The relationship of V˙O2 max to glucose disposal persisted after variables measured correlated with oxidative glucose disposal. Nevertheless, whole body and abdominal adiposity showed modest associations (range of r values from −0.32 to −0.46, P < 0.05 to P < 0.01). A similar pattern of correlations was observed for nonoxidative glucose disposal. None of the variables measured correlated with oxidative glucose disposal. The relationship of VO2 max to glucose disposal persisted after statistical control for FFM, percent body fat, and intra-abdominal fat (r = 0.40, P < 0.01). In contrast, correlations of total and regional adiposity measures to insulin sensitivity were no longer significant after statistical adjustment for VO2 max. VO2 max was the only variable to enter stepwise regression models as a significant predictor of total and nonoxidative glucose disposal. Our results highlight the importance of VO2 max as a determinant of glucose disposal and suggest that it may be a stronger determinant of variation in glucose disposal than total and regional adiposity in nonobese, nondiabetic, premenopausal women.

Physical activity modulates insulin sensitivity (23). Physical fitness, as reflected by maximal oxygen consumption (VO2 max), is positively associated with insulin sensitivity (34). Moreover, endurance exercise training regimens that improve physical fitness increase insulin-stimulated glucose disposal (31). One mechanism by which physical fitness or activity may alter insulin sensitivity is through the modulation of overall or regional body fat. For example, endurance training has been shown to decrease total body fat and abdominal adiposity (35). Despite the well known effect of physical activity on body fat regulation, few studies have considered simultaneously the relative importance of physical fitness and adiposity in the regulation of insulin sensitivity. Those studies that have attempted to address this question have yielded conflicting results (5, 6, 9, 18, 29). Moreover, to our knowledge, no study to date has examined the relationship of whole body and regional adiposity measures to insulin-stimulated glucose disposal. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
among individuals and their role in mediating the relationship between adiposity and insulin sensitivity has yet to be clearly defined. Our second objective, therefore, was to examine the relationship of \( \dot{V}O_2 \text{max} \) and physical activity energy expenditure (measured over 10 days by use of doubly labeled water) to insulin-stimulated glucose disposal. These measurements permit us to examine the relationship of insulin sensitivity to the physiological capacity for aerobic activity and the caloric expenditure of physical activity.

To accomplish our objectives, we examined data from a cohort of nonobese, nondiabetic, middle-aged women. Although this cohort was nonobese by design (body mass index \( \leq 30 \) kg/m\(^2\)), there was a wide range in total and regional adiposity. In addition, because we did not restrict recruitment to sedentary women, there was significant variability in both physical fitness and activity energy expenditure. The considerable degree of variation in adiposity, fitness, and activity in this cohort provided an ideal sample for us to examine the relative importance of these factors in determining insulin sensitivity. We limited our investigation to women to remove any confounding effects of gender on glucose disposal or the relationship of adiposity, physical fitness, or physical activity energy expenditure to insulin sensitivity.

METHODS

Materials. d-[6,6-^2H]glucose (98% ^2H), L-[1-^13C]leucine (99% ^13C), and sodium [1-^13C]bicarbonate (99% ^13C) were obtained from Cambridge Isotope Laboratories (Andover, MA). Chemical and isotopic purity was determined by gas chromatography-mass spectrometry (GC-MS). Solutions of each isotope were prepared using aseptic technique. Each compound was dissolved in weighed volumes of sterile, pyrogen-free saline and filtered through a 0.22-\( \mu m \) Millipore filter before use. An aliquot of the sterile solution was initially verified to be pyrogen free before administration. \( ^2H_2O \) (99% \(^2H_2O \)) and \( H_2^{18}O \) (10% \(^18H_2O \)) were obtained from Cambridge Isotope Laboratories. Isotopic purity was determined by isotope ratio-mass spectrometry (IRMS).

Subjects. Volunteers (\( n = 45 \)) in the present study were recruited from Burlington, Vermont, and surrounding areas to participate in the Vermont Longitudinal Study of the Menopause. Details of the recruitment of these volunteers and the purpose of this study have been described previously (46), where data from the first-year evaluation are presented. Data from this cohort have been published previously examining menopause-related differences in body composition (48) and insulin sensitivity (43) and correlates of energy expenditure (46), protein metabolism (47), and cardiovascular disease risk (41). The nature, purpose, and possible risks were explained to each subject before written consent to participate was given. The experimental protocol was approved by the Committee on Human Research at the University of Vermont.

The inclusion criteria were that subjects had to 1) be between 40 and 52 yr of age, 2) be premenopausal as defined by the occurrence of two menses in the 3 mo preceding testing, with no increase in cycle irregularity for 12 mo preceding testing and a follicle-stimulating hormone level <30 IU/L, 3) be nonsmoking, 4) have a normal electrocardiogram at rest and during an exercise test, 5) be weight stable (\( \pm 2 \) kg) during the 6 mo before testing, and 6) have a body mass index \( \leq 30 \) kg/m\(^2\). Women were excluded if they 1) were or planned on becoming pregnant, 2) had a personal history or current diagnosis of diabetes, heart disease, hypertension, or other chronic disease, 3) were taking hormone replacement therapy, oral contraceptives, chronic steroid therapy, neuroleptics, or other medication that could affect metabolic function, 4) had a history of alcohol or drug abuse, or 5) had impaired glucose tolerance or type 2 diabetes mellitus according to current guidelines (2).

