PET imaging reveals distinctive roles for different regional adipose tissue depots in systemic glucose metabolism in nonobese humans

Jason M. Ng, Koichiro Azuma, Carol Kelley, Richard Pencak, Zofia Radikova, Charles Laymon, Julie Price, Bret H. Goodpaster, and David E. Kelley

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Ng JM, Azuma K, Kelley C, Pencak R, Radikova Z, Laymon C, Price J, Goodpaster BH, Kelley DE. PET imaging reveals distinctive roles for different regional adipose tissue depots in systemic glucose metabolism. Am J Physiol Endocrinol Metab 303:E1134–E1141, 2012. First published September 11, 2012; doi:10.1152/ajpendo.00282.2012.—Excess amounts of abdominal subcutaneous (SAT) and visceral (VAT) adipose tissue (AT) are associated with insulin resistance, even in normal-weight subjects. In contrast, glutal–femoral AT (GFAT) is hypothesized to offer protection against insulin resistance. Dynamic PET imaging studies were undertaken to examine the contributions of both metabolic activity and size (volume) of these depots in systemic glucose metabolism. Nonobese, healthy volunteers (n = 15) underwent dynamic PET imaging uptake of \(^{[18}F\)FDG at a steady-state (20 mU·m\(^{-2}\)·min\(^{-1}\)) insulin infusion. PET images of tissue \(^{[18}F\)FDG activity were coregistered with MRI to derive \(K\) values for insulin-stimulated rates of fractional glucose uptake within tissue. Adipose tissue volume was calculated from DEXA and MRI. VAT had significantly higher rates of fractional glucose uptake per volume than SAT (P < 0.05) or GFAT (P < 0.01). \(K_{\text{GFAT}}\) correlated positively (r = 0.67, P < 0.01) with systemic insulin sensitivity [glucose disappearance rate (\(R_0\))] and negatively with insulin-suppressed FFA (r = −0.71, P < 0.01). SAT (r = −0.70, P < 0.01) and VAT mass (r = −0.55, P < 0.05) correlated negatively with \(R_0\), but GFAT mass did not. We conclude that rates of fractional glucose uptake within GFAT and VAT are significantly and positively associated with systemic insulin sensitivity in nonobese subjects. Furthermore, whereas SAT and VAT amounts are confirmed to relate to systemic insulin resistance, GFAT amount is not associated with insulin resistance. These dynamic PET imaging studies indicate that both quantity and quality of specific AT depots have distinct roles in systemic insulin resistance and may help explain the metabolically obese but normal-weight phenotype.

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MORE THAN 50 YEARS AGO, Vague (40) observed that upper body fat (the “apple” phenotype) confers greater cardiometabolic risk than lower body fat (the “pear” phenotype). Studies have since confirmed a distinct role for abdominal adipose tissue (AT) in insulin resistance (IR), type 2 diabetes, and metabolic syndrome (9). In the last 30 years, imaging studies have significantly increased our understanding of the role of AT in IR. However, this understanding is tempered by the wide variability in systemic IR seen in normal-weight individuals. Because adipocytes are highly responsive to insulin, insights into the roles of specific regional AT depots and skeletal muscle in IR independent of obesity is invaluable. The current study used dynamic PET imaging and MRI of regional AT and skeletal muscle to assess IR within specific tissue depots and their contribution to systemic glucose metabolism in nonobese subjects. This approach significantly increases our understanding of the role of glutal–femoral and abdominal AT with respect to systemic IR in nonobese individuals.

**MATERIALS AND METHODS**

**Study subject characteristics.** Nonobese young adults (n = 15) were recruited from the general community through flyers. The study was described and discussed in detail with all participants, with all questions answered. Informed, written consent was obtained, followed by a medical examination and blood collection to assure good health. All participants met the following inclusion criteria: body mass index (BMI) 20–27 kg/m\(^2\), blood pressure <140 and <90 mmHg, glucose <100 mg/dl, Hb A\(_1\)c <5.7%, triglycerides <150 mg/dl, total cholesterol 250 mg/dl, fasting insulin <12 \(\mu\)U/ml, no recent weight gain, no smoking, and no taking of medications known to affect adipose tissue metabolism. The protocol was approved by the University of Pittsburgh Institutional Review Board. Clinical characteristics are shown in Table 1.

