A novel minimal model to describe NEFA kinetics following an intravenous glucose challenge

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Boston RC, Moate PJ. A novel minimal model to describe NEFA kinetics following an intravenous glucose challenge. *Am J Physiol Regul Integr Comp Physiol* 294: R1140–R1147, 2008. First published January 30, 2008; doi:10.1152/ajpregu.00749.2007.—Dynamics of nonesterified fatty acid (NEFA) metabolism in humans requires quantification if we are to understand the etiology of such diseases as type 1 and 2 diabetes, as well as metabolic syndrome and obesity, or if we are to elucidate the mechanism of action of various interventions. We present a new compartmental model that employs the pattern of plasma glucose concentrations in healthy young adults to predict dynamic changes that occur in plasma NEFA concentrations during either a glucose-only intravenous glucose tolerance test, or an insulin-modified intravenous tolerance test, or a modified protocol during which variable-rate glucose infusions were administered to prevent plasma glucose from declining below 100 mg/dL. The model described all of the major features of NEFA response to an intravenous glucose tolerance test, including an initial latency phase, a phase during which plasma NEFA concentrations plummet to a nadir, and a rebound phase during which plasma NEFA concentrations may rise to a plateau concentration, which may be substantially higher than the initial basal NEFA concentration. This model is consistent with physiological processes and provides seven adjustable parameters that can be used to quantify NEFA production (lipolysis) and utilization (oxidation). When tested on data from the scientific literature, the range in estimated rate of lipolysis was 24–36 μmol·l⁻¹·min⁻¹ and for NEFA oxidation rate was 25–54 μmol·l⁻¹·min⁻¹. All model parameters were well identified and had coefficients of variation < 15% of their estimated values. It is concluded that this model is suitable to describe NEFA kinetics in human subjects.

 compartamental model; glucose tolerance test

PLASMA nonesterified fatty acids (NEFAs) are important substrates for triglyceride synthesis and gluconeogenesis, and they provide fuel for oxidation in nonadipose tissue (9, 14). Elevated plasma concentrations of NEFA are involved in the development of hypertriglyceridemia and insulin resistance as are often seen in pathological conditions, such as diabetes mellitus, obesity, hypertension, and coronary heart disease (6, 7, 8, 22, 25, 32, 35).

Blood NEFA concentrations may fluctuate considerably in the short term in response to eating, exercise, and stress (19, 26). The principal way in which NEFA concentrations are regulated involves the inhibition of hormone-sensitive lipase by insulin (17). When the concentration of glucose in plasma increases above a background level, insulin is released from the pancreas. Insulin mediates glucose disappearance from blood by increasing the availability of glucose transporters (GLUT-4) on the cell surface which enhances the uptake of glucose by tissue. Elevated plasma insulin levels also reduce the rate of hepatic endogenous glucose production. NEFA and glucose concentrations are related because they are both influenced by insulin (17, 20). Furthermore, NEFA and glucose metabolism are also linked because elevated plasma NEFA concentrations have a major role in inhibiting glucose metabolism (the Randle fatty acid cycle; 33) and because glucose and NEFA are known to be reciprocally regulated (the Sherringtonian metaphor; 40).

The intravenous glucose tolerance test (IVGTT) is a standard diagnostic procedure in which glucose (300 mg/kg) is injected over a time period of ~1 min, and frequent blood samples are collected during the subsequent 3 to 4 h. The IVGTT has long been used in humans to gauge the body’s capability of handling a glucose challenge and also the degree to which the pancreas can provide an appropriate insulin response to elevated plasma glucose apropos an IVGTT (24, 36). More recently, the IVGTT has been used to elucidate the relationship between plasma glucose and NEFA concentrations (39, 46).

A visual examination of the trajectories of plasma glucose, insulin, and NEFA concentrations that occur in response to an IVGTT indicates there is a systematic dynamic relationship between these entities. In 1979, Bergman and colleagues (4, 5) developed the glucose “Minimal Model” (MM) to facilitate kinetic analysis of plasma glucose and insulin concentrations apropos the IVGTT. Since that time, over 700 articles have been published based on the glucose MM, and the glucose MM has made some major contributions to the elucidation of the disordered interrelationship between glucose and insulin, especially in syndromes, such as diabetes mellitus, metabolic syndrome, and obesity (3). Clearly, a model of NEFA kinetics has the potential to also make a substantial contribution to the advancement of our understanding of NEFA metabolism in these syndromes. The glucose disposition index, which is derived from the glucose MM, is an integrated measure of the body’s ability to respond to hyperglycemia. The disposition index has been shown to be related to susceptibility to type 2 diabetes, and has also been shown to be genetically determined (1). Therefore, it seems reasonable to hypothesize that a NEFA model that contains parameters closely related to the rate of lipolysis in adipose tissue and the whole body rate of oxidation of fatty acids, and in which these parameters might also be genetically determined, would be useful for elucidating a number of metabolic diseases.

