard 5890 gas chromatograph coupled to a 5989A mass spectrometer was autotuned in Electron Impact (EI) mode according to the manufacturer's specifications. A

standard curve was prepared comparing unlabeled glycerol to varying known amounts of isotope-labeled glycerol ($^2\text{H}_5$, 98%). Ions representing natural or “unlabeled” glycerol ($m/z = 205$) and [^2H]glycerol ($m/z = 208$ $^2\text{H}_5$ as an $M + 3$ fragment) were monitored. The ratio of the total area counts for $m/z = 205$ and $m/z = 208$ is used to calculate the mole fraction.

[$6, 6 - ^2\text{H}_2$] glucose —Plasma samples were deproteinized with acetonitrile and were prepared as the aldonitrile pentaacetate derivative according to the method of Guo et al. (19). The same instrument as above in EI mode was used to quantify ion abundance. A standard curve was prepared along with in-house plasma control samples. Ions representing natural or unlabeled glucose ($m/z = 187$) and [$6, 6 - ^2\text{H}_2$] glucose ($m/z = 189$) were monitored. The ratio of the total area counts for $m/z = 187$ and $m/z = 189$ was used to calculate the mole fraction.

Statistics

Baseline group characteristics and both fasting and hyperinsulinemic physiological variables were compared between groups using Student's t -test. Within-group comparisons between fasted and insulin-stimulated conditions were made using paired t -tests. Data from both subject groups were combined, and linear correlation coefficients were generated to determine associations between measurements of body composition and metabolic parameters. In addition, stepwise multivariate regression models were constructed to assess potential predictors of insulin-stimulated glucose uptake into various tissues. All data are presented as means $\pm$ SE unless otherwise indicated. Nonnormally distributed variables were log transformed to approximate normality when indicated. A two-level α of 0.05 was used to determine statistical significance. Statistical analyses were performed using SAS JMP software, version 4.04 (SAS Institute, Cary, NC).

RESULTS

All HIV-infected subjects were on a stable antiretroviral regimen containing one or more nucleoside reverse transcriptase inhibitors (NRTIs) and a PI (2 lopinavir/ritonavir, 2 atazanavir/ritonavir, 1 nelfinavir, and 1 fosamprenavir) for ≥ 3 mo. Despite similarities in age, body mass index (BMI), and lean body mass (Table 1), HIV-infected subjects had significantly less body fat (DEXA total %body fat, $P = 0.008$), particularly in the subcutaneous fat compartment (SAT area $87.4 \pm 8.4 \text{ cm}^2$ vs. $176.6 \pm 26.9 \text{ cm}^2$, HIV vs. control, $P = 0.007$; and subcutaneous thigh fat area $23.0 \pm 4.3 \text{ cm}^2$ vs. $55.1 \pm 3.6 \text{ cm}^2$, $P = 0.0003$). Visceral adiposity was not significantly increased in the HIV-infected subjects with lipoatrophy ($106.0 \pm 25.0 \text{ cm}^2$ vs. $88.3 \pm 17.2 \text{ cm}^2$ in controls, $P = 0.59$). Although not statistically significant, HIV-infected subjects with lipoatrophy tended to have greater IMCL content of the tibialis anterior muscle compared with controls (104.4 ± 9.5 vs. $80.9 \pm 9.6 \text{ IU}$, $P = 0.12$).

Fasting glucose, insulin, HOMA-IR, and FFA levels were not significantly different between the two groups, whereas HIV-infected subjects had higher mean triglyceride levels and lower HDL cholesterol (Table 1). HIV-infected subjects tended to have lower levels of adiponectin (5.4 ± 2.2 vs. $9.5 \pm 1.2 \text{ } \mu\text{g/ml}$, $P = 0.15$) and had significantly lower leptin concentrations ($P = 0.02$) compared with control subjects. Resistin levels did not differ between the subject groups.

Basal endogenous glucose production (R_a glucose) was elevated in HIV-infected subjects but not significantly different from that in control subjects ($P = 0.07$; Table 1). Endogenous glucose production was completely suppressed with insulin administration in both subjects

groups. Although the basal rates of lipolysis measured by the R_a of glycerol were similar in HIV-infected and control subjects, suppression of lipolysis with insulin tended to be less in the HIV group (suppression of lipolysis: 43% HIV vs. 59% control, $P = 0.09$). REE did not differ between HIV-infected subjects and controls.

