Exercise Restores Skeletal Muscle Glucose Delivery But Not Insulin-Mediated Glucose Transport and Phosphorylation in Obese Subjects

L. Slimani, V. Oikonen, K. Hällsten, N. Savisto, J. Knuuti, P. Nuutila, and P. Iozzo

Turku PET Centre (L.S., V.O., K.H., N.S., J.K., P.N., P.I.) and Department of Medicine (P.N.), University of Turku, FIN-20521 Turku, Finland; and Institute of Clinical Physiology (P.I.), CNR National Research Council, 56124 Pisa, Italy

Insulin resistance in obese subjects results in an impaired use of glucose by skeletal muscle (1, 2); it is a strong predictor of the metabolic syndrome and of type 2 diabetes. In humans, insulin and exercise have synergistic increasing effects on glucose uptake (3). Exercise strongly promotes blood flow (4) and glucose uptake regardless of insulin stimulation (5, 6), thus counteracting insulin resistance. In most previous studies, the actions of insulin and exercise have been studied separately (7–9). Their simultaneous influence on skeletal muscle glucose uptake in vivo in humans has been addressed in a few studies (5, 10–12), but specific roles of single steps in the proximal pathway of glucose transport have not been addressed, and the confounding effect of perfusion, if any, was not separated from that of intrinsic tissue metabolism. Thus, it remains to be established whether the perfusion-independent, metabolic synergy between insulin and exercise stimulation is compromised in obesity.

Dynamic positron emission tomography (PET) with 2-deoxy-2-[18F]fluoro-D-glucose ([18F]FDG) provides a noninvasive description of the tissue-specific time radioactivity curve after tracer injection, which is used for the in vivo determination of glucose transport and phosphorylation in skeletal muscle (13). A three rate-constant ($k_3$) model, first developed for studying the brain, has been used in estimating the first steps of glucose metabolism in the heart (14, 15) and skeletal muscle (16, 17); it was modified by Bertoldo et al. (18) to take into account the concentration of tracer in the interstitial space, by the inclusion of four compartments and five rate-constant parameters.

The compartmental model in skeletal muscle must take into account the dependency of glucose transport on blood flow and the permeability of the sarcolemma. To simplify the model, it is assumed that $[18F]$FDG is in equilibrium between plasma and erythrocytes (17) and that the dephosphorylation of $[18F]$FDG-6-phosphate can be assumed to be negligible (19). Several studies have indicated that glucose transport is the rate-limiting step for glucose uptake in skeletal muscle in normal subjects (20–23). If the phosphorylation rate of $[18F]$FDG is high enough to prevent any noticeable outflow of tracer from myocytes, the transport across sarcolemma is virtually irreversible. The reduced $[18F]$FDG model for skeletal muscle can thus be described mathematically with the same three-compartmental model as for the brain (Fig. 1), albeit with a different interpretation.

The aim of the current study was to examine the in vivo effect of insulin and/or exercise on skeletal muscle glucose transport and phosphorylation in obese and lean subjects with dynamic PET imaging and compartmental modeling. In each group, there was no statistically significant difference between plasma flow and $K_1$. At rest, a significant defect in $K_1$ ($P = 0.0016$), $k_3$ ($P = 0.016$), and $K_3$ ($P = 0.022$) was found in obese subjects. Exercise restored $K_1$, improved but did not normalize $K_3$ ($P = 0.03$ vs. lean), and did not ameliorate the more than 60% relative impairment in $k_3$ in obese individuals ($P = 0.002$ vs. lean). The glucose oxidative potential tended to be reduced by obesity.

Conclusions/Interpretation: The study indicates that exercise restores the impairment in insulin-mediated skeletal muscle perfusion and glucose delivery associated with obesity but does not normalize the defect involving the proximal steps regulating glucose disposal in obese individuals. Our data support the use of 2-deoxy-2-[18F]fluoro-D-glucose positron emission tomography in the dissection between substrate supply and intrinsic tissue metabolism. (J Clin Endocrinol Metab 91: 3394–3403, 2006)
addition, we tested the information on skeletal muscle perfusion obtainable from $^{18}$F-FDG modeling. A subset of these studies was included in a previous publication (12), addressing the question of glucose uptake per se by a different method. Obese and lean subjects were studied with PET in combination with $^{18}$F-FDG during insulin stimulation and one-legged exercise. Perfusion and oxygen consumption were measured with PET and $^{15}$O-H$_2$O or $^{15}$O-O$_2$, respectively.

**Subjects and Methods**

Seven obese and 12 lean male untrained sedentary subjects were studied (Table 1). Maximal force of knee extensors was similar in both groups. Groups were well matched with respect to age, blood pressure, and workload during the PET study. Except for obesity, all subjects were in good general health, as judged from history, physical examination, and standard laboratory tests and were not taking any medications. Written informed consent was obtained after the nature, purpose, and potential risks of the study were explained to the subjects. The study protocol was approved by The Commission on Ethics of the University of Turku and Turku University Central Hospital.

