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**RESEARCH ARTICLE**

# Trafficking of nonesterified fatty acids in insulin resistance and relationship to dysglycemia

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**Walker RE, Ford JL, Boston RC, Savinova OV, Harris WS, Green MH, Shearer GC.** Trafficking of nonesterified fatty acids in insulin resistance and relationship to dysglycemia. *Am J Physiol Endocrinol Metab* 318: E392–E404, 2020. First published January 7, 2020; doi:10.1152/ajpendo.00331.2019.—In adipose, insulin functions to suppress intracellular lipolysis and secretion of nonesterified fatty acid (NEFA) into plasma. We applied glucose and NEFA minimal models (MM) following a frequently sampled intravenous glucose tolerance test (FSIVGTT) to assess glucose-specific and NEFA-specific insulin resistance. We used total NEFA and individual fatty acids in the NEFA MM, comparing the model parameters in metabolic syndrome (MetSyn) subjects ($n$ = 52) with optimally healthy controls (OptHC; $n$ = 14). Results are reported as mean difference (95% confidence interval). Using the glucose MM, MetSyn subjects had lower [−73% (−82, −57)] sensitivity to insulin ($S_i$) and higher [138% (44, 293)] acute insulin response to glucose ($AIR_g$). Using the NEFA MM, MetSyn subjects had lower [−24% (−35, −13)] percent suppression, higher [32% (15, 52)] threshold glucose ($g_s$), and a higher [81% (12, 192)] affinity constant altering NEFA secretion ($\phi$). Comparing fatty acids, percent suppression was lower in myristic acid (MA) than in all other fatty acids, and the stearic acid (SA) response was so unique that it did not fit the NEFA MM. MA and SA percent of total were increased at 50 min after glucose injection, whereas oleic acid (OA) and palmitic acid (PA) were decreased ($P < 0.05$). We conclude that the NEFA MM, as well as the response of individual NEFA fatty acids after a FSIVGTT, differ between OptHC and MetSyn subjects and that the NEFA MM parameters differ between individual fatty acids.

adipose; compartmental modeling; fatty acids; insulin resistance

## INTRODUCTION

The metabolic syndrome (MetSyn) is a prediabetic syndrome characterized by insulin resistance that clusters three or more of the following conditions: elevated fasting glucose, abdominal obesity, elevated plasma triglyceride (TG) levels, hypertension, and low HDL cholesterol (HDL-C) (10, 40). MetSyn results in increased risk for cardiovascular disease and other comorbidities (22). Along with liver and skeletal muscle, adipose tissue is an important target for insulin. Insulin acts to reduce blood glucose levels by facilitating uptake by the liver, skeletal muscle, and adipose tissue as well as stimulating glucose storage in liver. However, insulin's primary function in adipose tissue is to suppress the intracellular lipolysis catalyzed by hormone-sensitive lipase (HSL). The result is suppressed release of nonesterified fatty acid (NEFA) from adipose storage to the plasma. This suppression of NEFA limits availability of the fatty acids and increases systemic glucose utilization (15). Shearer et al. (36) hypothesized that the dyslipidemia of MetSyn is a result of increased NEFA flux from adipose tissue to the liver without significant elevation of circulating NEFA concentrations. This is supported by evidence that increased hepatic synthesis, not reduced clearance, of very low-density lipoprotein (VLDL) is the most common mechanism for hypertriglyceridemia in MetSyn (12, 13, 29). To assess NEFA flux, a dynamics approach must be applied.

Compartmental modeling is useful to describe the dynamic relationships between insulin, glucose, and NEFA metabolism. In many cases, a glucose challenge is administered as either an oral glucose tolerance test (OGTT) or a frequently sampled intravenous glucose tolerance test (FSIVGTT), and plasma glucose and insulin kinetics are followed over time. The glucose minimal model (MM) was developed by Bergman et al. (3) and Boston et al. (9) to study the interrelationship between insulin and glucose and has advanced current understanding of insulin resistance (30). Importantly, parameters and key indices derived from the glucose MM are quantitatively different between lean and obese subjects and between healthy and metabolic disease states (3, 30). This model adequately describes the glycemic response to insulin signaling, but it does not capture the effects of insulin on NEFA, so this model may not detect changes in adipocyte insulin sensitivity. To study the role of adipocyte responsiveness in the etiology of metabolic diseases, the NEFA MM was subsequently proposed by Boston and Moate (6). In this model, plasma glucose concentration and glucose in a functional compartment, presumably a proxy for insulin action in adipose tissue, are used to predict plasma NEFA response and describe NEFA kinetics (8). The NEFA MM has been used to describe the kinetics of adipose tissue lipolysis and whole body fatty acid oxidation/utilization in lean and obese individuals with and without type 2 diabetes utiliz-

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ing both the FSIVGTT and OGTT (1, 6, 7, 32). Although both the glucose MM and the NEFA MM have been utilized to compare insulin-resistant or obese subjects to apparently healthy or lean controls, no study to date has recruited definitively healthy subjects. In this study, we characterize the glucose MM and NEFA MM in MetSyn and optimally healthy control (OptHC) subjects recruited for optimal BMI, plasma TG, and TG/HDL-C. This ensures that we are comparing MetSyn with the healthy condition rather than a milder MetSyn phenotype. By utilizing both the glucose MM and the NEFA MM, we are able to validate that both models are useful for identification of MetSyn in the same population. We may also be able to identify subpopulations of MetSyn with predominant adipose tissue insulin resistance or predominant skeletal muscle and liver insulin resistance.

Plasma concentrations of individual NEFA species are also associated with insulin sensitivity. In a cohort of individuals at risk for diabetes, nonesterified palmitic acid (PA), oleic acid (OA), and linoleic acid (LA) were inversely related, and eicosapentaenoic acid (EPA) was positively related, to insulin sensitivity as measured by a number of different indices, including HOMA-IR, ISSI-2, and the Matsuda index (19). These three fatty acids, along with stearic acid (SA), make up the majority of the fatty acids in the NEFA fraction, with arachidonic acid (AA), vaccenic acid (VA), α-linolenic acid (ALA), palmitoleic acid (POA), and myristic acid (MA) also found in detectable concentrations (19, 42). In a different biomarker study, plasma nonesterified OA was a significant predictor of insulin resistance (39). This evidence suggests that individual fatty acids may be useful markers of insulin resistance, but little is known about the effects of insulin on individual fatty acid kinetics. To date, no study has attempted to model the response of individual fatty acids during a FSIVGTT.

Here, we used compartmental modeling to characterize differences in adipocyte insulin sensitivity among MetSyn subjects with subclinical insulin resistance and OptHC subjects following a FSIVGTT. Furthermore, by comparing results obtained from the NEFA MM (6) and the glucose MM (3, 9), we attempted to identify differences in adipocyte insulin sensitivity compared with glycemic measures of insulin sensitivity. Finally, we used the NEFA MM to investigate whether changes in NEFA response were similar across all fatty acids or whether some fatty acids responded differently from the total NEFA during a FSIVGTT. We hypothesize that using the response of individual fatty acids, such as OA, to a glucose challenge could provide a novel measure of adipose tissue insulin resistance.

## RESEARCH DESIGN AND METHODS

Details of the parent clinical trial, its design, and other methods have been reported previously (5, 33, 35). To distinguish between NEFA trafficking in optimal insulin sensitivity and MetSyn, we added to our original trial by recruiting age- and sex-matched OptHC individuals from the same population. We compare the NEFA response to glucose using a FSIVGTT in OptHC to MetSyn subjects during their baseline visit. Additionally, in a detailed analysis, we compare the response of individual fatty acids in a subset of both OptHC and MetSyn subjects.

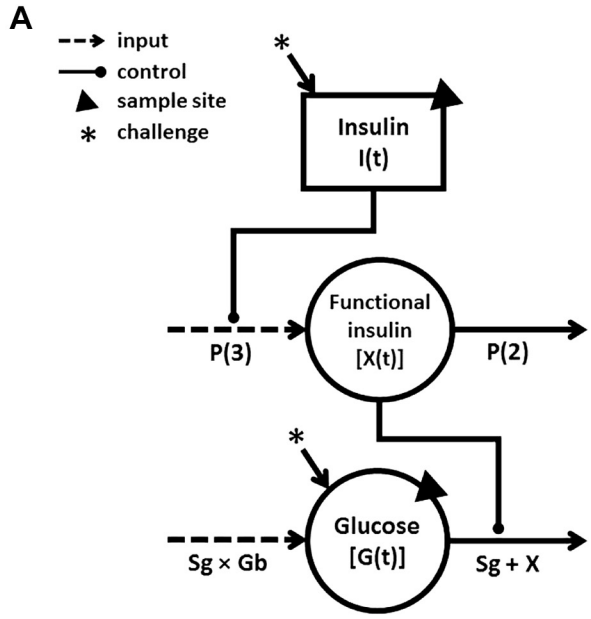
*Participants and study location.* The study was conducted in Sioux Falls, SD. Subjects with MetSyn were recruited using the following primary inclusion criteria: BMI between 25 and 40 kg/m$^2$, fasting TG between 1.7 and 8.5 mmol/L, HDL-C >0.26 mmol/L, and TG/HDL-C >3.5. These subjects continued participation in a randomized controlled trial (35). OptHC controls were co-recruited based on the following criteria: BMI between 21 and 25 kg/m$^2$, fasting TG <1.5 mmol/L, TG/HDL-C <3.0, and not being treated for any other condition. The protocols for both the parent trial and the OptHC subjects were approved by the Institutional Review Board at the University of South Dakota, and informed consent was required before participation. The study was registered at clinicaltrials.gov (NCT00286234).

*Study design.* The MetSyn subjects were recruited into a randomized, double-blind, placebo-controlled clinical trial with an additional arm of OptHC controls who were recruited alongside the MetSyn subjects during their baseline visit to provide reference measures. Only baseline data from these groups are considered here. After qualifying, OptHC subjects participated in only one visit. Subjects were asked to fast for ≥8 h before their visit. Upon arrival at the study unit, blood was drawn for fasting lipids, and then the FSIVGTT was administered. Plasma was separated from all blood samples within 30 min and stored at −70°C.