Experimental protocol. Each prospective volunteer underwent an outpatient screening visit, at which time medical history, physical examination, biochemical laboratory tests, treadmill test, and an oral glucose tolerance test were performed. Volunteers who met the eligibility criteria after screening and consented to participate were studied during two inpatient visits to the General Clinical Research Center (GCRC). The first inpatient visit occurred during the follicular phase and the second inpatient visit during the luteal phase of each woman's menstrual cycle. For 3 days before each admission, subjects consumed a standardized weight maintenance diet provided by the Metabolic Kitchen of the GCRC (1,988 \( \pm 193 \) kcal/day: 60% carbohydrate, 25% fat, 15% protein) that provided \( \geq 250 \) g of carbohydrate/day. Volunteers refrained from exercise for 24 h before each inpatient visit.

On the evening of the first inpatient visit (\( \leq 1700 \)), after providing a baseline urine sample, subjects consumed a mixed, oral dose of \(^2H_2O \) and \( H_2^{18}O \) (0.078 g \(^2H_2O/kg \) body mass and 0.092 g \( H_2^{18}O/kg \) body mass, respectively) and underwent computed tomography (CT) measurements. The following morning, two urine samples were obtained to mark the beginning of the doubly labeled water measurement period. Urine samples were frozen at \(-20^\circ \)C until analysis. Resting (\(-30 \) min) and 3-h postprandial energy expenditure measurements were performed, and body composition was measured.

On the evening of the second inpatient visit (10 days after the first inpatient visit), two urine samples were collected to mark the close of the doubly labeled water measurement period (\( \leq 1700 \)). After an overnight fast (12 h), insulin-stimulated glucose disposal was measured. Insulin-stimulated glucose disposal was divided into oxidative and nonoxidative pathways by use of a combination of \(^13C\)leucine tracer oxidation and indirect calorimetry data. In addition, \(^2H_2O\) was used to measure residual endogenous glucose production during hyperinsulinemia.

At \( \approx 0600 \), the subject was awakened and allowed to void. Teflon catheters were placed in an antecubital vein (18 gauge) for infusion and retrograde in a dorsal hand vein of the contralateral arm (20 gauge). Catheters were kept patent with a slow infusion of saline (30 ml/h). The hand was placed in a warming box to obtain arterialized venous blood. At 0630 (\( t = 0 \) min), priming doses of \(^{13C}\)leucine (4.5 \( \mu \)mol/kg) and sodium \(^{13C}\)bicarbonate (1.6 \( \mu \)mol/kg) were given, and a continuous infusion of \(^{13C}\)leucine (4.5 \( \mu \)mol/kg-h\(^{-1}\)) was started. At 90 min, a primed (4.1 \( mg/kg \)), continuous infusion of \(^{2H}_2\)leucine (3.4 \( mg/kg-h^{-1} \)) was started. A constant infusion of insulin (40 \( mU/m^2 \)min\(^{-1} \)) was started at 210 min. Ueuglucemia was maintained by a variable rate infusion of 20% dextrose (i.e., dextrose infusion). To minimize changes in plasma glucose enrichment during the clamp, \(^{2H}_2\)leucine (1 g; 98% \(^2H\) ) was added to the dextrose infusion. Plasma glucose levels were monitored every 5 min, and the dextrose infusion rate was adjusted to maintain euglucemia. All infusions were stopped at 330 min except for the dextrose infusion, which was continued and tapered until no longer required to maintain normal glycemia.
Blood and breath samples were drawn before the start of infusion, at 165, 180, 195, and 210 min for measurement of baseline leucine and glucose kinetics (data not presented) and at 285, 300, 315, and 330 min for measurement of leucine and glucose kinetics under euglycemic hyperinsulinemia. Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) rates were determined at 60 and 165 min for 15 min (data not presented) and at 300 min for 30 min with the use of the ventilated hood technique (DeltaTrac, Yorba Linda, CA).

Body composition. Body mass was measured on a metabolic scale (Scale-Tronix, Wheaton, IL) with the volunteer clothed in a hospital gown. Whole body fat mass, FFM, and bone mineral mass were measured by dual-energy X-ray absorptiometry using a Lunar DPX-L densitometer (Lunar, Madison, WI). All scans were analyzed using the Lunar Version 1.3y DPX-L extended analysis program for body composition. In our laboratory, the coefficient of variation (CV) for repeat determinations in seven older women was 1% for fat mass and 2% for FFM.

CT. Abdominal adipose tissue areas and thigh muscle composition were measured by CT with a GE High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI). Subjects were examined in the supine position with both arms stretched above the head. By use of a scout image to establish the correct position, scans were performed between the L₄ and L₅ vertebrae and approximately midway between the patella and the anterior superior iliac crest (ASIC) (average distance from ASIC to thigh slice: 295 ± 83 mm). Adipose tissue was highlighted and computed using an attenuation range from −190 to −30 Hounsfield units (HU) with commercially available software (GE Medical Systems). Intra-abdominal adipose tissue area was quantified by delineating the intra-abdominal cavity at the innermost aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body. Subcutaneous adipose tissue area was quantified by highlighting of adipose tissue located between the skin and the outermost aspect of the abdominal muscle wall. Abdominal subcutaneous adipose tissue was further divided into deep and superficial depots along the subcutaneous fascial plane, as described (20). Deep subcutaneous adipose tissue was defined as the area between the subcutaneous fascia and the abdominal muscle wall, and the superficial subcutaneous adipose tissue was defined as the area between the subcutaneous fascia and the skin. CT was also used to measure midtigh muscle fat content (17). Tissue areas were delineated using attenuation values of −190 to −30 HU for adipose tissue, 0–100 HU for muscle, and >200 HU for bone. The average attenuation value of skeletal muscle from the right and left thighs was used as an indicator of thigh skeletal muscle density.