**Body composition tissue volume measurements.** Dual-energy X-ray absorptiometry (DEXA; Lunar Prodigy, Madison, WI) measured fat
PET imaging in adipose tissue

Table 1. Clinical characteristics

<table>
<thead>
<tr>
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<th>All (n = 15)</th>
<th>Women (n = 9)</th>
<th>Men (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>31.5 ± 5.0</td>
<td>32.3 ± 6.1</td>
<td>30.2 ± 2.9</td>
</tr>
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<td>Weight, kg</td>
<td>73.7 ± 12.2</td>
<td>66.7 ± 7.7*</td>
<td>84.1 ± 10.0</td>
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<tr>
<td>BMI</td>
<td>25.1 ± 2.3</td>
<td>24.0 ± 2.0</td>
<td>26.7 ± 1.8</td>
</tr>
<tr>
<td>%Body fat</td>
<td>30.4 ± 8.4</td>
<td>35.2 ± 4.5**</td>
<td>23.3 ± 7.8</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>13 W/2 AA</td>
<td>8 W/1 AA</td>
<td>5 W/1 AA</td>
</tr>
<tr>
<td>Values are means ± SD</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(minimum/maximum values in parentheses). BMI, body mass index; W, white; AA, African-American. *P &lt; 0.05; **P &lt; 0.01, women vs. men.</td>
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</table>

PET imaging in adipose tissue

Dynamic PET imaging with [18F]FDG. Dynamic PET imaging was obtained at the University of Pittsburgh PET Center using a Siemens/CTI ECAT HR+ PET scanner with a 3D imaging mode (63 parallel planes, axial field of view, 15.2 cm, slice width of 2.4 mm, image resolution 6 mm). The [18F]FDG was synthesized using a method modified by Hamacher et al. (14). Volunteers were positioned in a supine position on the scanner body and abdomen so that the midjoint region or midabdominal area was in the center of the field of view. Subjects were positioned only after steady-state metabolic conditions were attained during the tracer clamp and continued during dynamic PET imaging. A transmission scan was performed to assess attenuation values (10–15 min). A 10-mCi dose of [18F]FDG was then injected at the beginning of 53 min of dynamic PET scanning with 27 total frames (8 frames for each 30-s duration, 9 frames for each 60-s duration, and 10 frames for each 4-min duration). Subsequently, the bed was repositioned to continue dynamic PET imaging of the other area (i.e., midjoint if abdominal area was imaged first and vice versa), and dynamic PET imaging continued for 40 min (10 frames for each 4-min duration), followed by a second transmission scan lasting 10–15 min. Arterial blood samples (0.5 ml) were hand-drawn to measure [18F]FDG arterial activity for a total of 95 min at approximately the following intervals: 10 samples in the 1st min, 8 samples from minutes 2 to 3, seven samples from minutes 3 to 10, and 20 samples from minutes 10 to 95. The blood was immediately centrifuged, and 100 μl of plasma was used for [18F] counting (>350 KeV). The study design is shown in Fig. 1.

PET image tissue activity measurements. PET images were coregistered with their corresponding MRI images to allow a region of interest (ROI) transfer to the PET image using a previously described method (29, 45). A representative illustration of MR and PET images with ROIs is shown in Fig. 2. ROIs allow for highly specific anatomic localization of the specific AT depot on the MR image to eliminate or minimize spillover from other regions, organs, and vessels on PET imaging. The ROIs were placed on subcutaneous AT (GFAT; n = 15) and muscle in midjoint PET images and subcutaneous AT (SAT; n = 15) and visceral AT (VAT; n = 12) in abdominal PET images. It was technically not feasible to assess visceral adipose [18F]FDG uptake in three women because ROIs could not be applied consistently in sequential images due to lack of VAT depots from image to image. The ROIs were used to measure [18F]FDG activity within adipose or muscle. To minimize scatter effect, the middle 30 of 63 planes of each leg and abdomen were used. Tracer activity within the ROI was converted to a radioactivity concentration (μCi/ml) using an empirical phantom-based calibration factor (μCi/ml–1·PET counts per pixel–1).