*Frequently sampled intravenous glucose tolerance test.* The test commenced at 8 AM following an overnight fast where fasting plasma was collected. At roughly 10 AM on the day of the test, two cannulae were placed in the same arm: one for infusion (forearm vein) and one for sampling (dorsal hand vein). After 30 min, samples were drawn from the antecubital vein at −10 and −1 min to determine baseline NEFA concentrations. At *time 0*, 0.3 g/kg glucose was injected intravenously from the opposite arm for 1 min. Starting at 20 min, 4 mU/kg insulin was infused into the opposite arm for 5 min. Venous samples were collected from the original arm at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min. Blood was analyzed for NEFA (Wako, Richmond, VA) and glucose (Sigma-Aldrich, St. Louis, MO) concentrations using standard colorimetry and for insulin and C-peptide using a Luminex 100/200 System (Austin, TX) and kits from Millipore (Billerica, MA).

*Individual fatty acids.* Fatty acids were extracted with a modified Bligh and Dyer (4) extraction, and NEFA were isolated using solid phase extraction (20). Briefly, plasma lipid extracts were loaded onto aminopropyl solid-phase extraction columns (Supelclean LC-$NH_2$; Supelco, Bellefonte, PA) conditioned with hexane. Neutral lipids were eluted and discarded, and then NEFA were eluted in 2% acetic acid in diethyl ether. Samples were dried under nitrogen and methylated with boron triflouride. Fatty acid methyl esters were analyzed by GC-MS using a Shimadzu GC-2010 gas chromatography system coupled with a QP2010 EI mass spectrometric detector (GCMS-QP2010; Shimadzu, Kyoto, Japan) using a Supelco SP-2560 fused silica column (100 m in length, 0.25 mm in diameter, 0.2-μm thickness; Supelco). Individual peaks were detected and quantified from mass spectrum data based on a multiple ion count and adjusted for response factors calculated from a standard fatty acid methyl ester mix. PA and SA concentrations were adjusted by subtracting background signal in blank samples.

*Compartmental modeling.* To describe the glucose response to a FSIVGTT, the glucose MM was applied using the MINMOD Millenium software (3, 9) (Fig. 1*A*). In the model shown in Fig. 1*A*, plasma insulin [I(*t*)] drives the plasma glucose response [G(*t*)] via its action in the functional insulin compartment [X(*t*)], previously called remote insulin (3). Using this software, insulin (mU/L) and glucose (mmol/L) concentration-time curves for each subject were fit to the model to obtain estimates for sensitivity to glucose ($S_g$), sensitivity to insulin ($S_i$), acute insulin response to glucose ($AIR_g$), disposition index (DI), glucose effectiveness at zero insulin (GEZI), and insulin-attributable glucose disposal (IAGD) (3); homeostatic assessment (HOMA) β-cell function and HOMA insulin resistance (HOMA-IR) were also calculated (26). For details on model outcomes and differential equations, see the APPENDIX.




| Parameter | OptHC | MetSyn |
|---|---|---|
| $G_0$ [mmol/L] | 9.65 | 11.5 |
| $G_b$ [mmol/L] | 5.18 | 5.65 |
| $I_b$ [mU/L] | 43.0 | 100 |
| P(2) [$min^{-1}$] | 0.109 | 0.147 |
| P(3) [$(mU/L)^{-1}\times min^{-2}$] | $1.7x10^{-5}$ | $6.7x10^{-6}$ |
| $S_g$ [$min^{-1}$] | 0.0238 | 0.0152 |
| $S_i$ [$(mU/L)^{-1}\times min^{-1}$] | 1.56 | 0.456 |
| AIRg [$mU\times L^{-1}\times min$] | 799 | 1824 |
| DI | 1242 | 832 |
| GEZI [$min^{-1}$] | 0.0171 | 0.0104 |
| HOMA β-cell function [mU/(mmol/L)] | 510 | 25.2 |

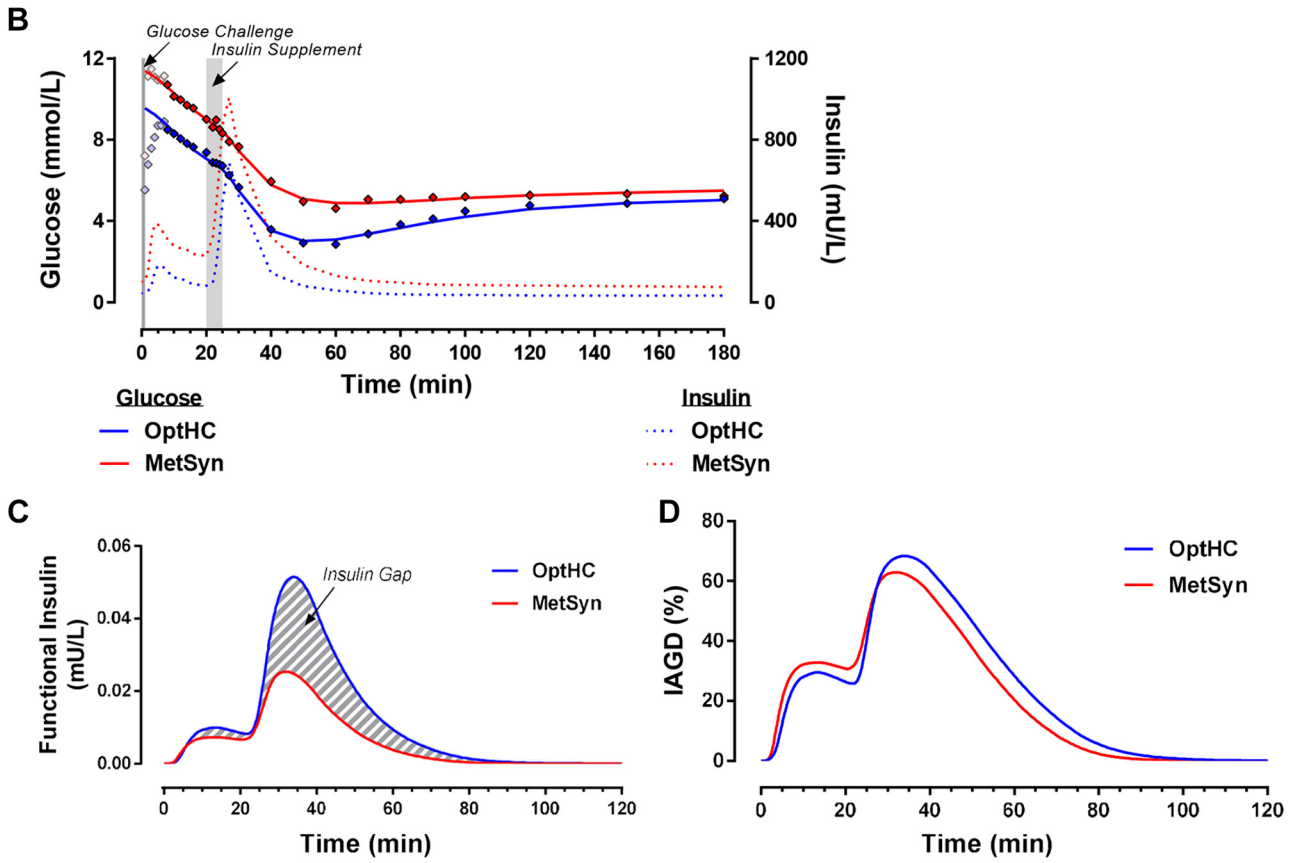



Fig. 1. Application of the minimal model of glucose and insulin dynamics following a frequently sampled intravenous glucose tolerance test (FSIVGTT) using the MinMod Millennium software (9). *A*: compartmental model for insulin and glucose kinetics. *B*: geometric mean data (symbols) and model predicted curves for plasma insulin (dotted lines) and glucose (solid lines) concentrations vs. time for 14 optimally healthy control (OptHC; blue) and 52 metabolic syndrome (MetSyn; red) subjects. A glucose challenge was injected at *time 0*, and starting at 20 min, insulin was infused for 5 min. Goodness of fit of model predictions to observed data was strong for both groups, with an $R^2$ of 99%. *C*: model-simulated insulin in the functional compartment vs. time for the OptHC (blue) and MetSyn (red) groups. *D*: insulin-attributable glucose disposal (IAGD) calculated as a function of time. IAGD starts higher in MetSyn, but OptHC subjects spike at a higher level following the insulin infusion at 20 min. $AIR_g$, acute insulin response to glucose; DI, disposition index; $G_0$, plasma glucose concentration immediately following glucose challenge; $G_b$, baseline plasma glucose concentration; GEZI, glucose effectiveness with zero insulin; HOMA, homeostatic model assessment; $I_b$, basal insulin; P(2), rate of insulin removal from the functional compartment; P(3), insulin removal from the functional compartment; $S_g$, glucose effectiveness; $S_i$, insulin sensitivity.

To describe the NEFA response to a glucose challenge, the NEFA MM (Fig. 2*A*) (6) was employed using the Windows version of Simulation, Analysis, and Modeling software [WinSAAM version 3.3.0; http://winsaam.org (38, 43)]. In the NEFA MM, plasma glucose [G(*t*)] drives the plasma NEFA response [NEFA(*t*)] via its action in the functional glucose compartment [F(*t*)], previously called remote glucose (6), after a latency period; note that glucose action is analogous to insulin action in the glucose MM [X(*t*) in Fig. 1*A*]. Parameters defined by the NEFA model (Fig. 2*B*) are described in Fig. 2 and the APPENDIX. For each subject, data for plasma glucose (mmol/L) and NEFA (μmol/L) were plotted versus time and fit to the model. Final parameter estimates and their uncertainty were obtained using weighted nonlinear regression analysis and were used to estimate glucose threshold for NEFA suppression ($g_s$), rate of glucose removal from plasma ($k_c$), NEFA secretion ($S_{NEFA}$), NEFA fractional clearance ($K_{NEFA}$), and the affinity constant altering NEFA secretion (Φ); baseline NEFA secretion from adipose by lipolysis ($LIP_0$), baseline NEFA clearance rate ($CL_0$), and percent NEFA suppression were also calculated. Bayesian constraints were applied to the model parameters when new estimates were calculated to ensure that historic information was not neglected and to improve the reliability of the estimates. See the APPENDIX for description of model outcomes and differential equations.