**Study design**

Before the PET study, maximal oxygen uptake (VO$_{2\text{max}}$) and maximal isometric contractile force of the quadriceps femoris muscle were determined. The design of this study is shown in Fig. 2. Alcohol and caffeine were prohibited for 1 d before the study, and the subjects were instructed to avoid strenuous physical activity for 48 h before the study. The PET study was performed after an overnight fast. Two catheters were inserted, one in an antecubital vein for infusion of glucose and insulin and injection of $^{15}$O-H$_2$O and $^{18}$F-FDG and one in the opposite radial artery for blood sampling. Muscle blood flow and oxygen consumption were measured at 60 and 90 min after the start of insulin infusion, respectively, in both femoral regions using $^{15}$O-H$_2$O infusion and $^{15}$O-O$_2$ inhalation techniques, as described in detail below. At 120 min, a bolus of $^{18}$F-FDG was injected for quantification of muscle glucose kinetics.

**Exercise**

All subjects were in supine position in the PET scanner with the femoral regions of both legs in the gantry and performed one-leg intermittent isometric exercise between 45 and 160 min (Fig. 2). Exercise consisted of 2 sec of isometric knee extension alternating with 2 sec of rest.

**Hyperinsulinemic clamp**

At 0 min, an iv infusion of insulin at a rate of 1 mU/kg body weight “min” was started. The study for each subject consisted of a 160-min normoglycemic hyperinsulinemic period. Normoglycemia was maintained by using a variable 20% glucose infusion rate adjusted according to frequent plasma glucose concentration measurements.

**TABLE 1. Clinical and biochemical characteristics of the subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>24 ± 3</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.6 ± 7.4</td>
<td>123.8 ± 15.9*</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>22 ± 2.4</td>
<td>35.6 ± 5.4*</td>
</tr>
<tr>
<td>Mean percent of body fat (%)</td>
<td>15.8 ± 6</td>
<td>28.8 ± 1.6*</td>
</tr>
<tr>
<td>Fasting plasma glucose (mM)</td>
<td>5.3 ± 0.3</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td>Fasting serum insulin (mU/liter)</td>
<td>6.2 ± 2</td>
<td>15.6 ± 7*</td>
</tr>
<tr>
<td>Maximal isometric force (n)</td>
<td>608 ± 173</td>
<td>718 ± 285</td>
</tr>
<tr>
<td>Work force during exercise (n)</td>
<td>85.7 ± 18</td>
<td>85.9 ± 19</td>
</tr>
<tr>
<td>VO$_{2\text{max}}$ (ml/kg BW/min)</td>
<td>50.8 ± 12</td>
<td>31.4 ± 6*</td>
</tr>
</tbody>
</table>

Data are means ± sd. BW, Body weight.

*P < 0.001.

*P < 0.01.
Production of PET tracers

$[^{18}F]FDG$ was synthesized with an automatic apparatus as described by Hamacher et al. (24). The specific radioactivity at the end of synthesis was 76 GBq/μmol $[^{18}F]FDG$ with radiochemical purity greater than 98%. For production of $[^{15}O]O_2$, a low-energy deuteron accelerator Cyclone 3 (Ion Beam Application Inc., Louvain-la-Neuve, Belgium) was used. $[^{15}O]O_2$ was produced using a dialysis technique in a continuously working water module (25). Sterility and pyrogenicity tests were performed daily to verify the purity of the product.

Image acquisition and processing

An ECAT 931/08 PET scanner (Siemens/CTI Corp., Knoxville, TN) was used for image acquisition. Each subject was positioned in the tomograph with the femoral regions in four consecutive cross-sectional slices, carefully scanned for 20 min with a removable ring $[^{68}Ge]$ source. All data were corrected for dead time and measured photon attenuation.

At 60 min after the start of insulin infusion, $[^{18}F]FDG$ (1.1–1.7 GBq) was injected iv over 30 sec, and a dynamic 6-min scan was started. To obtain the input function, arterial blood was withdrawn with a pump at a rate of 6 ml/min. Radioactivity was measured by using a two-channel detector system (Scanditronix, Uppsala, Sweden). Integrated $[^{18}F]FDG$ PET data were reconstructed by Bayesian iterative reconstruction algorithm using median root prior (26) with 150 iterations and a Bayesian coefficient of 0.3.

For the measurement of muscle oxygen consumption, a $[^{15}O]O_2$-bolus inhalation technique was used, as validated against Fick’s principle (5). $[^{15}O]O_2$ was administered at 90 min from the start of the clamp. A 120-min $[^{18}F]FDG$ (0.15–0.33 GBq) was injected iv over 2 min, and a 40-min dynamic scan was started ($12 \times 15$, 4 $\times$ 30, 3 $\times$ 60, 1 $\times$ 120, and thereafter 300-sec frames). Arterial blood samples for the measurement of plasma radioactivity were withdrawn during scan time, approximately one sample during each time frame. Radioactivity was assessed with an automatic y-counter (Wizard 1480, Wallac, Turku, Finland). Data from $[^{18}F]FDG$ images were reconstructed in a 128 $\times$ 128 matrix by standard algorithms with Hann filter of 0.5 cutoff frequency. The observed final in-plane resolution was 8 mm.