Patlak analysis and extrapolation to tissue mass. Patlak graphical model analysis modeled a macroscopic index of [18F]FDG uptake into AT and skeletal muscle, defined as a K (ml plasma/ml tissue –1·min–1) (26). Dynamic PET data acquired over 0–53 (1st imaging site) and 53–93 min (2nd imaging site) were averaged over 30 planes, and Patlak analysis was applied to the average for each table position, with a high degree of linearity shown in the Patlak plot data, with all regression values >0.9. Regional glucose uptake was calculated as the K product and arterial glucose concentration divided by the lumped glucose (FM) and fat-free mass (FFM). The DEXA images were analyzed (En Core for Windows version 9.30) to assess lower extremity FM and truncal FM. Abdominal WM was measured as described previously (18). The DEXA and MRI scans were combined to estimate abdominal AT quantity, and DEXA measurements were used to estimate GFAT quantity. Abdominal images obtained from MRI at the top of the diaphragm to the top of the femur were matched with DEXA images using the landmark of the greater edge of the superior trochanter that is easily identifiable on DEXA and has minimal adipose tissue. Lower and upper extremity lean body mass was used to estimate systemic skeletal muscle mass.

Metabolic studies. Research volunteers were admitted the night prior to dynamic PET imaging to the University of Pittsburgh General Clinical Research Center and fasted overnight after dinner. The next morning, a catheter was placed in the antecubital vein for insulin infusion, dextrose infusion, and fluorodeoxyglucose ([6,6-2H2]glucose) administration. A catheter was placed in the radial artery for blood sampling. All volunteers underwent an insulin infusion of (20 mU·m–2·min–1) for ~4 h to suppress hepatic glucose output and achieve nearly full suppression of lipolysis (32, 39). Euglycemia was maintained with an adjustable 20% dextrose infusion using the glucose clamp method (7). A primed (200 mg) continuous (2 mg/min) infusion of [6,6-2H2]glucose was initiated 2 h prior and continuing through the insulin infusion to determine systemic glucose utilization rate [glucose disappearance rate (Rd)] and calculate endogenous glucose production (EGP). Arterial glucose samples were measured every 5 min using a YSI (Yellow Springs, OH) Glucose Analyzer, and plasma insulin levels were collected every 30 min and measured by ELISA (Linco Research). Plasma fatty acid samples were measured using the Agilent Gas Chromatograph (Agilent, Santa Clara, CA). Glucose enrichment of [6,6-2H2]glucose was measured using gas chromatography-mass spectrometry. Blood samples for measurement of adipokines [adiponectin, retinol-binding protein 4 (RBP4), leptin, IL-6, and TNFα] were obtained 3 h prior to the insulin infusion being started and measured with ELISA (ALPCO Diagnostics for adiponectin and RBP4, Linco Research for leptin, and R & D Systems for IL-6 and TNFα).

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Fig. 2. Representative PET and MRI. Imaging with region of interest (ROI) placement. An example illustrating MRI and dynamic PET imaging after injection of [18F]FDG. Top pictures represent abdominal imaging, and bottom pictures represent thigh imaging, with MR images on the left and PET images on the right. After MR-PET coregistration, ROI (shown as red ovals) were generated on the MR image and applied to the corresponding coregistered PET image (shown as white ovals) to generate regional FDG time activity curves. GFAT, gluteal-femoral adipose tissue.

RESULTS

Systemic and hepatic insulin sensitivity measurements. Insulin infusion increased arterial insulin from 5.4 ± 2.0 μU/ml fasting to 23.8 ± 4.6 μU/ml with insulin (P < 0.01, n = 13). Free fatty acids were suppressed from 362 ± 120 μM fasting to 93 ± 16 μM with insulin (72 ± 11% suppression, P < 0.01). However, there was a broad range from 48 to 87%. Systemic glucose utilization rates during steady-state conditions doubled from 2.07 ± 0.34 (fasting) and 4.19 ± 1.45 mg-min⁻¹·kg⁻¹ (insulin stimulated). There was a broad, three-fold range of insulin-stimulated systemic glucose utilization between 2.33 and 6.77 mg-min⁻¹·kg⁻¹. Greater FFA suppression was correlated with an increase in percentage stimulation of glucose R₄ (r = −0.60, P < 0.05). EGP was suppressed to 91.4 ± 14.2%, whereas glucose R₄ was stimulated to 98.4 ± 54.7% with insulin stimulation from baseline (n = 13). No sex differences were found with EGP suppression (P = 0.29) or glucose R₄ (P = 0.65) with insulin stimulation.