*Compartmental modeling for individual fatty acid analysis.* To study the response of individual fatty acids in total plasma NEFA, eight OptHC subjects and eight MetSyn subjects were selected based on their NEFA MM glucose threshold ($g_s$); MetSyn subjects with the lowest and OptHC subjects with the highest values for $g_s$ were included. Seven fatty acids in the NEFA fraction with sufficient data quality were used for modeling: MA, PA, POA, SA, OA, LA, and ALA. To compare kinetics of individual fatty acids, we plotted concentration-time curves for each fatty acid, and data were fit to the NEFA model (Fig. 2*B*), following the process described above. Final parameter estimates were compared with identify differences in trafficking of the individual fatty acids for this subset of OptHC and MetSyn subjects.

To determine whether the trafficking of fatty acids differed from total NEFA, total NEFA and individual fatty acid concentration data were normalized to baseline. Geometric means were calculated for the normalized data and fit to the NEFA MM, using an approach to test the minimal change theory/hypothesis by comparing the weighted residual sum of squares for different model fits. First, total NEFA was

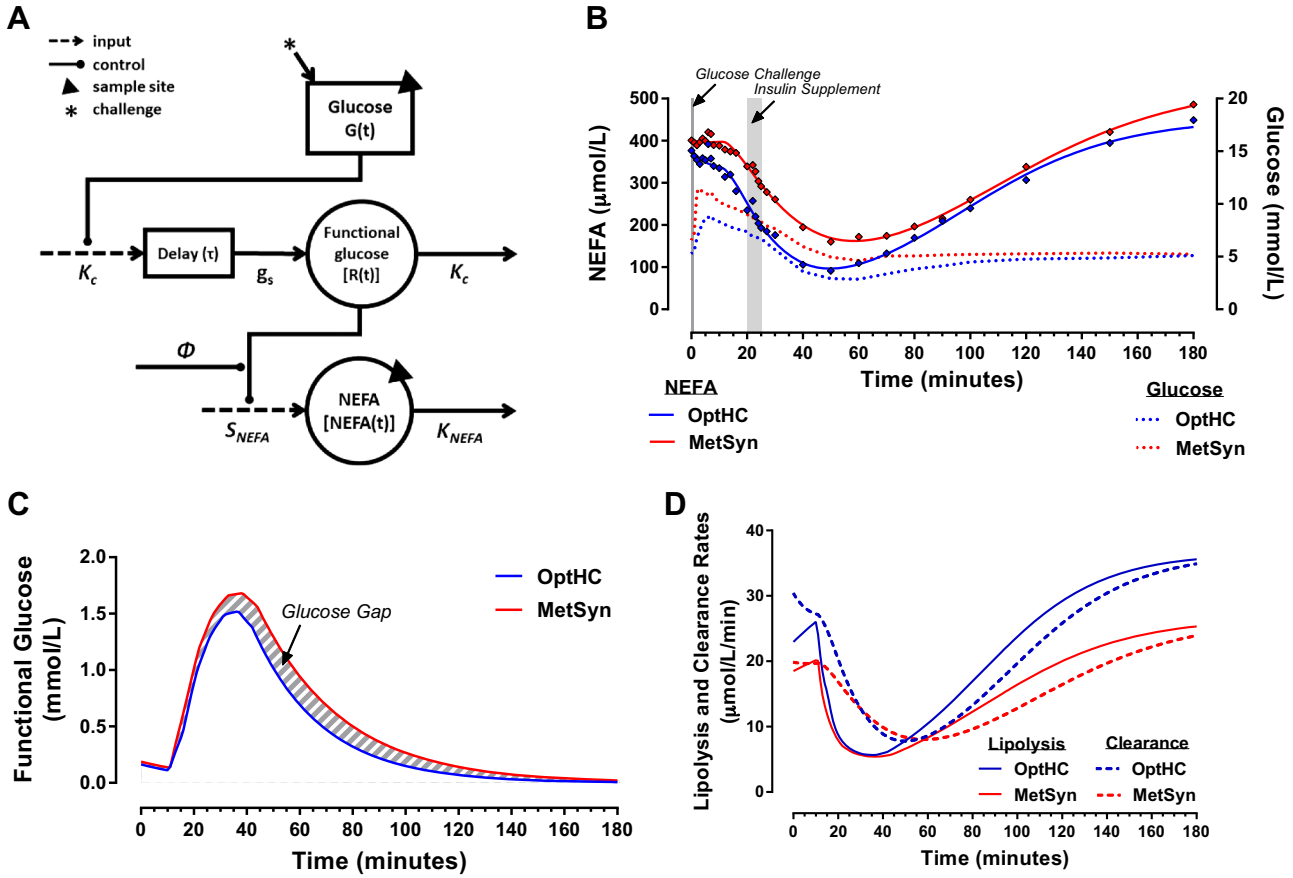



Fig. 2. Application of the minimal model of nonesterified fatty acid (NEFA) dynamics following a frequently sampled intravenous glucose tolerance test (FSIVGTT). *A*: NEFA minimal models (MM) (6) for glucose and NEFA kinetics. *B*: geometric mean data (red and blue diamonds) and model predicted curves for plasma glucose (dotted lines) and NEFA (solid lines) concentrations vs. time for 14 optimally healthy control (OptHC; blue) and 52 metabolic syndrome (MetSyn; red) subjects. A glucose challenge was injected at *time 0*, and starting at 20 min, insulin was infused for 5 min. A fractional standard deviation (FSD) of 0.02–0.08 was used as a weighting factor on the NEFA data. Parameter identifiability was assessed and a FSD of $< 0.5$ was considered well-identified. Goodness of fit was evaluated by comparing the model predictions with the observed values; $R^2$ was calculated using linear regression analysis. Model results were then used to calculate the rates of lipolysis ($LIP_0$) and clearance at *time 0* ($CL_0$) and as a function of time. NEFA %suppression was calculated from initial NEFA concentration ($NEFA_0$) and NEFA concentration at the nadir. *C*: model simulated glucose action in the functional compartment vs. time for the OptHC (blue) and MetSyn (red) groups. *D*: lipolysis and clearance rates were calculated as a function of time. Both lipolysis and clearance rates are generally lower and are suppressed less after insulin infusion in MetSyn subjects. $g_s$, threshold glucose concentration; $k_c$, rate of %transfer of glucose into the functional compartment from the plasma compartment; $K_{NEFA}$, NEFA %clearance rate; Φ, Michaelis-Menton affinity constant; $S_{NEFA}$, initial rate of NEFA secretion; τ, delay time before NEFA suppression begins.

fit to the model, and the weighted residual sum of squares were obtained. Then, kinetic parameters for each individual fatty acid were fixed at the value derived for the total NEFA. Next, several parameters were tested in this analysis by fixing all parameters at the values calculated for the total NEFA and making one specified parameter adjustable at a time. The best fit model for each fatty acid was determined based on the weighted residual sum of squares and an F-statistic; using this strategy, model complexity was increased only when it resulted in a significant improvement in the sums of squares (23). A more complex model indicates that a fatty acid requires kinetic parameters different from the total NEFA to achieve the best fit model.

*Statistical methods.* For each model, parameter estimates and calculated indices were compared using a mixed-model ANOVA adjusting for age and sex. Residuals were examined for normality and homogeneity, and a natural log transformation was used as needed for improved model assumptions. $P < 0.05$ was considered statistically significant. Analyses were performed using JMP software (version 13.1.0; SAS Institute Inc., Cary, NC).

## RESULTS

*Subject characteristics.* The baseline demographic values for OptHC and MetSyn subjects have been reported previously (34). FSIVGTT data were available for 14 OptHC and 54 MetSyn subjects. Acceptable model convergences were not achieved in two MetSyn subjects, resulting in a final sample size of 14 OptHC and 52 MetSyn subjects (Table 1). Although our recruitment criteria focused on TG and HDL levels and BMI, MetSyn subjects were also mildly hyperglycemic and had high blood pressure.

*Comparison of optimally healthy and metabolic syndrome subjects.* The response to glucose and insulin challenges in the MetSyn and OptHC subjects is depicted in Fig. 1 (glucose MM) and Fig. 2 (NEFA MM). Glucose MM parameter results for both the MetSyn and OptHC groups are summarized in Table 2, and NEFA MM results are in Table 3. MetSyn subjects had higher fasting and final concentrations of both insulin and glucose. The glucose MM showed that MetSyn subjects had a greater $AIR_g$, slower acceleration of functional insulin [P(3)], and a faster rate of insulin removal from the functional pool [P(2)]. β-Cell function was also higher in this group compared with OptHC, which corresponded with higher plasma insulin concentrations, whereas concentration in the functional insulin pool was reduced in MetSyn compared with the OptHC group. MetSyn subjects had lower $S_g$ and $S_i$, but GEZI and DI was unchanged between groups. Change in IAGD over time was distinct between the two groups, as depicted in Fig. 1*D*. Overall, these results show that MetSyn subjects had reduced insulin action and glucose response to insulin. Insulin resistance was also strongly evidenced in the NEFA MM, which showed blunted timing, speed, and intensity with MetSyn, corresponding to reduced adipocyte sensitivity to glucose (Fig. 2*B*). The key model-estimated parameters contributing to these changes were $k_c$, $K_{NEFA}$, $g_s$, and Φ. $K_{NEFA}$, $k_c$, and percent suppression were lower, and $g_s$ and Φ were higher in MetSyn subjects. However, $LIP_0$, $CL_0$, and $S_{NEFA}$ were unchanged between OptHC and MetSyn subjects. Parameters from both the glucose MM and NEFA MM were able to discriminate significantly between OptHC and MetSyn.

*Results from individual fatty acid analysis.* The individual fatty acid responsea for the eight MetSyn and eight OptHC subjects were analyzed. Data quality for one MetSyn subject was insufficient for model fitting, possibly due to sample degradation or experimental error. Therefore, our final sample size was 7 for the MetSyn subgroup. The subgroups had similar baseline characteristics to the large groups (Table 4), except that the MetSyn subgroup had a higher proportion (71 vs. 60%) and the OptHC subgroup had a lower proportion (50% vs. 64%) of male subjects compared with the large groups.