PET scanner and other devices for the determination of radioactivity were cross-calibrated with the automated y-counter. Radioactivity was corrected for decay to the time of injection and expressed in units of $\mu$Ci/ml.

Regions of interest (ROIs)

ROIs were drawn in the anteromedial muscle compartments of both femoral regions in four consecutive cross-sectional slices, carefully avoiding large blood vessels. Localization of the muscle compartments was verified by comparing the $[^{18}F]FDG$ and $[^{15}O]O_2$ images with the transmission image, which provides a topographical distribution of tissue density. The same ROIs were placed in the $[^{18}F]FDG$ and $[^{15}O]O_2$ images to generate respective time-activity curves. At rest, the total volume of the regions was about 120 ml. In the exercise studies, only the ROIs on the exercising rectus femoris with the highest workload were used (34 ml).

Perfusion analysis

For the calculation of parametric perfusion images, the autoradiographic method with an integration time of 250 sec was applied. This method has been previously validated in skeletal muscle (27).

Oxygen analysis

Oxygen consumption was quantified with nonlinear fitting (12). Regional muscle oxygen uptake is given by the product of arterial oxygen concentration, regional oxygen extraction fraction, and blood flow. A theoretical maximal glucose oxidative capacity was estimated based on the knowledge that 6 mol oxygen are needed for the complete oxidation of 1 mol glucose.

Compartmental modeling of $[^{18}F]FDG$

Skeletal muscle $[^{18}F]FDG$ time-activity curves were analyzed with a three-compartmental model (18, 28–30), as shown in Fig. 1B, which comprises one extracellular (arterial plasma $[^{18}F]FDG$) and two tissue compartments. Three rate constant parameters characterize the exchange of $[^{18}F]FDG$ among the three compartments. $K_i$ (ml/min $^{-1}$ per ml) represents inward transport of $[^{18}F]FDG$ from arterial plasma into muscle, mainly reflecting plasma flow; therefore, it is expressed in units of ml/min $^{-1}$ per ml of ROI. $K_o$ describes the transport rate of unphosphorylated $[^{18}F]FDG$ from muscle to the venous vasculature (min $^{-1}$), including $[^{18}F]FDG$ returning to plasma from the extracellular space because it has not been transported into myocyte and/or from the intracellular space because it has been transported into the myocyte but not phosphorylated; thus, $K_o$ is influenced by both $K_i$ and $K_o$, and it is not per se informative on single processes, but it permits to estimate the distribution volume of nonphosphorylated $[^{18}F]FDG$, as given below. Skeletal muscle inward transport and phosphorylation rates of $[^{18}F]FDG$ are characterized by $k_1$ (min $^{-1}$). Given a high phosphorylation rate under the conditions of this study and a relatively brief duration of emission scanning (19), tracer outflow into the interstitial compartment is assumed to be negligible ($K_i = 0$).

After administration of $[^{18}F]FDG$, the total $[^{18}F]FDG$ tissue concentration (C) is the sum of the free $[^{18}F]FDG$ ($C_a$) and $[^{18}F]FDG$-P ($C_b$) skeletal muscle concentrations and is expressed for each PET scan at time (t) as:

$$C(t) = C_a(t) + C_b(t)$$

This 3K model can be described mathematically by the following equations:

$$\frac{dC_a(t)}{dt} = K_i C_b(t) - [k_1 + k_2] C_a(t)$$

$$\frac{dC_b(t)}{dt} = \frac{k_1 C_a(t)}{k_2}$$

$$\frac{dC_d(t)}{dt} = 0$$

The model assumes that the arterial blood volume is negligible. A vascular volume ($V_b$), representing the fraction of blood pool volume in the tissue ROI, was incorporated into the model as:

$$C(t) = (1 - V_b)[C_a(t) + C_b(t)] + V_b C_d(t)$$

where $C_p$ is the $[^{18}F]FDG$ plasma arterial concentration.

The arterial plasma time course of $[^{18}F]FDG$ activity, $C_p$, is used as an input function in the model. All four model parameters, $K_i$, $k_1$, $k_2$, and $V_b$, are a priori uniquely identifiable (18).

With the use of the individual kinetic parameters, three additional combined parameters were calculated: 1) the distribution volume of nonphosphorylated extracellular (DVCE) $[^{18}F]FDG$ ($DVCE = K_i/k_2$ (ml/min $^{-1}$)); 2) the combined transport and phosphorylation fraction (PF) of $[^{18}F]FDG$ ($PF = k_1 + k_2$); and 3) the fractional uptake of $[^{18}F]FDG$ ($k = k_1 + k_2$, (ml/min $^{-1}$)).

Thus, K results in clearance units of ml/min $^{-1}$ per unit volume of ROI. For comparison, the fractional $[^{18}F]FDG$ uptake was estimated by graphical methods. It was then multiplied by steady-state plasma glucose levels and divided by a lumped constant of 1.2 to estimate skeletal muscle glucose uptake (8).