VO₂ max. VO₂ max was assessed by a progressive and continuous test to exhaustion on a treadmill, as previously described (44). A comfortable initial walking or jogging speed was determined for each individual and was maintained throughout the test. After the first 2 min, the incline was increased by 2.5% every 2 min until volitional fatigue. Heart rate was monitored throughout the test with a 12-lead electrocardiogram. VO₂ and VCO₂ were monitored continuously using an open-circuit gas analysis system (Ametek, Pittsburgh, PA). A volunteer was judged to have reached VO₂ max if she reached a respiratory quotient > 1.1 and her age-predicted maximal heart rate was ≥10 beats/min. All volunteers met this criteria. VO₂ max was defined as the highest 30-s average VO₂ reached during the final 2 min of the test. Test-retest conditions (±1 wk) for VO₂ max in a previous group of 18 female volunteers had yielded an intraclass correlation of 0.98 and a CV of 3.4%.

Energy metabolism. Daily energy expenditure (DEE) was measured on 29 volunteers by means of the doubly labeled water technique, as previously described (45). DEE was calculated from VCO₂, data obtained from isotope decay curves by use of the equations outlined by Tchernof et al. (40). Resting (REE) and postprandial energy expenditure were determined using indirect calorimetry, as previously described (46). The thermic effect of the liquid meal (TEM; 10 kcal/kg FFM of Ensure Plus, Ross Laboratories, Columbus, OH: 53.3% carbohydrate, 32% fat, 14.7% protein) was calculated by measuring the area under the curve (AUC) with the trapezoid method and was expressed as a fraction of the energy content of the liquid meal by dividing the AUC by the caloric content of the liquid meal. Physical activity energy expenditure (PAEE) was calculated as

\[ \text{PAEE (kcal/day)} = [\text{DEE (kcal/day)}] \times (1 - \text{TEM}) \]  

Analytical methods. Serum insulin was determined with a double antibody radioimmunoassay (Diagnostic Products, Los Angeles, CA). The intra- and interassay CVs for insulin were 4% and 10%, respectively. Plasma glucose concentrations were measured by a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma glucose enrichment was measured by electron impact ionization GC-MS, as previously described (15). Before measurement by GC-MS, glucose was derivatized to the butane boronate acetyl derivative, as described (4). Injection of butane boronate-glucose were made isothermally into the GC-MS (model 5971A, Hewlett-Packard, Palo Alto, CA) while the [M − 57]⁺ ions were monitored at a mass-to-charge ratio (m/z) of 297 and 299 for unlabeled and [1³H]glucose, respectively. Plasma α-ketoisocaproate (KIC) enrichment was measured by electron impact ionization GC-MS, as previously described (47). Before measurement by GC-MS, keto acids were isolated from plasma and derivatized to tert-butylidimethylsilyl (t-BDMS)-quinoxalinol derivatives (26). The t-BDMS derivative of the keto acids was also measured by GC-MS with selected monitoring of the [M − 57]⁺ ion at m/z = 259 and 260 for unlabeled and [1³C]KIC, respectively. The enrichment of expired 1³CO₂ [molar percent excess (MPE) × 1,000] was measured by IRMS (VG Sira II, Middletown, Cheshire, UK). Urine samples were analyzed for ¹⁸O enrichment by the CO₂ equilibration technique (10) and for ³H enrichment by the zinc catalyst method of Wong et al. (51) by IRMS.

Calculations. During hyperinsulinemia, steady state was achieved for plasma glucose concentrations and achievement. Thus the rate of insulin-stimulated glucose disposal corresponded to the removal of glucose from two sources: the dextrose infusion used to maintain euglycemia and residual endogenous glucose production [rate of appearance (Ra)]. To calculate the residual endogenous glucose production, we had to consider the input of [²H₂]glucose into the plasma pool from two sources: 1) the [²H₂]glucose infusion and 2) the dextrose infusion. Thus Ra was calculated as

\[ R_a = \frac{(E_{\text{int}} - E_p)_{\text{int}} + (E_{\text{dex}} - E_p)_{\text{dex}}}{E_p} \]  

where E is the enrichment of [²H₂]glucose (MPE) in the [²H₂]glucose infusion (inf), plasma glucose (P), and the dextrose infusion (dex), and i is the infusion rate of [²H₂]glucose tracer (mg/min) from the inf and dex infusions, respectively. Any negative values for glucose R_a during hyperinsulinemia
were assumed to indicate complete suppression of endogenous glucose production.

