Dose-responsive insulin regulation of glucose transport in human skeletal muscle

R. Richard Pencek, Alessandra Bertoldo, Julie Price, Carol Kelley, Claudio Cobelli, and David E. Kelley. Dose-responsive insulin regulation of glucose transport in human skeletal muscle. *Am J Physiol Endocrinol Metab* 290: E1124–E1130, 2006. First published January 3, 2006; doi:10.1152/ajpendo.00598.2004.—Glucose transport is regarded as the principal rate control step governing insulin-stimulated glucose utilization by skeletal muscle. To assess this step in human skeletal muscle, quantitative PET imaging of skeletal muscle was performed using 3-O-methyl-[11C]glucose (3-[11C]OMG) in healthy volunteers during a two-step insulin infusion \[\text{n = 8}; 30 \text{ and 120 mU} \cdot \text{min}^{-1} \cdot \text{m}^{-2}, \text{low (LO)} \text{ and high (HI)}}\] and during basal conditions \[\text{n = 8}]. Positron emission tomography images were coregistered with MRI to assess 3-[11C]OMG activity in regions of interest placed on oxidative (soleus) compared with glycolytic (tibialis anterior) muscle. Insulin dose-responsive increases of 3-[11C]OMG activity in muscle were observed \[P < 0.01\]. Tissue activity was greater in soleus than in tibialis anterior \[P < 0.05\]. Spectral analysis identified that two mathematical components interacted to shape tissue activity curves. These two components were interpreted physiologically as likely representing the kinetics of 3-[11C]OMG delivery from plasma to tissue and the kinetics of bidirectional glucose transport. During low compared with basal, there was a sixfold increase in \(k_s\), the rate constant attributed to inward glucose transport, and another threefold increase during HI \[0.012 \pm 0.003, 0.070 \pm 0.014, 0.272 \pm 0.059 \text{ min}^{-1}, P < 0.001\]. Values for \(k_i\) were similar in soleus and tibialis anterior, suggesting similar kinetics for transport, but compartmental modeling indicated a higher value in soleus for \(k_i\), denoting higher rates of 3-[11C]OMG delivery to soleus than to tibialis anterior. In summary, in healthy volunteers there is robust dose-responsive insulin stimulation of glucose transport in skeletal muscle.

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**INSULIN STIMULATES GLUCOSE UTILIZATION** by skeletal muscle in a dose-responsive manner \(14, 13, 26, 28\). It is generally considered that substantial control over this response resides within the proximal steps of metabolism, these being delivery, transmembrane transport, and trapping of glucose within muscle by phosphorylation. Of these, glucose transport is regarded as the pivotal locus of control. This concept is based on the molecular physiology of insulin-stimulated translocation of GLUT4 proteins \(23\), findings obtained in animal studies and cell culture systems \(17, 20\), and absence of the accumulation of free glucose in muscle during stepwise increases in insulin \(14\). Estimations of free glucose in skeletal muscle performed using nuclear magnetic resonance spectroscopy during insulin-stimulated conditions in animals and in humans also indicate minimal accumulation \(6, 7\). These findings support the concept that glucose transport limits insulin-stimulated glucose utilization in muscle, but the findings are also consistent with limitation at the preceding step, that of glucose delivery. Blood flow and substrate delivery are considered to have more control over rates of substrate uptake when tissue permeability is heightened and less effect when the fractional extraction is low \(22\). In this regard, GLUT4-mediated glucose transport might be regarded as a “permeability” factor. Animal studies have indicated that insulin-mediated activation of glucose transport in muscle does distribute control over rates of glucose uptake toward delivery \(10, 11\).

