Oral minimal model-based estimates of insulin sensitivity in obese youth depend on oral glucose tolerance test protocol duration

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ABSTRACT

Introduction: The Oral Minimal Model (OMM), a differential-equations based mathematical model of glucose-insulin dynamics, utilizes data from a frequently sampled oral glucose tolerance test (OGTT) to quantify insulin sensitivity (S_I). OMM-based estimates of S_I can detect differences in insulin resistance (IR) across population groups and quantify effects of clinical or behavioral interventions. These estimates of S_I have been validated in healthy adults using data from OGTTs with durations from 2 to 7 hours. However, data demonstrating how protocol duration affects S_I estimates in highly IR populations such as adolescents with obesity are limited.

Methods: A 6-hour frequently sampled OGTT was performed in adolescent females with obesity. Two, 3-, and 4- hour implementations of OMM assuming an exponentially-decaying rate of glucose appearance beyond measured glucose concentrations were compared to the 6-hour implementation. A 4- hour OMM implementation with truncated data (4h tr) was also considered.

Results: Data from 68 participants were included (age 15.8 ± 1.2 years, BMI 35.4 ± 5.6 kg/m^2). Although S_I values were highly correlated for all implementations, they varied with protocol duration (2h: 2.86 ± 3.31, 3h: 2.55 ± 2.62, 4h: 2.81 ± 2.59, 4h tr: 3.13 ± 3.14, 6h: 3.06 ± 2.85 $\times 10^{-4}$ dl/kg/min). S_I estimates based on 2 or 3 hours of data underestimated S_I values, whereas 4-hour S_I estimates more closely approximated 6-hour S_I values.

Discussion: These results suggest that OGTT protocol duration should be considered when implementing OMM to estimate S_I in adolescents with obesity and other IR populations.

1. INTRODUCTION

Measures of insulin sensitivity (S_I) quantify the ability of insulin to both suppress endogenous glucose release and promote glucose uptake in response to a glucose challenge. Decreased S_I is a critical component of metabolic syndrome, polycystic ovary syndrome (PCOS), type 2 diabetes mellitus and type 1 diabetes, and it contributes significantly to morbidity and mortality in these populations [1-4]. Decreased S_I is also present in adolescents with obesity [5-7]: adolescents display a puberty related reduction in S_I and greater insulin secretion rates when compared to adults [8, 9]; excess weight is known to further decrease the S_I reduction associated with pubertal status; and S_I is markedly lower in teenage girls compared to boys [10-12].

The hyperinsulinemic euglycemic (HE) clamp remains the gold standard for measuring S_I in adults and youth with and without obesity [13, 14, 2, 15-17], but it is invasive, labor intensive, and does not represent physiological conditions [18-20]. Similarly, the intravenous glucose tolerance test (IVGTT) is non-physiological due to the IV infusion that bypasses the gut [21]. Several surrogate indices, including HOMA [22], QUICKI [23], and the Matsuda index [24] have been developed to efficiently quantify S_I in the clinical setting [25, 26, 23, 27-29]. Alternatively, the Oral Minimal Model (OMM), a mathematical model of glucose-insulin dynamics in the more physiological conditions of an oral glucose tolerance test (OGTT) [30-32], may be used to estimate S_I. The OMM describes an individual's plasma glucose response to a glucose challenge, and model parameters are used to derive a model-based measure of S_I that demonstrates increased

sensitivity to group differences compared to fasting and OGTT-based indices of S_I [33-37]

The OMM was developed in healthy adults and validated against the HE clamp using data from a 7-hour OGTT, but some implementations of OMM have been applied in adults with type 2 diabetes and adolescent cohorts [30, 38-43]. S_I estimates from shorter OGTT/OMM protocols have been validated in adult populations without type 2 diabetes [30, 38], lean to obese adolescents [40], and adolescent girls [42, 43]. However, insulin resistance (IR) alters the glucose-insulin dynamics represented by OMM, and protocol duration dependence of OMM S_I estimates has not been rigorously assessed in populations expressing diverse metabolic phenotypes.