Nonoxidative glucose disposal was calculated by subtracting glucose oxidation from glucose disposal. Indirect calorimetry was used to measure glucose oxidation during the hyperinsulinemic euglycemic clamp. This method requires a correction for protein oxidation (36). Urinary nitrogen excretion has commonly been used to estimate protein oxidation. This technique, however, is subject to errors (42), due to insulin-induced changes in urea pool size (38) and the inability to collect a representative sample over the short time period of the clamp. To overcome these problems, [13C]leucine was used to measure protein oxidation under hyperinsulinemic euglycemic conditions. Leucine oxidation data obtained from infusion of [13C]leucine have been shown to accurately reflect whole body protein oxidation (39). Leucine oxidation (C, expressed as μmol/h) was calculated as

\[ C = \frac{F_{13C}(1/E_p - 1/E_i)}{E_{CO2}/0.81} \times 100 \]  

where \( F_{13C} \) is the rate of \(^{13}CO_2\) excretion into expired air (μmol \(^{13}C/\)h), and \( E_i \) and \( E_p \) are the enrichment (MPE) of leucine in the infused and plasma KIC, respectively. \( F_{13C} \) was calculated as

\[ F_{13C} = \frac{F_{CO2} - E_{CO2}/0.81}{1/E_{i}} \]  

where \( F_{CO2} \) is the V\( \dot{O}_2 \) rate (μmol/min) and \( E_{CO2} \) is the \(^{13}C\) enrichment of expired CO\(_2\) (atom percent excess). The constant 0.81 accounts for the retention of \(^{13}CO_2\) in the bicarbonate pool. Changes in \(^{13}CO_2\) natural abundance due to the hyperinsulinemic euglycemic clamp were accounted for by measuring \(^{13}CO_2\) natural abundance in an independent cohort of women (\( n = 6 \)) undergoing an identical clamp with no infusion of \([^{13}C]\)leucine. \(^{13}CO_2\) excretion data were adjusted for natural abundance changes, as described (47). Leucine oxidation data were then used to define protein oxidation, with the assumption that body proteins are 8% leucine. Protein oxidation was used together with \( \dot{V}_{O2} \) and \( \dot{V}_{CO2} \) data to calculate carbohydrate and fat oxidation (36).

Statistics. Relationships between variables were determined by Pearson correlation coefficients. Partial correlation analysis was performed to examine the relationship between glucose disposal measures and predictor variables after statistical control for covariates. Because intra-abdominal fat was not normally distributed (Shapiro-Wilk test, \( P < 0.01 \)), data were log\(_{10}\) transformed [Shapiro-Wilk test, not significant (NS)]. For all correlation analysis, glucose disposal data were expressed relative to FFM (mg·kg\(^{-1}\)·min\(^{-1}\)). The adequacy of this mathematical adjustment of glucose disposal data rests on the assumptions that the y-intercept of the relationship between glucose disposal and FFM is not different from zero and that adjusted glucose disposal data (mg·kg·FFM\(^{-1}·\)min\(^{-1}\)) are not related to FFM (kg). Although glucose disposal (mg/min) and FFM (kg) were related (\( r = 0.42; P < 0.01 \)), the y-intercept of this relationship was not different from zero (\( r = -181 \pm 204 \) mg/min, \( P = 0.38 \)). More importantly, no correlations between adjusted glucose disposal data (mg·kg·FFM\(^{-1}·\)min\(^{-1}\)) and FFM were found (total glucose disposal: \( r = 0.12 \); nonoxidative: \( r = 0.14 \); oxidative: \( r = 0.12 \)), suggesting that this method of data expression removed the effect of FFM on glucose disposal measures. \( \dot{V}_{O2,\max} \) was expressed as liters per minute, given that we (44) and others (21) have shown that expression of \( \dot{V}_{O2,\max} \) per kilogram of body weight or per kilogram of FFM can lead to erroneous results. However, to ensure that the relationship of \( \dot{V}_{O2,\max} \) to glucose disposal was not due to the effects of FFM, we examined this relationship by use of partial correlation analysis, with FFM as a covariate, after adjusting \( \dot{V}_{O2,\max} \) for FFM with regression techniques, as previously described (44), and after dividing \( \dot{V}_{O2,\max} \) by FFM. To control for the effects of body size, physical activity energy expenditure was adjusted for body mass by means of partial correlation analysis and was divided by body mass\(^{0.65} \), as described (32). Correlation coefficients from bivariate and partial correlation analysis are expressed as r values. Stepwise regression analysis was used to determine which variables predicted glucose disposal measures. Possible predictor variables were entered into the stepwise model if a physiological basis for explaining variation in glucose disposal was supported by previous studies and if a significant bivariate relationship was observed between the glucose disposal measure and the predictor variable. The cumulative variation in glucose disposal data accounted for by the stepwise model is expressed as an \( r^2 \) value. All data are expressed as means ± SD unless otherwise specified.

RESULTS

Physical characteristics are shown in Table 1 and energy metabolism data in Table 2. These data show that the cohort is relatively homogeneous with respect to age, body mass, and FFM. In contrast, the cohort showed a considerable degree of variability in total and regional body fat, \( \dot{V}_{O2,\max} \), and physical activity energy expenditure, as indicated by CVs >20%.