An examination of the scientific literature indicates there has been a number of metabolic models developed for the purpose of describing NEFA kinetics (10, 16, 34, 37, 41). The 1970
model of Srinivasan et al. (37) contains a large number of parameters and compartments and is described by an extensive set of differential equations, which may limit its routine use for research and clinical purposes. In contrast, the much more practical and potentially clinically useful model recently developed by Roy and Parker (34), contains just four differential equations and 18 parameters, nine of which are adjustable and the remaining nine fixed parameters have their values estimated from the scientific literature. Future research investigating the utility of these models may be warranted.

The early model of Fabian et al. (16) (the Fabian model) employs a simple exponential slope to describe the decrease in plasma NEFA concentrations that occur following an administration of glucose. Furthermore, the Fabian model uses a zero constant order to describe the rebound in plasma NEFA concentrations following their nadir. Thus, the Fabian model has the laudable quality of simplicity, but it does not have a structure that permits an elucidation of the dynamic interaction between plasma NEFA concentrations and plasma concentrations of either glucose or insulin. Furthermore, the Fabian model cannot describe the initial latency period before plasma NEFA concentrations begin their precipitous decline, and it cannot describe the rebound to a plateau concentration that occurs after NEFA concentrations reach nadir.

The more recent compartmental dynamic model of Thomaseth and Pavan (41) (the Thomaseth model), utilizes plasma insulin concentrations to predict the plasma concentrations of NEFA apropos an IVGTT. Like the Fabian model, the Thomaseth model cannot describe the initial latency period (28). Furthermore, the Thomaseth model assumes that the rebound plateau concentration of NEFA is the same as the initial or pre-IVGTT NEFA concentration (28). This assumption is a serious defect since NEFA concentrations often rebound to a plateau concentration of NEFA that substantially greater than the initial or pre-IVGTT NEFA concentration (39). We speculate that with a number of changes, especially related to assumptions regarding initial conditions, the Thomaseth model might be modified so that it can overcome these defects. Nevertheless, despite extensive efforts, our attempts in this area have as yet, been unsuccessful.

Recently, Boston et al. (10) have presented a novel minimal model to describe NEFA kinetics apropos the IVGTT in individual dairy cows (the Boston model). In the Boston model, glucose in a compartment “remote” from plasma, is used to predict the plasma concentrations of NEFA. Boston et al. (10) considered that this “remote” glucose might be a proxy for the action of insulin in adipose tissue or that it might also reflect the fact that elevated levels of glucose per se, in a remote compartment, may also reduce the net rate of lipolysis (2, 12, 15, 31). When applied to diverse data sets from individual dairy cows, the Boston model is able to accurately predict the plasma NEFA profile, including the initial latency period, the nadir in NEFA concentrations, and the rebound phase where plasma NEFA concentrations may reach a plateau concentration substantially greater than the initial or pre-IVGTT plasma NEFA concentration (10). The Boston model provides a number of parameters related to rate of lipolysis and oxidation of NEFA, and since in dairy cows, the plasma NEFA response to an IVGTT is similar to the NEFA response that occurs in humans, we speculate that the Boston NEFA model (10) or a slight variation on it, might be useful for modeling NEFA kinetics in humans. In this paper, we will evaluate a modified version of the Boston model in terms of its ability to describe NEFA kinetics in normal human subjects following a range of experimental protocols.