Analysis of the kinetics of a minute mass of tracer introduced during steady-state conditions can provide insight into the rates of metabolic reactions that underlie steady-state substrate flux. In the current study, steady-state euglycemic insulin clamp conditions were combined with dynamic positron emission tomography (PET) imaging to obtain temporal resolution and organ-specific measurements of 3-O-methyl-[11C]glucose (3-[11C]OMG) tracer activity in the tissue bed of human skeletal muscle. Because 3-OMG is not metabolized, this tracer isolates the step of bidirectional transmembrane glucose transport \(5\), although when used in vivo there is the added component of tracer delivery from plasma to tissue. Bonaonna et al. \(4\) used a brachial artery injection of 3-[14C]OMG and measured the venous washout curve across the forearm in healthy volunteers at multiple doses of insulin stimulation. Dose-responsive increases in the rate constant ascribed to the step of glucose transport were observed \(4\). The glucose analog deoxy-[18F]glucose has been used with dynamic PET imaging to study the kinetics of glucose uptake into skeletal muscle \(15, 16, 21\); however, it remains uncertain whether separate estimations of delivery, transport, and phosphorylation can be obtained from the activity curve of a single tracer \(2\). A recent study from our laboratory \(3\) demonstrated technical feasibility in using 3-[14C]OMG for PET imaging of human skeletal muscle during basal and insulin-stimulated conditions. Spectral analysis \(\text{SA}\) indicated that tissue tracer activity represented the interaction of two kinetic processes \(3\). These two processes were interpreted to represent the sequential steps of glucose delivery and transport \(3\). To extend this line of investigation, the current study was undertaken to examine dose-response effects of insulin on the uptake of 3-[11C]OMG and to compare tracer activity in soleus, a pre-

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Address for reprint requests and other correspondence: D. E. Kelley, 807N Montefiore-University Hospital, 3459 Fifth Ave., Pittsburgh, PA 15213 (e-mail: kelley@dom.pitt.edu). 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.
dominately oxidative muscle, with tibialis anterior, a predomi-
nately glycolytic fiber type muscle.

METHODS

Research volunteers. Lean, healthy young adults were recruited by
advertisement in the general community. Before participating, each
volunteer had medical and laboratory examinations to verify good
health. Informed, written consent was obtained, and the University of
Pittsburgh Institutional Review Board reviewed and approved this
clinical investigation.

Hyperinsulinemic euglycemic clamp. Research volunteers were
admitted to the University of Pittsburgh General Clinical Research
Center on the evening before PET studies, received dinner, and then
fasted overnight. Volunteers had been instructed to refrain from
exercise on the day of admission. On the following morning, a
catheter was placed in an antecubital vein for infusions and in a radial
artery for blood sampling. To study insulin-stimulated metabolism, a
two-step insulin infusion was used, infusing insulin at 30
mU·min⁻¹·m⁻² (LO) for 3 h followed by 120 mU·min⁻¹·m⁻² (HI) for
3 h. To study fasting metabolism (Basal), insulin was not infused;
these results have been reported earlier (3). Arterial glucose was
measured at 5-min intervals, and euglycemia was maintained using an
adjustable infusion of 20% dextrose. Samples were obtained for
determinations of insulin and fatty acids at baseline and hourly
thereafter. Plasma glucose was measured using a YSI Glucose Ana-
lyzer (Yellow Springs, OH); plasma insulin was measured using a
radioimmunoassay, and plasma fatty acids were measured using a
colorimetric enzymatic assay (NEFA C test kit; Wako Chemicals,
Richmond, VA).

PET image acquisition. Dynamic PET imaging of tissue activity
of 3-[11C]OMG in skeletal muscle was performed for 90 min at Basal,
LO, and HI and was carried out at the University of Pittsburgh PET
Center. A Siemens/CTI ECAT HR+ PET scanner in 3-D imaging
mode (63 parallel planes; axial field of view 15.2 cm; slice width 2.4
mm) was used. The final reconstructed PET image resolution was
= 6
mm. Volunteers were positioned in the PET scanner so that the
midcalf corresponded to the midpoint axial field of view. To minimize
movement during scanning and to prevent compression of muscle
tissue, the legs were supported by foam blocking at the ankles and
knees. Radiosynthesis of 3-[11C]OMG was performed as previously
described (3). PET imaging (36 frames: 8 × 15 s, 8 × 15 s, 4 × 1 min,
16 × 5 min) began 1 h after the onset of insulin infusion simulta-
neously with the injection of 5 mCi of 3-[11C]OMG, administered
over 20 s. Blood for measuring the arterial 3-[11C]OMG activity curve
was obtained manually, drawing 0.5-ml samples from the radial artery
catheter (10 samples every 6 s, 8 samples every 15 s, 7 samples every
1 min, 10 samples every 5 min, and 3 samples every 10 min). Each
blood sample was immediately centrifuged, and 200 µl of plasma
were removed for immediate 11C counting using a COBRA Auto-
Gamma model 5003 gamma counter (Packard Instruments, Meriden,
CT). The PET data were corrected for radioactive decay and scatter
(24).