Table 1. *Baseline characteristics*

| Parameter | Optimally Healthy Controls (14) | Metabolic Syndrome (52) | *P* Value | Test |
|---|---|---|---|---|
| Demographic | | | | |
| Age, yr | 45 (12) | 48 (11) | 0.38 | W |
| Sex (%male) | 9 (64%) | 31 (60%) | | |
| **BMI** | **23 (1)** | **32 (4)** | **<0.0001** | **W** |
| Anti-hypertensive | . | 14 (27%) | | |
| Statin Use | . | 7 (13%) | | |
| Smoker | . | 3 (6%) | | |
| Vascular | | | | |
| **Heart rate, beats/min** | **54 (8)** | **68 (9)** | **<0.0001** | **W** |
| **BP systolic, mmHg** | **112 (9)** | **134 (11)** | **<0.0001** | **W** |
| **BP diastolic, mmHg** | **69 (7)** | **83 (8)** | **<0.0001** | **W** |
| **Pulse pressure, mmHg** | **44 (6)** | **51 (9)** | **0.002** | **W** |
| **Mean arterial pressure, mmHg** | **87 (8)** | **100 (8)** | **<0.0001** | **W** |
| Glycemia | | | | |
| **Glucose, mmol/L** | **4.9 (0.5)** | **5.6 (0.6)** | **0.0003** | **W** |
| **Insulin, μU/mL** | **3.0 [2.0, 3.5]** | **14.0 [10.0, 19.0]** | **<0.0001** | **$X^2$** |
| **HOMA** | **0.63 [0.49, 0.77]** | **3.27 [2.44, 4.43]** | **<0.0001** | **$X^2$** |
| Lipemia | | | | |
| **Total cholesterol, mmol/L** | **4.6 [4.0, 5.5]** | **5.1 [4.6, 5.8]** | **0.04** | **$X^2$** |
| **HDL cholesterol, mmol/L** | **1.4 (0.2)** | **1.1 (0.2)** | **<0.0001** | **W** |
| **LDL cholesterol, mmol/L** | **2.8 (0.7)** | **3.4 (1.0)** | **0.009** | **W** |
| **VLDL cholesterol, mmol/L** | **0.41 [0.36, 0.52]** | **0.72 [0.59, 0.98]** | **<0.0001** | **$X^2$** |
| **Triglyceride, mmol/L** | **0.86 (0.25)** | **2.53 (1.23)** | **<0.0001** | **W** |

BP, blood pressure; W, Welch's *t* test (assuming unequal variances), reported as means (SD); $X^2$, Wilcoxon's nonparametric test, reported as median [interquartile range]. Rows in boldface are significant at $P < 0.05$.

Table 2. *Group differences in glucose MM parameters*

| Glucose MM Parameters | Optimally Healthy Controls | Metabolic Syndrome | %Difference (Adjusted) | *P* Value | FSD [Means (SD)] |
|---|---|---|---|---|---|
| **$G_0$, mmol/L*** | **10.0 (9.4, 10.6)** | **11.7 (11.3, 12.1)** | **17% (9, 25)** | **<0.0001** | 0.037 (0.023) |
| **$G_b$, mmol/L*** | **5.2 (4.9, 5.4)** | **5.7 (5.5, 5.8)** | **10% (4, 17)** | **0.002** | |
| **$I_b$, mU/L*** | **42 (31, 58)** | **98 (83, 116)** | **131% (61, 229)** | **<0.0001** | |
| P(2), min* | 0.08 (0.05, 0.14) | 0.11 (0.08, 0.14) | 31% (−31, 149) | 0.40 | 0.121 (0.145) |
| **P(3), $(mU/L)^{-1} \times min^{-2}$*** | **$1.3 \times 10^{-5}$ ($7.1 \times 10^{-6}$, $2.4 \times 10^{-5}$)** | **$4.7 \times 10^{-6}$ ($3.4 \times 10^{-6}$, $6.4 \times 10^{-6}$)** | **−64% (−82, −29)** | **0.004** | 0.111 (0.150) |
| **HOMA-IR, (mmol/L) × $mu/L^2$ *** | **9.7 (7.0, 13.6)** | **24.7 (20.7, 29.5)** | **154% (75, 269)** | **<0.0001** | |
| **$AIR_g$, $mU \cdot L^{-1} \cdot min^{-1}$*** | **654 (416, 1026)** | **1,556 (1,226, 1,975)** | **138% (44, 293)** | **0.001** | |
| GEZI, min | 0.048 (0.037, 0.061) | 0.053 (0.046, 0.061) | 11% (−15, 46) | 0.44 | 0.110 (0.221) |
| **β-Cell function, $mU \cdot mmol^{-1} \cdot L^{-1}$*** | **528 (382, 730)** | **919 (774, 1090)** | **74% (21, 149)** | **0.003** | |
| DI* | 1052 (623, 1777) | 686 (520, 905) | −35% (−64, 17) | 0.15 | 0.027 (0.031) |
| **$S_g$, min** | **0.027 (0.022, 0.032)** | **0.018 (0.016, 0.021)** | **−44% (−74, −13)** | **0.003** | 0.062 (0.096) |
| **$S_i$, $mU \cdot L^{-1} \cdot min^{-1}$*** | **1.61 (1.08, 2.40)** | **0.46 (0.36, 0.54)** | **−73% (−82, −57)** | **<0.0001** | 0.027(0.031) |

Values are means (95% confidence interval). $AIR_g$, acute insulin response to glucose; DI, disposition index; FSD, fractional standard deviation; $G_0$, plasma glucose concentration immediately following glucose challenge. $G_b$, baseline plasma glucose concentration; GEZI, glucose effectiveness at zero insulin; HOMA-IR, homeostatic assessment insulin resistance; $I_b$, baseline plasma insulin concentration; MM, minimal models; P(2), insulin removal; P(3), insulin secretion; $S_g$, sensitivity to glucose; $S_i$, sensitivity to insulin. Effect size is difference adjusted for age and sex, with $P < 0.05$ considered significant. Optimally healthy controls (OptHC), $n = 14$; metabolic syndrome (MetSyn), $n = 52$; $R^2$ [means (SD; range)] = 95.1% (4.6; 72.7, 99.1). Rows in boldface indicate a significant difference between OptHC and MetSyn. *Log-transformed to satisfy test assumptions.

During analysis, we found significant contamination of the PA and SA peaks in blank samples. The level of contamination varied from sample to sample, but it was ~25–75% of the PA and SA peaks at the nadir. We found an unidentified contaminant that was strongly correlated with the PA and SA contamination peaks, which we used to calculate an adjustment for each PA and SA peak. In some cases, this adjustment resulted in values of PA and SA that were nonphysiologically low and had to be excluded from the analysis.

The fatty acids MA, PA, POA, SA, OA, LA, and ALA were quantified, and acceptable model fits were achieved for all except SA. Final parameter estimates for the individual fatty acid analysis are presented in Table 5. For all fatty acids, the nadir occurred between 40 and 70 min, and suppression was less in the MetSyn group compared with OptHC (Fig. 3). All unsaturated fatty acids (POA, OA, LA, and ALA) had an increased $g_s$ in MetSyn compared with OptHC, meaning that more glucose was required to trigger suppression. The values of $g_s$ for the saturated fatty acids (MA and PA) were not significantly different by group. Visual inspection of the data showed that SA responded differently to glucose challenge from both total NEFA and the other fatty acids, potentially explaining why the model was not identifiable (Fig. 3). When fitting SA data, the model did not converge, and thus parameters are not reported.

To investigate which parameters might be responsible for differences in response between different fatty acids, $g_s$, $S_{NEFA}$, $K_{NEFA}$, τ, and Φ were estimated independently, using normalized data for each fatty acid to determine which parameter(s) improved the fit for the individual fatty acids when compared with total NEFA (Table 6). Only $K_{NEFA}$ and Φ improved the fit for individual fatty acids, so they were also considered in combination. In OptHC subjects, one or more parameters had to be adjusted from the total NEFA value to obtain the best fit model for all six fatty acids. This suggests that NEFA clearance and/or the suppression of NEFA secretion in response to FSIVGTT may not occur consistently across all fatty acids.

For all fatty acids for which the model converged, percent suppression was consistently lower in MetSyn subjects, and in both groups, MA suppression was the lowest (Table 5 and Fig. 4*A*). Note that SA appeared to be even less suppressed (Fig. 3),

Table 3. *Group differences in NEFA MM parameters*

| NEFA MM Parameters | Optimally Healthy Controls | Metabolic Syndrome | %Difference (Adjusted) | *P* Value | FSD [means (SD)] |
|---|---|---|---|---|---|
| τ, min, * | 9.9 (7.4, 13.2) | 11.0 (9.4, 12.8) | 10.9% (−19.4, 52.7) | 0.52 | 0.126 (0.113) |
| $R_0$, mmol/L* | 0.055 (0.020, 0.154) | 0.081 (0.048, 0.135) | 47% (−53, 356) | 0.51 | 2.452 (4.014) |
| $NEFA_0$, μmol/L* | 378 (302, 473) | 407 (364, 458) | 8% (−16, 38) | 0.55 | 0.020 (0.019) |
| **$k_c$, %/min*** | **6.8 (4.8, 9.6)** | **3.6 (3.0, 4.3)** | **−47% (−64, −22)** | **0.002** | 0.259 (0.316) |
| $S_{NEFA}$, $\mu mol \cdot L^{-1} \cdot min^{-1}$* | 35.7 (29.2, 43.7) | 31.5 (28.3, 35.0) | −12% (−29, 10) | 0.27 | 0.378 (0.395) |
| ***$K_{NEFA}$*, %/min*** | **6.7 (5.4, 8.2)** | **5.1 (4.6, 5.8)** | **−23% (−39, −2)** | **0.03** | 0.285 (0.329) |
| **$g_s$, mmol/L*** | **5.2 (4.6, 5.9)** | **6.9 (6.5, 7.4)** | **32% (15, 52)** | **0.0002** | 0.306 (0.521) |
| **Φ, mmol/L*** | **0.14 (0.09, 0.22)** | **0.26 (0.20, 0.32)** | **81% (12, 192)** | **0.02** | 1.645 (3.249) |
| $LIP_0$, $\mu mol \cdot L^{-1} \cdot min^{-1}$ | 23.4 (17.5, 29.2) | 23.5 (20.4, 26.5) | 0% (−27, 28) | 0.98 | |
| $CL_0$, $\mu mol \cdot L^{-1} \cdot min^{-1}$ | 27.9 (21.9, 33.9) | 23.5 (20.4, 26.6) | −19% (−47, 9) | 0.19 | |
| **%Suppression** | **78.1 (71.8, 84.4)** | **62.9 (59.7, 66.2)** | **−24% (−35, −13)** | **<0.0001** | |

Values are means (95% confidence interval). $CL_0$, baseline nonesterified fatty acid (NEFA) clearance rate; FSD, fractional standard deviation; $g_s$, threshold plasma glucose concentration above which results in NEFA suppression after a delay of τ (latency, or delay time until NEFA levels begin to drop; min); $LIP_0$, baseline NEFA secretion from adipose by lipolysis; MM, minimal models; $NEFA_0$, baseline NEFA; $k_c$, rate constant for movement of glucose from plasma to functional pool; $K_{NEFA}$, rate constant for clearance of NEFA from the plasma; Φ, affinity constant altering rate of NEFA secretion in the plasma ($S_{NEFA}$); $R_0$, initial concentrations of glucose in the functional pool. Effect size is difference adjusted for age and sex, with $P < 0.05$ considered significant. Optimally healthy controls (OptHC), $n = 14$; metabolic syndrome (MetSyn), $n = 52$; $R^2$ [means (SD; range)] = 90.1% (8.0; 55.1, 98.3). Rows in boldface indicate a significant difference between OptHC and MetSyn. *Log-transformed to satisfy test assumptions.