**MATERIALS AND METHODS**

**NEFA model.** The NEFA compartmental model presented here, is encapsulated in the following differential and ancillary equations. A schematic of the model is presented in Fig. 1.

\[
\frac{dR}{dt} = \frac{K}{100}[G'(t) - R(t)]
\]

\[
\frac{d\text{NEFA}}{dt} = S_{\text{NEFA}}[1 - h(t)] - \frac{K_R}{100} \text{NEFA}(t)
\]

where \(G(0) = G_0; g(t) = G(t - \tau), \) if \(t \geq \tau, \) else \(G_0; G'(t) = g(t) - \)gs, if \(g(t) > \)gs, else zero; \(R(0) = R_0
\]

\[
h(t) = \frac{1}{1 + \frac{\Phi}{R(t)}}
\]

\(\text{NEFA}(0) = \text{NEFA}_0, \) where \(r \) represents the time in minutes after an intravenous injection of glucose; \(G(t) \) [mmol/l] is a function describing the plasma glucose concentration and it is obtained by linear interpolation of the plasma glucose data; \(G_0 \) (mmol/l) is the initial or basal glucose concentration, \(g_0 \) (mmol/l) is a parameter that defines a threshold or set point in plasma glucose concentration above which elevated levels of plasma glucose result after a delay of \(\tau \) (min), in entry of plasma glucose into a remote or inaccessible compartment denoted by \(R(t) \) (mmol/l). In this model, \(R(t) \) is the principal driver of NEFA concentrations, and \(R(t) \) is analogous to \(X(t) \) or insulin action, which drives glucose concentrations in the Bergman minimal model of glucose (4, 5). The rate constant \(k_c \) (min), describes the movement of plasma glucose (above \(g_0 \)) into the remote compartment and also describes the clearance of glucose from the remote compartment.

\(\text{NEFA}(t) \) represents the plasma NEFA concentration (\(\mu\text{mol/l}) \) at \(t \) time.

The initial NEFA concentration, \(\text{NEFA}_0 \) (\(\mu\text{mol/l}) \) is the NEFA concentration measured at \(t = 0 \). The unitless function \(h(t) \) which takes values \(>0 \) and \(<1 \), is used to modulate the rate of NEFA production. The parameter \(\Phi \) (\(\mu\text{mol/l}) \) is an adjustable Michaelis Menten-type affinity constant. The two main parameters that can be obtained from this

![Fig. 1. A schematic of the mechanistic model developed to describe the pattern of change in plasma nonesterified fatty acid (NEFA) concentrations (\(\mu\text{mol/l}) \) following an intravenous glucose tolerance test. \(t \), time; \(G(t), \) linear interpolation of plasma glucose concentration (mmol/l); \(R(t), \) glucose concentration (mmol/l) in a remote compartment; \(\tau, \) delay in minutes.](http://ajpregu.physiology.org/DownloadedFrom:H11002/H9270/H3170/H8262/H11022/H20849/H20873/H20874)
model are $S_{\text{FFA}}$ (μmol/l/min) and $K_{\text{FFA}}$ (%/min). $S_{\text{FFA}}$ is a parameter describing the rate of provision of NEFA to the plasma pool. Because patients undergoing a standard IVGTT have been fasted for 12 h, it is assumed that the rate at which NEFA appear in blood as a result of intestinal absorption must be insignificant. Therefore, $S_{\text{FFA}}$ at time 0 primarily represents the rate of lipolysis of adipose tissue and $S_{\text{FFA}}$ is the potential maximum rate of lipolysis. $K_{\text{FFA}}$ is a rate constant which describes the rate at which NEFAs leave the plasma pool. Again, since patients undergoing a standard IVGTT have been fasted for 12 h, $K_{\text{FFA}}$ must primarily represent oxidation of fatty acids. We speculate that if an IVGTT were to be carried out in nonfasted patients, $K_{\text{FFA}}$ would, under the latter circumstances, represent both oxidation of fatty acids and sequestration of fatty acids into adipose tissue.

As well as these model parameters, a number of indices describing specific aspects of NEFA metabolism, may be derived from combinations of model parameters. For example, the instantaneous net rate of production of NEFA at time 0, LIP$_0$ (μmol/l/min), i.e., the rate of lipolysis is given by:

$$LIP_0 = S_{\text{FFA}} \left(1 - \left(\frac{R_0}{\Phi - R_0}\right)\right) \quad (3)$$

Similarly, the net rate of oxidation of NEFA at time 0, OX$_0$ (μmol/l/min) is given by

$$OX_0 = NEFA_0 K_{\text{FFA}} \quad (4)$$

Note that instantaneous rates of lipolysis and oxidation at any specific time may be determined by evaluating Eqs. 3 and 4 for those times. One assumption of this NEFA model which is reflected in Eqs. 3 and 4, is that before the glucose injection, i.e., just at time 0, the system may not necessarily be in a steady state with respect to the production and utilization of NEFA. Thus, the difference between LIP$_0$ and OX$_0$, which we term net transient lipolytic rate (NTLR$_0$, μmol/l/min), is a measure of the degree to which the system is not in steady state at time 0, NTLR$_0 = LIP_0 - OX_0$.