On the same day, by use of the same alignment of foam blocking
at the ankles and knees, a T1-weighted MRI of the midcalf was
obtained. MRI was obtained only on participants in the two-step
insulin infusion.

Analysis of PET images. PET and MRI were aligned using a
previously described method (18, 27). Briefly, summation PET im-
ages were created from the frames of the initial 15 min of the scanning
period and coregistered with MRI, so that precise anatomic alignment
was achieved. By use of the coregistered MR image, a region of
interest (ROI) was placed on skeletal muscle, avoiding major vessels
and the bones of the lower leg, as earlier described (3). ROIs were also
placed on anterior tibialis and soleus muscles, as shown in Fig. 1.
3-[11C]OMG activity within an ROI is termed “tissue activity” and
represents tracer within extracellular and intracellular spaces. Tissue
activity was measured in each of the 36 frames for a given ROI, across
the middle 50 of the 63 planes. Tissue activity was converted to units
of radioactivity concentration (µCi/ml) using an empirical phantom-
based calibration factor (µCi/ml per PET counts per pixel). The
tissue-to-plasma ratio (TPR) for tracer activity, the quotient of tissue
to arterial radioactivity (each in units of µCi/ml), at each time was
calculated and plotted for a qualitative examination of the kinetics of
3-[11C]OMG uptake.

Spectral analysis. Tissue activity was analyzed with SA (15),
which describes this as the sum of M components, consisting of the
convolution integrals of the arterial tracer activity (input function)
with decaying exponentials: Ate⁻µ
. The number of components, M,
yields the number of compartments needed to describe the system
with a compartmental model and starts by using M = 1, estimating
amplitudes and eigenvalues of the decaying exponential, and then tries
M = 2, and so on. Akaike information criteria (AIC) were used to
derive the optimal M on the basis of the principle of parsimony, i.e.,
the fewest number of parameters that can best fit the data. AIC is
computed as AIC = N ln WRSS(µ) + 2P, where N is the number of
PET frames (observations), P is the number of parameters, and
WRSS(µ) is the weighted residual sum of squares evaluated in
correspondence to the estimated value of P, µ. The model of choice is
associated with the lowest AIC value.

Compartmental model. Compartmental modeling was next used to
examine the kinetics of tissue tracer activity, using a model compris-
ing a plasma compartment (i.e., the measured arterial activity) and two
tissue compartments, developed on the basis of SA results as earlier
described (3). The compartment model is described mathematically by
the following equations:

\[
\text{Activity}_{tissue} = \sum_{i=1}^{M} A_i e^{-\lambda_i t} \]

where \( A_i \) are the amplitudes and \( \lambda_i \) are the eigenvalues of
the decaying exponentials.
where \( C_p \) is 3\(^{[11]C}\)OMG arterial plasma radioactivity (\( \mu Ci/ml \)), \( C_t \) is the first tissue compartment 3\(^{[11]C}\)OMG radioactivity per unit volume of tissue, \( C_b \) is 3\(^{[11]C}\)OMG radioactivity in the second tissue compartment, and \( C_t \) is overall 3\(^{[11]C}\)OMG activity per unit volume of tissue. The rate constants \( k_1 \) (\( ml^{-1} \cdot m^{-1} \)) and \( k_2 \) (\( m^{-1} \)) describe exchange of 3\(^{[11]C}\)OMG between plasma and the first tissue compartment. The rate constants \( k_3 \) (\( m^{-1} \)) and \( k_4 \) (\( m^{-1} \)) describe 3\(^{[11]C}\)OMG exchange between the first and second tissue compartments. All five model parameters \( k_1, k_2, k_3, k_4, \) and \( V_b \) are a priori uniquely identifiable (2).  

The clinical characteristics of the lean, healthy adults are shown in Table 1. Metabolic parameters during the PET studies are shown in Table 2. Arterial glucose was similar during Basal, LO, and HI clamp studies. ANOVA was used to examine for within-subject differences between LO and HI clamp studies.  