Parameter estimation

All kinetic parameters were determined by iterative curve fitting and the minimization of an objective function implemented in the SAAM II software (31). Compartmental model parameters were estimated by weighted nonlinear least squares and the measured PET activity, $C_{obs}$, was described as:

$$C_{obs}(t) = C(t) + e(t) \quad j = 1, 2, \ldots, N$$

where $e(t)$ is the measurement error at time $t$, assumed to be independent, Gaussian, zero mean. The parameters’ precision was judged by the residual error evaluation calculated at the different sample time as:

$$\text{wres}_j = \sqrt{\sum_{i=1}^{N} \left( w_j \left(s(\hat{p}, t), \gamma_i \right) - y_j \right)^2}$$

where $w_j$ is the weight associated with each datum, $s(\hat{p}, t)$ is the value predicted by the model, $\gamma_i$ is the measured experimental value, and $p$ is the unknown parameter estimated. SAAM II calculates a weight $w_j$
associated with each datum $y_{ij}$ from the sample that has been assigned to the datum by the user, a fractional sample (FSD) of 0.1 was assigned to each datum.

**Statistical analysis**

Results are expressed as mean ± sd. Values of model parameters with low precision, as defined by a coefficient of variation $CV = \text{sd}(p_i)/p_i \times 100$, where $p_i$ is the parameter estimate and $\text{sd}(p_i)$ is its sd greater than 100%, were excluded from the calculation of the group mean. Data comparison was determined by using the Student’s $t$ test. Correlations and linear regression were used to examine associations between variables. $P < 0.05$ was considered to be significant. Statistical analysis was performed using Statview for Windows (SAS Institute, Cary, NC).

**Results**

**Metabolic characteristics**

Clinical and biochemical characteristics of the groups are shown in Table 1. Fasting serum insulin concentrations were slightly higher in obese than lean subjects, whereas fasting plasma glucose concentrations were similar (5.3 ± 0.2 vs. 5.4 ± 0.2 mmol/liter$^{-1}$). Maximal aerobic capacity (VO$_2$max) was significantly higher in lean than obese subjects ($P < 0.01$).

**Compartmental modeling**

A representative example of the measured plasma and muscle activity curves for subject 4 (lean) at rest and during exercise is shown in Fig. 3. The shape of the [$^{18}$F]FDG activity curves documented faster tracer uptake during exercise. Model parameters were estimated with good precision in most studies; individual results are shown in Tables 2 and 3.

**Simulation**

The 3K model was able to predict the time course of tracer concentrations in each compartment. An example is given in Fig. 3, delineating model predicted time activity curves of extracellular ($q_2$), intracellular, and phosphorylated [$^{18}$F]FDG ($q_3$) in subject 4, showing an increase in $q_3$ in exercising (Fig. 3B) as compared with resting skeletal muscle (Fig. 3C).

**Effects of insulin and exercise**

The rate constant for [$^{18}$F]FDG transport from the extracellular compartment into tissue ($K_3$) showed a significant increase during exercise ($P < 0.01$) in lean subjects (Tables 2 and 3); a 3.5-fold stimulation was observed in obese individuals ($P < 0.014$) (Tables 2 and 3). A significant difference between rest and exercise was observed in the [$^{18}$F]FDG outward transport parameter, $k_2$, in lean subjects ($P < 0.05$). The distribution of $k_2$ values covered a wide range and was slightly skewed, with median values of 0.191 and 0.141 min$^{-1}$ (141 and 107% of the mean) in obese individuals during exercise and rest, respectively, as compared with 0.554 and 0.322 min$^{-1}$ in lean subjects (92 and 97% of mean, respectively). Exercise determined a more than 3-fold increase in $k_3$ in lean and obese subjects ($P < 0.001$). Exercise increased PF (0.469 ± 0.248 and 0.707 ± 0.265, $P < 0.05$) in lean but not obese subjects. It did not affect DV$_{CE}$. Estimated values for the $V_a$ were 3- and 5-fold higher in lean and obese individuals, respectively, in the exercising leg than in the resting leg ($P < 0.0001$).

**Lean vs. obese subjects**

Parameter comparisons between lean and obese subjects are shown in Fig. 4, A–E. In resting skeletal muscle, $K_3$, representing blood flow and substrate delivery in skeletal muscle (Fig. 4A), was 2.5-fold higher ($P < 0.01$); $k_2$ (transport and phosphorylation) was 3-fold higher ($P < 0.05$) (Fig. 4C); and $K$ (fractional uptake, Fig. 4D) was two times higher in lean than obese subjects ($P < 0.05$). In exercising skeletal muscle, $k_3$ was 4-fold higher ($P < 0.01$), and $k_4$ was significantly higher in lean than obese subjects ($P < 0.01$). The fractional uptake of [$^{18}$F]FDG was 79% higher in lean as compared with obese subjects ($P < 0.05$).