Regional AT mass and IS. There was a negative association between total FM and glucose R₄ (r = −0.51, P < 0.05). Truncal FM by DEXA was strongly and negatively associated with glucose R₄ (r = −0.74, P < 0.01). When abdominal FM was subdivided using MRI, both SAT (r = −0.70, P < 0.01) and VAT (r = −0.55, P = 0.05) were associated with systemic glucose R₄ (Fig. 4, E and F). However, absolute GFAT mass did not correlate with systemic glucose R₄ [r = −0.28, not significant (NS)], although GFAT accounted for ~40% of overall FM. A higher proportion of GFAT relative to total body fat tended to correlate positively with systemic glucose R₄ (r = 0.45, P = 0.09). Interestingly, a higher proportion of GFAT was significantly correlated with systemic glucose R₄ when measured as a proportion of combined regional AT: %GFAT/(GFAT + SAT) (r = 0.63, P = 0.01), %GFAT/(GFAT + SAT + VAT) (r = 0.64, P = 0.01).

AT PET imaging. The [18F]FDG tissue activity within specific ROIs and arterial activity were used to calculate fractional FDG uptake expressed as K. The K value of [18F]FDG summarizes net effects of tracer delivery, transmembrane transport, and intracellular trapping of [18F]FDG 6-phosphate (2, 16). The mean values for K with insulin stimulation were similar and nonsignificant in GFAT (K_GFAT) and SAT (K_SAT) at 1.47 ± 0.79 and 1.72 ± 0.97 10⁻³ ml plasma-ml tissue⁻¹·min⁻¹, respectively, but VAT (K_VAT) was significantly higher than both K_GFAT (P < 0.01) and K_SAT (P < 0.05) at 2.67 ± 1.23 (Fig. 3). Skeletal muscle (K_MUS) was three to five times higher at 8.12 ± 3.19 10⁻³ ml plasma-ml tissue⁻¹·min⁻¹ (P < 0.001) than any AT depot (Table 2). K_VAT could not be measured in three women with minimal VAT (<1 kg) due to the technical challenges of assigning proper ROIs within VAT. No sex differences in [18F]FDG uptake in any AT depot were observed in K_VAT (P = 0.38) or K_GFAT (P = 0.85). K_SAT trended toward (P = 0.07) a higher uptake in women (2.07 ± 1.00 vs. 1.20 ± 0.69 10⁻³ ml plasma-ml tissue⁻¹·min⁻¹). No sex differences were observed in systemic glucose R₄ [4.10 ± 1.69 (men) vs. 4.24 ± 1.37 mg-min⁻¹·kg⁻¹ (women), P = 0.87]. Each fat depot had a broad range in values, with a sixfold range in K_GFAT and a

Statistical analysis. Statistical analysis was performed using Sigma Stat 3.0 (SAS institute). Data are presented as means ± SD unless otherwise indicated. To examine sex differences and differences between depots, analysis of variance was used. Pairwise comparisons were calculated with the Holm-Sidak multiple-comparison method procedure. Association between variables was examined with a Pearson or Spearman correlation. Spearman and Pearson correlations were used when appropriate to examine associations between variables. Stepwise regression analyses were used to adjust for other variables. A P value of <0.05 was considered significant.

constant, using 1.14 for adipose tissue and 1.2 for skeletal muscle (22, 42, 43).

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sevenfold range in $K_{SAT}$. $K_{VAT}$ also had a range, but this should be interpreted with caution due to the small cohort of nonobese, healthy, young adults (~10–15% truncal adiposity).

**AT metabolic activity and IS measurements.** Values for $K_{GFAT}$ were positively correlated with systemic glucose $R_d$ (Fig. 4A) and skeletal muscle ($K_{MUS}$; $r = 0.53$, $P < 0.05$), indicating salutary physiological cross-talk between GFAT metabolism and systemic insulin sensitivity. Additionally, $K_{GFAT}$ values correlated negatively with insulin-suppressed FFA ($r = -0.63$, $P < 0.05$), but there was no association between $K_{GFAT}$ and GFAT mass ($r = 0.02$, NS). These observations further support the concept of dissociation between metabolic activity and quantity of GFAT and their role in systemic insulin sensitivity.

In contrast to $K_{GFAT}$, $K_{SAT}$ did not correlate with systemic $R_d$ (Fig. 4B). Interestingly, $K_{VAT}$ was positively correlated with glucose $R_d$. However, total estimated glucose uptake within VAT mass was much less than other fat depots. $K_{MUS}$ was strongly correlated with glucose $R_d$ ($r = 0.89$, $P < 0.01$). Moreover, $K_{MUS}$ had a similar pattern of association with FM and the amounts of SAT, VAT, and GFAT, as described above with glucose $R_d$ (data not shown). As a technical consideration, regression values ($r^2$) as a fit of the Patlak $K$ plot were determined for the following: $K_{GFAT} 0.97 \pm 0.04$, $K_{SAT} 0.96 \pm 0.06$, $K_{VAT} 0.93 \pm 0.06$, and $K_{MUS} 1.00 \pm 0.00$. As expected, the Patlak line fit was superior in muscle, owing to a much higher signal intensity and higher signal/noise ratio than in adipose tissue.

