Insulin Activation of Plasma Nonesterified Fatty Acid Uptake in Metabolic Syndrome

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Objective—Insulin control of fatty acid metabolism has long been deemed dominated by suppression of adipose lipolysis. The goal of the present study was to test the hypothesis that this single role of insulin is insufficient to explain observed fatty acid dynamics.

Methods and Results—Fatty acid kinetics were measured during a meal tolerance test and insulin sensitivity assessed by intravenous glucose tolerance test in overweight human subjects (n=15; body mass index, 35.8 ± 7.1 kg/m²). Non–steady state tracer kinetic models were formulated and tested using ProcessDB software. Suppression of adipose release alone could not account for nonesterified fatty acid concentration changes postprandially, but when combined with insulin activation of fatty acid, uptake was consistent with the nonesterified fatty acid data. The observed insulin $K_c$ for nonesterified fatty acid uptake was inversely correlated with both insulin sensitivity of glucose uptake (intravenous $r=-0.626; P=0.01$) and whole body fat oxidation after the meal ($r=-0.538; P=0.05$).

Conclusion—These results support insulin regulation of fatty acid turnover by both release and uptake mechanisms. Activation of fatty acid uptake is consistent with the human data, has mechanistic precedent in cell culture, and highlights a new potential target for therapies aimed at improving the control of fatty acid metabolism in insulin–resistant disease states. (Arterioscler Thromb Vasc Biol. 2012;32:XX-XX.)

Key Words: insulin mathematical modeling nonesterified fatty acids regulation

The intravenous glucose tolerance test (IVGTT), in all its incarnations, remains a staple analytical procedure in clinical endocrinology. Systems biology and modeling tools have a long history in this field and have yielded the clinically important measure of insulin sensitivity, $(S_i)$, widely used to measure the whole body $S_i$ in glucose metabolism. In recent years, pioneering efforts to extend this systems biology approach to insulin regulation of fatty acid metabolism have appeared. These studies have addressed key issues of insulin–inhibited adipose lipolysis and parameter identifiability but have not addressed the potential complication, also faced by models of glucose metabolism, that insulin regulates entry and removal of plasma fatty acids. The relationship between insulin and nonesterified fatty acid (NEFA) clearance has been explored in pioneering studies by Carpentier et al., who provided evidence that insulin per se does not stimulate plasma NEFA clearance at the whole body level. Instead, these investigators demonstrated an inverse relationship between NEFA appearance rate and NEFA clearance and concluded that “…insulin stimulates plasma NEFA clearance by reducing the endogenous appearance rate of NEFA.” Studies on the specific uptake of fatty acids by different organs have been recently investigated by this group.

The paucity of other data on the physiological control of fatty acid uptake is understandable in the light of the widely held view that fatty acid removal is passive. But passivity does not necessarily imply absence of regulation because it has been reported that, at least in rat adipocytes, long chain fatty acid uptake is predominantly transporter mediated and where a protein is involved control is always possible. There are, for example, persistent hints that insulin accelerates fatty acid uptake. Early kinetic studies of fatty acid clearance in human subjects during glucose consumption revealed fatty acid fractional catabolic rates (FCRs) =60% greater than FCRs measured 6 to 8 hours after glucose consumption ends. More recently, elegant studies taking advantage of stable isotope tracer technology have reported fatty acid flux and mass data, or fatty acid metabolic clearance rates, in normal human subjects during insulin infusions of 2 or 3 hours in duration. When FCRs are calculated from these results it can be determined that fatty acids are removed as much as...
Another way to approach the question of insulin-mediated NEFA uptake is to contrast absolute values of fatty acid FCR as measured in different subject groups with different prevailing plasma insulin concentrations. Healthy subjects exhibited fatty acid FCRs of 0.22 per minute measured with radiotracer or stable isotope administration. These low FCRs were reported while mean insulin concentrations were also low (4.2 ± 0.3 μU/mL). By contrast, in an obese, nonobese Indian population, a much higher fatty acid FCR was reported (0.71 per minute) concurrent with a higher average fasting insulin concentration of 56 μU/mL. Importantly, a potential cellular mechanistic basis for greater in vivo fatty acid FCR with higher insulin values is that fatty acid transporter content in the cell membrane is increased by insulin and that these transporters regulate a substantial fraction of cellular fatty acid uptake. Physiological roles for membrane fatty acid transporters have recently been the subject of a comprehensive review.

Insulin is already well established as a potent regulator of glucose entry into plasma from liver and of fatty acid entry into plasma from adipose tissue. If insulin regulation of NEFA removal pathways could be convincingly demonstrated as a feature of human physiology, the prevailing paradigm for regulation of human fat metabolism would undergo a substantial revision. In return for the added complexity, investigators would gain new explanatory power and possibly new therapeutic targets. Moreover, the existence of insulin-sensitive NEFA uptake would increase the structural similarity between the insulin–glucose control system and the insulin–NEFA control system.

The primary goals of the present study were to test the hypothesis that insulin activation of fatty acid uptake is a significant feature of human physiology, and to develop, in humans, a method for estimating the separate S, of 2 key determinants of plasma NEFA concentration: (1) adipose tissue release and (2) peripheral fatty acid uptake. As a practical application of these methods, we quantified S, for the principal determinants of plasma fatty acid balance in 15 overweight subjects with metabolic syndrome during a meal tolerance test (MTT) and an IVGTT.