Under basal fasted conditions, PET imaging demonstrated that glucose uptake into the abdominal subcutaneous fat compartment was significantly greater in HIV-infected compared with control subjects (3.8 ± 0.4 vs. $2.3 \pm 0.5 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P < 0.05$), whereas uptake into the visceral fat compartment and muscle did not differ between groups (Fig. 3). With insulin stimulation, glucose uptake into both fat depots and muscle increased compared with the fasted state (Fig. 3), but there was no difference between HIV-infected and control subjects. Although not statistically significant, insulin-stimulated glucose uptake into muscle was decreased in HIV-infected subjects (54.9 ± 10.9 vs. $86.0 \pm 16.1 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $P = 0.1$) compared with control subjects.

To evaluate the potential relationship among body fat distribution, glucose metabolism, and adipocytokine concentrations, data from HIV-infected subjects with lipoatrophy and control subjects were combined for linear regression analyses. Importantly, in both the fasted and insulin-stimulated states, VAT glucose uptake was a strong predictor of overall insulin sensitivity (e.g., correlation with M: $r = 0.72$, $P = 0.01$, during insulin-stimulated conditions; Table 2). Furthermore, there were strong, statistically significant inverse correlations between the amount of VAT and insulin-stimulated whole body glucose disposal, thigh muscle glucose uptake, and VAT glucose uptake, and there was a trend toward a significant relationship with SAT glucose uptake ($r = -0.56$, $P = 0.07$; Table 3). For example, VAT area was highly predictive of M ($r^2 = 0.94$, $P < 0.0001$; Fig. 4). In addition, VAT area was significantly inversely correlated with percent FFA suppression with insulin ($r = 0.77$, $P = 0.006$).

Abdominal SAT area was inversely related to glucose uptake into subcutaneous fat ($r = -0.72$, $P = 0.01$) and muscle ($r = -0.58$, $P = 0.06$) in the fasted state. However, SAT glucose uptake did not correlate with general measurements of insulin sensitivity or tissue-specific glucose uptake in other depots.

There was a strong inverse correlation between adiponectin and VAT area ($r = -0.75$, $P = 0.008$). Adiponectin levels were significantly positively correlated with measurements of whole body insulin sensitivity. For example, M was strongly associated with adiponectin ($r = 0.80$, $P = 0.003$). Although muscle glucose uptake was also directly related to adiponectin levels under insulin-stimulated conditions ($r = 0.77$, $P = 0.005$), no significant relationships were identified between adiponectin and VAT or SAT glucose uptake. Resistin levels were positively correlated with total percent body fat ($r = 0.62$, $P = 0.04$) and negatively associated with glucose uptake into muscle in the fasted state ($r = -0.67$, $P = 0.02$) but not related to VAT or SAT area.

To assess the relative contribution of adipocytokines and body fat distribution to tissue-specific insulin-stimulated glucose uptake, multivariate stepwise regression analyses were performed. Variables eligible for inclusion in each model were VAT area and SAT area and adiponectin, resistin, and leptin concentrations. VAT area was the only significant independent predictor of whole body insulin-stimulated glucose disposal ($\beta = -0.053$, $P < 0.0001$). Similarly, VAT area was the only significant predictor of insulin-stimulated glucose uptake into visceral fat ($\beta = -0.080$, $P < 0.005$), whereas VAT area and SAT area were independently associated with glucose uptake into subcutaneous fat (VAT area $\beta = -0.034$, $P = 0.02$; SAT area $\beta = -0.026$, $P = 0.03$). Interestingly, adiponectin, and not VAT

area, was the significant predictor of insulin-mediated glucose uptake into muscle ($\beta = 5.6$, $P = 0.005$) in the multivariate model.

DISCUSSION

Using PET, we identified fasting elevations in subcutaneous glucose uptake in subjects with HIV-lipoatrophy. This unexpected finding was present despite ongoing antiretroviral therapy containing a PI, marked lipoatrophy, and hyperlipidemia. In contrast, glucose uptake into the visceral fat was similar in the HIV and control groups, and measurements of VAT area were linearly correlated with overall insulin sensitivity. Moreover, VAT area was highly related to glucose uptake into the muscle and fat depots, suggesting a schema whereby increased glucose uptake in subcutaneous fat may be compensatory for diminished SAT stores, but VAT largely regulates insulin-mediated glucose uptake across multiple depots in patients with lipodystrophy. Among lipoatrophic patients, increased glucose uptake into subcutaneous fat may partially compensate for reduced glucose uptake into muscle, but overall insulin sensitivity is maintained if the visceral fat area is normal. In this regard, VAT area was highly positively related to adiponectin and impaired suppression of lipolysis. These observations help to identify the individual roles played by specific fat depots in the regulation of tissue-specific and whole body glucose metabolism among HIV-infected patients with lipoatrophic changes in subcutaneous fat.