### Table 1. Baseline characteristics of research volunteers in Basal and LO-and HI-insulin clamp experiments

| Age, yr | 31±4 | 28±3 |
| Weight, kg | 67±5 | 66±4 |
| Sex, F/M | 5:3 | 4:4 |
| BMI, kg/m² | 22.7±0.5 | 22.6±0.9 |

Data are means ± SE. LO, low insulin; HI, high insulin. BMI, body mass index.

Arterial insulin and plasma FFA differed significantly across Basal, LO, and HI clamps and in an expected manner. There was a highly significant difference in steady-state rates of glucose infusion during LO vs. HI (\( P < 0.001 \)), with values being approximately double during HI.

### RESULTS

**Systemic glucose metabolism.** The clinical characteristics of the lean, healthy adults are shown in Table 1. Metabolic parameters during the PET studies are shown in Table 2. Arterial glucose was similar during Basal, LO, and HI clamps.

![PET 3-OMG Imaging of Glucose Transport](http://ajpendo.physiology.org/)

**Table 2. Arterial glucose, insulin, FFA, and \( G_{INF} \) from BASAL, LO, and HI experiments**

<table>
<thead>
<tr>
<th>( G_{INF} ), mg/min/( 10^3 )kg(^{-1} )</th>
<th>BASAL</th>
<th>LO, 30</th>
<th>HI, 120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Art. glucose, mg/dl</td>
<td>88±1</td>
<td>92±0.9</td>
<td>91.6±0.9</td>
</tr>
<tr>
<td>Art. insulin, ( \mu U/ml )</td>
<td>9±1</td>
<td>63±5*</td>
<td>278±15†</td>
</tr>
<tr>
<td>Art. FFA, ( \mu M/l )</td>
<td>627±42</td>
<td>98±14*</td>
<td>74.7±7*</td>
</tr>
<tr>
<td>( G_{INF} ), mg/min/( 10^3 )kg(^{-1} )</td>
<td>NA</td>
<td>5.3±0.6</td>
<td>9.7±0.5‡</td>
</tr>
</tbody>
</table>

Data are means ± SE. Art., arterial; FFA, free fatty acids; \( G_{INF} \), glucose infusion rates; *\( P < 0.05 \) vs. Basal; †\( P < 0.05 \) vs. Basal and LO insulin; ‡\( P < 0.05 \) vs. LO insulin. NA, not applicable.

Arterial insulin and plasma FFA differed significantly across Basal, LO, and HI clamps and in an expected manner. There was a highly significant difference in steady-state rates of glucose infusion during LO vs. HI (\( P < 0.001 \)), with values being approximately double during HI.

**Noncompartmental analysis of 3\(^{[11]C}\)OMG tissue activity.** An ROI was placed on skeletal muscle to measure tissue activity for 3\(^{[11]C}\)OMG. Representative curves are shown in Fig. 2A. Higher tissue activity was present during LO and HI compared with Basal, and there was a change in the shape of tissue activity curves. Qualitatively, during Basal conditions there was an initial brisk increase in tissue activity followed by a concavity that was not evident during LO or HI. TPR plots are shown in Fig. 2B and reveal dose-dependent insulin stimulation.

SA was used to examine which among a one-, two-, or three-exponential equation achieved the best statistical fit to tissue activity, assessed on the basis of the lowest AIC value. A three-exponential model yielded a high AIC value and low precision in parameter estimation (i.e., coefficient of variation, >100\%), confirming our earlier report (3), and was not further considered. AIC values were lower with a two-exponential than one-exponential equation. This was evident during Basal (\( −5.74 ± 0.31 \) vs. \( −4.12 ± 0.31, P < 0.001 \)), LO (\( −5.30 ± 0.34 \) vs. \( −4.15 ± 0.67, P < 0.001 \)), and HI (\( −5.55 ± 0.38 \) vs. \( −4.87 ± 0.67, P < 0.01 \)) conditions. These findings indicate that a compartment model with two tissue compartments is more appropriate than one with a single compartment. However, it is of interest that, in comparing a one- with a two-exponential model, differences in AIC values were greatest during Basal and narrowed during LO and HI. Closer approximation between a one- and a two-exponential model in fitting tissue activity during LO and HI compared with Basal conditions suggests accelerated tracer exchange during insulin-stimulated conditions.