Patients and Methods

Human Subjects

The present project was part of a larger study on the effect of body weight (BW) on liver health in Hispanic and black subjects, and a portion of the data (acyl carnitine concentrations) has been published recently. Subjects (n=15) were recruited from health fairs and by physician referrals, and the inclusion criteria were overweight or obese with elevated liver enzymes (alanine aminotransferase >30; aspartate aminotransferase >30) or characteristics of the metabolic syndrome. Subjects were nondiabetic (fasting glucose <125 mg/dL), age 20–67 years, with stable BW, and maintenance of preenrollment physical activity. The intensive nature of the tracer and modeling studies precluded a larger population study. Each subject participated in 2 metabolic tests, with the first designed to determine metabolic flux during an IVGTT, and the second designed to measure changes in metabolites after a standardized meal. The present study was approved by the Institutional Review Board at UT Southwestern Medical Center (approval No. 062007-025).

Study Design, IVGTT, and MTT

Subjects consumed weight-maintaining diets formulated with comparison to 3-day dietary recall of usual intake for 3 days before undergoing an IVGTT (admission No. 1) and for 7 days before the MTT (admission No. 2). For admission No. 1, each subject reported to the Clinical and Translational Research Center at 7 AM on the day of the study. One antecubital IV line was placed in each arm. The subject underwent an IVGTT, and blood was collected at standard intervals of up to 180 minutes (Figure 1 for exact times) for measurement of non–steady state plasma glucose, NEFA, and insulin concentrations. The other IV line was used to administer a bolus of 50% dextrose (Hospira Inc, Lake Forest, IL) at 0.3 g/kg BW at 0 minute and a bolus of regular insulin (Humulin R U-100, Eli Lilly, Indianapolis, IN) at 0.03 units/kg BW at 20 minutes. The IVGTT started between 8:00 and 9:45 AM after a 12-hour fast and after at least 30 minutes from the IV line placement. Within 2 weeks of the IVGTT, the subject was readmitted to the Clinical and Translational Research Center and underwent a constant infusion of 13C3-palmitate (7 μg/kg per minute bound to albumin) to measure the total rate of appearance of NEFA in plasma (RaNEFA) compartment during the fasted and fed states. Breakfast was withheld in the morning to bring the subjects to a significant fast to test adipose fatty acid release. At noon, the subject consumed a meal consisting of a cocoa-flavored drink (cocoa, corn oil, heavy cream, sucrose, and skim milk), cereal, banana, and...
to a significant fast to test adipose fatty acid release. At noon, the subjects were fasting in the morning to bring the subjects to a fed state. Breakfast was withheld in the morning to bring the subjects to a fed state. Breakfast was withheld in the morning to bring the subjects to a fed state.

For details of meal, see Patients and Methods. The lines on graphs A to C represent simple linear interpolations between data points. By contrast, the lines on graph D are model solutions. The dotted line represents the model with constant plasma NEFA fractional catabolic rate (FCR) and is incapable of accounting for the experimental data (filled triangles) representing the mean plasma NEFA concentration time course after the test meal. This model was constrained using the directly measured total RaNEFA as the only input to plasma NEFA, and the value of the constant FCR was optimized during the fitting process. The solid line, by contrast, is the model solution that includes insulin-sensitive fatty acid uptake. Parameter values for this fit for the $P_{intra}$ rate law (see text) are $\text{DelayFacUptake} = 11$ minutes; $k_{\text{FacUptake}} = 0.063$ per minute; $k_{\text{RefacUptake}} = 14$ pmol/kg BW; $h_{\text{FacUptake}} = 4.2$; and $k_{\text{diff}} = 0.112$ per minute. $P_{\text{intra}}$ indicates the flux of fatty acids taken up by peripheral tissues; DelayFacUptake, insulin signaling delay; FacUptake, facilitated uptake; $k_{\text{FacUptake}}$, $k_{\text{diff}}$, $k_{\text{RefacUptake}}$, $K_{\text{max}}$ of fatty acid uptake; $K_{\text{mFacUptake}}$, $K_{\text{mInsulin}}$ for insulin; $h_{\text{FacUptake}}$, Hill cooperativity coefficient; $k_{\text{diff}}$, diffusive fatty acid uptake.

Figure 2. Concentrations of glucose and insulin and rate of appearance of NEFA into plasma (RaNEFA) before and after a standardized meal. Concentrations of nonesterified fatty acid (NEFA) with model fits. Mean plasma concentrations of glucose (A), and insulin (B), and mean total fatty acid delivery to the plasma (RaNEFA) (C) in subjects with metabolic syndrome ($n=15$) fed a standardized meal at t=0 (12 noon). For details of meal, see Patients and Methods. The lines on graphs A to C represent simple linear interpolations between data points. By contrast, the lines on graph D are model solutions. The dotted line represents the model with constant plasma NEFA fractional catabolic rate (FCR) and is incapable of accounting for the experimental data (filled triangles) representing the mean plasma NEFA concentration time course after the test meal. This model was constrained using the directly measured total RaNEFA as the only input to plasma NEFA, and the value of the constant FCR was optimized during the fitting process. The solid line, by contrast, is the model solution that includes insulin-sensitive fatty acid uptake. Parameter values for this fit for the $P_{intra}$ rate law (see text) are $\text{DelayFacUptake} = 11$ minutes; $k_{\text{FacUptake}} = 0.063$ per minute; $k_{\text{RefacUptake}} = 14$ pmol/kg BW; $h_{\text{FacUptake}} = 4.2$; and $k_{\text{diff}} = 0.112$ per minute. $P_{\text{intra}}$ indicates the flux of fatty acids taken up by peripheral tissues; DelayFacUptake, insulin signaling delay; FacUptake, facilitated uptake; $k_{\text{FacUptake}}$, $k_{\text{diff}}$, $k_{\text{RefacUptake}}$, $K_{\text{max}}$ of fatty acid uptake; $K_{\text{mFacUptake}}$, $K_{\text{mInsulin}}$ for insulin; $h_{\text{FacUptake}}$, Hill cooperativity coefficient; $k_{\text{diff}}$, diffusive fatty acid uptake.