![Fig. 3. Example of compartmental model fit to the measured tissue activity curves in one lean subject (no. 4), showing a good agreement between estimated (continuous lines) and measured values (squares). A, Model interpolation of plasma activity was used as a forcing function. B and C, Model estimate (continuous lines) and measured tissue data (squares) obtained in skeletal muscle in exercising (B) and resting legs (C), where line $q_2$ represents the model fit to total tissue radioactivity over time, and lines $q_2$ and $q_3$ are model simulations of free and phosphorylated [$^{18}$F]FDG concentrations in tissue compartments 2 and 3 (C2 and C3 in Fig. 1).](image-url)
TABLE 2. Rate constants from three-compartment modeling of dynamic [18F]FDG PET in skeletal muscle in lean and obese subjects during exercise plus insulin

<table>
<thead>
<tr>
<th>Lean exercise + insulin</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.032 (25)</td>
<td>0.767 (79)</td>
<td>0.084 (36)</td>
<td>0.458 (33)</td>
<td>0.031 (6)</td>
</tr>
<tr>
<td>2</td>
<td>0.015 (29)</td>
<td>0.672 (76)</td>
<td>0.089 (27)</td>
<td>0.531 (36)</td>
<td>0.039 (4)</td>
</tr>
<tr>
<td>3</td>
<td>0.024 (98)</td>
<td>= 0</td>
<td>0.258 (146)</td>
<td>0.601 (42)</td>
<td>0.024 (4)</td>
</tr>
<tr>
<td>4</td>
<td>0.042 (8)</td>
<td>= 0</td>
<td>0.058 (4)</td>
<td>0.251 (95)</td>
<td>0.058 (5)</td>
</tr>
<tr>
<td>5</td>
<td>0.043 (9)</td>
<td>0.017 (223)</td>
<td>0.076 (8)</td>
<td>0.111 (227)</td>
<td>0.066 (9)</td>
</tr>
<tr>
<td>6</td>
<td>0.039 (8)</td>
<td>= 0</td>
<td>0.030 (10)</td>
<td>0.901 (56)</td>
<td>0.030 (4)</td>
</tr>
<tr>
<td>7</td>
<td>0.017 (17)</td>
<td>0.724 (57)</td>
<td>0.072 (23)</td>
<td>0.458 (27)</td>
<td>0.028 (4)</td>
</tr>
<tr>
<td>8</td>
<td>0.022 (12)</td>
<td>0.554 (44)</td>
<td>0.049 (19)</td>
<td>0.279 (24)</td>
<td>0.017 (5)</td>
</tr>
<tr>
<td>9</td>
<td>0.040 (8)</td>
<td>0.294 (13)</td>
<td>0.026 (33)</td>
<td>0.346 (66)</td>
<td>0.014 (6)</td>
</tr>
<tr>
<td>10</td>
<td>0.044 (15)</td>
<td>0.162 (476)</td>
<td>0.060 (39)</td>
<td>0.834 (52)</td>
<td>0.050 (4)</td>
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<tr>
<td>11</td>
<td>0.024 (8)</td>
<td>= 0</td>
<td>0.025 (7)</td>
<td>0.633 (356)</td>
<td>0.025 (6)</td>
</tr>
<tr>
<td>12</td>
<td>0.054 (7)</td>
<td>= 0</td>
<td>0.029 (13)</td>
<td>1.05 (839)</td>
<td>0.029 (5)</td>
</tr>
</tbody>
</table>

Mean ± SD
0.033 ± 0.012 * | 0.602 ± 0.19 | 0.055 ± 0.024 | 0.518 ± 0.23 b | 0.034 ± 0.013 a | 0.094 ± 0.03 | 0.707 ± 0.288

Values in parentheses represent fractional coefficient of variation. $K_0$ and $k_2$. Exchange parameters between plasma and intracellular space (free [18F]FDG pool); $K_a$, transport/phosphorylation parameter; $V_h$, fraction of total blood volume occupied by blood pool; $K$, fractional uptake of [18F]FDG; $DV_{CR}$, distribution volume of free [18F]FDG. Values in italics were excluded from the calculation of the group mean due to bad precision, as based on coefficient of variation greater than 100.

a $P < 0.05$.
b $P < 0.01$.

Unpaired Student’s t test, lean vs. obese.

Blood flow

Plasma flow values were calculated from blood flow PET data by using the hematocrit. There was no significant difference between plasma flow and $K_a$ values estimated by [18F]FDG compartmental modeling (Fig. 4A), although the correlation between individual values did not reach statistical significance.