Total glucose disposal averaged 10.74 ± 2.92 mg·kg\(^{-1}·\)min\(^{-1}\). Oxidative glucose disposal accounted for 37 ± 2% (3.63 ± 0.88 mg·kg·FFM\(^{-1}·\)min\(^{-1}\)) of total glucose disposal, with nonoxidative glucose disposal comprising the remaining 63 ± 2% (7.11 ± 3.11 mg·kg·FFM\(^{-1}·\)min\(^{-1}\)). Fat oxidation during the final 30 min of the clamp averaged 0.89 ± 0.41 mg·kg·FFM\(^{-1}·\)min\(^{-1}\). Insulin levels averaged 598 ± 163 pmol/l, and glucose levels averaged 4.76 ± 0.27 mmol/l during the last 30 min of the clamp.

Correlation coefficients for the relationships between total, nonoxidative, and oxidative glucose disposal and selected physiological variables are shown in Table 3. Of particular note are the strong correlations of \( \dot{V}_{O2,\max} \) (l/min) to total and nonoxidative glucose disposal. These correlations were similar when \( \dot{V}_{O2,\max} \) was adjusted for FFM with a regression-based approach (44).

Table 1. Physical characteristics of 45 middle-aged, premenopausal women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>47 ± 3</td>
<td>40–52</td>
<td>6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165 ± 5</td>
<td>154–173</td>
<td>3</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>62 ± 8</td>
<td>47–84</td>
<td>13</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>41 ± 4</td>
<td>34–54</td>
<td>10</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>18 ± 8</td>
<td>8–40</td>
<td>44</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>30 ± 9</td>
<td>16–50</td>
<td>30</td>
</tr>
<tr>
<td>Intra-abdominal fat, cm(^2)</td>
<td>62 ± 34</td>
<td>24–148</td>
<td>55</td>
</tr>
<tr>
<td>Subcutaneous fat, cm(^2)</td>
<td>246 ± 116</td>
<td>32–573</td>
<td>48</td>
</tr>
<tr>
<td>Superficial</td>
<td>132 ± 51</td>
<td>27–271</td>
<td>39</td>
</tr>
<tr>
<td>Deep</td>
<td>114 ± 65</td>
<td>5–301</td>
<td>57</td>
</tr>
<tr>
<td>Thigh muscle attenuation, HU</td>
<td>47 ± 3</td>
<td>41–52</td>
<td>6</td>
</tr>
<tr>
<td>( \dot{V}_{O2,\max} ), l/min</td>
<td>1.97 ± 0.44</td>
<td>1.05–3.10</td>
<td>22</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; \( \dot{V}_{O2} \), oxygen consumption; HU, Hounsfield units.
Table 2. Energy metabolism data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily energy expenditure, kcal/day</td>
<td>2,598 ± 398</td>
<td>1,709–3,279</td>
<td>15</td>
</tr>
<tr>
<td>Resting energy expenditure, kcal/day</td>
<td>1,346 ± 152</td>
<td>1,010–1,810</td>
<td>11</td>
</tr>
<tr>
<td>Thermic effect of liquid meal, %</td>
<td>6.0 ± 1.8</td>
<td>2.8–10.3</td>
<td>30</td>
</tr>
<tr>
<td>Physical activity energy expenditure</td>
<td>1,095 ± 344</td>
<td>347–1,748</td>
<td>31</td>
</tr>
</tbody>
</table>

Daily and physical activity energy expenditure data were available on 29 volunteers. Thermic effect of liquid meal was expressed as a percentage of the caloric content of the liquid meal consumed.

When expressed as a relative percentage of total abdominal fat area or statistically adjusted for total abdominal fat area by use of partial correlation analysis, none of the subcompartments of abdominal fat were related to glucose disposal variables. All correlations between subcompartments of abdominal adiposity and glucose disposal became nonsignificant after statistical control for VO\(_2\)\(_{\text{max}}\). No correlation was found between glucose disposal data and physical activity energy expenditure (\(n = 29\)) after adjustment for body mass using partial correlation analysis (\(r = 0.30\)) or by dividing by body mass\(^0.5\) (\(r = 0.27\)). Neither age (\(r = 0.05\)) nor FFM (\(r = 0.12\)) was related to insulin-stimulated glucose disposal.

The relationships of VO\(_2\)\(_{\text{max}}\) (L/min) to total, nonoxidative, and oxidative glucose disposal are shown in Fig. 1. The regression equation for the relationship of VO\(_2\)\(_{\text{max}}\) to total glucose disposal was:

\[
\text{Glucose disposal} = 0.63\cdot V\dot{O}_2\text{max} - 6.0
\]

\[r = 0.63\]  
P < 0.01

When expressed as a relative percentage of total abdominal fat area or statistically adjusted for total abdominal fat area by use of partial correlation analysis, none of the subcompartments of abdominal fat were related to glucose disposal variables. All correlations between subcompartments of abdominal adiposity and glucose disposal became nonsignificant after statistical control for VO\(_2\)\(_{\text{max}}\). No correlation was found between glucose disposal data and physical activity energy expenditure (\(n = 29\)) after adjustment for body mass using partial correlation analysis (\(r = 0.30\)) or by dividing by body mass\(^0.5\) (\(r = 0.27\)). Neither age (\(r = 0.05\)) nor FFM (\(r = 0.12\)) was related to insulin-stimulated glucose disposal.