Stepwise regression analysis was performed to address more specifically how the amounts of specific tissue depots and specific tissue insulin sensitivities were associated with systemic IS. In GFAT, tissue IS ($K_{GFAT}$) correlated with systemic glucose $R_d$ and insulin-suppressed FFA even after adjustment for GFAT volume and age, sex, BMI, and ethnicity ($P < 0.01$ and $P < 0.05$, respectively). In contrast, SAT tissue volume negatively correlated with systemic $R_d$ ($P < 0.05$) even after adjustment for SAT tissue IS.

**Contributions of AT and skeletal muscle glucose uptake to overall systemic IS.** Estimated glucose uptake into each depot was performed by extrapolating [18F]FDG uptake to glucose using the lumped constant and the estimate of specific depot $K$ values. FDG uptake in GFAT was similar to SAT ($K_{GFAT}$, $K_{SAT}$) but significantly less than other adipose depots ($K_{VAT}$ and percentage of systemic glucose uptake into SAT (8.0% vs. 3.0% and 3.0%, $P < 0.05$) compared with men. VAT glucose uptake was significantly less than other adipose depots ($P < 0.01$). In total, these adipose depots represented ~90% of FM, noting that upper extremity FM could not be estimated, and accounted for ~13% of systemic glucose $R_d$ in women and ~6% of systemic glucose $R_d$ in men ($P < 0.05$). The order in

### Table 2. Adipose tissue mass and extrapolated tissue glucose uptake

<table>
<thead>
<tr>
<th>Tissue mass, kg</th>
<th>All (n = 15)</th>
<th>Women (n = 9)</th>
<th>Men (n = 6)</th>
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</thead>
<tbody>
<tr>
<td>GF-FM, kg</td>
<td>8.6 ± 2.7</td>
<td>9.8 ± 2.3*</td>
<td>6.9 ± 2.5</td>
</tr>
<tr>
<td>ABD SAT, kg</td>
<td>9.9 ± 3.3</td>
<td>10.1 ± 2.5</td>
<td>9.5 ± 4.4</td>
</tr>
<tr>
<td>VAT, kg</td>
<td>1.2 ± 0.7</td>
<td>1.2 ± 0.8</td>
<td>1.3 ± 0.6</td>
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<tr>
<td>Skeletal muscle, kg</td>
<td>25.6 ± 7.9</td>
<td>20.3 ± 2.9**</td>
<td>33.6 ± 5.9</td>
</tr>
<tr>
<td>$K$ values, $10^{-3}$ ml plasma·ml tissue$^{-1}$·min$^{-1}$</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$K_{GFAT}$, min</td>
<td>1.47 ± 0.79</td>
<td>1.50 ± 0.90</td>
<td>1.42 ± 0.68</td>
</tr>
<tr>
<td>$K_{SAT}$, min</td>
<td>1.72 ± 0.97</td>
<td>2.07 ± 1.00†</td>
<td>1.20 ± 0.69</td>
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<tr>
<td>$K_{VAT}$, min [n = 12 (6 men and 6 women)]</td>
<td>2.67 ± 1.23</td>
<td>2.99 ± 1.27</td>
<td>2.34 ± 1.21</td>
</tr>
<tr>
<td>$K_{MUS}$, min</td>
<td>8.12 ± 3.19</td>
<td>8.15 ± 3.12</td>
<td>8.07 ± 3.59</td>
</tr>
<tr>
<td>Glucose uptake, mg/min</td>
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<tr>
<td>$R_a$, mg/min</td>
<td>306 ± 116</td>
<td>278 ± 80</td>
<td>347 ± 155</td>
</tr>
<tr>
<td>$R_a$, mg·min$^{-1}$·kg body wt$^{-1}$</td>
<td>4.19 ± 7.3</td>
<td>4.24 ± 1.37</td>
<td>4.10 ± 1.69</td>
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<tr>
<td>GFAT, mg/min</td>
<td>11.3 ± 7.3</td>
<td>13.4 ± 8.7</td>
<td>8.2 ± 3.1</td>
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<tr>
<td>%Glucose uptake</td>
<td>3.9 ± 2.7</td>
<td>4.3 ± 3.1</td>
<td>2.5 ± 0.8</td>
</tr>
<tr>
<td>ABD SAT, mg/min</td>
<td>15.0 ± 10.3</td>
<td>18.7 ± 11.6*</td>
<td>8.7 ± 2.3*</td>
</tr>
<tr>
<td>%Glucose uptake</td>
<td>5.7 ± 4.7</td>
<td>7.6 ± 5.3*</td>
<td>3.0 ± 1.3</td>
</tr>
<tr>
<td>VAT, mg/min [n = 12]</td>
<td>2.7 ± 1.3</td>
<td>3.1 ± 1.6</td>
<td>2.3 ± 1.1</td>
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<tr>
<td>%Glucose uptake</td>
<td>1.0 ± 0.6</td>
<td>1.2 ± 0.8</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Skeletal muscle, mg/min</td>
<td>210 ± 115</td>
<td>163 ± 60</td>
<td>280 ± 147</td>
</tr>
<tr>
<td>%Glucose uptake</td>
<td>65.4 ± 20.2%</td>
<td>57.4 ± 11.9%</td>
<td>77.3 ± 25.1%</td>
</tr>
</tbody>
</table>