To address this gap, we compared S_I estimates computed using a 6-hour protocol to S_I estimates computed with data from shorter protocols in a cohort of sedentary, highly IR adolescent girls with obesity. In this cohort and other IR populations, glucose may remain elevated for extended periods reflecting dysglycemia [44]. For these individuals, longer OGTT protocols may facilitate a more reliable OMM-based estimation of S_I [41]. However, in IR individuals this constraint may require extending OGTT protocols to 6 or 7 hours [30]. Given the extended period of elevated glucose concentrations in our cohort, we hypothesized that estimates of S_I would depend on protocol duration and that at least 4 hours of data are required to accurately estimate S_I using OMM.

2. MATERIALS AND METHODS

2.1 Participants

This is a secondary analysis of data collected in the APPLE (Liver and Fat Regulation in Overweight Adolescent Girls; NCT02157974) and PLUM (Post-Prandial Liver Glucose Metabolism in PCOS; NCT03041129) studies performed to explore abnormalities in PCOS. These studies consisted of two visits: 1) consent/screening for eligibility; 2) overnight monitored fast followed by an OGTT for metabolic assessment. Inclusion criteria were female sex, overweight/obese status (BMI ≥ 90th percentile for age and sex), post pubertal status (Tanner Stage 5) and a sedentary lifestyle (< 3 hours routine exercise per week, validated with both a 3-day activity recall and 7-day accelerometer use). Sixty-eight participants were included in this analysis (18 with normal menses and 50 with PCOS). Participants with PCOS were medication naïve and defined according to the NIH criteria: 1) an irregular menstrual cycle, 2) ≥18 months post-menarche and, 3) clinical and biochemical evidence of hyperandrogenism. Exclusion criteria for the studies included the following: confirmed diagnosis of diabetes, pregnancy, anemia, liver diseases other than non-alcoholic fatty liver disease (NAFLD), an alanine transferase (ALT) level greater than 125 (IU/L) and use of medications known to affect insulin sensitivity or glucose metabolism (including systemic steroids, antipsychotics and treatment with hormonal contraception or metformin) in the last six months. The study was approved by the Colorado Multiple Institutional Review Board. Informed consent was obtained from all participants 18-21 years old, and parental consent and participant assent from all participants between 12 - 18 years old.

2.2 OGTT Protocol

Participants refrained from physical activity 3 days prior to the metabolic stay, and consumed an isocaloric diet (65% carbohydrate, 15% protein, 20% fat) the afternoon and evening prior to the OGTT. Following a monitored inpatient 12-hour fast, a frequently sampled OGTT was performed. Blood was sampled for glucose and insulin concentrations at the following time points: 0, 10, 20, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 210, 240, 300 and 360 minutes after ingesting 75 g plus an additional 40 mg/kg of glucose and 25 grams of fructose. Fructose was included to drive de novo lipogenesis [45] and did not contribute to plasma glucose concentrations [46]. Blood glucose was measured at the bedside with the StatStrip® Hospital Glucose Monitoring System (Novo Biomedical, Waltham, MA, USA). Serum insulin was measured with radioimmunoassay (Millipore, Billerica, MA).

2.3 Oral Minimal Model

The OMM was used to simulate glucose-insulin dynamics and estimate S_I [30]. The OMM is a one-compartment model represented by the following equations:

$$\begin{cases} \dot{G}(t) = -[S_G + X(t)] \cdot G(t) + S_G \cdot G_b + \dfrac{Ra_{meal}(\boldsymbol{\alpha}, t)}{V} & G(0) = G_b \\ \dot{X}(t) = -p_2 \cdot X(t) + p_3 \cdot [I(t) - I_b] & X(0) = 0 \end{cases}$$

with

$$Ra_{meal}(\boldsymbol{\alpha}, t) = \begin{cases} \alpha_{i-1} + \dfrac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}} \cdot (t - t_{i-1}) \ for\ t_{i-1} \le t \le t_i, i = 1, \dots, n \\ 0 \qquad\qquad otherwise \end{cases}$$

where $G(t)$ is glucose concentration; $X(t)$ is insulin action; $I(t)$ is insulin concentration; G_b and I_b are basal glucose and insulin concentrations, respectively; S_G, p_2 and p_3 are rate constants; and $Ra_{meal}(\boldsymbol{\alpha}, t)$ is a piecewise-linear function describing the rate of

appearance of exogenous glucose. The time breakpoints t_i were specified by the sampling times and protocol duration, and parameter $\boldsymbol{\alpha} = [\alpha_0, \alpha_1, \ldots, \alpha_n]^{\mathrm{T}}$ with $\alpha_0 = 0$ are unknown amplitudes [30, 31, 39]. The breakpoints for the 6-hour protocol represent the sampled time points for the full glucose profile, and the breakpoints for the shorter protocols represent truncated subsets of the 6-hour breakpoints based on protocol duration (see Supplementary Material for details) [38].