**Compartmental modeling of 3\(^{[11]C}\)OMG tissue activity.** Values for the four rate constants describing tracer exchange between plasma and the two tissue compartments are shown in Table 3 along with the error of parameter estimation and the precision in parameter estimation (i.e., coefficient of variation, \( P < 0.05 \) vs. Basal; †\( P < 0.05 \) vs. Basal and LO insulin; ‡\( P < 0.05 \) vs. LO insulin. NA, not applicable.

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**Compartmental modeling of 3\(^{[11]C}\)OMG tissue activity.** Values for the four rate constants describing tracer exchange between plasma and the two tissue compartments are shown in Table 3 along with the error of parameter estimation and the partition coefficients. The rate constant \( k_1 \) (\( ml^{-1} \cdot m^{-1} \)) describes the kinetics of 3\(^{[11]C}\)OMG delivery from plasma into the first tissue compartment, was estimated with excellent precision and did not change during LO and HI compared with BASAL. The rate constants \( k_2, k_3, \) and \( k_4 \) (\( m^{-1} \)) signify the fraction of tracer leaving a compartment per minute. \( k_2 \) describes the egress of 3\(^{[11]C}\)OMG from the first tissue compartment to plasma. There were no significant changes in \( k_2 \) across BASAL, LO, and HI. \( k_3 \) describes the
movement of 3-[11C]OMG from the first into the second compartment and is attributed to inward transmembrane glucose transport. $k_3$ increased sixfold from Basal to LO ($P < 0.001$) and another threefold from LO to HI ($P < 0.01$). Thus, during Basal conditions, $\sim 1\%$ per minute of [11C]3-OMG within the first compartment entered the second compartment, and this increased to $\sim 7\%$ per minute during LO and to $\sim 21\%$ per minute during HI. $k_4$ describes egress from the second to the first tissue compartment, and this rate constant increased significantly with insulin, about threefold from Basal through HI. The error of estimation for $k_1$ and $k_4$ remained low during LO and HI but increased for $k_2$ ($P < 0.05$) and $k_3$ ($P < 0.01$) compared with Basal.

The rate constants were used to calculate the partition coefficient for 3-[11C]OMG. The overall partition coefficient for 3-[11C]OMG from plasma to tissue ($V_a$) increased in a dose-dependent manner across Basal, LO, and HI (0.21 ± 0.01, 0.63 ± 0.04, and 0.74 ± 0.03 ml/ml, $P < 0.01$). Identical values for $V_a$ were obtained using SA (data not shown). $V_a$ can be separated into a partition coefficient for 3-[11C]OMG from plasma into the first tissue compartment ($V_{ac}$) and one for the first to the second tissue compartment ($V_{ec}$). $V_{ec}$ did not change during LO and HI compared with Basal, but there were significant increases in $V_{ec}$ ($P < 0.001$), by fivefold during LO and sevenfold during HI. Time plots for estimations of 3-[11C]OMG within the first and the second compartments are shown in Fig. 3 and were calculated using the rate constants and the measured tracer activity in plasma and tissue. Compared with Basal, 3-[11C]OMG activity in the second tissue compartment increased more rapidly during LO and HI, whereas tracer activity in the first tissue compartment decreased more rapidly. These plots illustrate that the main effect of insulin was to accelerate exchange of 3-[11C]OMG between the first and second compartments.

3-[11C]OMG tracer activity in soleus and tibialis anterior. A comparison of 3-[11C]OMG tracer activity in soleus and tibialis anterior muscles was made to explore the potential influence of muscle fiber type (predominantly oxidative vs. predominantly glycolytic). Muscle-specific data, obtained by coregistration of PET with MRI, were available only for LO and HI. TPR plots revealed higher 3-[11C]OMG activity in soleus compared with tibialis anterior muscle, as shown in Fig.