Skim milk formulated to provide 38% of total daily energy needs. The test meal composition, analyzed using the Nutrition Data System for Research (2009), was (mean±SD) 790±119 kcal, 44.3±9.3 g fat, 15.8±8.2 g protein, 90.7±22.7 g carbohydrate, 4.22±2.11 g fiber, and 83.3±11.9 mg cholesterol. The subject was given 15 minutes to consume the meal. Blood samples were collected, and fasting data are presented from 6:00 AM (denoted 6 hours on Figure 2) and fed state data are 12:00 to 6:00 PM (time denoted 0–6 hours postprandially on Figures 2 and 3). Indirect calorimetry was performed using a metabolic cart (VMax Encore; Viasys Healthcare Inc, Yorba Linda, CA) in the hooded mode; data were obtained for 30 minutes while subjects were fasting (8:00–8:30 AM) and again 2.5 hours (2:30 PM) after the initiation of the meal (12:00 PM).

Sample Analysis
Blood was collected in tubes containing sodium fluoride for plasma glucose and tubes containing EDTA for plasma insulin and NEFA. Plasma from the EDTA tubes was separated immediately by centrifugation at 2850g for 10 minutes at 5°C and stored at −20°C. Plasma NEFA concentrations were measured by enzymatic reaction (Wako Diagnostics, Richmond, VA) and insulin concentrations by ELISA (Millipore Corporation, Billerica, MA) within 2 days of the study. The specimens in sodium fluoride tubes taken during the IVGTT were maintained on ice after centrifugation and analyzed with a YSI 2300 Stat Plus analyzer within 1 hour of collection.

Glucose Minimal Modeling and Calculations of RaNEFA
Glucose and insulin responses during an IVGTT were analyzed using the minimal model (MINMOD) technique and the MINMOD

Millenium software. Four indices were reported from the fitting of the glucose data. Glucose effectiveness is the capacity of glucose to mediate its own disposal. Acute insulin response to glucose, the insulin release during the first 10 minutes after dextrose bolus, $\delta$, the capacity of insulin to promote glucose disposal, and disposition index the product of acute insulin response to glucose and $\delta$, which represents the interaction between insulin secretion and action. For the calculations of time-varying RaNEFA, the fatty acid infuse composition, palmitate enrichments in infusates, and plasma NEFA concentrations were analyzed by gas chromatography and gas chromatography/mass spectrometry, as described by Barrows et al. Plasma concentrations of long-chain acylcarnitines (LCAC) were measured as described previously.

Systems Biology and NEFA Modeling, Calculations, and Statistics
Model development and testing were carried out as previously described. Briefly, models were formulated as systems of nonlinear ordinary differential equations using standard principles of chemical kinetics, enzyme kinetics, and transport kinetics. The resulting models were subjected to simulated protocols corresponding to the experimental clinical protocols described above. Simulations of the combined models of experiments were carried out using standard techniques of numerical integration, and simulated time courses were compared with the experimental data as a means of testing the hypothesis represented by the model. To give each tested model its best chance to account for a given experimental data set, model parameters, such as rate constants, maximal velocities, substrate constants, and inhibition constants, were adjusted using standard–weighted least squares optimization. As in nearly all published
models of insulin-regulated metabolism, the measured time course of plasma insulin concentration is used as a known input or forcing function. These methods were implemented in the ProcessDB software (Integrative Bioinformatics Inc, www.integrativebioinformatics.com/processdb.html). Models were combined with appropriate experimental protocols (infusion rates, meal contents, bolus glucose amounts, etc) in ProcessDB and exported to the Berkeley Madonna solver (www.berkeleymadonna.com) for numerical integration and parameter optimization. Postsimulation calculations were performed using Microsoft Excel (version 2000; Seattle, WA) and statistical analyses on Statview for Windows (version 5.0.1, SAS Institute Inc, Berkeley, CA). P<0.05 was considered statistically significant.

There are many approaches to kinetic modeling of biological systems. Some investigators focus on parameters, some on predictions, and some on hypothesis testing. A focus on parameters results in MINMODs for which each parameter value is formally identifiable with coefficients of variation, given that the model is correct, often <30%. A focus on prediction is often desirable because it helps guide experimental design. The approach taken in the present article falls into the third category. The model presented here is a mechanistic working hypothesis whose goal is to account successfully for complex data sets. It is not intended as a MINMOD and the optimization process does not yield fully identified parameters. Instead, the optimizer’s search for parameter space is used to give each hypothesis its best chance to account for the experimental data. Hence, the principal benefits of the hypothesis testing mode of modeling are quantitative rejection of inadequate hypotheses and quantitative corroboration of successful hypotheses.