Modeling vs. graphical analysis

The rate constant, $K$, reflecting the net use of [18F]FDG from the arterial compartment into skeletal muscle was estimated by both 3K modeling and Gjedde-Patlak graphical analysis (32, 33). We found a strong correlation between these two methods ($P < 0.0001, R^2 = 0.96$) in each group (obese and

TABLE 3. Rate constants from three-compartment modeling of dynamic [18F]FDG PET in skeletal muscle in lean and obese subjects during rest plus insulin

<table>
<thead>
<tr>
<th>Lean rest + insulin</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0145 (17)</td>
<td>0.322 (51)</td>
<td>0.035 (16)</td>
<td>0.226 (37)</td>
<td>0.015 (8)</td>
</tr>
<tr>
<td>2</td>
<td>0.010 (9)</td>
<td>0.013 (285)</td>
<td>0.011 (9)</td>
<td>0.089 (325)</td>
<td>0.0095 (15)</td>
</tr>
<tr>
<td>3</td>
<td>0.0037 (25)</td>
<td>0.344 (23)</td>
<td>0.024 (10)</td>
<td>0.088 (20)</td>
<td>0.0049 (9)</td>
</tr>
<tr>
<td>4</td>
<td>0.02 (6)</td>
<td>0.039 (86)</td>
<td>0.0085 (12)</td>
<td>0.036 (159)</td>
<td>0.0015 (40)</td>
</tr>
<tr>
<td>5</td>
<td>= 0</td>
<td>0.052 (26)</td>
<td>0.35 (881)</td>
<td>0.052 (8)</td>
<td>1 (32)</td>
</tr>
<tr>
<td>6</td>
<td>= 0</td>
<td>0.107 (9)</td>
<td>0.236 (9)</td>
<td>0.0083 (4)</td>
<td>0.038 (7)</td>
</tr>
<tr>
<td>7</td>
<td>0.019 (23)</td>
<td>0.191 (62)</td>
<td>0.05 (16)</td>
<td>0.186 (41)</td>
<td>0.024 (6)</td>
</tr>
</tbody>
</table>

Mean ± SD
0.021 ± 0.011 | 0.135 ± 0.134 | 0.043 ± 0.032 | 0.202 ± 0.077 | 0.019 ± 0.016 | 0.166 ± 0.095 | 0.529 ± 0.280

Values in parentheses represent fractional coefficient of variation. $K_0$ and $k_2$. Exchange parameters between plasma and intracellular space (free [18F]FDG pool); $K_a$, transport/phosphorylation parameter; $V_h$, fraction of total blood volume occupied by blood pool; $K$, fractional uptake of [18F]FDG; $DV_{CR}$, distribution volume of free [18F]FDG. Values in italics were excluded from the calculation of the group mean due to bad precision, as based on coefficient of variation greater than 100.

a $P < 0.05$.
b $P < 0.01$.

Unpaired Student’s t test, lean vs. obese.

See legend to Table 2.

a $P < 0.01$.
b $P < 0.05$.

Unpaired Student’s t test, lean vs. obese subjects.

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lean) and in both resting and exercising legs (Fig. 5). No correlation was found between $K$ and individual parameters derived by 3K modeling.

**Oxidative capacity**

The maximal glucose oxidative potential was significantly increased by exercise in both groups (lean subjects $16 \pm 3$ vs. $277 \pm 40 \mu$mol$\cdot$kg$^{-1}$$\cdot$min$^{-1}$ in resting and exercising leg, $P = 0.0002$; obese subjects $12 \pm 3$ vs. $200 \pm 40 \mu$mol$\cdot$kg$^{-1}$$\cdot$min$^{-1}$, respectively, $P = 0.005$). During exercise, a tendency toward defective glucose oxidative potential was observed in obese vs. lean subjects, which fell short of statistical significance ($P = 0.2$).

**Discussion**

The main results of the present study indicate that skeletal muscle perfusion, glucose transport, and phosphorylation defects are concomitant findings in obesity during insulin stimulation and that, by restoring perfusion but not the proximal metabolic steps of glucose disposal, exercise unravels the predominance of the metabolic impairment in the obese population. To the best of our knowledge, this is the first...
report describing the combined effects of exercise and insulin stimulation on skeletal muscle glucose kinetics, as analyzed by compartmental modeling of PET data. Under euglycemic hyperinsulinemia, exercise enhanced skeletal muscle perfusion to a similar extent in lean and obese subjects but did not ameliorate the relative defect in glucose transport and phosphorylation. The oxidative capacity tended to be lower in obese subjects during exercise.

The 3K modeling of dynamic \([^{18}\text{F}]\text{FDG}\) data used in our study has been adopted to quantify glucose metabolism rates in skeletal muscle in humans by other authors (13–15). Recently Bertoldo et al. (18) proposed a four-compartment model with five parameters, which enable dissection of glucose transport and phosphorylation. This model was applied by Williams et al. (34, 35), revealing that insulin resistance involves both metabolic processes in obesity and type 2 diabetes. The reduced 3K model used in our study has been shown to provide accurate fits to muscle data in both fasting and insulin-stimulated conditions (8, 17). Reinhardt et al. (17) tested the 3K model against more reduced (two-compartment model) and complex (four-compartment model) approaches by using the Akaike and Schwarz information criteria; their results favored the present reduced model, suggesting that the fourth compartment, i.e. phosphorylated \([^{18}\text{F}]\text{FDG}\), cannot be clearly identified from \([^{18}\text{F}]\text{FDG}\) kinetics in the skeletal muscle, especially given a relatively limited study duration (17), similar to that in the present design. Given the overall study length required for the sequence of measurements performed here after the achievement of metabolic steady state, we opted for a 40-min \([^{18}\text{F}]\text{FDG}\) PET scanning time, and we cannot exclude that a prolonged experiment may have been compatible with a more complex and informative model, as described by others (18). In our hands, the current model permitted a good estimation and unique identification of parameters in most subjects in both groups, with few exceptions, due to the original data quality.