Values are means ± SD. GF-FM, gluteal-femoral fat mass; ABD, abdominal; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; GFAT, gluteal-femoral adipose tissue; $K_{MUS}$, skeletal muscle; $R_a$, glucose disposal. *$p < 0.05$; **$p < 0.01$; †$p = 0.07$; ‡$p = 0.06$, women vs. men.

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which fat depots were scanned with PET did not significantly affect glucose uptake (data not shown).

Estimation of skeletal muscle glucose uptake took into account appendicular skeletal muscle mass, representing ~75% of total skeletal muscle mass in the body estimated from a whole body DEXA scan (31). Skeletal muscle accounted for ~65% of systemic glucose utilization (Table 2). Skeletal muscle accounted for ~57% of systemic $R_d$ in women and ~77% in men, but this difference was not statistically significant ($P = 0.11$). Considering that glucose uptake in the central nervous system is ~1.0 mg·min$^{-1}$·kg body wt$^{-1}$ (100 g/day in a 70-kg person), the central nervous system accounts for the vast majority of the small remaining fraction of systemic glucose uptake (3). Of note, women had a higher percent body fat (41.5 ± 5.0 vs. 35.8 ± 5.1%, $P = 0.05$) and a greater amount of GFAT than men, but men had more skeletal muscle.

Fig. 4. Systemic insulin sensitivity correlations. ●, Men; ○, women. A: positive significance between the glucose rate of disappearance ($R_d$) and the overall activity ($K$) of GFAT. B: no significance between the overall activity of SAT and $R_d$. C: significance between the activity of VAT and $R_d$. D: no significance between the amount of GFAT and insulin sensitivity. E: negative correlation between SAT volume and insulin sensitivity. F: a negative correlation between VAT volume and insulin sensitivity.
mass, with SAT and VAT being similar and nonsignificant (Table 2).

**AT sensitivity and plasma adipokine levels.** \( K_{\text{GFAT}} \) did not correlate with adipokines. \( K_{\text{SAT}} \) correlated with RBP4 \( (r = -0.59, P < 0.05) \) and tended to correlate with TNF\( \alpha \) \( (r = -0.42, P = 0.11) \). \( K_{\text{MUS}} \) correlated positively with adiponectin \( (r = 0.53, P < 0.05) \) and negatively with IL-6 \( (r = -0.62, P < 0.05) \). The percent gluteal-femoral FM correlated positively with adiponectin \( (r = 0.78, P < 0.01) \) and negatively with RBP4 \( (r = -0.76, P < 0.01) \). Other body composition indices were not correlated with these adipokines. Adiponectin and RBP4 correlated with each other \( (r = -0.59, P < 0.05) \). Other significant correlations with body composition included leptin and total adiposity \( (r = 0.84, P < 0.01) \), IL-6 and trunk SAT \( (r = 0.55, P < 0.05) \), IL-6 and SAT \( (r = 0.53, P < 0.05) \), and TNF\( \alpha \) and VAT \( (r = 0.57, P < 0.05) \). In regard to adipokine and systemic insulin sensitivity, adiponectin positively correlated with \( R_d \) \( (r = 0.58, P < 0.05) \). IL-6 negatively correlated with \( R_d \) \( (r = -0.52, P < 0.05) \), and adiponectin was negatively correlated with IL-6 \( (r = -0.52, P < 0.05) \).