Results

Subjects studied were of Hispanic (2 men and 7 women) and black (1 man and 5 women) ethnicity. Clinical and laboratory values for the subjects (aged 46±8 years) revealed characteristics common of obesity. Mean body mass index was 35.8±7.1 kg/m², plasma triacylglycerol 1.24±0.42 mmol/L, high-density lipoprotein–cholesterol 1.30±0.33 mmol/L.
mmol/L, low-density lipoprotein–cholesterol 3.15 ± 3.12 mmol/L, glucose 5.3 ± 0.8 mmol/L, and waist circumference 114 ± 27 cm for men and 108 ± 14 cm for women. The subjects’ homeostasis model assessment of insulin resistance was 2.3 ± 1.2 (range, 0.9–4.4) and hemoglobin A1c was 5.9 ± 0.3%. The data above were not different between the 2 ethnic groups nor did ethnicity influence any of the kinetic data presented below. Thus, the results are presented for the group as a whole.

NEFA Dynamics

We first tested the null hypothesis that changes in NEFA release from adipose were able to account for the observed reductions in plasma NEFA concentrations after an MTT. Figure 2A and 2B show the concentrations of glucose and insulin before and after the mixed meal. Total RaNEFA was measured using continuous IV infusion of [1-13C]palmitate (Figure 2C). If inhibition of adipose fatty acid release is sufficient to explain the observed decreases in plasma NEFA concentration then a model consisting of a constant NEFA FCR and a time-varying NEFA input into plasma measured by the RaNEFA would be capable of accounting for the observed changes. As shown in Figure 2D, this was not the case (dotted line). Plasma NEFA concentrations declined faster and further than could be accounted for by the measured decreases in RaNEFA. We, therefore, tested a more complex hypothesis by augmenting the simple mass action (constant FCR) uptake flux with an insulin–facilitated uptake flux as follows:

\[ P_{\text{AdipRel}} = \frac{V_{\text{AdipRel}}}{1 + \left( \frac{I_{\text{plasma}}}{I_{\text{insulin}}} \right)^{V_{\text{AdipRel}}}} \]

where \( P_{\text{AdipRel}} \) is the flux (\( \mu \text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1} \)) of fatty acids released into plasma as measured by the total RaNEFA technique. This includes fatty acids released from both intraadipocyte lipolysis (mediated by triacylglycerol lipase and hormone-sensitive lipase) and extracellular lipolysis, mediated by the spillover of fatty acids from lipoprotein lipase–mediated lipoprotein hydrolysis, primarily occurring at the adipose.32,33 The term \( I_{\text{plasma}} \) represents the maximal flux of insulin–sensitive adipose fatty acid release. The term \( I_{\text{insulin}} \) represents the time course of plasma insulin concentration delayed by time, and \( Delay_{\text{AdipRel}} \) accounts for delays encountered in propagating the insulin signal from the plasma into the interstitial space, receptor binding, and intracellular signaling cascades. \( K_{\text{insulin}} \) represents the sensitivity of fatty acid release to insulin (half-maximally affected). Finally, \( h_{\text{AdipRel}} \) is the Hill cooperativity coefficient for \( I_{\text{insulin}} \) of fatty acid release; increasing values of the Hill coefficient correspond to more and more on-or-off, switch-like behavior. Both nonlinear rate laws were derived on the basis of parallels to the classic rapid equilibrium methods of enzyme kinetics.25

The equations for \( P_{\text{AdipRel}} \) and \( P_{\text{uptake}} \) represent the input and output processes for the INEFA model. For each individual subject these equations were fitted simultaneously to the non–steady state plasma NEFA concentrations and total RaNEFA data. The individual fits for 3 subjects are shown in Figure 3A (NEFA concentrations) and Figure 3B (RaNEFA), and the fits for each of the 15 subjects are shown in Figure I in the online-only Data Supplement for NEFA concentrations and Figure II in the online-only Data Supplement for the RaNEFA models. This procedure yielded quantitative parameter estimates for all the INEFA model parameters, which are summarized in the Table.

One goal of this study was to establish the feasibility of quantifying the \( S_{\text{f}} \) of fatty acid release and fatty acid uptake in individual subjects based on single protocol involving 1 tracer infusion and 1 non–steady state physiological perturbation. Because the resulting data provide 1 time course (plasma NEFA concentration) containing all the information on the 4 parameters of fatty acid uptake and another time course (total RaNEFA) containing all the information on the 3 parameters characterizing fatty acid release, the method appears well suited to future work targeting detailed parameter identifiability and characterization.
## Table. Parameter Values Obtained for the Insulin-Sensitive Processes of Plasma NEFA Metabolism Derived From MTT and IVGTT