We confirm the previous observation of an impaired glucose uptake in obese vs. lean subjects in both resting and exercising muscle and showed a strong correlation between compartmental modeling and linearly derived fractional \([^{18}\text{F}]\text{FDG}\) uptake values in all study groups and conditions. In our previous report, skeletal muscle glucose uptake rates in a subgroup of this population have been estimated by using graphical methods (32, 33). These methods do not allow resolve between flow and metabolic modulation of the target process. Because the influence of glucose delivery on glucose uptake remains a matter of debate (36) and it may vary on study population and conditions, it was not possible to define to which extent an intrinsic metabolic process was responsible for the observed defect. The lack of a correlation between glucose uptake and the individual inward parameters of the model in this study underlines this limitation and its intersubject dependency. Notably, the current analysis method contributes a relevant piece of information, in that it allows an almost fully separate effect of perfusion and substrate delivery from the intrinsic ability of the tissue to transport and phosphorylate glucose.

The evidence of reduced insulin-mediated perfusion in obesity in resting skeletal muscle has been previously reported (37), and it concordantly resulted from two independent methods in the current study, in which the use of PET allowed selectively target skeletal muscle with no influence of surrounding structures. The parameter \(k_3\) is interpreted, as described by other authors (13, 15, 34), as an aggregate parameter reflecting both skeletal muscle glucose transport and intracellular phosphorylation. The rate of this process was decreased by approximately 65 and 62% in resting and exercising muscle, respectively, in obese as compared with lean subjects despite a significant effect of exercise in promoting both perfusion and glucose transport and phosphorylation in both study groups during euglycemic hyperinsulinemia. Therefore, skeletal muscle insulin resistance is associated with an impaired

![Linear Regression](linear_regression.png)

**Fig. 5.** Linear regression between Ki values, as estimated by graphical analysis and calculated by using the 3K model-derived parameters.
response of the proximal steps responsible for the metabolic disposal of glucose in obese subjects due to an intrinsic tissue defect, which persists with similar severity during exercise stimulation. In fact, the $k_f$ difference between groups did not parallel that in tissue perfusion, which was impaired at rest and restored during exercise, documenting the independence of the intrinsic component of insulin resistance from substrate delivery in obese subjects during exercise.

A single bout of exercise has been long recognized to enhance insulin-mediated whole-body glucose uptake (3, 5), an effect that is protracted for several subsequent hours; consequently, exercise has been considered as one cornerstone of treatment in insulin-resistant conditions. Although confirming that acute exercise strongly accelerates the rate of glucose entry in skeletal muscle, more than doubling the effect of insulin stimulation in lean and obese individuals, our data document that such stimulation does not normalize this process, thus perpetuating the same greater than 60% defect in muscle glucose transport and phosphorylation in obese patients. Notably, our results refer to a single-bout exercise in untrained individuals and cannot be directly extrapolated to the case of regular training or single-bout exercise in trained subjects. Moreover, because glucose disposal results from the fractional uptake rate, which is a composite parameter accounting for perfusion and transport/phosphorylation (in graphical analysis) or $K_1$ and $k_f$ (in the 3K model), the above considerations are not to be taken to indicate that exercise does not alleviate insulin resistance in obesity. However, they indicate that $K_1$, rather than $k_f$, was responsible for a less than 20% reduction in the relative fractional uptake gap between lean and obese subjects in our study. Accordingly, $k_f$ was up-regulated during exercise and was higher in lean than obese individuals; this parameter showed interindividual variability, partly because it reflects a balance of several processes (see Subjects and Methods), among which perfusion appeared to be predominant in our study.

The obesity-related defect in glucose transport and phosphorylation has been interpreted as being of a secondary origin because it was reversed by weight reduction (34). Extrapolating from our previous and current findings, we calculated a theoretical maximum glucose oxidation rate by assuming that all oxygen extracted by skeletal muscle was used for the oxidation of this substrate. Under conditions of insulin stimulation, such as in the present study, circulating fatty acids are suppressed, and their storage in complex lipids is favored. Thus, glucose is the main fuel undergoing oxidation, the latter process accounting for approximately 30% of glucose uptake in healthy individuals (38). Consistent with this knowledge, the glucose oxidative capacity in healthy subjects was on average 16 $\mu$mol$\cdot$min$^{-1}\cdot$kg$^{-1}$ in resting skeletal muscle, corresponding to 28 ± 5% of glucose uptake in this organ; it was enhanced by approximately 15-fold in both groups in the exercising situation, thus exceeding the disposal of plasma glucose, suggesting internal storage breakdown. A tendency toward a lower glucose oxidative capacity in obese subjects was observed, falling short of statistical significance during exercise. Given the more pronounced reduction in glucose uptake than oxidative potential in obese subjects, the above evidence may indicate that either a larger proportion of delivered glucose is oxidized leading to a deficient glycogen incorporation or, more likely, the oxidation of alternative substrates or fuel sources operates to compensate for the limited availability of glucose in these individuals in this high energy-requiring condition.