The relationships of VO\(_2\)\(_{\text{max}}\) (L/min) to total, nonoxidative, and oxidative glucose disposal are shown in Fig. 1. The regression equation for the relationship of VO\(_2\)\(_{\text{max}}\) to total glucose disposal was:

\[
\text{Glucose disposal} = 0.63\cdot V\dot{O}_2\text{max} - 6.0
\]

\[r = 0.63\]  
P < 0.01

When expressed as a relative percentage of total abdominal fat area or statistically adjusted for total abdominal fat area by use of partial correlation analysis, none of the subcompartments of abdominal fat were related to glucose disposal variables. All correlations between subcompartments of abdominal adiposity and glucose disposal became nonsignificant after statistical control for VO\(_2\)\(_{\text{max}}\). No correlation was found between glucose disposal data and physical activity energy expenditure (\(n = 29\)) after adjustment for body mass using partial correlation analysis (\(r = 0.30\)) or by dividing by body mass\(^0.5\) (\(r = 0.27\)). Neither age (\(r = 0.05\)) nor FFM (\(r = 0.12\)) was related to insulin-stimulated glucose disposal.

The relationships of VO\(_2\)\(_{\text{max}}\) (L/min) to total, nonoxidative, and oxidative glucose disposal are shown in Fig. 1. The regression equation for the relationship of VO\(_2\)\(_{\text{max}}\) to total glucose disposal was:

\[
\text{Glucose disposal} = 0.63\cdot V\dot{O}_2\text{max} - 6.0
\]

\[r = 0.63\]  
P < 0.01

When expressed as a relative percentage of total abdominal fat area or statistically adjusted for total abdominal fat area by use of partial correlation analysis, none of the subcompartments of abdominal fat were related to glucose disposal variables. All correlations between subcompartments of abdominal adiposity and glucose disposal became nonsignificant after statistical control for VO\(_2\)\(_{\text{max}}\). No correlation was found between glucose disposal data and physical activity energy expenditure (\(n = 29\)) after adjustment for body mass using partial correlation analysis (\(r = 0.30\)) or by dividing by body mass\(^0.5\) (\(r = 0.27\)). Neither age (\(r = 0.05\)) nor FFM (\(r = 0.12\)) was related to insulin-stimulated glucose disposal.

The relationships of VO\(_2\)\(_{\text{max}}\) (L/min) to total, nonoxidative, and oxidative glucose disposal are shown in Fig. 1. The regression equation for the relationship of VO\(_2\)\(_{\text{max}}\) to total glucose disposal was:

\[
\text{Glucose disposal} = 0.63\cdot V\dot{O}_2\text{max} - 6.0
\]

\[r = 0.63\]  
P < 0.01

When expressed as a relative percentage of total abdominal fat area or statistically adjusted for total abdominal fat area by use of partial correlation analysis, none of the subcompartments of abdominal fat were related to glucose disposal variables. All correlations between subcompartments of abdominal adiposity and glucose disposal became nonsignificant after statistical control for VO\(_2\)\(_{\text{max}}\). No correlation was found between glucose disposal data and physical activity energy expenditure (\(n = 29\)) after adjustment for body mass using partial correlation analysis (\(r = 0.30\)) or by dividing by body mass\(^0.5\) (\(r = 0.27\)). Neither age (\(r = 0.05\)) nor FFM (\(r = 0.12\)) was related to insulin-stimulated glucose disposal.

The relationships of VO\(_2\)\(_{\text{max}}\) (L/min) to total, nonoxidative, and oxidative glucose disposal are shown in Fig. 1. The regression equation for the relationship of VO\(_2\)\(_{\text{max}}\) to total glucose disposal was:

\[
\text{Glucose disposal} = 0.63\cdot V\dot{O}_2\text{max} - 6.0
\]

\[r = 0.63\]  
P < 0.01

When expressed as a relative percentage of total abdominal fat area or statistically adjusted for total abdominal fat area by use of partial correlation analysis, none of the subcompartments of abdominal fat were related to glucose disposal variables. All correlations between subcompartments of abdominal adiposity and glucose disposal became nonsignificant after statistical control for VO\(_2\)\(_{\text{max}}\). No correlation was found between glucose disposal data and physical activity energy expenditure (\(n = 29\)) after adjustment for body mass using partial correlation analysis (\(r = 0.30\)) or by dividing by body mass\(^0.5\) (\(r = 0.27\)). Neither age (\(r = 0.05\)) nor FFM (\(r = 0.12\)) was related to insulin-stimulated glucose disposal.

The relationships of VO\(_2\)\(_{\text{max}}\) (L/min) to total, nonoxidative, and oxidative glucose disposal are shown in Fig. 1. The regression equation for the relationship of VO\(_2\)\(_{\text{max}}\) to total glucose disposal was:

\[
\text{Glucose disposal} = 0.63\cdot V\dot{O}_2\text{max} - 6.0
\]

\[r = 0.63\]  
P < 0.01

When expressed as a relative percentage of total abdominal fat area or statistically adjusted for total abdominal fat area by use of partial correlation analysis, none of the subcompartments of abdominal fat were related to glucose disposal variables. All correlations between subcompartments of abdominal adiposity and glucose disposal became nonsignificant after statistical control for VO\(_2\)\(_{\text{max}}\). No correlation was found between glucose disposal data and physical activity energy expenditure (\(n = 29\)) after adjustment for body mass using partial correlation analysis (\(r = 0.30\)) or by dividing by body mass\(^0.5\) (\(r = 0.27\)). Neither age (\(r = 0.05\)) nor FFM (\(r = 0.12\)) was related to insulin-stimulated glucose disposal.
et al. (29) showed that VO₂ max was a stronger predictor of insulin sensitivity (5, 6, 29, 34). Bogardus et al. (5, 6) and Nyholm et al. (9, 18) found, by use of statistical modeling, a stronger relationship of V0₂ max to nonoxidative glucose disposal was glucose disposal (mg·kg FFM⁻¹·min⁻¹) = 3.771 × [VO₂ max (l/min)] + 0.011. The slopes for these relationships were indistinguishable [3.811 ± 0.761 vs. 3.771 ± 0.895 (mg·kg FFM⁻¹·min⁻¹)/l(min)].