To our knowledge, this is the first human study to measure both fasting and insulin-stimulated glucose uptake in regional fat and muscle compartments. In prior studies using PET among healthy obese patients, adipose tissue was estimated to account for a greater proportion of whole body glucose disposal compared with nonobese individuals (13 vs. 8%, respectively), but a pattern was seen in which the uptake of glucose into abdominal SAT per unit volume was reduced (50). In contrast, we observed the opposite phenomenon among patients selected for lipoatrophy, i.e., increased subcutaneous glucose uptake, primarily in the fasted state but with a similar pattern in hyperinsulinemic conditions. In the otherwise healthy obese patients studied by Virtanen et al. (50), both SAT and VAT areas were increased. In contrast, abdominal SAT area was markedly decreased whereas VAT area was similar to that in healthy control subjects in our study. Taken together, our data and those of Virtanen et al. describe two opposite ends of a fat distribution spectrum and show differential behaviors of SAT, depending on the relative amount of SAT present.

Among diabetic subjects, abdominal SAT may account for 4% of glucose disposal and overall adipose tissue for 22-26% (49). As peripheral SAT was markedly attenuated in our study, measurements of nonabdominal SAT could not be made, and therefore we could not determine the overall relative contribution of SAT to whole body glucose uptake, except to note that glucose uptake into SAT was significantly increased compared with controls.

It is unlikely that the observed increased glucose uptake in SAT is a result of PIs, as they have been shown to decrease glucose transport (32, 36). Instead, our results may be due to a NRTI effect. In a small study assessing mitochondrial toxicity of thymidine analogs on adipocyte physiology, Bleeker-Rover et al. (6) observed increased whole body peripheral fat glucose uptake associated with stavudine in patients with HIV-lipodystrophy. Furthermore, they describe normalization of peripheral fat glucose uptake in three individuals who switched from stavudine to zidovudine or abacavir. In the present study, no subject was on a stavudine-containing regimen, but all subjects were receiving an NRTI, and subcutaneous fat glucose uptake was increased compared with controls in the fasted state.

Heightened fasting glucose uptake in abdominal SAT may be a marker of metabolic dysfunction. SAT from subjects with HIV-lipodystrophy manifests inflammatory cell

infiltration (14, 24), increased adipocyte apoptosis (12), and elevated expression of proinflammatory cytokines (24), and in vitro evidence suggests that NRTIs compromise mitochondrial membrane function and differentiated adipocyte cell survival (8). Subcutaneous adipose mitochondrial DNA content is depleted in fat from lipotrophic individuals (42) and in those exposed to NRTIs such as stavudine or zidovudine (39).

Ample evidence exists for mitochondrial dysfunction that may account for increased glucose transport in the fasted state. For example, nonoxidative glucose disposal via GLUT1 may increase in the context of compromised mitochondrial function, as there is in vitro evidence suggesting that mitochondrial oxidative inhibition results in increased translocation of GLUT1 and increased anaerobic ATP production via increased glycolysis (2, 15). Similarly, NRTIs can impair mitochondrial function in adipocytes (29) and therefore may shift metabolism toward GLUT1-mediated glucose uptake and increased glycolysis in the fasted state in vivo. This pattern may represent a stress response as a result of mitochondrial dysfunction and may explain the increase in SAT glucose uptake during the fasted state, when GLUT1 is important. With hyperinsulinemia, stimulation of GLUT4 trafficking may increase despite compromised mitochondrial function, lessening the difference in SAT glucose uptake between controls and HIV-infected patients, as suggested by our data.

The precise mechanism by which glucose uptake into SAT among lipotrophic patients is increased during a fasted, low-insulin state is not known. Factors such as regulation of the GLUT4 or GLUT1 glucose transporters may be involved. Further studies will be necessary to determine the mechanisms controlling this potentially compensatory increase in glucose uptake into SAT in lipotrophic patients and to determine whether this novel finding extends to patients with congenital or generalized lipodystrophy. Furthermore, the fate of increased glucose into SAT in lipotrophic patients has not been quantified, and it remains unknown if glucose disposition and fuel cycling is normal.

Under insulin-stimulated conditions, visceral adipose depot size was robustly correlated with whole body as well as regional muscle and fat glucose uptake. Furthermore, our data are in agreement with those of Virtanen et al. (50), who demonstrated that visceral fat glucose uptake was highly correlated with overall glucose disposal. However, we show for the first time that VAT area is inversely correlated with glucose uptake into specific tissue depots, including muscle and visceral and subcutaneous fat, as well as with overall whole body glucose disposal. These observations point to the important role of VAT in glucose homeostasis. The relatively normal amounts of VAT in the patients selected for lipotrophy may explain the relatively normal whole body glucose disposal found in this group.