<table>
<thead>
<tr>
<th>Process</th>
<th>Parameter Description</th>
<th>Parameter Name in Model</th>
<th>Units</th>
<th>MTT Mean*</th>
<th>MTT SD</th>
<th>IVGTT Mean*</th>
<th>IVGTT SD</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty acid release</td>
<td>Insulin signalling delay</td>
<td>DelayAdipRel</td>
<td>min</td>
<td>47.4</td>
<td>27.0</td>
<td>36.7</td>
<td>16.4</td>
<td>0.101</td>
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<tr>
<td></td>
<td>$V_{\text{max}}$, adipose release</td>
<td>$V_{\text{max}}$</td>
<td>μmol·min$^{-1}$·kgBW$^{-1}$</td>
<td>5.61</td>
<td>2.6</td>
<td>5.9</td>
<td>3.5</td>
<td>0.407</td>
</tr>
<tr>
<td></td>
<td>Insulin inhibition constant</td>
<td>$K_{\text{i}}$</td>
<td>pmol/kgBW</td>
<td>11.0</td>
<td>9.5</td>
<td>9.7</td>
<td>9.7</td>
<td>0.360</td>
</tr>
<tr>
<td></td>
<td>Hill cooperativity coefficient</td>
<td>$h_{\text{AdipRel}}$</td>
<td>unitless</td>
<td>1.59</td>
<td>1.6</td>
<td>1.2</td>
<td>0.6</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td>Diffusive fatty acid uptake</td>
<td>$k_{\text{diff}}$</td>
<td>min$^{-1}$</td>
<td>0.08</td>
<td>0.04</td>
<td>0.09</td>
<td>0.06</td>
<td>0.307</td>
</tr>
<tr>
<td>Fatty acid uptake</td>
<td>Insulin signalling delay</td>
<td>DelayFacUptake</td>
<td>min</td>
<td>24.1</td>
<td>25.9</td>
<td>29.4</td>
<td>26.7</td>
<td>0.293</td>
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<td></td>
<td>$k_{\text{max}}$ of fatty acid uptake</td>
<td>$k_{\text{facUptake}}$</td>
<td>min$^{-1}$</td>
<td>0.12</td>
<td>0.12</td>
<td>0.10</td>
<td>0.03</td>
<td>0.348</td>
</tr>
<tr>
<td></td>
<td>$K_{\text{m}}$ for insulin</td>
<td>$K_{\text{infacUptake}}$</td>
<td>pmol/kgBW</td>
<td>12.3</td>
<td>9.2</td>
<td>13.0</td>
<td>10.2</td>
<td>0.421</td>
</tr>
<tr>
<td></td>
<td>Hill cooperativity coefficient</td>
<td>$h_{\text{facUptake}}$</td>
<td>Unitless</td>
<td>4.4</td>
<td>3.8</td>
<td>3.8</td>
<td>2.8</td>
<td>0.312</td>
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</tbody>
</table>

NEFA indicates nonesterified fatty acid; MTT, meal tolerance test; IVGTT, intravenous glucose tolerance test; BW, body weight; FacUptake, facilitated uptake.

*Mean* and SD are the mean ± SD of the parameter values generated from the MTT and IVGTT.

†Paired t test for parameter values generated from the MTT and IVGTT.

of additional subject populations. The INEFA model is shown in Figure 4 and the complete INEFA model equations are as follows:

\[
\frac{dN_{\text{plasma}}}{dt} = P_{\text{AdipRel}} - P_{\text{uptake}}
\]

\[
P_{\text{AdipRel}} = \frac{V_{\text{AdipRel}}}{1 + \left(\frac{I_{\text{plasma}} (t - \text{DelayAdipRel})}{K_{\text{insulin}}}\right)^{h_{\text{AdipRel}}}}
\]

\[
P_{\text{uptake}} = k_{\text{diff}} N_{\text{plasma}} + \frac{K_{\text{facUptake}}}{K_{\text{max facUptake}}} \left(1 - \text{DelayFacUptake}\right)
\]

where $N_{\text{plasma}}$ is plasma NEFA concentration, $P_{\text{AdipRel}}$ is the fatty acid flux delivered to plasma via adipose lipolysis and release (from adipose lipoprotein lipase, triacylglycerol lipase, hormone-sensitive lipase, and monoglyceride lipase), $P_{\text{uptake}}$ is the flux of fatty acids taken up by peripheral tissues, $I_{\text{plasma}}$ is plasma insulin concentration, $V_m$ is maximal velocity or maximal tissue capacity, $K_i$ and $K_m$ are inhibition and Michaelis constants, $k_{\text{max}}$ is the maximum insulin-stimulated rate constant for fatty acid uptake, DelayAdipRel and DelayFacUptake are delays associated with insulin signaling, and $h$ are Hill coefficients permitting cooperativity in regulatory mechanisms.