**DISCUSSION**

Systemic IR is associated with generalized obesity but is also well known to manifest in normal-weight men and women. Body fat distribution in both normal-weight and obese people exhibits a stronger relationship with IR than total adiposity. Indeed, there is widespread acceptance in the literature that abdominal fat mass is associated with IR. In addition, studies in our laboratory have shown that subcutaneous thigh AT mass either did not associate with IR or is positively associated with higher systemic insulin sensitivity (1, 8, 11). Indeed, body fat distribution variations are often cited as key evidence for the “metabolically obese but normal-weight phenotype” (35). Our dynamic PET imaging studies revealed novel findings that intrinsic metabolic activity with GFAT and VAT can vary considerably among normal-weight subjects, and in combination with body fat distribution they may play key roles in determining systemic IR.

Our results indicated that higher metabolic activity \( (K_{\text{GFAT}}) \) within GFAT is associated with better overall systemic glucose disposal (glucose \( R_d \)). A potential explanation for this observation is that subcutaneous adipose depots act as a protective “sink” by trapping excess fatty acids and preventing exposure to elevated lipid levels. In our nonobese individuals, greater \( K_{\text{GFAT}} \) activity correlated with a lower absolute FFA value with insulin stimulation. Prior studies have shown that meal-derived fatty acids have greater uptake into abdominal adipose vs. lower body AT (23, 34). Our novel findings add to these studies by demonstrating that greater \( K_{\text{GFAT}} \) activity was associated with lower systemic FFA values and improved IS. To the best of our knowledge, this has not been reported previously. In contrast, fractional glucose uptake within \( K_{\text{SAT}} \) was not correlated with glucose \( R_d \) or insulin-stimulated decreased FFAs, indicating that the mass but not the intrinsic metabolic activity within this depot contributes to systemic IS, as reported previously using other methodologies (12).

Our novel data also indicate that higher VAT intrinsic metabolic activity \( (K_{\text{VAT}}) \) is associated with systemic insulin sensitivity and more complete suppression of lipolysis, as reflected by the insulin-stimulated FFA levels. This was similar to GFAT and suggests that variation in VAT metabolic activity \( (K_{\text{VAT}}) \) is related to systemic IS. To the best of our knowledge, this has not been reported previously. However, it appears that excess VAT mass may offset any potential positive influence that its intrinsic metabolic activity may have on systemic insulin sensitivity. Indeed, our study is in agreement with prior studies showing that VAT mass, but not GFAT mass, negatively correlated with systemic glucose \( R_d \) in nonobese individuals (35). Hence, our studies suggest opposing influences of metabolic activity and mass in VAT. However, we note that a limitation of this study is that we could not quantify different areas within VAT such as mesenteric, perirenal, and epididymal white AT depots. Moreover, additional studies are warranted to examine these associations among overweight and obese individuals.

Glucose uptake within AT accounted for \( \sim 11\% \) of total systemic glucose uptake, with GFAT and SAT contributing \( 4-6\% \) each. This is similar to previous studies employing PET, which reported that total body AT accounted for 8% of systemic glucose uptake (42). This study, however, reported a slightly lower contribution of SAT \( (1.3\%) \) to total systemic uptake (42). This previous study examined only men, and our higher contribution by AT was likely due to the women in our study who had more AT. Although this contribution by AT to total body glucose uptake might seem relatively minor, it could be important in the context of fat mass expansion, i.e., weight gain in which this contribution would increase. Further investigations are needed to examine how AT depot-specific metabolic activity may play a role in cardiometabolic risk according to sex difference. Prior isotope labeling studies in nonobese subjects showed meal fat uptake over 24 h did not predict regional fat body distribution over 8 wk (44). Because our study demonstrated differences only in regional glucose uptake and body fat distribution, other mechanisms need to be explored to further understand how regional AT distribution and “quality” of AT affect the variability in insulin sensitivity. For example, estrogen has been shown to promote insulin sensitivity in animal and human models through suppression of hepatic gluconeogenesis and enhancement of skeletal muscle glucose transport (27, 28, 33). We speculate that estrogen could also have direct effects on promoting glucose uptake directly into adipose tissue. Taken together, this would complement prior studies that have observed sex-related differences in AT distribution and mass related to insulin resistance, type 2 diabetes, and cardiovascular disease risk.