Two empirical indices that can be obtained from model predictions are the nadir of plasma NEFA concentrations [NEFA$_{\text{min}}$, μmol/l] and the time of the nadir (T$_{\text{min}}$) (min). One index, which we postulate as providing a measure of the flexibility of the system to alternate between using glucose or NEFA as an energy source, is the Suppression%:

$$\text{Suppression}\% = \frac{100(\text{NEFA}_0 - \text{NEFA}_{\text{min}})}{\text{NEFA}_0}$$

Experimental data. The data used to develop this model came from two previously reported experiments involving a range of subject types and experimental protocols (Table 1). Briefly, the experiment of Sumner et al. (39) involved 13 healthy, African-Americans who were administered an insulin-modified frequently sampled IVGTT (IM-FSIGT), and then, 4 wk later were administered a glucose-only IVGTT (GO-FSIGT). The experiment of Brehm et al. (11) involved 13 lean, healthy, subjects who underwent in random order, an IM-FSIGT and a modified protocol (IM-FSIGT-CLAMP) during which variable-rate glucose infusions were administered to prevent plasma glucose from declining below 100 mg/dl. Full details of patient demographics and experimental protocols have been described previously (op. cit.).

The mean glucose and NEFA data reported in Fig. 1, A and C in Sumner et al. (39), and Fig. 1, A and C in Brehm et al. (11) were used in this analysis. For each experiment, the relevant figure was electronically scanned and the data digitally extracted by means of UN-SCAN-IT, version 5.0 software (Silk Scientific, Orem UT).

Implementation and analyses. This nonlinear model was implemented using WinSAAM (http://www.winsaam.org), and the model was fitted to the NEFA and glucose data as described previously (10). In fitting the model to the data, G$_0$ and NEFA$_0$ were fixed at the time 0 values for plasma glucose and NEFA, respectively. To recognize that plasma glucose concentrations and glucose concentrations in remote compartments are in a continual state of dynamic change with small minute-to-minute fluctuations, the initial concentrations of glucose in the remote compartments is assumed to be $G_0$, an estimated parameter. The seven adjustable parameters in this model are: $k_C$, $S_{\text{FFA}}$, $K_{\text{FFA}}$, $g$, $R_0$, $\Phi$, and $\tau$, while the fixed or set parameters are $G_0$, and NEFA$_0$. Goodness of fit of model predictions to data was assessed by plotting measured data against model predictions and residuals against model predictions (38). Sensitivity analysis using the relative (or logarithmic) sensitivity function (18, 43) as the specific sensitivity measure, was performed to relate dependency of predicted NEFA concentrations on model parameters. Sensitivity analysis exposes the time-varying pattern of the fractional change in the predicted response per fractional change in the parameter under investigation.

### RESULTS AND DISCUSSION

Figures 2–5 show mean plasma concentrations of glucose and NEFA obtained from the publications by Sumner et al. (39) and Brehm et al. (11), and model-derived predictions of plasma NEFA concentrations.

Our objective was to develop a simple compartmental/kinetic model that could accurately describe all of the dynamic features present in the NEFA response to an intravenous glucose challenge in humans and provide sensitive and plausible estimates of parameters describing physiological processes. The Boston model (10) presented here was able to well describe the entire trajectory of plasma NEFA responses to a GO-FSIGT (Fig. 2), an IM-FSIGT (Figs. 3 and 4), and an IM-FSIGT-CLAMP (Fig. 5). In all cases, the R$^2$s were in excess of 0.99, the root mean square errors were < 12 μmol/l, and there were no substantial systematic deviations of model predictions from the observed data (Fig. 6). As can be seen in Figs. 2–5, and as has been reported previously (39, 46), when an FSIGT is conducted in humans, especially overweight or obese humans, there is often an extended latency period before NEFA concentrations begin a precipitous decline. In contrast, in lactating dairy cows there is generally only a short latency period. The particular strength of this model is that it can describe the four phases of the NEFA response, including the initial latency phase (a period of from 0 to 15 min). The