Our data suggest that adiponectin may mediate the effects of increased VAT area. Adiponectin was significantly inversely correlated with VAT area and significantly positively correlated with whole body glucose disposal and even more strongly with glucose uptake into muscle by PET. Furthermore, in a multivariate regression model, adiponectin was the strongest predictor of muscle glucose uptake. Adiponectin is known to increase mitochondrial oxidation (54), and reduced adiponectin in association with increased VAT may thereby reduce insulin sensitivity via this mechanism. In addition, we show that resistin levels correlated positively with percent body fat; thus resistin may also affect glucose homeostasis in lipotrophic patients.

VAT area was significantly inversely correlated with percent suppression of lipolysis by insulin. In non-HIV-infected patients, elevated FFA and impaired suppression of lipolysis in response to insulin may be a direct consequence of excess VAT and thereby contribute to whole body and tissue-specific insulin resistance (26, 35). In HIV-infected patients, we have shown increased lipolytic rates and improved insulin sensitivity with suppression of lipolysis

with acipimox (20, 22). Behrens et al. (4) previously found a defect in lipolytic suppression in HIV-infected patients on HAART, but we extend these findings by demonstrating a relationship with VAT. Increased VAT area was strongly associated with impaired FFA suppression in the insulin-stimulated state, suggesting that FFAs may mediate the effects of VAT on insulin resistance.

Our data extend the findings of Behrens et al. (4) but differ in a number of respects. Behrens et al. compared HIV patients receiving a PI with those not receiving a PI and demonstrated marked differences in whole body glucose uptake and reduced uptake into the muscle by PET. In contrast, we chose HIV patients on the basis of an objective marker of lipoatrophy (BIA <20%) and did not see global differences in whole body glucose disposal, but we did show a trend toward reduced glucose uptake into the muscle during hyperinsulinemia. Behrens et al. did not measure glucose uptake into any of the fat depots and thus did not characterize overall glucose homeostasis in the fasting and hyperinsulinemic state across multiple depots. We speculate that overall whole body glucose disposal was not different between the HIV patients and controls in our study for two reasons. First, as discussed above, increased glucose uptake into SAT may partially compensate for anticipated reduction in insulin sensitivity associated with lipoatrophy. Second, VAT was not significantly different between the groups. As we have shown, VAT area largely drives overall and depot-specific glucose uptake in the insulin-stimulated state and thus may coordinate glucose trafficking among the different depots, in part through adiponectin or differences in suppression of lipolysis.

The use of PET to classify glucose trafficking into multiple fat and muscle depots simultaneously under fasted and insulin-stimulated states is a novel method to determine depot-specific insulin sensitivity and to unmask components that contribute to whole body glucose uptake. We demonstrate a compensatory increase in glucose uptake into subcutaneous fat not previously seen with more global measurements of insulin sensitivity and demonstrate the clear predominance of the visceral compartment to determine whole body insulin sensitivity. The results of this study suggest the potential utility of this technique to determine the behavior of regional fat depots in other patient populations. Our data from a lipoatrophic model suggest that fat depots may act independently to contribute to the overall glucose regulation, including the uptake of glucose into the muscle.

In this study, we were limited in our ability to determine glucose uptake into a nonabdominal SAT depot due to severe lipoatrophy. In this regard, future studies should determine whether assessment of whole body and regional fat volume with MRI is a better index of glucose uptake by PET than regional cross-sectional fat area by CT scan. In this crosssectional study, we could not determine causality with certainty, and it remains unclear whether lipoatrophy in general, an effect of specific antiretrovirals, or another factor contributed to the observed findings with respect to SAT in our patient population.

In summary, this is the first quantitative observation of increased glucose uptake into SAT in lipoatrophic HIV-infected patients. These data have implications beyond the model studied, and it will be useful to determine whether increased glucose transport is mediated by GLUT1 independently or as a result of factors related to antiretroviral medications, e.g., mitochondrial damage from NRTIs. This study is the first to directly demonstrate the clear relationship between the VAT depot and glucose trafficking across multiple fat and muscle depots of HIV-infected subjects with lipoatrophy, and we highlight the significant potential role of adiponectin in this process. Additional studies of simultaneous glucose trafficking into multiple fat and muscle depots by use of PET may be useful to determine the mechanisms of insulin resistance in type 2 diabetes mellitus and obesity.

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Fig. 1. Protocol schema for simultaneous hyperinsulinemic clamp and positron emission tomography (PET) 2-deoxy-[¹⁸F]fluoro-D-glucose (¹⁸FDG) testing. IC, indirect calorimetry.