Our adipokine data provide additional evidence that GFAT plays a positive role in systemic glucose metabolism. The percent GFAT mass positively correlated with adiponectin, a signaling protein secreted from AT known to affect insulin sensitivity and obesity by increasing glucose uptake, increasing IS, and promoting weight loss (6, 19). Percent GFAT mass negatively correlated with RBP4, a known protein secreted by AT that is elevated in IR and possibly contributes to IR (13, 46). Our data suggest that GFAT may have more than one mechanism influencing systemic insulin sensitivity, because both metabolic activity and secretion of specific adipokines affect systemic IS. Body composition indices of SAT did not show any significance, but \( K_{\text{SAT}} \) correlated negatively with RBP4, indicating that SAT activity may play a role in altering systemic IS through adipokines. Increasing VAT mass correlated positively with TNF\( \alpha \) as expected, indicating that in-
creasing VAT mass may play a role in increasing TNFα, which is known to play a role in the pathogenesis of obesity (15).

There were several limitations to this study. Although our high-resolution MRIs allowed us to distinguish segment tissue activity specific to adipose tissue and muscle, it was technically challenging to assign proper ROIs within VAT for several of these nonobese subjects; thus we did not analyze [18F]FDG activity within VAT for all subjects. Future studies in obese subjects with greater VAT mass would allow for more precise discrimination of VAT activities. Prior studies have shown differences in SAT subdepot effects on systemic IS, but in our nonobese population it was technically difficult to properly coregister superficial and deep subcutaneous AT on MR and PET imaging, and additional studies with obese subjects could further investigate these subdepots (10). To address possible tissue activity contamination, we used the Bayesian approach that is also applied to account for tracer activity in blood. Another limitation was FDG signal decay depending upon the order of tissue imaging. Taking into account the long half-life of FDG (~109 min), we applied a decay correction to the PET imaging studies, and our glucose uptake data revealed no significant differences depending on which depot was imaged first. We also recognize that ROIs are estimates of whole organ/tissue metabolism by means of extrapolation. However, the linear regression in the PATLAK modeling suggests this estimate is reasonable under the steady-state conditions provided by the euglycemic insulin clamp. We recognize that extrapolation of glucose uptake from metabolic activity relies on the lumped constant, and this has been verified independently for both muscle and AT in prior PET studies (22, 43). We recognize that a 20 mU·m·min⁻¹ insulin infusion likely does not maximally stimulate peripheral glucose uptake, but our main emphasis was suppressing lipolysis and hepatic glucose metabolism to exert effects on AT metabolism. Further studies at higher insulin infusion rates may further delineate the specific roles of AT, muscle, and liver in regional glucose uptake. Additionally, we considered the possibility of the timing of the menstrual cycles affecting the female participants’ insulin sensitivity, but evidence suggests that IS did not vary significantly with menstrual cycles (4).

In summary, these dynamic PET studies provide novel in vivo information regarding distinct roles of regional AT depot mass and metabolic activity affecting systemic glucose metabolism within nonobese subjects. This supports the hypothesis that AT “quality” is an important component in systemic IS in nonobese individuals, particularly thigh AT, as greater metabolic activity in thigh AT is associated with greater overall systemic glucose disposal, possibly through a greater suppression of FFAs with insulin stimulation. These findings potentially explain part of the interindividual variability in systemic IS among nonobese individuals of the same body phenotype (fat mass distribution). Future studies in lean and obese individuals may help further our understanding of the role of regional AT in systemic glucose metabolism through suppression of FFAs, release of adipokines, glucose uptake, or a combination of these mechanisms and may help explain the wide variation in cardiometabolic risk among nonobese men and women not fully explained by general adiposity or body fat distribution.

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DISCLOSURES

J. Ng, K. Azuma, C. Kelley, Z. Radikova, C. Laymon, J. Price, and B. H. Goodpaster have no conflicts of interests to declare. R. Pencek is currently employed by Amylin Pharmaceuticals. D. E. Kelley is currently employed by Merck, Sharp, and Dohme Corp.

AUTHOR CONTRIBUTIONS

J.N., K.A., C.K., R.P., and Z.R. performed the experiments; J.N., K.A., C.L., and J.P. analyzed the data; J.N., K.A., B.H.G., and D.E.K. interpreted the results of the experiments; J.N. and K.A. prepared the figures; J.N. drafted the manuscript; J.N., J.P., and B.H.G. edited and revised the manuscript; J.N., B.H.G., and D.E.K. approved the final version of the manuscript; J.P. and D.E.K. did the conception and design of the research.

REFERENCES


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