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>No. Subjects</th>
<th>Age, yr</th>
<th>BMI, kg/m$^2$</th>
<th>Protocol/Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sumner et al. (39)</td>
<td>AA</td>
<td>13</td>
<td>36 ± 6</td>
<td>31.4 ± 8.3</td>
<td>FSIGT</td>
</tr>
<tr>
<td>Sumner et al. (39)</td>
<td>AA</td>
<td>13</td>
<td>36 ± 6</td>
<td>31.4 ± 8.3</td>
<td>IM-FSIGT</td>
</tr>
<tr>
<td>Brehm et al. (11)</td>
<td>NS</td>
<td>13</td>
<td>26 ± 1</td>
<td>22.1 ± 0.7</td>
<td>IM-FSIGT</td>
</tr>
<tr>
<td>Brehm et al. (11)</td>
<td>NS</td>
<td>13</td>
<td>26 ± 1</td>
<td>22.1 ± 0.7</td>
<td>IM-FSIGT with glucose clamp</td>
</tr>
</tbody>
</table>

Values are means ± SD; BMI, body mass index; AA, African Americans; NS, race not specified; FSIGT, intravenous glucose tolerance test; IM-FSIGT, insulin-modified FSIGT.

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**Table 1. Source of data, description of subjects, and experimental protocols**

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_AJP-Regul Integr Comp Physiol • VOL 294 • APRIL 2008 • www.ajpregu.org_
The purpose of the insets in Figs. 2–5 is to clearly show the NEFA response and the goodness of fit of the model predictions during the latency period. NEFA concentrations during the latency period may either rise slightly (see Fig. 2, inset), persist at an approximately constant level, or even decline (Figs. 3 and 4).

In developing the compartmental model presented here, we essentially employed the same NEFA model that we previously developed for lactating dairy cows (10), but to accommodate the extended latency period that sometimes occurs in humans, we modified the original model by including an explicit delay ($\tau$). Note, in the insets to Figs. 2 and 3, the apparent delay is somewhat larger than the corresponding values for $\tau$ shown in Table 2. This is because, in the model, the total apparent delay is distributed across three parameters: the explicit delay $\tau$, the threshold parameter $g_s$, and the rate constant $k_c$, which is in effect an implicit delay. We speculate that $\tau$ may be related to the time it takes glucose and insulin concentrations within adipocytes to reflect glucose and insulin concentrations in the systemic plasma. A number of researchers have investigated the effects of including in the glucose minimal model, an explicit time delay associated with the transfer of the insulin signal from plasma to the remote insulin action compartment X (30, 42). They found that inclusion of an explicit time delay resulted in a better fit of model predictions to data and better identification of minimal model parameters. There is mounting experimental evidence that an important contributor toward this delay may be associated with transcapillary insulin transport (44).

The threshold parameter $g_s$ may also contribute to an apparent delay since $g_s$, in effect, prevents the system from responding until the glucose concentration exceeds the threshold concentration. A number of the physiological processes involved in the NEFA response to glucose might involve an effect manifested after a glucose threshold concentration. It has been
reported that glucose concentrations above a threshold of 5.5 mM stimulated the release in vitro of insulin from perfused rat pancreas (21). Since $g_s$ in the Boston model (10) ranged from 4.32 to 5.74 mM, it is tempting to speculate that $g_s$ may be related to the in vivo threshold glucose concentration in humans at which islet cells release insulin.

The rate constant $k_c$ describes both the rate of entry and exit of glucose (or possibly insulin action apropos of lipolysis) into the remote compartment R. Thus parameter $k_c$ can be regarded as being the rate of dissipation of glucose action (or insulin action) and is therefore analogous to parameter $P_2$ in the Bergman glucose minimal model (4, 5). However, $k_c$ necessarily confers a delay to this process. Since in the Boston model (10), we have included the explicit delay $\tau$ “upstream” of compartment R, this serves to more closely couple $\tau$ to the actual delay that occurs between when plasma glucose is first elevated and when lipolysis begins to be switched off. Thus, the inclusion of both $\tau$ and $g_s$ in the Boston model serve to some degree to uncouple the delay effect from $k_c$. We speculate that future application of this model to patients with poor circulation and metabolic disorders, such as insulin resistance, may help to elucidate the roles and specific nature of each of the delay-related parameters in the Boston model.