Fig. 2. Representative abdominal cross-sectional magnetic resonance images (*left*) at L4 and corresponding PET images (*right*) of a control subject and a subject with HIV and lipodatrophy.

Fig. 3.

Depot-specific glucose uptake in fasted and insulin-stimulated conditions in healthy controls (empty bars) and HIV lipoatrophy (filled bars). *A*: abdominal subcutaneous adipose tissue uptake. *B*: abdominal visceral adipose tissue (VAT) uptake. *C*: thigh muscle uptake.

Fig. 4.
Linear correlation between VAT and whole body glucose disposal (M) under insulin-stimulated steady state.

Table 1

General clinical characteristics, body composition, and metabolic parameters for HIV-lipoatrophy and control subjects

	Lipoatrophy (n = 6)	Control (n = 5)	P Value
Age, yr	49.2±1.7	44.4±2.1	0.11
BMI, kg/m ²	22.2±0.5	23.7±1.1	0.22
DEXA			
Lean body mass, kg	58.6±2.1	57.2±3.1	0.7
Lower extr. fat, kg	2.5±0.4	5.6±0.6	0.002
Total fat, kg	10.7±0.7	16.6±1.9	0.01
% Body fat	14.9±0.8	21.6±1.9	0.008
Abdominal CT			
VAT, cm ²	106.2±25.0	88.3±17.2	0.59
SAT, cm ²	87.4±8.4	176.6±26.9	0.007
IMCL _W , IU	104.4±9.5	80.9±9.6	0.12
<i>Metabolic parameters</i>			
Triglycerides, mg/dl	253±49	97±18	0.02
Cholesterol, mg/dl	180±11	210±15	0.14
HDL cholesterol, mg/dl	33±4	52±4	0.006
LDL cholesterol, mg/dl	104±15	134±14	0.18
Adiponectin, µg/ml	5.4±2.2	9.5±1.2	0.15
Leptin, ng/ml	6.5±1.1	14.3±2.9	0.02
Resistin, ng/ml	4.8±0.6	4.9±0.9	0.89
M, µmol·kg ⁻¹ ·min ⁻¹	56.0±8.4	63.0±6.3	0.54
HOMA-IR	2.4±0.6	1.7±0.3	0.36
REE, kcal/24 h	1,595±90	1,566±95	0.83
FFA, meq/l			
Fasting	0.47±0.07	0.47±0.06	0.93
Insulin	0.17±0.06	0.08±0.01	0.21
%Change	-65±9	-83±3	0.12
R _a glycerol, µmol·kg ⁻¹ ·min ⁻¹			
Fasting	1.4±0.1	1.4±0.1	0.82
Insulin	0.83±0.15	0.60±0.11	0.27
%Change	-43±7	-59±5	0.09
R _a glucose fasting, µmol·kg ⁻¹ ·min ⁻¹	12.4±0.5	10.6±0.7	0.07

Values represent means ± SE unless otherwise indicated. *P* values represent Student's *t*-test between groups. VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; IMCL_W, intramyocellular lipid; M, glucose disposal; HOMA-IR, homeostasis model assessment of insulin resistance; REE, resting energy expenditure; FFA, free fatty acid; Ra, rate of appearance.

Table 2

Correlation coefficients between PET-determined regional glucose uptake and whole body measures of insulin sensitivity (n = 11)

	M	HOMA-IR
Fasting glucose uptake		
SAT	0.10	0.01
VAT	0.78 [*]	−0.82 [*]
Muscle, thigh	−0.06	0.17
Insulin-stimulated glucose uptake		
SAT	0.57	−0.48
VAT	0.72 [*]	−0.75 [*]
Muscle, thigh	0.82 [*]	−0.74 [*]

^{*} $P \leq 0.01$.

Table 3

Correlation coefficients between PET-determined regional glucose uptake, whole body measures of insulin sensitivity, and abdominal adipose tissue area (n = 11)

	SAT Area	VAT Area
Fasting glucose uptake		
SAT	-0.72 [*]	-0.08
VAT	0.07	-0.85 [†]
Muscle, thigh	-0.58 [‡]	0.08
Insulin-stimulated glucose uptake		
SAT	-0.52	-0.56
VAT	0.03	-0.78 [*]
Muscle, thigh	0.23	-0.75 [*]
Whole body insulin sensitivity		
M	0.05	-0.97 [†]
HOMA-IR	-0.25	0.95 [†]

^{*} P ≤ 0.01

[†] P < 0.001

[‡] P = 0.06.