Using stepwise regression analysis, we found that VO₂ max was the only significant predictor of total (r² = 0.40, P < 0.01) and nonoxidative glucose disposal (r² = 0.32, P < 0.01).

**DISCUSSION**

This study examined the relationship of insulin sensitivity to adiposity, physical fitness, and physical activity energy expenditure in a cohort of nonobese, non-diabetic, middle-aged, premenopausal women. Our principal finding is that VO₂ max was a stronger correlate of glucose disposal than total or regional adiposity measurements. This finding is supported by several lines of evidence. 1) The positive relationship between VO₂ max and insulin sensitivity persisted after adjustment for FPM, percent body fat, and intra-abdominal fat; 2) VO₂ max was the only variable to enter stepwise regression models as a significant predictor of glucose disposal; and 3) correlations of total and regional adiposity measures to insulin sensitivity were no longer significant after statistical adjustment for VO₂ max.

**Insulin sensitivity and VO₂ max.** Our results agree with several studies that have shown a strong, positive relationship between aerobic capacity and insulin sensitivity (5, 6, 29, 34). Bogardus et al. (5, 6) and Nyholm et al. (29) showed that VO₂ max was a stronger predictor of insulin sensitivity than overall adiposity. These early studies did not, however, measure abdominal or thigh muscle fat content. Recent studies that have combined aerobic capacity measurements with imaging techniques have shown that intra-abdominal, subcutaneous abdominal, and thigh muscle fat are more closely associated with insulin sensitivity than VO₂ max (9, 18). Although these results appear to disagree with the present study and previous work (5, 6, 29), some important differences among studies should be considered. Specifically, divergent results have been derived from studies examining sedentary (18) and/or obese (9, 18) individuals. Limiting the range of VO₂ max by recruiting sedentary subjects may reduce the correlation between VO₂ max and glucose disposal by restricting the range of the independent variable (37). Additionally, the relationship between VO₂ max and insulin sensitivity may be less apparent in obese individuals (9, 18) as the effect of adiposity on glucose disposal becomes predominant. Differences in subject selection, therefore, may explain variable results among studies.

The relationship of VO₂ max to insulin-stimulated glucose disposal was explained through its association with nonoxidative glucose disposal (Fig. 1). Thus, if VO₂ max regulates insulin sensitivity, it likely does so by altering nonoxidative glucose disposal. In support of this notion, previous studies have shown that differences in insulin-stimulated glucose disposal between athletes and sedentary individuals (i.e., individuals with high and low VO₂ max, respectively) are due to differences in nonoxidative glucose disposal (13, 19). These studies also showed that athletes were characterized by greater insulin-induced stimulation of blood flow, greater proportions of oxidative muscle fibers, increased GLUT-4 expression, and increased glycogen synthase activity (13, 19). These physiological and biochemical factors have been shown to modulate insulin-stimulated glucose disposal (3, 7, 19, 24). Moreover, these factors are positively related to VO₂ max (13, 19, 49) and are increased with exercise training (1, 11, 12). Thus the relationship between VO₂ max and glucose disposal may be explained by the fact that VO₂ max reflects the activity or responsiveness of various physiological and biochemical processes that regulate insulin-stimulated glucose disposal.

The relationship between VO₂ max and insulin sensitivity could relate to the acute effect of previous physical activity on insulin-stimulated glucose disposal (27, 28). For several reasons we do not believe this to be the case in our study. First, we instructed volunteers to refrain from exercise for 24 h before their admission. Thus the measurement of insulin sensitivity was removed from their last bout of exercise by ≥36 h. Second, we provided ≥250 g of dietary carbohydrate for 3 days before the measurement to ensure replenishment of glycogen stores (25). Finally, evidence suggests that previous exercise of sufficient intensity to reduce skeletal muscle glycogen content by 21% has no effect on whole body glucose disposal (13).

**Insulin sensitivity and physical activity energy expenditure.** To our knowledge, this is the first study to examine the relationship between physical activity energy expenditure measured by doubly labeled water and insulin-stimulated glucose uptake in humans. Physical activity energy expenditure was not related to insulin-stimulated glucose disposal or its components. The extent to which our measurement of physical activity energy expenditure reflects the “amount” of physical activity is not known, because differences in body size among individuals can alter the energetic cost of a given amount of physical activity. No correlation was found, however, between glucose disposal data and physical activity energy expenditure after statistical adjustment for body mass with partial correlation analysis or when physical activity energy expenditure was divided by body mass⁰.⁵. Thus, we conclude from these findings that insulin sensitivity was more closely related to the physiological capacity for aerobic activity, as reflected by VO₂ max than the caloric expenditure of physical activity, as measured by doubly labeled water over a 10-day period.