Table 4. *Baseline characteristics from individual fatty acid analysis*

| Parameter | Optimally Healthy Controls (8) | Metabolic Syndrome (7) | *P* Value | Test |
|---|---|---|---|---|
| Demographic | | | | |
| Age, yr | 45 (11) | 45 (7) | 0.87 | W |
| Sex (%male) | 4 (50%) | 5 (71%) | | |
| **BMI** | **23 (1)** | **32 (4)** | **0.0007** | **W** |
| Anti-hypertensive | | 1 (14%) | | |
| Statin Use | | . | | |
| Smoker | | 1 (14%) | | |
| Vascular | | | | |
| **Heart rate, beats/min** | **53 (7)** | **68 (11)** | **0.0096** | **W** |
| **BP systolic, mmHg** | **112 (7)** | **139 (11)** | **0.0002** | **W** |
| **BP diastolic, mmHg** | **70 (6)** | **86 (8)** | **0.0009** | **W** |
| Pulse pressure, mmHg | 44 (7) | 53 (13) | 0.13 | W |
| **Mean arterial pressure, mmHg** | **84 (8)** | **103 (6)** | **0.0001** | **W** |
| Glycemia | | | | |
| **Glucose, mmol/L** | **4.8 (0.3)** | **5.8 (0.9)** | **0.027** | **W** |
| **Insulin, μU/mL** | **3 [3, 3]** | **11 [5, 14]** | **0.0015** | $X^2$ |
| **HOMA** | **0.63 [0.59, 0.69]** | **2.99 [1.28, 3.81]** | **0.0017** | $X^2$ |
| Lipemia | | | | |
| Total cholesterol, mmol/L | 4.3 [3.8, 5.6] | 5.6 [3.5, 5.7] | 0.45 | $X^2$ |
| **HDL cholesterol, mmol/L** | **1.6 (0.2)** | **1.1 (0.2)** | **0.0039** | **W** |
| LDL cholesterol, mmol/L | 2.6 (0.7) | 3.4 (1.0) | 0.23 | W |
| **VLDL cholesterol, mmol/L** | **0.39 [0.36, 0.47]** | **0.62 [0.52, 0.75]** | **0.0026** | $X^2$ |
| **Triglyceride, mmol/L** | **0.78 (0.19)** | **2.04 (0.58)** | **0.0007** | **W** |

BP, blood pressure; BMI, body mass index; HOMA; homeostatic model assessment; W, Welch's *t* test (assuming unequal variances), reported as means (SD); $X^2$, Wilcoxon's nonparametric test, reported as median [interquartile range]. Rows in boldface are significant at $P < 0.05$.

but because the model did not converge, percent suppression could not be calculated. Additionally, differences in fatty acid profiles over time are not the same in the MetSyn group as the OptHC group (Fig. 4, *B* and *C*). SA increased and OA decreased as percent of total at 50 min in both MetSyn and OptHC groups. However, MA increased and PA decreased as percent of total fatty acids at 50 min in OptHC subjects but not MetSyn subjects.

## DISCUSSION

There were significant differences in both the glucose MM and the NEFA MM between the OptHC and MetSyn subjects. In the glucose MM, MetSyn subjects had altered $S_g$, $S_i$, HOMA-IR, and $AIR_g$ compared with OptHC, but no change in GEZI, demonstrating no impairment in insulin-independent glucose clearance. In the NEFA MM, $k_c$, $K_{NEFA}$, $g_s$, and $\Phi$ differed between groups, and percent suppression was significantly lower in MetSyn subjects. Because baseline NEFA concentration was unchanged between groups, this indicates that MetSyn subjects could maintain basal NEFA in steady-state conditions. However, when challenged, NEFA secretion was not suppressed adequately, likely providing the liver with excess fatty acids in the presence of insulin. Plasma NEFA is the primary source of fatty acids used by the liver to synthesize VLDL TGs (41), and both plasma insulin and NEFA levels affect the rate of VLDL synthesis (24). Therefore, the insulin sensitivity of the adipose tissue may be critical to understand hypertriglyceridemia in MetSyn. In general, both the glucose MM and NEFA MM were useful for describing differences between the MetSyn and OptHC groups. One of our study aims was to determine whether we could identify subpopulations of MetSyn subjects with either adipose tissue (lipemic) or glycemic insulin resistance. We conducted an exploratory cluster analysis of the glucose MM and NEFA MM parameters to evaluate the possibility of identifying MetSyn subpopulations. However, there was little evidence that these models could be used to classify MetSyn subjects as having either glycemic insulin resistance or lipemic insulin resistance in this population. MetSyn subjects in this sample tended to be different from OptHC subjects based on parameters from both models. Future studies with larger sample sizes and carefully selected populations would be useful to bring more clarity to this topic.

Insulin does not affect the response of all fatty acids equally. Specifically, SA responded differently from the other fatty acids, and the NEFA MM could not be used to describe its response to the glucose challenge. This suggests that insulin has a limited effect on SA suppression and that other mechanisms mediate the SA response. This finding supports previous work that shows that nonesterified SA concentration in plasma is approximately double the predicted amount based on its release from adipose tissue (17). Mittendorfer et al. (27) also found a similar result in an isotope infusion study. In their study, the percent contribution of SA during insulin treatment was significantly higher than in the basal state or during epinephrine treatment. SA also had significantly lower measured rate of appearance and clearance rate than all other fatty acids when subjects were treated with insulin.

In the current study, SA percent suppression could not be calculated since no acceptable model fit was available. Percent suppression differed across fatty acids, with MA being suppressed less than all others. These differences in kinetics between fatty acids prompts the following question: What mechanism explains the differential response of individual fatty acids to glucose challenge? One possible explanation is that some adipose depots are less (or more) sensitive to insulin than others, and since fatty acid profiles (28) can differ between visceral and subcutaneous, more effective suppression in one depot could alter the net overall profile of released NEFA. However, differences in depot FA composition are not extreme