### Table 3. Rate constants from PET imaging of 3-[11C]OMG in skeletal muscle in healthy volunteers at Basal, LO, and HI conditions

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>LO</th>
<th>HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$, ml/ml/min</td>
<td>0.018±0.002 (2±0.3)</td>
<td>0.017±0.001 (3±1)</td>
<td>0.019±0.004 (4±1)</td>
</tr>
<tr>
<td>$k_2$, min$^{-1}$</td>
<td>0.137±0.007 (4±1)</td>
<td>0.104±0.023 (13±3)</td>
<td>0.205±0.115 (33±13)</td>
</tr>
<tr>
<td>$k_3$, min$^{-1}$</td>
<td>0.012±0.003 (14±1)</td>
<td>0.074±0.014 (26±11)</td>
<td>0.210±0.067† (44±17)</td>
</tr>
<tr>
<td>$k_4$, min$^{-1}$</td>
<td>0.021±0.003 (14±1)</td>
<td>0.032±0.006 (14±8)</td>
<td>0.206±0.009† (12±4)</td>
</tr>
<tr>
<td>$V_a$, ml/ml</td>
<td>0.21±0.01</td>
<td>0.64±0.02</td>
<td>0.79±0.02†</td>
</tr>
<tr>
<td>$V_{ac}$, ml/ml</td>
<td>0.13±0.01 (2±0.3)</td>
<td>0.21±0.05 (10±2)</td>
<td>0.21±0.05 (29±2)</td>
</tr>
<tr>
<td>$V_{ec}$, ml/ml</td>
<td>0.08±0.01 (6±2)</td>
<td>0.42±0.04 (7±3)</td>
<td>0.53±0.07† (14±6)</td>
</tr>
</tbody>
</table>

Data are means ± SE. Experimental errors are in parentheses. See text for explanations of parameters. 3-[11C]OMG, 3-O-methyl-[11C]glucose. *Within-subject paired t-test for LO vs. HI; $P < 0.05$; †one-way ANOVA across doses (Basal, LO, and HI); $P < 0.01$.
Applying SA, AIC values in soleus during LO (−3.93 ± 0.65 vs. −5.28 ± 0.30, \( P < 0.001 \)) and HI (−4.80 ± 0.50 vs. −5.46 ± 0.14, \( P < 0.01 \)) favored a two-exponential rather than a one-exponential model. This was also found for tibialis anterior during LO (−4.83 ± 0.20 vs. −4.21 ± 0.24, \( P < 0.05 \)) and HI (−5.04 ± 0.21 vs. −4.48 ± 0.17, \( P < 0.05 \)). This similarity of findings in soleus and tibialis anterior muscles indicates that a similar approach to compartment modeling is warranted.

Mean values for the rate constants for soleus and tibialis anterior muscle are shown in Table 4. The key difference between soleus and tibialis anterior muscle is that values for \( k_1 \) are higher in soleus muscle. In both muscles, values for \( k_1 \) remained stable at HI compared with LO. Apart from \( k_1 \), there were no significant differences between soleus and tibialis anterior muscles for the remaining three rate constants at either LO or HI.

**DISCUSSION**

In the current study, dynamic PET imaging was used to examine the effect of insulin on uptake of 3-[11C]OMG into skeletal muscle in lean, healthy research volunteers. Dose-responsive increases were observed. 3-OMG is a transport-specific tracer and does not undergo additional metabolism (5). Accordingly, tracer activity in tissue beds of skeletal muscle measured during PET imaging represents 3-[11C]OMG that is either within interstitial space or within myocytes. Spectral analysis was performed to deconvolute tissue activity curves for 3-[11C]OMG with respect to arterial activity. This mathematical approach does not contain a priori assumptions about either tissue characteristics (e.g., interstitial and intracellular spaces) or metabolic processes. In the present study, SA identified that a two-exponential equation achieved a more robust fit than either a single-exponential equation or one with more than two exponentials. Taking this into account, we analyzed the tissue activity curves for 3-[11C]OMG by using a model with two compartments.