We implemented both "truncated" and "exponential" versions of OMM. For sufficiently long protocols that showed glucose and insulin concentrations returning to baseline values (4- and 6- hours of data), we directly applied the OMM to compute S_I ("truncated models"). For shorter protocols, it has been recommended to assume an exponential decay of $Ra_{meal}(\boldsymbol{\alpha}, t)$ after the last time breakpoint [47]. In our IR population, we explored this approach for 2-, 3- and 4-hour OGTT protocols using both fixed and estimated decay time constants ("exponential models," see Supplementary Material for details). In the exponential models, S_I was estimated directly, and in the other model implementations S_I was defined to be

$$S_I = \frac{p_3}{p_2} \cdot V$$

with units dl/kg/min per μU/ml [31]. To improve numerical identifiability of OMM, we applied two constraints that have been described previously [30, 31]. Details regarding these constraints are included in Supplementary Material.

The OMM was implemented for each participant using 2, 3, 4 and 6 hours of data. For exponential models, exponential decay of the rate of appearance of glucose was assumed

following the last time point for 2, 3, and 4 hours of data. All model equations were implemented in SAAM II (SAAM II software v 2.2, The Epsilon group, Charlottesville, VA, USA).

2.4 Statistical Analysis and Model Comparison

All model comparisons were performed in MATLAB (Mathworks, Natick, MA). Data are reported as the mean ± SD. ANOVA was used to compare S_I values obtained from each method. The 6-hour OMM S_I estimates were taken to be the reference estimates because glucose concentrations returned to baseline for all participants by 6 hours [30]. Six hour estimates were compared to the S_I estimates from shorter OMM protocol durations using Pearson's correlation coefficients, linear regressions, and Bland-Altman plots [48] to quantify the differences between estimates obtained using different methods. The Williams correlation test was used for pairwise comparison of the strengths of the correlations [49]. Additionally, the mean squared error (MSE) between 6-hour OMM S_I estimates and S_I estimates from protocols of shorter duration were computed, and we compared these errors using paired t-tests. For Bland-Altman plots the bias, the 95% confidence intervals of the bias, and the 95% limits of agreement between the S_I estimates obtained using the two methods are reported.

To investigate the differences in S_I values estimated using the 4-hour truncated model and the 4-hour exponential model, participants were divided into subgroups based on 4 hour glucose concentrations being above, below or within 10 mg/dL of baseline glucose. For each subgroup, the MSE between 4-hour S_I estimates associated with the exponential and

truncated models and 6-hour estimates were calculated. The errors associated with the S_I estimates obtained from 4-hour exponential and 4-hour truncated models were compared using paired t-tests. Correlations between the S_I estimates from the 6-hour OMM and the S_I estimates from the 4-hour exponential and 4-hour truncated models by subgroup were calculated and compared for each subgroup [50].

The error between the glucose data and simulated 6-hour glucose concentrations for each of the four models was computed as the square root of the squared sum of differences between the data and simulated glucose at each sample time. To elucidate how the exponential assumption for $Ra_{meal}(\boldsymbol{\alpha}, t)$ translates to glucose dynamics across the OGTT, the 2-, 3- and 4-hour exponential models were simulated for 6 hours. Predicted glucose concentrations associated with each model were plotted with the glucose concentration data and the 6-hour model simulations. Model simulations were performed using the MATLAB built-in ode solver **ode45.** P-values < 0.05 were considered significant; we report p-values greater than 0.001 and otherwise report p-values as <0.001.