The model can also describe the second phase when NEFA concentrations decline precipitously and then the rate of decline slows to a “soft-landing” at the nadir of NEFA concentrations (see Figs. 2–4). The model can also accurately describe the third phase during which time NEFA concentrations gradually increase from nadir and then rise steeply until they reach the preglucose infusion level. Finally, the Boston model (10) is able to describe the fourth phase during which NEFA concentrations often continue to rise to a suprabasal plateau concentration (see Figs. 2–4). We are aware of no other NEFA kinetic model that has the structure or flexibility to describe all of these features. The results presented here with data from humans, duplicate our findings in dairy cows (10).

All parameters of this model were well identified with most parameters having coefficients of variation < 15% of the estimated value (Table 2). The Boston model (10) was easily fitted to each data set, with essentially the same parameter estimates being determined, regardless of initial estimates of model parameters.

### Table 2. NEFA model parameters and indices obtained by analysis of data extracted from two published experiments

<table>
<thead>
<tr>
<th>Experiment Protocol</th>
<th>Sumner et al. (39)</th>
<th>Brehm et al. (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
<td>FSIGT</td>
<td>IM-FSIGT</td>
</tr>
<tr>
<td>$G_b$, mmol/l</td>
<td>4.83</td>
<td>4.85</td>
</tr>
<tr>
<td>NEFA$_{b0}$, µmol/l</td>
<td>454</td>
<td>491</td>
</tr>
<tr>
<td>$R_0$, mmol/l</td>
<td>0.568±0.039</td>
<td>0.416±0.027</td>
</tr>
<tr>
<td>$k_c$, %/min</td>
<td>2.26±0.05</td>
<td>2.25±0.05</td>
</tr>
<tr>
<td>$S_{NEF}A$, µmol/l/min</td>
<td>45.0±1.8</td>
<td>40.8±1.3</td>
</tr>
<tr>
<td>$K_{FFA}$, %/min</td>
<td>5.44±0.18</td>
<td>5.48±0.16</td>
</tr>
<tr>
<td>$g_s$, mmol/l</td>
<td>4.32±0.016</td>
<td>4.41±0.037</td>
</tr>
<tr>
<td>$\tau$, min</td>
<td>3.54±0.46</td>
<td>5.48±0.38</td>
</tr>
<tr>
<td>$\Phi$, mmol/l</td>
<td>0.809±0.014</td>
<td>0.608±0.016</td>
</tr>
<tr>
<td><strong>Indices</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIP$_{b0}$, µmol/l/min</td>
<td>26.4±0.8</td>
<td>24.2±0.71</td>
</tr>
<tr>
<td>OX$_{b0}$, µmol/l/min</td>
<td>24.7±0.8</td>
<td>26.9±0.7</td>
</tr>
<tr>
<td>NTLR$_{b0}$, µmol/l/min</td>
<td>1.7±0.8</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>LIP$_{b060}$, µmol/l/min</td>
<td>26.9±0.7</td>
<td>30.7±0.8</td>
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<tr>
<td>LIP$_{b030}$, µmol/l/min</td>
<td>39.9±0.8</td>
<td>39.3±1.3</td>
</tr>
<tr>
<td>NEFA$_{min}$, µmol/l</td>
<td>179.3±0.7</td>
<td>159.2±0.7</td>
</tr>
<tr>
<td>$T_{min}$, min</td>
<td>73±4.4</td>
<td>64±5.5</td>
</tr>
<tr>
<td>Suppression, %</td>
<td>60.5±0.2</td>
<td>67.6±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SD. $G_b$, initial or basal glucose concentration; NEFA$_{b0}$, nonesterified fatty acid; $R_0$, the initial concentrations of glucose in the remote compartments; $k_c$, rate constant describing the movement of plasma glucose into the remote compartment and the clearance of glucose from the remote compartment; $S_{NEF}A$, rate of provision of NEFA to the plasma pool; $K_{FFA}$, rate constant which describes the rate at which NEFAs leave the plasma pool; $g_s$, a threshold or set point in plasma glucose concentration above which elevated levels of plasma glucose result after a delay of $\tau$ (min), in entry of plasma glucose into a remote or inaccessible compartment; $\Phi$, adjustable Michaelis Menten-type affinity constant; LIP$_{b0}$, the rate of lipolysis; OX$_{b0}$, net rate of oxidation of NEFA at time 0; NTLR$_{b0}$, net transient lipolytic rate; $T_{min}$, time of the nadir; Suppression, index providing a measure of the flexibility of the system to alternate between using glucose or NEFA as an energy source.
An important assumption imbedded in this model, and one which distinguishes it from a number of other metabolic models is that it is assumed that the system is not necessarily in steady state at time 0. As stated above, this assumption recognizes the fact that even in a fasted patient at rest, plasma NEFA concentrations may undergo substantial minute-to-minute fluctuations. The index NTLR0 provides an indication of whether at time 0, the production rate of NEFA exceeds the utilization rate (see Fig. 2, inset) or if the utilization rate exceeds the production rate (see Figs. 3–5, insets). As can be seen in Figs. 2–5, insets, if the slope of the NEFA curve at time 0 is positive, this indicates that NTLR0 is positive, while a negative slope to the NEFA curve at time 0 indicates that NTLR0 is negative.