On the basis of the modeling of tissue activity, there were three key findings of the present study. The first was that in healthy volunteers insulin strongly stimulates exchange of 3-[11C]OMG between the first and second compartments. This effect was manifested by dose-responsive insulin-stimulated increases in the rate constant \( k_3 \). Compared with basal conditions, values for \( k_3 \) increased sixfold at a low physiological level of insulin stimulation and 20-fold at a supraphysiological level. Based on the fact that metabolism of 3-[11C]OMG is restricted to transport, our interpretation is that this robust effect reflects insulin stimulation of bidirectional, transmembrane glucose transport in skeletal muscle of healthy, lean men and women. This is consistent with the tenet that glucose transport is an important locus of insulin action and in the control of glucose uptake into skeletal muscle (20, 23). The second finding was that insulin did not significantly change values for \( k_1 \), the rate constant describing tracer delivery from plasma (12). \( k_1 \) is mainly determined by mathematical fitting of the proximal portion of the tissue-time activity curve (with relation to arterial activity), and this is a key reason it is widely attributed to tracer delivery (12). The third finding relates to interaction between delivery (\( k_1 \)) and transport (\( k_3 \)) in integrating the effect of insulin to govern rates of glucose uptake. Although values for \( k_1 \) did not change in response to insulin, whereas those for \( k_3 \) did increase during insulin-stimulated conditions, \( k_1 \) appeared to influence 3-[11C]OMG uptake to a greater extent during insulin-stimulated than during basal conditions. This relationship between delivery and transport was best discerned in comparing soleus and anterior tibialis mus-
cles. Insulin-stimulated uptake of 3-\[^{11}\text{C}\]OMG was significantly greater in soleus, and with regard to the individual rate constants the main difference from anterior tibialis was a significantly greater in soleus, and with regard to the individual rate constant.

It is axiomatic that glucose must be transported into muscle before it can be oxidized, stored as glycogen, or otherwise metabolized. Clear dose-responsive insulin stimulation of muscle glucose metabolism is well established (13, 26). Thus the finding of dose-responsive insulin stimulation of glucose transport in skeletal muscle of healthy volunteers is not unexpected. Nonetheless, there have been few prior human studies that have specifically examined this step. One clinical investigation that is perhaps the most specific was performed by Bonadonna et al. (4) and used a bolus injection of 3-\[^{14}\text{C}\]OMG, given into the brachial artery, following which venous washout curves were measured across the forearm. The modeling of these data indicated a fourfold stimulation of glucose transport at supraphysiological insulin (4). Although both the study by Bonadonna et al. and the current study evaluated skeletal muscle in humans by use of 3-OMG, data obtained by arteriovenous isotope washout and by PET imaging are not exactly the same, as PET measures tracer activity within the tissue bed itself. Despite this difference, there was similarity in findings in that in the current study the overall partition coefficient for 3-\[^{11}\text{C}\]OMG uptake from plasma into tissue increased above basal three- and fourfold, respectively, at low physiological and supraphysiological elevations of insulin.

The rate constant \(k_1\) delineates the fraction of tracer entering the second tissue compartment from the first, and it increased from 1% during Basal to 7% and 21% during LO and HI, respectively. This effect is consistent with the known effect of insulin to stimulate translocation of glucose transporter proteins in muscle (23). In contrast, the rate constant \(k_s\), which describes the kinetics of 3-\[^{11}\text{C}\]OMG delivery from plasma into the tissue bed of skeletal muscle, did not change in response to insulin. This finding is consistent with our earlier study using 3-\[^{14}\text{C}\]OMG, in which it was also observed that \(k_s\) did not change from basal values during midphysiological insulin stimulation (3). Similar findings were made in PET imaging of muscle using \[^{18}\text{F}\]FDG (25). In prior studies that modeled tissue activity of \[^{18}\text{F}\]FDG in skeletal muscle, it was found that \(k_1\) correlated well with values for tissue perfusion measured using \[^{15}\text{O}\]H\(_2\)O, a tracer that is specific for this parameter (25). This type of comparison with \[^{15}\text{O}\]H\(_2\)O should also be performed in future studies with 3-\[^{11}\text{C}\]OMG to gain a more complete understanding of the physiological attributes of the \(k_1\) rate constant. At high levels of insulin stimulation, particularly with prolonged duration, increases in muscle blood flow have been observed (1). At low physiological elevations, increases of overall blood flow to muscle generally do not occur, but there may be an important effect to initiate capillary recruitment (9). Unfortunately, measurement of capillary recruitment is beneath the spatial resolution of PET, since a pixel, the smallest divisible unit of PET imaging, can be estimated to contain thousands of muscle capillaries.