3. RESULTS

3.1 Participants

Sixty-eight participants were included in this study. Participant demographics are summarized in Table 1. The mean 2-hour glucose is close to the pre-diabetes cut-off of > 140 mg/dL demonstrating that dysglycemia was common in this cohort. The average

glucose and insulin concentrations across the OGTT illustrate the moderate variability in glucose and large variability of insulin concentrations for this participant group (Fig. 1).

3.2 OMM Implementations

Glucose and insulin data are presented with simulated glucose time traces for each model for four representative participants, ordered from more to less insulin sensitive (Fig. 2). The measured insulin concentration data describe each individual's insulin response to the OGTT and are used to force the insulin action dynamics in OMM.

All of the models accurately described glucose concentrations over the duration for which data were specified as assessed by least squares error. By contrast with the 6-hour and 4-hour truncated models, the exponential models predicted glucose concentrations beyond the duration for which data were specified using the assumption of the exponentially decaying $Ra_{meal}(\boldsymbol{\alpha}, t)$. However, in general, exponential assumptions for $Ra_{meal}(\boldsymbol{\alpha}, t)$ did not accurately reproduce glucose profiles. Error generally decreased with model duration with mean errors of 60.73 ± 36 (mean ± SD), 40.80 ± 23, 29.61 ± 17 and 19.43 ± 6 mg/dL for the 2-, 3- and 4- hour exponential models and the 6-hour model, respectively.

The estimated profiles of $Ra_{meal}(\boldsymbol{\alpha}, t)$ also varied with model duration as seen for the 4 representative participants (Fig. 3**)**. In general, the assumption of exponential decay in the 2-, 3- and 4-hour exponential methods resulted in less variability in $Ra_{meal}(\boldsymbol{\alpha}, t)$ compared to the rates predicted in the 6-hour OMM implementation.

3.3 Model Comparisons

To investigate the effect of model duration on estimated S_I, we compared S_I values calculated using the 6-hour OMM to S_I values calculated from OMM implementations with shorter durations over all participants. For each model, average S_I estimates and average precision of S_I estimates given as the coefficient of variation (CV) were determined (Table 2). Mean S_I values for all participants were not significantly different across methods (ANOVA, $p = 0.79$). However, the MSE between the 6-hour S_I estimates and S_I estimates from shorter OMM implementations decreased with protocol duration with the lowest errors associated with the 4-hour truncated model. Specifically, the errors for S_I estimates from the 4-hour truncated model were lower compared to the errors for the S_I estimates from other models with the exception of the 2-hour exponential model (4-hour exponential: $p < 0.001$; 3-hour exponential: $p = 0.01$; 2-hour exponential: $p = 0.07$). Similarly, the errors for S_I estimates from the 4-hour exponential model were lower compared to the errors for S_I estimates from the 3-hour exponential model ($p = 0.01$) and not significantly different from the errors for S_I estimates from the 2-hour exponential model ($p = 0.78$).

The correlations between the S_I estimates from OMM implementations with shorter durations and the S_I estimates from the 6-hour OMM were high and increased monotonically with protocol duration from the 2-hour exponential model ($R = 0.93$) to the 4-hour truncated model ($R = 0.97$) (Fig. 4). Comparisons of correlations showed that the correlations between the 6-hour OMM S_I estimates and the 2-hour and 3-hour exponential S_I estimates, respectively, were generally weaker than the correlations

between the 6-hour OMM S_I estimates and the 4-hour exponential or 4-truncated S_I estimates (2-hour exponential v. 4-hour exponential, $p = 0.008$; 2-hour exponential v. 4-hour truncated, $p < 0.001$; 3-hour exponential v. 4-hour truncated, $p = 0.02$). However, in general, correlations between the 6-hour OMM S_I estimates and OMM S_I estimates from OMM exponential implementations were not significantly different (2-hour exponential v. 3-hour exponential, $p = 0.07$; 3-hour exponential v. 4-hour exponential, $p = 0.29$). Moreover, the correlation between the 4-hour truncated S_I estimates and the 6-hour OMM S_I estimates was not significantly different from the correlation of the 4-hour exponential S_I estimates and the 6-hour OMM S_I estimates ($p = 0.09$).