Table 5. *Parameter group differences for individual fatty acid analysis*

| | Optimally Healthy Controls ($n$ = 8) | Metabolic Syndrome ($n$ = 7) | %Difference (Adjusted) | $P$ Value |
|---|---|---|---|---|
| MA Parameters | | | | |
| τ, min | 9.6 (4.3, 21.7) | 8.3 (3.3, 20.9) | −13% (−71, 162) | 0.80 |
| $R_0$, mmol/L | 0.069 (0.018, 0.260) | 0.018 (0.004, 0.080) | −74% (−96, 56) | 0.16 |
| $NEFA_0$, μmol/L | 12.4 (9.3, 16.6) | 12.1 (8.7, 16.8) | −2% (−34, 45) | 0.91 |
| $k_c$, %/min | 4.0 (2.9, 5.5) | 5.9 (4.1, 8.4) | 47% (−4, 124) | 0.10 |
| $S_{NEFA}$, μmol·L$^{-1}$·min$^{-1}$ | 1.03 (0.51, 2.10) | 0.72 (0.32, 1.59) | −31% (−73, 82) | 0.47 |
| $K_{NEFA}$, %/min | 7.5 (3.6, 15.5) | 4.5 (2.0, 10.3) | −39% (−77, 63) | 0.34 |
| $g_s$, mmol/L | 6.1 (4.9, 7.7) | 8.6 (6.7, 11.1) | 40% (3, 91) | 0.05 |
| Φ, mmol/L | 0.09 (0.02, 0.40) | 0.24 (0.05, 1.30) | 172% (−64, 1958) | 0.35 |
| **%Suppression** | **69.2 (60.5, 77.9)** | **43.6 (33.8, 53.4)** | **−37% (−54, −20)** | **0.0013** |
| PA parameters | | | | |
| τ, min | 12.4 (4.8, 32.2) | 8.6 (2.9, 25.1) | −31% (−81, 152) | 0.58 |
| $R_0$, mmol/L | 0.069 (0.018, 0.260) | 0.018 (0.004, 0.080) | −74% (−96, 56) | 0.16 |
| $NEFA_0$, μmol/L | 98.0 (73.3, 131.0) | 130.0 (93.8, 180.3) | 33% (−11, 97) | 0.18 |
| $k_c$, %/min | 4.0 (2.9, 5.5) | 5.9 (4.1, 8.4) | 47% (−4, 124) | 0.10 |
| $S_{NEFA}$, μmol·L$^{-1}$·min$^{-1}$ | 14.4 (8.5, 24.3) | 11.8 (6.6, 21.3) | −18% (−60, 67) | 0.59 |
| $K_{NEFA}$, %/min | 11.9 (7.0, 20.3) | 6.0 (2.7, 3.3) | −49.5% (−76, 4) | 0.09 |
| $g_s$, mmol/L | 5.7 (4.1, 7.9) | 8.8 (6.1, 12.8) | 56% (0, 144) | 0.07 |
| Φ, mmol/L | 0.05 (0.01, 0.21) | 0.09 (0.02, 0.42) | 62% (−76, 993) | 0.63 |
| **%Suppression** | **85.2 (76.3, 94.0)** | **54.2 (44.2, 64.1)** | **−36% (−50, −22)** | **0.0003** |
| POA parameters | | | | |
| τ, min | 10.4 (6.0, 17.9) | 14.6 (7.9, 27.2) | 41% (−33, 198) | 0.38 |
| $R_0$, mmol/L | 0.069 (0.018, 0.260) | 0.018 (0.004, 0.080) | −74% (−96, 56) | 0.16 |
| $NEFA_0$, μmol/L | 14.1 (9.6, 20.8) | 16.3 (10.5, 25.3) | 16% (−32, 96) | 0.60 |
| $k_c$, %/min | 4.0 (2.9, 5.5) | 5.9 (4.1, 8.4) | 47% (−4, 124) | 0.10 |
| $S_{NEFA}$, μmol·L$^{-1}$·min$^{-1}$ | 2.73 (1.62, 4.58) | 1.28 (0.71, 2.30) | −53% (−77, −5) | 0.06 |
| $K_{NEFA}$, %/min | 11.5 (6.3, 21.0) | 6.3 (3.1, 12.3) | −46% (−76, 22) | 0.16 |
| **$g_s$, mmol/L** | **5.4 (4.3, 6.7)** | **8.6 (7.0, 11.4)** | **66% (24, 123)** | **0.005** |
| Φ, mmol/L | 0.05 (0.01, 0.16) | 0.14 (0.03, 0.57) | 193% (−47, 1525) | 0.24 |
| **%Suppression** | **86.5 (80.9, 92.2)** | **62.9 (56.5, 69.3)** | **−27% (−36, −18)** | **<0.0001** |
| OA Parameters | | | | |
| τ, min | 12.8 (8.6, 19.1) | 13.0 (8.3, 20.4) | 1% (−41, 75) | 0.80 |
| $R_0$, mmol/L | 0.069 (0.018, 0.260) | 0.018 (0.004, 0.080) | −74% (−96, 56) | 0.16 |
| $NEFA_0$, μmol/L | 129.4 (94.4, 177.4) | 155.7 (109.1, 222.1) | 20% (−22, 85) | 0.41 |
| $k_c$, %/min | 4.0 (2.9, 5.5) | 5.9 (4.1, 8.4) | 47% (−4, 124) | 0.10 |
| $S_{NEFA}$, μmol·L$^{-1}$·min$^{-1}$ | 19.8 (8.7, 45.2) | 11.2 (4.4, 28.3) | −44% (−82, 73) | 0.33 |
| $K_{NEFA}$. %/min | 11.6 (5.6, 24.3) | 5.0 (2.2, 11.5) | −57% (−84, 17) | 0.12 |
| **$g_s$, mmol/L** | **5.6 (4.5, 6.9)** | **8.9 (7.0, 11.4)** | **60% (20, 115)** | **0.008** |
| Φ, mmol/L | 0.07 (0.02, 0.29) | 0.11 (0.02, 0.59) | 72% (−76, 1135) | 0.60 |
| **%Suppression** | **85.4 (79.9, 90.8)** | **52.7 (46.5, 58.8)** | **−38% (−47, −30)** | **<0.0001** |
| LA Parameters | | | | |
| τ, min | 14.1 (9.6, 20.6) | 12.3 (8.0, 18.9) | −13% (−48, 46) | 0.61 |
| $R_0$, mmol/L | 0.069 (0.018, 0.260) | 0.018 (0.004, 0.080) | −74% (−96, 56) | 0.16 |
| $NEFA_0$, μmol/L | 76.2 (57.8, 100.3) | 84.2 (61.7, 114.8) | 10% (−96, 56) | 0.61 |
| $k_c$, %/min | 4.0 (2.9, 5.5) | 5.9 (4.1, 8.4) | 47% (−4, 124) | 0.10 |
| $S_{NEFA}$, μmol·L$^{-1}$·min$^{-1}$ | 9.7 (4.3, 22.2) | 6.3 (2.5, 15.9) | −35% (−79, 97) | 0.45 |
| $K_{NEFA}$, %/min | 11.7 (6.2, 22.1) | 5.3 (2.6, 10.8) | −55% (−81, 7) | 0.10 |
| **$g_s$, mmol/L** | **5.8 (4.5, 7.4)** | **9.5 (7.2, 12.6)** | **65% (18, 130)** | **0.01** |
| Φ, mmol/L | 0.09 (0.02, 0.38) | 0.09 (0.02, 0.42) | −7% (−87, 537) | 0.94 |
| **%Suppression** | **82.6 (76.3, 88.8)** | **52.5 (45.4, 59.5)** | **−37% (−47, −26)** | **<0.0001** |
| ALA Parameters | | | | |
| τ, min | 9.2 (4.9, 17.2) | 10.4 (5.1, 21.1) | 13% (−52, 165) | 0.78 |
| $R_0$, mmol/L | 0.069 (0.018, 0.260) | 0.018 (0.004, 0.080) | −74% (−96, 56) | 0.16 |
| $NEFA_0$, μmol/L | 4.2 (3.0, 5.8) | 4.9 (3.4, 7.0) | 15% (−26, 80) | 0.53 |
| $k_c$, %/min | 4.0 (2.9, 5.5) | 5.9 (4.1, 8.4) | 47% (−4, 124) | 0.10 |
| $S_{NEFA}$, μmol·L$^{-1}$·min$^{-1}$ | 0.56 (0.21, 1.46) | 0.49 (0.17, 1.45) | −12% (−76, 226) | 0.85 |
| $K_{NEFA}$, %/min | 11.0 (5.6, 21.3) | 6.8 (3.2, 14.1) | −39% (−75, 50) | 0.30 |
| **$g_s$, mmol/L** | **5.9 (4.8, 7.2)** | **8.5 (6.7, 10.7)** | **44% (9, 90)** | **0.02** |
| Φ, mmol/L | 0.08 (0.02, 0.28) | 0.13 (0.03, 0.58) | 76% (−70, 948) | 0.54 |
| **%Suppression** | **82.5 (76.6, 88.5)** | **59.6 (52.9, 66.3)** | **−28% (−38, −18)** | **0.0002** |

Values are mean (95% confidence interval; model did not converge for stearic acid parameters). ALA, α-linolenic acid; $g_s$, threshold plasma glucose concentration above which results in nonesterified fatty acid (NEFA) suppression after a delay of τ (latency, or delay time until NEFA levels begin to drop; min); $k_c$, rate constant for movement of glucose from plasma to functional pool; $K_{NEFA}$, rate constant for clearance of NEFA from the plasma; LA, linoleic acid; MA, myristic acid; $NEFA_0$, baseline NEFA; OA, oleic acid; $R_0$, initial concentrations of glucose in the functional pool; $S_{NEFA}$, rate of NEFA secretion in the plasma; Φ, affinity constant altering $S_{NEFA}$; PA, palmitic acid; POA, palmitoleic acid. Effect size is difference adjusted for age and sex, with $P < 0.05$ considered significant. Optimally healthy controls (OptHC), $n$ = 14; metabolic syndrome (MetSyn), $n$ = 52; $R^2$ [means (SD; range)] = 90.1% (8.0; 55.1, 98.3). All parameter data, except for %suppression, were log-tranformed to satisfy test assumptions. Rows in boldface indicate a significant difference between OptHC and MetSyn.

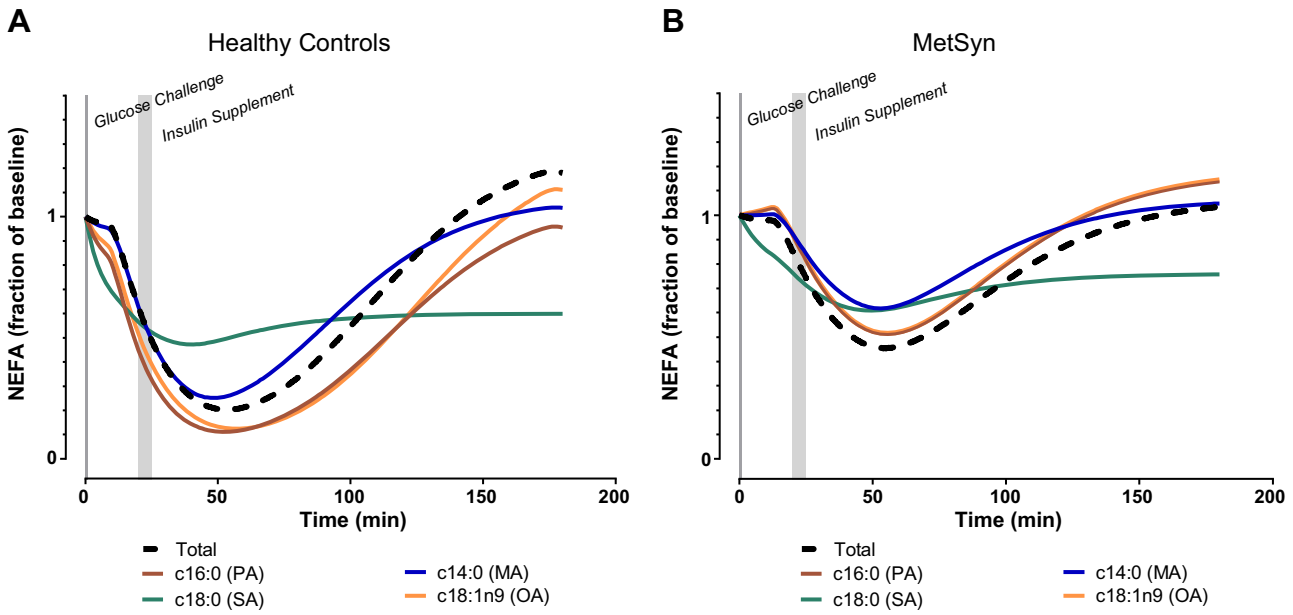



Fig. 3. Best-fit models for each individual fatty acid. Using the nonesterified fatty acid (NEFA) minimal models (MM), data for each individual fatty acid were normalized to baseline and are reported as fraction of baseline. Calculated stearic acid (SA) values from the NEFA MM are shown here, although acceptable model convergence could not be achieved. Values shown are visually representative of the SA data. *A*: for optimally healthy control (OptHC) subjects, the best-fit model was different from the total for all fatty acids. Suppression of palmitic acid (PA) and oleic acid (OA) was greater, and suppression of SA and myristic acid (MA) was lower compared with total NEFA. *B*: in metabolic syndrome (MetSyn) subjects, individual fatty acid response was closer to the total NEFA response. Data represented here are geometric mean data from MetSyn and OptHC groups (MetSyn $n = 7$; OptHC $n = 8$).

enough to explain the high proportion of SA in plasma NEFA, since SA constitutes a relatively large proportion of the plasma NEFA profile (~10%) but a comparatively smaller proportion of adipose total fatty acids (3–6%) (25, 42).