### Model Extension to Analysis of Standard IVGTT

Finally, we tested the hypothesis that the INEFA model, including the insulin–sensitive fatty acid uptake required to model the MTT, could predict the changes in NEFA dynamics during an insulin-modified IVGTT. Presented in Figure 1 are the mean concentrations of glucose, insulin, and NEFA during the IVGTT. The nadir for NEFA concentration occurred at 70 minutes and was 0.21±0.11 mmol/L, representing a 72.7±14.9% suppression of NEFA from fasting. For the glucose/insulin system, metabolic parameters calculated using the MINMOD Millennium program revealed that the acute insulin response to glucose was 603±396 μU/mL per minute, the disposition index was 1238±644, whereas the glucose effectiveness was 0.010±0.007 per minute. Mean whole body $\beta$ was 2.51±1.46×10−4 min$^{-1}$·μU$^{-1}$·mL$^{-1}$, range [0.89−6.25], which was slightly >2.5×10−4 [0.89−6.25], a commonly used cutoff, below which subjects are characterized as insulin resistant.34

Using the measured IVGTT plasma insulin concentrations (Figure 1) as a known input, the INEFA model was capable of accounting for the full range of plasma NEFA concentration time courses recorded during IVGTT. Data and model fits for the 3 representative subjects are shown in Figure 3C. Model fits for all the 15 subjects are shown in Figure III in the online-only Data Supplement. Importantly, the parameter values for these fits were constrained by knowledge of the parameters extracted from the MTT data in the same subjects. Standard
least squares fitting of the IVGTT NEFA data was undertaken with upper and lower bounds on adipose fatty acid release parameters \( V_{m_{\text{AdipRel}}} \), \( K_{\text{AdipRel}} \), DelayAdipRel, and tissue fatty acid uptake parameters \( k_{\text{diff}}, K_{\text{FacUpake}}, k_{\text{FacUpake}} \text{DelayFacUpake}, \text{and } h_{\text{FacUpake}} \) set to \( \pm 50\% \) of the values obtained for each individual subject in the MTT analysis above. Because the INEFA model uses plasma insulin as a known input, it was occasionally also useful to allow the optimizer to adjust the basal value of \( I_{\text{plasma}} \) that was used as the model input before and after the period when insulin measurements were actually taken. It should be emphasized that the resolution of the 2 insulin-sensitive processes depends on measuring RaNEFA. The time course of plasma NEFA concentrations alone, in either MTT or IVGTT, can neither demonstrate nor quantify insulin–sensitive fatty acid uptake.

Thus, a single model (INEFA) with 4 parameters characterizing adipose fatty acid release and 5 parameters characterizing tissue fatty acid uptake provided a mechanistic accounting for observed NEFA dynamics in both MTT and IVGTT. We validated the model derived from the meal studies by showing that it could also account for the NEFA response to an IVGTT. As shown in the Table, a paired \( t \) test on each parameter (comparing meal-derived and IVGTT-derived values) reveals no significant difference. Development of the INEFA model thus uncovered the presence of a physiological control mechanism that activates tissue fatty acid uptake at the same time adipose release is inhibited. By testing against MTT and IVGTT data in 15 overweight subjects, we corroborated the hypothesis that insulin is the required regulator of human fatty acid uptake.

## Correlations Between MINMOD and INEFA Parameters

Given the wide use of the IVGTT in clinical research to generate measures of glucose metabolism, we determined whether any relationships existed between the standard MINMOD parameters, subject characteristics, and parameters of fatty acid metabolism obtained from the INEFA model. As expected, NEFA concentrations after the meal were a function of the release rates (Figure 5A) and the greater the body fat mass of the subjects the lower the \( S_i \) (Figure 5B). However, a better relationship was observed between the \( K_i \) for effect of insulin on adipose fatty acid release after the meal (\( K_{\text{AdipRel}} \)) and the natural log of the IVGTT \( S_i \) supporting the concept that 1 mediator of the negative association between body fat and \( S_i \) is a lack of suppression of fatty acid release (Figure 5C; \( r=-0.626; P=0.013 \)). Further, whole body oxidation of fatty acids after the meal correlated positively with the MTT \( K_{\text{AdipRel}} \) (Figure 5D) and negatively with the IVGTT \( K_{\text{FacUpake}} \) (Figure 5E). These data support the effect of insulin on both fatty acid release rates and peripheral uptake rates to influence the oxidation of lipid in tissues. Last, plasma LCAC represent the products of incomplete fatty acid oxidation in the mitochondria. The present results connect higher \( K_{\text{AdipRel}} \) to reduce fatty acid release to overload of mitochondrial \( \alpha \)-oxidation (Figure 5F).

## Discussion

Modeling and systems biology are particularly well suited to the complexities of metabolism and metabolic disease, explaining, perhaps, the extensive literature linking these 2 disciplines. In this article, we have applied these techniques to human fatty acid metabolism and have demonstrated unequivocally that the classic (constant FCR) model of peripheral fatty acid uptake is incomplete. The constant FCR model fails to account for plasma NEFA dynamics during an MTT when the total RaNEFA is known. Some additional mechanism is inescapably required.

Because there is evidence, based on studies in cultured cells and that insulin promotes fatty acid uptake, we tested the hypothesis that fatty acid uptake is, in part, regulated by insulin in a group of subjects with a wide range of \( S_i \). This hypothesis was corroborated using measured plasma insulin as the stimulus. The results thus make a strong case for insulin activation of human fatty acid uptake from plasma. However, not all published works favor a significant contribution of insulin–stimulated NEFA uptake in tissues. NEFA uptake in perfused rodent skeletal muscle was shown to be independent of insulin, and NEFA concentration itself appears to play a role in its clearance rate. Insulin increases cellular uptake of glucose, which through glycolysis could potentially increase fatty acid reesterification and reduce lipolysis by providing \( \alpha \)-glycerol phosphate to form the glycerol backbone for triacylglycerol synthesis. However, Caruso et al found no insulin-independent effect of hyperglycemia to reduce adipose lipolysis in humans in vivo.