To further characterize the correlations between S_I estimates obtained using different OMM implementations, we compared the regression lines associated with S_I estimates from different methods to the identity line. The slopes of the regression lines for the 3- and 4- hour exponential model S_I estimates compared to the 6-hour model S_I estimates were 1.03 and 1.05, respectively, and these slopes were not significantly different from 1 (both $p<0.001$). The slope of the regression line was 0.79 for the 2-hour exponential model S_I estimates and 0.88 for the 3-hour exponential model S_I estimates. The intercept for the regression line for the 4-hour exponential model S_I estimates was computed as 8.69×10^{-6}, but it was not significantly different from 0 ($p<0.001$). All other intercepts were significantly different from 0 (all $p<0.001$) with intercepts of 3.03×10^{-5}, 4.24×10^{-5}, and 7.77×10^{-5} for the S_I estimates associated with the 4-hour truncated, 3-hour exponential, and 2-hour exponential models, respectively.

Bland-Altman plots demonstrated the differences in the S_I estimates from shorter OMMs compared to the 6-hour S_I estimates (Fig. 5). Differences between S_I estimates obtained using different models do not show systematic change with average S_I values, indicating that the variability in the differences is not related to the size of the measurement. The biases for the S_I estimates from the 2-, 3-, and 4-hour exponential models were of order 10^{-5}, positive, and, for the 3- and 4-hour exponential models, significantly different from zero, indicating that these models underestimate S_I. By contrast, the bias for the 4-hour truncated model was an order of magnitude smaller than the bias for the other models, negative, and the CI of the bias contained zero. Similar results were observed when analyzing Bland-Altman plots for the percent differences with the smallest bias in the percent differences occurring for the 4-hour truncated model (data not shown).

3.4 Baseline glucose affects variability in S_I estimates

To better understand the effects of delayed return to baseline and reactive hypoglycemia on the 4-hour OMM implementations, we considered glucose concentrations 4 hours after drink ingestion relative to return to baseline glucose for each participant. After dividing participants into subgroups based on whether they were above, below, or within 10 mg/dl of their baseline glucose concentrations at 4 hours we compared 4-hour S_I estimates from both the exponential and truncated models for each subgroup. Approximately 54% (n = 37) of participants had 4-hour glucose concentrations at least 10 mg/dL below their baseline glucose levels; 10% (n = 7) of participants had 4-hour glucose concentrations at least 10 mg/dL above their baseline glucose levels; and the remaining participants (n = 24) had 4-hour glucose concentrations within 10 mg/dL of their baseline glucose levels.

We compared S_I estimates from the 4-hour exponential and 4-hour truncated models relative to the 6-hour S_I estimates using MSE. The MSE of the 4-hour exponential S_I estimates was 1.07×10^{-8}, 1.27×10^{-8}, and 2.21×10^{-9} for subgroups with 4-hour glucose concentrations that were 10 mg/dL above, within, or below baseline glucose concentrations, respectively. The MSE of the 4-hour truncated S_I estimates was 3.50×10^{-9}, 9.98×10^{-9}, and 3.50×10^{-9} for the above, within, and below 10 mg/dL subgroups, respectively. For each subgroup, the errors in S_I estimates from the 4-hour exponential and 4-hour truncated models were significantly different: errors in S_I estimates associated with the 4-hour exponential model were lower for the 10 mg/dL below baseline subgroup ($p = 0.003$), and errors in S_I estimates from the 4-hour truncated model were lower for the 10 mg/dL above ($p = 0.002$) and within ($p = 0.03$) subgroups.

We also compared the correlations for S_I estimates from both 4-hour models to S_I estimates from the 6-hour model by subgroup. We did not detect differences in correlations of S_I estimates based on the 4-hour model used; however, we did observe differences by subgroup. Specifically, we found that for the 4-hour exponential model, the correlation in S_I estimates for the 10 mg/dL above baseline subgroup ($R = 0.74$) was weaker compared to the correlations for other subgroups (all $R \geq 0.97$; within: $p = 0.02$; below: $p = 0.02$) (Fig. 6). Similarly, for the 4-hour truncated model, the correlation in S_I estimates for the 10 mg/dL above baseline subgroup ($R = 0.80$) was weaker compared to the correlation for the 10 mg/dL within baseline subgroup ($R = 0.97$; $p = 0.05$) and marginally weaker compared to the correlation for the 10 mg/dL below baseline subgroup

(R = 0.97; p = 0.06). There were no differences in the correlations of S_I estimates from either 4-hour model for the 10 mg/dL below or within subgroups (4-hour exponential: p = 0.92, 4-hour truncated: p = 0.73). These results indicate that the S_I values estimated using either 4-hour model were less reliable for the 10 mg/dl above baseline subgroup compared to the other subgroups.