At first appraisal, the current findings of unchanged values for \(k_1\) during insulin-stimulated compared with basal conditions might seem to indicate that glucose delivery is not a strong locus of control in the effect of insulin to govern rates of glucose uptake into muscle. However, our interpretation is different and is that, as insulin acts to increase glucose transport, glucose delivery emerges to have greater importance in governing glucose uptake than it exerts during basal conditions, when the kinetics of glucose transport are at their bottommost. Renkin et al. (22) postulated that the influence of substrate delivery upon its uptake is a function of tissue permeability and that flow is least likely to modulate substrate uptake when permeability is low. In this context, bidirectional transmembrane glucose transport functions as the permeability factor. Previous observations concerning the interaction of delivery and transport were based on experiments in a conscious rat model that also used 3-OMG and, like the current studies in humans, revealed interdependence of transport and delivery in control of glucose uptake (10, 11). In those studies, the transsarcomembrmal glucose gradient fell progressively with increasing insulin, as an increased efficiency of glucose transport brought extra- and intracellular glucose to closer equilibration and overall control of glucose uptake shifted toward rates of delivery (19).

Comparison of 3-\[^{11}\text{C}\]OMG uptake into the tissue beds of soleus and tibialis anterior muscles provides further perspective on the interaction between delivery and transport and how this is potentially modulated by insulin. On the basis of ex vivo and animal studies, it is generally considered that oxidative muscle fibers have greater insulin sensitivity than glycolytic fibers (13). This was observed in the current study, as insulin-stimulated tissue activity of 3-\[^{11}\text{C}\]OMG was significantly higher in soleus than in tibialis anterior. Spectral analysis indicated that a similar two-exponential model was applicable to both muscles. In compartmental modeling of 3-\[^{11}\text{C}\]OMG activity in the two muscles, values for \(k_3\) were similar in soleus and tibialis anterior muscles during LO and HI insulin and therefore did not account for higher uptake of 3-\[^{11}\text{C}\]OMG in soleus. Instead, the main difference between soleus and tibialis anterior.

### Table 4. Rate constants from PET imaging of 3-\[^{11}\text{C}\]OMG in soleus and anterior tibialis muscles groups in healthy volunteers at LO and HI (paired studies) conditions

<table>
<thead>
<tr>
<th></th>
<th>Soleus</th>
<th>Tibialis anterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_1), ml/ml</td>
<td>0.020±0.001* (3±0.4)</td>
<td>0.015±0.002 (5±1)</td>
</tr>
<tr>
<td>(k_1), min(^{-1})</td>
<td>0.122±0.028 (10±2)</td>
<td>0.111±0.028 (18±5)</td>
</tr>
<tr>
<td>(k_s), min(^{-1})</td>
<td>0.062±0.012 (22±9)</td>
<td>0.096±0.033 (31±10)</td>
</tr>
<tr>
<td>(k_s), min(^{-1})</td>
<td>0.027±0.005 (14±6)</td>
<td>0.034±0.006 (21±10)</td>
</tr>
<tr>
<td>(V_a), ml/ml</td>
<td>0.67±0.05</td>
<td>0.60±0.06</td>
</tr>
<tr>
<td>(V_a), ml/ml</td>
<td>0.22±0.05 (8±2)</td>
<td>0.21±0.06 (14±4.0)</td>
</tr>
<tr>
<td>(V_a), ml/ml</td>
<td>0.45±0.04 (7±3)</td>
<td>0.40±0.05 (11±4)</td>
</tr>
</tbody>
</table>

Data are means ± SE. Error of estimation for the parameter is shown in parentheses. *\(P < 0.05\) vs. anterior tibialis; †\(P < 0.05\), LO vs. HI.
anterior was that $k_1$ was $\sim 25\%$ higher in soleus at midphysiological insulin and $33\%$ higher at supraphysiological insulin. Although higher $k_1$ and, hence, presumably higher rates of delivery occur in soleus, the fact that the overall partition coefficient for the 3-[11C]OMG was just $10\%$ greater in soleus than in tibialis anterior suggests that rate control over glucose uptake is distributed between delivery and transport even at supraphysiological insulin stimulation.

In summary, bioimaging of skeletal muscle using dynamic PET imaging of the tracer 3-[11C]OMG provides a novel approach to assess glucose transport in human skeletal muscle. This method reveals clear dose-responsive insulin stimulation in healthy volunteers. This method begins to establish a technical feasibility for integrated physiology studies of the interdependence between glucose delivery and transport in mediating the action of insulin to govern glucose uptake into human skeletal muscle.

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