Because it has been proposed that insulin increases skeletal muscle and adipose blood flow, an alternative hypothesis might include insulin-mediated control of vascular fatty acid delivery. This mechanism would require that fatty acid uptake is flow limited, but the literature as a whole appears to support diffusion/transport as the limiting determinant of fatty acid uptake. This position, combined with the increasingly prominent cellular biological evidence for insulin–activated plasma membrane fatty acid transport, led us to focus on the parenchymal plasma membrane as the site of insulin action.

Clinical research is most practical and least expensive when only blood samples are required, but just as with glucose, plasma NEFA data alone are insufficient to calculate the absolute magnitude of fatty acid fluxes entering and leaving the plasma compartment. Indeed, concentration changes can, at most, yield the integrated difference between input and output fluxes. This inescapable fact requires that we find additional constraints to support data analysis. Frequently, as in the present work, those constraints have come from tracer kinetics. Our use of tracer–based RaNEFA flux measurements can be conceived as a 2-step process. First, the RaNEFA flux data were used as known inputs to the incompletely known fatty acid uptake system. This permits analysis of the factors regulating fatty acid uptake by tissues. Second, the RaNEFA data themselves were fitted to a rate law representing fatty acid release, which permitted characterization of adipose \( S_r \).

Other modeling approaches to this need for constraints have included carefully selected priors during parameter optimization and an explicit reliance on estimates from the literature. Remarkably, despite very different approaches to this issue, our range of maximal adipose release rates...
(2.04–10.38 $\mu$mol·min$^{-1}$·kgBW$^{-1}$) includes the value calculated from Table 4 of Periwal et al as $(l_0+l_2)$ (0.045 L/kg BW) = 2.04 $\mu$mol/min per kilogram.4 The INEFA mean value, 5.61 $\mu$mol/min per kilogram is greater, but this general agreement suggests that different methods are converging on the same answer. Useful constraints come in many forms. In the early literature on insulin and glucose kinetic modeling, Sherwin et al38 identified the utility of extravascular insulin concentration as the direct regulator of glucose uptake. Because these investigators suggested that the same interstitial insulin concentration is obtained in skeletal muscle and adipose tissue, we tested the hypothesis that extravascular insulin (calculated by applying our measured plasma insulin to Model A of Sherwin et al)38 would provide the requisite delays and dynamics to account for the observed NEFA data. This would have been a considerable simplification because insulin kinetics have been shown to vary little in human subjects over a large range of body mass index.45 Unfortunately, if extravascular insulin dynamics were modeled as rate determining for adipocyte release rate and myocyte fatty acid uptake, then the FCR of plasma NEFA would be forced to be 10-fold slower than FCRs measured in our subjects by the $^{13}$C-palmitate RaNEFA technique or as reported by others.6,10,11,13 This inconsistency suggests that the rate-determining steps in controlling plasma NEFA reside in the cellular insulin signaling pathways. This is desirable, because it offers the possibility of inferring changes in insulin signaling from plasma NEFA measurements during IVGTT and MTT. We conclude that the dynamics of extravascular insulin, by themselves, are insufficient to account for the required delays in insulin signaling. For this reason, we do not include an explicit extravascular insulin compartment; to do so would needlessly increase the number of parameters to be estimated from the insulin and NEFA data.

The INEFA model builds on the foundation laid by pioneering models of NEFA metabolism. Consequently, it has many structural features in common with those models. All published models include insulin inhibition of adipose lipolysis.3,4 This is well known as a major physiological determinant of the plasma NEFA time course. Another feature shared by all models is some method of delaying the action of insulin. One useful approach is the remote insulin compartment.1 We adopted the closely related first-order delay from Jelic.4

Figure 5. Relationships between insulin activation of plasma NEFA uptake (INEFA) model parameters, body fat, and metabolic variables: The figures demonstrate the relationships between parameters obtained from the INEFA model ($K_{i_{\text{Insulin}}}$ for insulin suppression of fatty acid release and $K_{m_{\text{FacUptake}}}$ for insulin stimulation of fatty acid uptake), those obtained during the intravenous glucose tolerance test (IVGTT; insulin sensitivity [SI]), and during the meal tolerance test (MTT; rate of appearance of NEFA into plasma [RaNEFA], body composition, and plasma concentrations of nonesterified fatty acid [NEFA] and long-chain acylcarnitines [LCAC]). FatOx is whole body fat oxidation assessed by indirect calorimetry, and LCAC represent the saturated, mono-, and polyunsaturated acylcarnitines with chain lengths from 12 to 18. $K_{i_{\text{Insulin}}}$ indicates insulin inhibition constant; $K_{m_{\text{FacUptake}}}$, $K_m$ for insulin; FacUptake, facilitated uptake; BW, body weight; FatOx, fat oxidation.
model presented by Jelic included insulin activation of lipoprotein lipase. Unfortunately, we were unable to resolve a lipoprotein lipase contribution, and this would be a goal for future studies. We adopted the concept of first-order delays, not only for insulin inhibition of adipose lipases but also for insulin activation of fatty acid uptake. Other models treat this delay by including a remote insulin compartment, driven by plasma insulin and turning over at some slower rate to be determined by fitting the NEFA data. Each method has its strengths; the first-order delay is arguably more physiological, and the parameters of a delayed or remote insulin compartment may be more readily identifiable.