The first step of glucose uptake in tissue is the delivery by perfusion, which was shown here to be relevant in regulating the synergy between exercise and insulin in obese subjects. One salient finding of this study was that in each group and experimental condition, we observed no significant difference between plasma flow, as estimated from $^{[15]O}$H$_2$O PET analysis and the $^{[18]}$FDG inward rate constant $K_f$. Mean values in lean subjects were 2.5 vs. 3.1 in the resting leg and 5.6 vs. 5.5 ml$\cdot$100 g$^{-1}\cdot$min$^{-1}$ in the exercising leg, respectively, by the two methods; corresponding figures in obese subjects were 1.1 vs. 1.2 and 3.1 vs. 4.3 ml$\cdot$100 g$^{-1}\cdot$min$^{-1}$.

The current results are consistent with estimated $K_f$ values in previous $^{[18]}$FDG PET studies and similar to the known perfusion rates of 2–4 ml$\cdot$100 g$^{-1}\cdot$min$^{-1}$ in resting skeletal muscle (39). After unit conversion, published values ranged between 1.1–5 and 0.6–2.2 ml$\cdot$100 g$^{-1}\cdot$min$^{-1}$ in resting skeletal muscle in lean and obese subjects, respectively (7, 8, 16, 17). A significant correlation between leg blood flow and $K_f$ has been previously reported (8), suggesting an influence of tissue perfusion on $K_f$. Despite the above evidence, $K_f$ has been variably interpreted as predominantly reflecting glucose transport (8) or blood flow (34). The former concept was inherited from the original application of the reduced 3K model to the quantification of brain glucose uptake in which: 1) the capillary endothelium forms a tight blood-brain barrier and is rate limiting for glucose transport; thus, the interstitial space and intracellular space can be combined in one compartment (28, 29); and 2) the permeability surface area product is less than blood flow, and the transport of glucose to tissue is nearly independent of blood flow; thus, the effect of blood flow has been eliminated in the model (28, 29). Conversely, in skeletal muscle, the capillary wall allows free diffusion of small hydrophilic solutes (40), the sarcolemma becomes more rate limiting for the transport of glucose than the endothelium, and the permeability surface area product for glucose is approximately 5 ml$\cdot$100 g$^{-1}\cdot$min$^{-1}$ (40) and is in the same order of magnitude as blood flow (39), which represents one further rate-limiting factor in this tissue.

The equivalence between $K_f$, i.e., the first rate-limiting parameter in the model, and the $^{[15]}$OH$_2$O-derived tissue perfusion in this and previous studies is in keeping with the above knowledge. Our finding demonstrates that modeling of $^{[18]}$FDG can be used as a reliable surrogate for the evaluation of skeletal muscle perfusion, as previously surmised (34), also incorporating the influence of transendothelial diffusion, thus reflecting the integrated process of substrate delivery. In PET scanning, the assessment of perfusion needs to precede that of glucose uptake by at least 15 min. Because insulin action is time dependent, the
lack of simultaneity unpredictably affects the evaluation of the glucose extraction fraction. This, together with the influence of transendothelial transport on $K_1$ likely explains the lack of a correlation against $[\text{15O}]\text{H}_2\text{O}$ measurements in our study. Thus, $[\text{18F}]\text{FDG}$ modeling provides simultaneous information on intrinsic glucose metabolism and substrate delivery (including perfusion and endothelial transport) in skeletal muscle.

In summary, the current study showed that, under insulin-stimulated conditions, exercise promotes skeletal muscle perfusion and glucose transport/phosphorylation in lean and obese subjects; it restores the impairment in muscle perfusion associated with obesity but does not normalize the defect involving the proximal steps regulating skeletal muscle glucose disposal in obese individuals. Oxidative and non-oxidative glucose metabolic defects likely coexist in skeletal muscle under combined exercise and insulin stimulation in these subjects. Our data support the use of $[\text{18F}]\text{FDG}$-PET in the evaluation of skeletal muscle perfusion and glucose delivery and in the dissection between the substrate supply and the inherent metabolic dependency of insulin resistance in skeletal muscle.

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Address all correspondence and requests for reprints to: Patricia Iozzo, M.D., Ph.D., Turku PET Centre, University of Turku, P.O. Box 52, FIN-20521 Turku, Finland. E-mail: patricia.iozzo@ffc.cnr.it.

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References

31. SAAM Institute 1998 SAAM II user’s guide, version 1.1.11. Seattle, WA: SAAM Institute

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