Another source of NEFA in plasma, lipoprotein lipase (LpL)-mediated release of NEFA from TGs in chylomicrons and VLDL, may provide an explanation for the differential effect of insulin on SA and MA. Although adipocyte lipolysis by HSL accounts for the majority of plasma NEFA, LpL release from TGRL is responsible for ≤24% of plasma NEFA in the fasting state (2). Because SA makes up a larger proportion of fatty acids in chylomicrons and VLDL and a smaller proportion of adipose (11, 42), it is plausible that the nonesterified SA concentration in plasma is less sensitive to insulin because it is not primarily HSL derived. Additionally, LpL in the adipose tissue is activated, not suppressed, by insulin, which would increase relative levels of fatty acids originating from the lipoproteins (31). A similar explanation was proposed by Mittendorfer et al., (27) who suggested that differences in SA kinetics could be explained by an increased contribution of phospholipid turnover to the NEFA composition during insulin-stimulated suppression of adipocyte lipolysis. The SA concentration in phospholipids is generally higher, and OA is generally lower, compared with the fatty acid profile in TG's (27). SA clearance is also slower than OA and LA, as measured by the veno-arterial difference (17) and isotope infusion during euglycemic clamp (27). Slower clearance, combined with different dominant NEFA sources during adipocyte lipolysis suppression, may explain the differential response of SA compared with other fatty acids.

Table 6. *Differences in model parameters by individual fatty acids*

| Fatty Acid | Parameters Investigated | Optimally Healthy Controls | |
|---|---|---|---|
| | | Parameter Values | Changes |
| Total NEFA | $K_{NEFA}$ (in %/min), ϕ (in mM) | 6.7, 0.16 | Reference value |
| MA | $K_{NEFA}$,ϕ | 8.0, 0.31 | 18%, 98% |
| PA | $K_{NEFA}$,ϕ | 8.1, 0.10 | 21%, −34% |
| POA | $K_{NEFA}$,ϕ | *, 0.07 | *, −57% |
| SA† | $K_{NEFA}$,ϕ | | |
| OA | $K_{NEFA}$,ϕ | *, 0.08 | *, −51% |
| LA | $K_{NEFA}$,ϕ | *, 0.08 | *, −47% |
| ALA | $K_{NEFA}$,ϕ | 9.1, 0.23 | 36%, 50% |

ϕ, affinity constant altering NEFA secretion, or the glucose concentration (in mM) required to suppress NEFA by 50%; ALA, α-linolenic acid; $K_{NEFA}$, nonesterified fatty acid (NEFA) %clearance rate; LA, linoleic acid; MA, myristic acid; OA, oleic acid; PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid. Data were geometric mean data from the fatty acid analysis subjects (metabolic syndrome, $n = 7$; optimally healthy controls, $n = 8$). *Changing parameter did not significantly improve fit; fixed to reference value; †model did not converge.

It has been proposed that adipose insulin resistance may precede insulin resistance of the skeletal muscle and liver (14), making accurate measurement of adipose-specific insulin resistance critical to understanding the progression of MetSyn. However, few studies measure both lipidemic and glycemic measures of insulin resistance in cohorts of MetSyn and healthy subjects. Extensive work has been done to develop hyperinsulinemic euglycemic clamp methods with infused stable isotope-labeled fatty acid tracers. These methods provide the gold standard for measuring the rate of appearance of fatty acids released from adipose at steady state, but such methods are invasive and costly (16, 18). Simplified indices of adipose insulin resistance have also been developed but have not been extensively validated (37). Therefore, there is a need for ongoing research using measures of lipidemic insulin resistance to optimize more clinically feasible methods. This study provides support for the concept of using individual fatty acids as a measure of adipose insulin sensitivity in future studies.

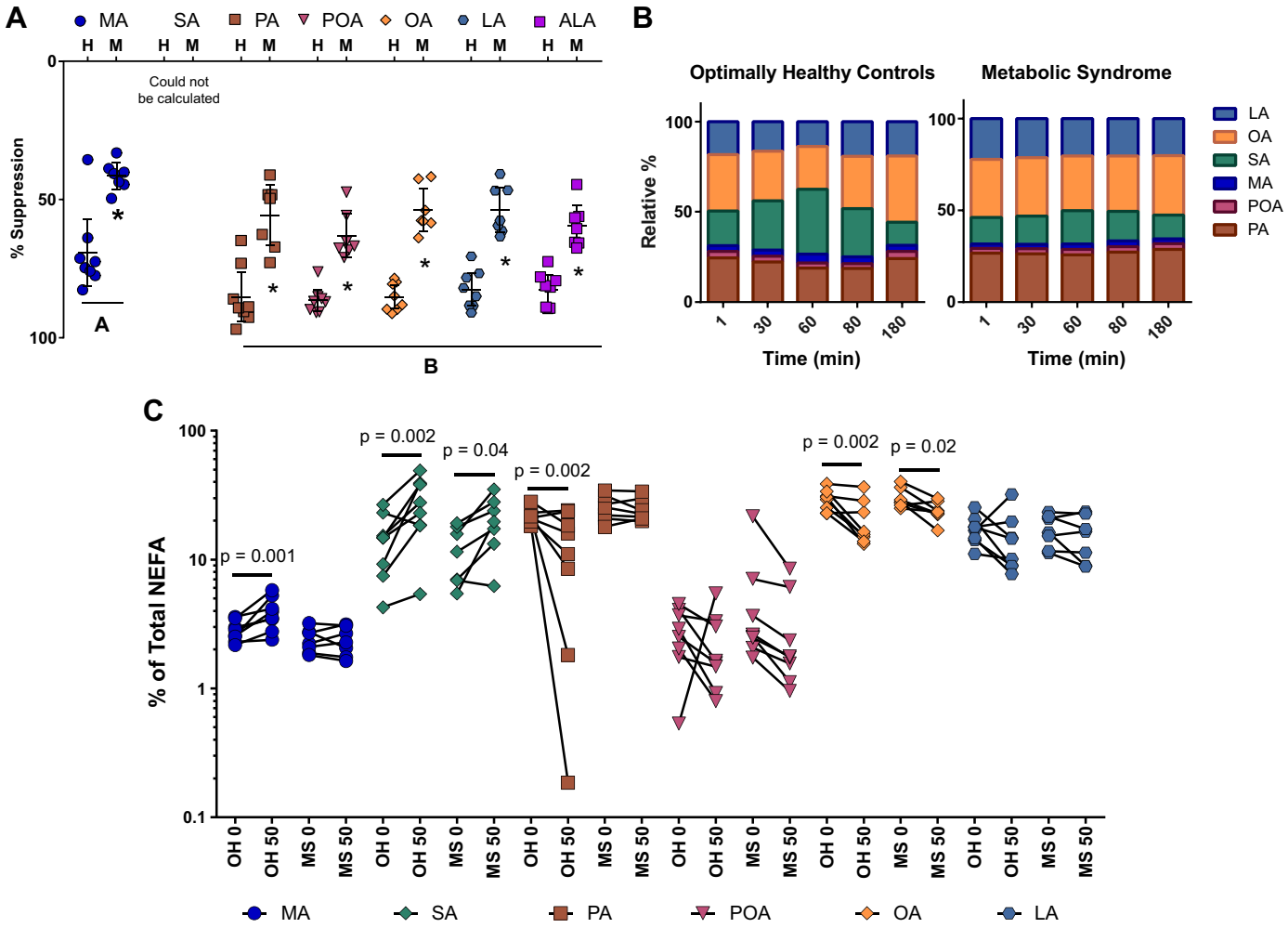



Fig. 4. Fatty acid composition of nonesterified fatty acid (NEFA) fraction changes with time following glucose challenge. *A*: %suppression of each fatty acid was reduced in in metabolic syndrome (MetSyn); suppression of myristic acid (MA; blue) was lower than all other fatty acids using Tukey post hoc analysis. H, optimally healthy; M, metabolic syndrome. [A,B]Groups with different letters are statistically different from each other. *B*: changes in fatty acid %total are most pronounced in optimally healthy control (OptHC) subjects at 50–60 min and then return to baseline. Stearic acid (SA; green) increases as %total, whereas oleic acid (OA; orange) and palmitic acid (PA; brown) decrease. *C*: MA and SA increase and PA and OA decrease as %total NEFA at 50 min following glucose challenge in OptHC subjects. However, MA and PA do not change in MetSyn subjects. Differences were tested by repeated-measures ANOVA, adjusted for age and sex and considered significant at $P < 0.05$. *%Suppression was significantly different in optimally healthy (OH) compared with metabolic syndrome (MS) subjects.

Although we believe the NEFA MM is the best available dynamic model of the NEFA response, an insulin-driven model, possibly including C-peptide as a measure of insulin secretion, could improve on the model. This would allow the interpretation of insulin as a direct driver of the NEFA response instead of the indirect nature of the current NEFA MM. The development of such a compartmental model could be a useful aim for future studies.

*Strengths and limitations.* This study has several strengths, including the careful selection of healthy subjects who were identified not by self-declaration of health but by multiple biomarker-based measures. This careful selection of the subject groups increases power to see subtle differences with smaller sample sizes. Another strength of the study is the large volume of FSIVGTT-based NEFA and glycemic data points collected for each subject, allowing for multivariate analyses and appropriate data adjustments to ensure robust results. Finally, the careful measurement of individual fatty acids at enough time points to perform compartmental modeling has not been done previously in the context of a glucose challenge.

This study also had important limitations. The NEFA MM, although an elegant and novel method for describing the NEFA response to glucose (and indirectly to insulin), has some inherent limitations. In some cases, collinearity between adjustable parameters was present, meaning that values for one were not independent of values for another. However, using parameter estimation predicated on the method of generalized least squares (38) offers some protection against covariance collapse with highly correlated parameters. To clarify the robustness of our parameter estimates, we used the method described by Boston et al. (8) to explore the stability of the estimation, which was acceptable for all parameters. Another limitation was extraneous background signal introduced by the solid-phase extraction columns that overlapped with PA and SA peaks. Because of this, PA and SA peaks had to be adjusted for blank levels, and some data had to be excluded when

background and specific signals could not be resolved. Despite these contamination issues, we found a highly repeatable, time-dependent pattern for both fatty acids with NEFA profiles similar to those in other published reports. Therefore, we believe our primary results are still valid. Different NEFA extraction methods should be investigated to address this limitation. Finally, the small sample size in individual fatty acid analysis limits the generalizability this method for assessing insulin resistance. The sample size of the individual fatty acid analysis was especially small (MetSyn $n = 7$; OptHC $n = 8$) due to the time- and resource-intensive nature of measuring multiple fatty acids for 31 time points per subject. Selection for the subgroup analysis also altered our sex distribution. Females have higher fasting NEFA concentration than males (21), so it is possible that this affected our findings in the subgroups. To help correct for this, we adjusted for sex in all statistical models. Future studies should be powered and designed to specifically test sex differences in NEFA insulin response. Studies with larger sample sizes could also better define the differences between the insulin response of the individual fatty acids and strengthen the results of the current study.