Some published models control all insulin responsive processes with the same insulin-derived signal, as in the model XH in Periwal, whereas Jelic permitted different signaling and response times for different processes, an aspect also highlighted in the Model YH of Periwal. We found different response times to be both necessary and reasonable in terms of the underlying molecular cell biology. An innovative approach by 1 group extends the MINMOD concept even further by treating plasma glucose, rather than plasma insulin, as the driver of the surrogate control signal, obviating the need for insulin measurements altogether. Thus, the spectrum of models ranges from those with a focus on parameter estimation, to those endeavoring to represent explicitly the relevant physiological and molecular processes as they are understood.

Various rate laws have been used to quantify the lipolytic flux of fatty acids from adipose tissue. Periwal et al. have done a careful evaluation of at least 11 such rate laws and favor one that is the sum of an insulin-insensitive basal flux and a standard Hill term for the insulin-sensitive component. This same rate law, without a Hill coefficient, was also used by Jelic et al. For our subjects, the constant term was consistently driven to 0 during parameter optimization so that the rate law is identical to the H1(=0) model type in Periwal, except that we also include the first-order delay for insulin signaling as proposed in Periwal’s value of X, which corresponds to our Kiinsulin as 3.1-fold smaller. Hill coefficients are close; our MTT mean is 1.59 and theirs is 1.88. Differences in parameter values may well follow from our conclusion that an insulin-insensitive component of adipose lipolysis is unnecessary. Our values for the hormone-sensitive lipase signaling delay (mean, 55 minutes; range, 3–162 minutes) surround the value of 30 minutes reported for normal subjects but again reflect a wide range of strengths; the first-order delay is arguably more physiological.

Remarkably, mean insulin Ki for adipose release (Kiinsulin) and the K values for insulin activation of fatty acid uptake in the INEFA model were found to be similar numbers (11.0 and 12.3 pmol/kgBW). If these were distributed in plasma (0.045 L/kgBW) they would correspond to ~290 pmol/L or 42 µU/mL. If distributed in the extravascular, extracellular fluid (0.097 L/kg BW), then 140 pmol/L or 20 µU/mL. For either volume of distribution, our Ki for release is substantially greater than the values in either Jelic (7.2 µU/mL) or Periwal (11.3 µU/mL). This is consistent with reduced S in our metabolic syndrome subjects. Importantly, however, Ki and K values in a given subject were sometimes different. This suggests that in some subjects adipose NEFA release is more sensitive to insulin than is fatty acid uptake, whereas in other subjects the opposite is true. It may be interesting to determine whether this ratio of S correlates with any of the phenotype variables. If the Ki/Ki ratio is high (>2), then this would indicate more insulin resistance at the adipose, whereas if this ratio is closer to 1, it would indicate good suppression of adipose fatty acid release after a meal and relatively poor activation of fatty acid uptake after a meal.

From a clinical perspective, we found that the sensitivity of the glucose system to insulin is also reflected in the ability of insulin to reduce fatty acid release into plasma (Figure 5C). Further, plasma LCAC represent the products of incomplete fatty acid oxidation in the mitochondria, and we have shown previously in these subjects that the greater the fatty acid flux after a meal, the higher the plasma concentration of LCAC occurring at the same time. That result is extended here by showing that in the setting of poor, insulin-stimulated glucose disposal (Figure 5C), higher quantities of insulin were also needed to suppress adipose fatty acid release (Kiinsulin). In these same subjects, higher postmeal fat oxidation (Figure 5D) was observed along with higher concentrations of products reflecting incomplete fatty acid oxidation (LCAC, Figure 5F). The data are internally consistent and offer an integrated view of the impact of adipose insulin resistance on plasma NEFA flux and concentration. The sum of these relationships reveals that the S values calculated from an IVGTT with MINMOD mirror the more physiological interrelationships observed between glucose and fatty acid metabolism occurring after consumption of a mixed meal in obese subjects.

In summary, we have developed and tested a new model (INEFA) of human fatty acid metabolism whose structure is, in part, informed by recent molecular and cell biological findings. By doing so we have provided evidence, in a direct parallel to insulin regulation of glucose metabolism, that insulin is a significant activator of fatty acid uptake from plasma to tissues in humans with metabolic syndrome. Importantly, the methods described here permit quantification of the separate S of adipose fatty acid release and peripheral fatty acid uptake in single clinical protocol. It will, of course, be important to confirm the generality of this control mechanism in other groups of subjects and in different disease states.

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Disclosures

R.D. Phair and S.A. Lapidot are employed by Integrative Bioinformatics, Inc, developer of the ProcessDB modeling software
used in the present study. R.D. Phair is Chief Science Officer of Integrative Bioinformatics, Inc. The other authors have no conflicts to report.

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Supplementary Figure I. Data and model solutions for NEFA concentrations after the MTT

Subject 1

Subject 2

Subject 3

Subject 4

Subject 5

Subject 6

Subject 7

Subject 8

Subject 9

Subject 10

Subject 11

Subject 12

Subject 13

Subject 14

Subject 15
Supplementary Figure II. Data and model solutions generated from the rate of appearance of NEFA after the MTT.
Supplementary Figure III. Data and model solutions generated from the NEFA concentrations during the IVGTT