One aim of the work presented here was to determine the influence of experimental protocol on the parameters of the Boston NEFA model (10). As can be seen in Table 2, in the experiment by Sumner et al. (39), the mean NEFA0 was slightly higher when the patients were administered the IM-FSIGT. Similarly, in the experiment of Brehm et al. (11), NEFA0 was substantially higher in the subjects receiving the glucose clamp compared with those receiving the IM-FSIGT only. These differences in NEFA0 rather than experimental protocol per se, might be responsible for the small differences in the magnitudes of model parameters when the patients received the different protocols. LIP0 and OX0, the indices describing, respectively, the rate of lipolysis and rate of oxidation at the start of the experiment, were not influenced by experimental protocol in either the experiment by Sumner et al. (39) or the experiment by Brehm et al. (11).

From Table 2, we can make some very preliminary observations and speculations regarding model parameters, as well as rates of lipolysis and oxidation. First, despite the experiments of Sumner et al. (39) and Brehm et al. (11) having been carried out at different times and on different continents, probably with subjects of different races, but certainly of different mean age and body mass index (BMI), the parameters of the NEFA model were not inordinately different for both groups. This observation supports the general applicability of this model. Second, it appears that LIP0 is unrelated to NEFA0, demonstrating that plasma NEFA concentration per se cannot be used as an index of rate of lipolysis. Third, it is apparent that the estimated LIP0 in the subjects in the experiment of Brehm et al. (11) is ~50% higher than in the patients in the experiment of Sumner et al. (39). We speculate that part of the explanation for this difference might be the higher BMI of the patients in the Sumner experiment compared with the lower BMI in the patients in the Brehm experiment, as total body rates of lipolysis tend to be related to the fat-to-lean body mass ratio rather than to total body mass per se (27).

As is shown in Fig. 7, although rates of lipolysis and oxidation of NEFA can vary considerably, they are generally approximately balanced or somewhat out of phase. In Fig. 7, it can also be seen that the nadir in NEFA concentration occurs when the rate of NEFA production (lipolysis) is equal to the rate of NEFA utilization (oxidation), and that during the terminal plateau phase, the rates of NEFA production and utilization are also equivalent.

Previously, to estimate whole body rates of lipolysis and oxidation of NEFA, researchers have used intravenous infusions of isotopic tracers to measure glycerol rate of appearance and NEFA oxidation rate (μmol·kg⁻¹·min⁻¹) (26, 28). The assumptions involved with the tracer technique are that glycerol has a volume of distribution of 0.2 l/kg bodyweight and that 3 mol of NEFA are produced for every mole of glycerol released during lipolysis in adipose tissue. Rates of glycerol appearance in healthy, young, fasted adults, at rest and with normal plasma glucose concentrations, have ranged from ~3.0 to 3.5 μmol/kg/min (13, 23, 26). These rates of

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Innovative Methodology

A MINIMAL MODEL OF NEFA KINETICS

glycerol appearance translate to rates of lipolysis of between 45.0 and 52.5 μmol/l/min. Our estimates for minimum and maximum rates of lipolysis (24–61 μmol·kg⁻¹·min⁻¹) straddle the literature estimates for rates of lipolysis. Similarly, our estimate for the range in rates of NEFA oxidation (25–54 μmol·kg⁻¹·min⁻¹) are consistent with a value of 35 μmol·kg⁻¹·min⁻¹ that can be calculated from published data (28), if it is assumed the volume of distribution of NEFA is 40 ml/kg.