*Conclusions.* Insulin resistance associated with MetSyn has become a public health concern because of comorbidities and increased risk of chronic disease. However, current clinical testing methods do not assess the role of adipose insulin resistance in MetSyn. Methods using a glucose challenge, such as the MinMod Millenium, provide a wealth of information but still do not target the contribution of the adipose in the insulin response. Here, we demonstrate that the MetSyn and OptHC have differences in apparent adipose insulin sensitivity using the NEFA MM. We further showed that insulin does not affect all fatty acids the same, and some fatty acids were suppressed differently, especially in OptHC. We provide evidence that measuring specific NEFA fatty acids following a glucose challenge may be useful for identifying adipose insulin resistance.

## APPENDIX

*Model parameters and calculations.* Differential equations underlying the Bergman Minimal Model of insulin and glucose dynamics (3) shown in Fig. 1*A*.

$$\frac{dG(t)}{dt} = -G(t) \times \left[S_g + X(t)\right] + G_b \times S_g, \quad (A1)$$

where $G(0) = G_0$,

$$\frac{dX(t)}{dt} = -P(2) \times X(t) + P(3) \times F(t), \quad (A2)$$

where $F(t) = 0$ if $I(t) \leq I_b$, or else $I(t) - I_b$; $X(0) = 0$.

In *Eqs. A1* and *A2*, $G(t)$ is plasma glucose at time $t$; $S_g$ is a parameter that describes glucose effectiveness, or the capacity for glucose to mediate its own disposal and suppress its own production; $X(t)$ is insulin in the functional compartment at time $t$ and is the principal driver of the glucose response; $G_b$ is basal glucose concentration; $G(0)$ is the glucose level following distributional equilibration after the glucose challenge; $P(2)$ is the rate of insulin removal from the functional compartment; $P(3)$ describes insulin delivery to the functional compartment; and $F(t)$ is a time variant function that represents an increase in plasma insulin concentration [$I(t)$] above basal insulin concentration [$I_b$].

*Calculated indices.*

$$S_I = \frac{P(3)}{P(2)} \times 10^4, \quad (A3)$$

where $S_i$ is insulin sensitivity, or the capacity for insulin to promote glucose disposal, and is calculated as fractional insulin secretion from the functional pool [P(3)]/the rate of functional insulin removal [P(2)].

$$AIG_g = \int_0^{10} [I(t) - I_b]dt, \quad (A4)$$

where $AIR_g$ is acute insulin response to glucose, or the first phase insulin response, and is calculated as the area under the plasma insulin concentration time curve from 0 to 10 min. $I(t)$ is plasma insulin at time $t$ and $I_b$ is basal insulin.

$$DI = AIR_g \times S_I, \quad (A5)$$

where DI is the disposition index, or the ability for the β-cells to secrete insulin under the condition of normal insulin resistance, and is calculated as the product of acute insulin response to glucose ($AIR_g$) and insulin sensitivity ($S_i$).

$$GEZI = S_G - S_I \times I_b, \quad (A6)$$

where GEZI is glucose effectiveness at zero insulin, or glucose effectiveness ($S_g$) independent of changes in insulin. $S_i$ is insulin sensitivity and $I_b$ is basal insulin.

$$IAGD = \frac{X(t)}{X(t) + S_G} \times 100, \quad (A7)$$

where IAGD is insulin attributable glucose disposal or the time variant descriptor of the instantaneous glucose disposal that is attributable to insulin action in the functional compartment as a percentage of total glucose disposal. $X(t)$ is insulin in the functional compartment at time $t$, and $S_g$ is glucose effectiveness.

*Homeostatic model assessment parameters.*

$$\text{HOMA } \beta\text{-cell function} = \frac{20 \times I_b}{G_b - 3.5}, \quad (A8)$$

where $I_b$ and $G_b$ are basal plasma insulin and glucose concentrations, respectively.

$$\text{HOMA insulin resistance} = \frac{G_b \times I_b}{22.5}, \quad (A9)$$

where $I_b$ and $G_b$ are basal plasma insulin and glucose concentrations, respectively.

Differential equations underlying the Boston and Moate (6) minimal model for glucose and NEFA dynamics shown in Fig. 2*A*:

$$\frac{dR(t)}{dt} = \frac{k_c}{100} \times \left[G^*(t) - R(t)\right], \quad (A10)$$

$$\frac{dNEFA(t)}{dt} = S_{NEFA}\left[1 - h(t)\right] - \frac{K_{NEFA}}{100} \times NEFA(t), \quad (A11)$$

where $G(0) = G_b$; $g(t) = G(t - \tau)$ if $t \geq \tau$, or else $G_b$; $G^*(t) = g(t) - g_s$, if $g(t) > g_s$, or else zero; $R(0) = R_0$.

$$h(t) = \frac{1}{1 + \frac{\Phi}{R(t)}}. \quad (A11a)$$

In *Eqs. A10* and *A11*, $R(t)$ is glucose in the functional (inaccessible) compartment at time $t$ and is the principal driver of the NEFA response; $G(t)$ is linear interpolation of plasma glucose concentration; $G^*(t)$ is a function that represents an increase in $g(t)$ above the

threshold glucose concentration ($g_s$) that results in entry of glucose into the functional compartment after a delay of $\tau$ min, and g($t$) is a function that represents glucose concentrations after the time delay; $k_c$ is the percent transfer of glucose from the plasma compartment into the functional compartment above $g_s$; NEFA($t$) is plasma NEFA concentration at *time t*; $S_{NEFA}$ describes the rate of NEFA secretion into plasma; $K_{NEFA}$ is a rate constant that describes plasma NEFA percent clearance; h($t$) is a unitless function that regulates NEFA production rate; and $\Phi$ is a Michaelis-Menton affinity constant.

*Calculated indices.*

$$LIP_0 = S_{NEFA} \times \left[1 - \left(\frac{R_0}{\Phi - R_0}\right)\right], \quad (A12)$$

where $LIP_0$ describes the instantaneous net rate of NEFA production by adipose lipolysis at baseline; $S_{NEFA}$ is the rate of NEFA secretion; $R_0$ is functional glucose at *time 0*; and $\Phi$ is a Michaelis-Menton affinity constant.

$$CL_0 = NEFA_0 \times \frac{K_{NEFA}}{100}, \quad (A13)$$

where $CL_0$ describes the net rate of NEFA clearance at baseline; $K_{NEFA}$ is the percent clearance rate of plasma NEFA.

$$\text{Suppression}\% = \frac{100 \times (NEFA_0 - NEFA_{min})}{NEFA_0}, \quad (A14)$$

where $NEFA_0$ represents plasma NEFA concentration at *time 0*; $NEFA_{min}$ is the NEFA concentration at the time of the nadir ($T_{min}$).

## GLOSSARY

| | |
|---|---|
| $\Phi$ | Affinity constant altering NEFA secretion |
| $\tau$ | Latency, or delay time until NEFA levels begin to drop |
| AA | Arachidonic acid |
| ALA | α-linolenic acid |
| $AIR_g$ | Acute insulin response to glucose |
| DI | Disposition index |
| EPA | Eicosapentaenoic acid |
| FSIVGTT | Frequently sampled intravenous glucose tolerance test |
| F($t$) | Functional glucose model compartment |
| $G_0$ | Plasma glucose concentration immediately following glucose challenge |
| $G_b$ | Baseline plasma glucose concentration |
| GEZI | Glucose effectiveness with zero insulin |
| $g_s$ | Threshold glucose |
| G($t$) | Plasma glucose model compartment |
| HOMA-IR | Homeostatic assessment insulin resistance |
| HSL | Hormone-sensitive lipase |
| IAGD | Insulin-attributable glucose disposal |
| $k_c$ | %Glucose transfer rate from plasma to the functional compartment |
| $K_{NEFA}$ | NEFA % clearance rate |
| LA | Linoleic acid |
| $LIP_0$ | Baseline NEFA secretion from adipose by lipolysis |
| I($t$) | Plasma insulin model compartment |
| MA | Myristic acid |
| MetSyn | Metabolic syndrome |
| MM | Minimal model |
| NEFA | Nonesterified fatty acids |
| $NEFA_0$ | Baseline NEFA |
| NEFA($t$) | Plasma NEFA model compartment |
| OA | Oleic acid |
| OGTT | Oral glucose tolerance test |
| OptHC | Optimally healthy controls |
| $CL_0$ | baseline NEFA clearance rate |
| P(2) | Insulin removal from the functional compartment |
| P(3) | Insulin secretion into the functional compartment |
| PA | Palmitic acid |
| POA | Palmitoleic acid |
| $R_0$ | Initial concentration of glucose in the functional pool |
| SA | Stearic acid |
| $S_g$ | Sensitivity to glucose |
| $S_i$ | Sensitivity to insulin |
| $S_{NEFA}$ | NEFA secretion |
| TG | Triglyceride |
| VA | Vaccenic acid |
| VLDL | Very low-density lipoprotein |
| X($t$) | Functional insulin model compartment |

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## DISCLOSURES

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## AUTHOR CONTRIBUTIONS

W.S.H. and G.C.S. conceived and designed research; R.E.W. and O.V.S. performed experiments; R.E.W., J.L.F., R.C.B., and M.H.G. analyzed data; R.E.W., J.L.F., R.C.B., M.H.G., and G.C.S. interpreted results of experiments; R.E.W. and J.L.F. prepared figures; R.E.W. and J.L.F. drafted manuscript; R.E.W., J.L.F., R.C.B., O.V.S., W.S.H., and G.C.S. edited and revised manuscript; R.E.W., J.L.F., R.C.B., W.S.H., and G.C.S. approved final version of manuscript.

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