Our findings differ from those of Wareham et al. (50), who found, by use of statistical modeling, a stronger effect of physical activity on oral glucose tolerance compared with VO₂ max. Differences between studies probably relate to the techniques used to assess insulin sensitivity and physical activity. In the present study, insulin sensitivity was measured by hyperinsulinemic
euglycemic clamp and physical activity energy expenditure by doubly labeled water. These techniques are considered criterion methods for assessing insulin sensitivity and physical activity energy expenditure, respectively. In contrast, Wareham et al. used oral glucose tolerance tests to assess insulin sensitivity and estimated physical activity energy expenditure from heart rate monitoring.

Physical activity, no doubt, plays a role in mediating the relationship between \( V_{\text{O}_2} \text{max} \) and insulin sensitivity, because \( V_{\text{O}_2} \text{max} \) is partially dependent on the amount of physical activity performed. To effect \( V_{\text{O}_2} \text{max} \), however, physical activity must be of sufficient intensity to elicit a training response. Therefore, the relationship between physical activity and insulin sensitivity is likely more a function of the intensity of activity than of caloric expenditure. This finding may have implications for the prescription of exercise to improve insulin sensitivity. Although low-intensity exercise may be beneficial through its ability to increase energy expenditure and reduce adiposity, greater improvements in insulin sensitivity may be observed with higher-intensity exercise that increases \( V_{\text{O}_2} \text{max} \).

**Insulin sensitivity and adiposity.** Measures of total and regional adiposity were negatively related to insulin sensitivity. Total adiposity, expressed on an absolute (kg) or relative basis (%body mass), was a stronger correlate of glucose disposal measures than regional body fat measurements. Our results agree with Bonoro et al. (8), who showed that total adiposity was a stronger correlate of glucose disposal than either abdominal subcutaneous or intra-abdominal fat in nonobese, premenopausal women. Our findings differ, however, from recent studies showing stronger relationships of intra-abdominal, deep subcutaneous abdominal, and thigh muscle fat to insulin sensitivity compared with total adiposity (9, 18, 22). One explanation for differences among studies is subject selection. Previous studies that have found relatively strong correlations between regional adiposity measures and insulin sensitivity have included obese subjects (9, 18, 22). Regional adiposity measures have been shown to be stronger determinants of glucose disposal in obese compared with nonobese individuals (8, 18). Thus the absence of an association between insulin sensitivity and regional adiposity measurements in the current study may relate to the fact that nonobese women were studied.

Reasons for the differing relationship of insulin sensitivity to total and regional adiposity in the nonobese and obese states are not clear (8). This observation could reflect differences in the effect of overall and regional adiposity on pathways of insulin-stimulated glucose disposal throughout the adiposity spectrum. For example, as demonstrated by Bogardus et al. (5) in Pima Indian men, total adiposity may antagonize insulin sensitivity up to some threshold level and then lose its effect at higher levels of body fat (>28% body fat). This threshold may mark the point at which fat accumulates in specific anatomic depots (i.e., intra-abdominal, deep subcutaneous abdominal, thigh muscle) to a level where it affects glucose disposal. At this point, the distribution of fat would become a more important modulator of insulin sensitivity than overall adiposity. This threshold level of adiposity, however, may vary among individuals, depending on age, gender, and ethnic admixture.

An equally tenable explanation, however, is that differences in the predictors of insulin sensitivity between lean and obese individuals are a function of the variation in total and regional adiposity measures in these two populations. In studies that have examined obese individuals (9) or combined obese and lean volunteers (18, 22), the CV for total adiposity measures is less than that for regional adiposity markers. In contrast, in nonobese populations, the CVs for total adiposity measures are nearly equivalent to regional adiposity measures (Table 1). Considering that, mathematically, the correlation coefficient is a function of the standard deviations of the dependent and independent variable, the reduced variability in overall adiposity in obese individuals could limit the strength of its association to insulin sensitivity (37).

In conclusion, our findings suggest that \( V_{\text{O}_2} \text{max} \) is a stronger correlate of insulin-stimulated glucose disposal than indexes of total or regional adiposity. Moreover, correlations between \( V_{\text{O}_2} \text{max} \) and insulin sensitivity persisted after statistical control for fat-free mass, percent fat, and intra-abdominal fat. In contrast, physical activity energy expenditure was not related to insulin-stimulated glucose disposal, suggesting that insulin sensitivity is more closely related to the physiological capacity for aerobic activity than the caloric expenditure of physical activity. Although measures of total and regional adiposity were related to insulin sensitivity, none of these correlations remained significant after statistical control for \( V_{\text{O}_2} \text{max} \). Collectively, these results highlight the potentially important role of aerobic fitness in the regulation of insulin sensitivity in nonobese, nondiabetic, premenopausal women.

The authors thank all the participants who volunteered their time for this study. We are grateful to Chris Potter for skilled assistance. This work was supported by grants from National Institutes of Health (AR-02125, AG-13978, MO1 RR-1093252, and AG-151121), the US Department of Agriculture (96-35200-3488), and the General Clinical Research Center (RR-00109).

**REFERENCES**

E120

\[ \text{\textbf{VO}_2\text{max AND INSULIN SENSITIVITY}} \]