Figure 8 depicts the relative sensitivity of the five major parameters of the model applied to the model solution of the Summer IM-FSIGT data. From Fig. 8, it can be seen that $K_{FFA}$ has a strongly moderated peaked negative influence on the response prior to the nadir, but after the nadir, its negative effect, although blunted, persists for the duration of the solution. $K_c$ also has a negative effect up to the nadir, and then following a slight positive peak, its effect becomes vanishingly small. $Φ$ and $S_{FFA}$ both have only positive effects on the response with $Φ$ having a small peak around the time of the NEFA nadir, and thereafter, the influence of $Φ$ also becomes vanishingly small. Parameter $S_{FFA}$ has a positively increasing involvement in the shape of the NEFA profile up to the nadir, and beyond this has a steady effect. The most interesting profile observed among the relative sensitivities is that for $g_s$. The sensitivity of the response to $g_s$ in the time region between the NEFA nadir and the point at which NEFA crosses its baseline concentration, reaches a peak value, which is positive and at least twice the magnitude attained by any of the other system parameters considered here. Following this positive peak of influence of $g_s$ on the NEFA response, the influence of $g_s$ declines steadily to time 300 min.

The usefulness of a biological model depends not only upon its ability to characterize a system and whether or not it can be used to diagnose abnormally functioning systems, but also on its practicability, simplicity, and cost of implementation. The NEFA model presented here can certainly characterize NEFA kinetics well. Furthermore, unlike techniques that rely on complicated and expensive tracer technology, it is practicable and relatively inexpensive since it relies on analysis of data from a standard or modified FSIGT. A further cost advantage of the Boston model (10) is the fact that it employs just plasma glucose and NEFA and unlike the models of Thomas-eth and Pavan (41) or Roy and Parker (34), does not require expensive data on plasma insulin. Accurate estimation of NEFA kinetics depends upon accurate measurement of NEFA concentrations in plasma. Therefore, blood samples should not be collected into heparinized containers, as heparin has been shown to stimulate lipolysis of triglycerides in vitro (45). For this reason, specific steps should be taken to minimize the artificial elevation of plasma NEFA due to in vitro lipolysis (45).

Perspectives and Significance

The NEFA model presented here is based on known physiology and is a "minimal" model in that it is the simplest model we could devise, containing the smallest number of compartments and parameters necessary to describe all of the features of the NEFA response to an intravenous glucose challenge. Although the Boston model (10) holds great promise as a research and diagnostic tool, much research is required before it can have practical clinical application. The modeling described in this paper was on mean data from normal subjects. It is possible that in severely diabetic patients, where the relationship between elevated plasma glucose and insulin breaks down, that the model described here might not adequately describe a NEFA response. Therefore, future research will focus on challenging the model with datasets from insulin-resistant and severely diabetic patients and patients with obesity and metabolic syndrome. An important outcome of such work would be the determination of means and ranges for parameter estimates of these populations. It is also desirable that experiments are conducted in which rates of lipolysis and oxidation as determined by the Boston model are directly compared with rates of lipolysis and oxidation as determined by using tracer techniques. Furthermore, although in this investigation data from standard protocols for FSIGT without additional insulin, the IM-FSIGT, and the IM-FSIGT with glucose clamp were successfully analyzed, further work may be needed to identify sampling protocols (number of samples and timing of samples) specifically optimized for estimating parameters of this NEFA model. Further research could also examine the suitability of this model for analysis of data resulting from oral glucose tolerance tests.

In conclusion, the new NEFA minimal model presented here was able to characterize all of the major features of a NEFA response to either a GO-FSIGT, an IM-FSIGT, or an IM-FSIGT-CLAMP. The model contains seven adjustable parameters that describe various aspects of the mechanisms that control NEFA kinetics. All model parameters can be easily estimated and are well identified. Further research is required to establish normal ranges for model parameters and to identify ranges for specific parameters that may either indicate the presence of metabolic abnormality or perhaps even presage the emergence of abnormal syndromes.

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REFERENCES