4. DISCUSSION

We investigated differences in S_I estimates obtained from 2-, 3- and 4-hour implementations of OMM as compared to a 6-hour OMM implementation to describe OGTT glucose concentration data in adolescent girls with obesity. Insulin sensitivity is reduced in adolescents compared to adults [51, 9], and the estimates of S_I in our cohort of adolescent girls with obesity are consistent with reduced insulin sensitivity as reported in previous studies [52]. However, our results established that, in this cohort, OMM-based S_I estimates depend on the duration of the data considered and generally improve with inclusion of more data up to 6 hours. OMM-based estimates of S_I utilize the full dynamics of glucose and insulin during the course of an OGTT to describe S_I [53]. In the current analysis, we took the S_I estimates from a 6-hour OMM to represent the reference estimate against which other S_I estimates are compared because the 6-hour protocol was sufficiently long that glucose concentrations returned to baseline levels for all participants, a feature associated with optimal numerical identifiability of OMM. Furthermore, longer protocol durations enabled the inclusion of the full glucose profile, so more features of the data were represented in these estimates of S_I.

Of the shorter implementations, the 4-hour truncated OMM provided the most reliable estimates of S_I compared to S_I estimates from other OMM implementations. For the majority of participants, glucose concentrations return to baseline after 4 hours, and, therefore, 4- and 6-hour S_I estimates reflect similar data features. Estimates of S_I using implementations of OMM for shorter durations of data were highly correlated with the 6-hour OMM S_I estimates consistent with results in healthy adult populations [38]. However, in our IR cohort, S_I estimates from 2-, 3-, and 4-hour exponential OMM implementations showed significant bias indicating that these methods may underestimate S_I compared to 6-hour OMM S_I estimates, thereby potentially misrepresenting the degree of metabolic disease experienced by a particular individual or patient population. These results suggest that, in a cohort of adolescent girls with obesity, reliable estimates of S_I using OMM require OMM implementations based on OGTT data of at least 4 hours. These findings are consistent with results suggesting that glucose and insulin concentrations at the 240 min time point may help estimate S_I in adults with severe type 2 diabetes [41].

By contrast with our results, previous studies that investigated the effect of protocol duration on OMM-based estimates of S_I did not find that S_I was sensitive to protocol duration [38]. However, these studies focused on healthy adult populations or small populations of adolescents [40]. The protocol duration-dependence we observed may arise from assumptions in the model that do not apply to our cohort of adolescent girls with obesity. For example, we observed more variability in the rate of appearance of glucose ($Ra_{meal}(\boldsymbol{\alpha}, t)$) in our cohort compared to published estimates from adults [31].

This difference reflected the increased variability in glucose trajectories following drink ingestion, longer durations of elevated glucose, and frequent incidence of reactive hypoglycemia (in which glucose concentrations fall below baseline levels during the recovery from the glucose challenge) in our cohort compared to the glucose profiles of healthy adults [9]. To account for these features of the glucose profiles in our cohort, we included more breakpoints in our linear approximation of $Ra_{meal}(\boldsymbol{\alpha}, t)$. This approach produced robust simulated glucose profiles, however, it may have contributed to overfitting of $Ra_{meal}(\boldsymbol{\alpha}, t)$ in our participants. More work is needed to establish optimal breakpoints for the representation of $Ra_{meal}(\boldsymbol{\alpha}, t)$ in populations, such as adolescent girls with obesity, with atypical glucose trajectories following drink ingestion.

Relatedly, we found that the glucose-insulin dynamics in our cohort were not well described by implementations of OMM that assumed exponential decay of $Ra_{meal}(\boldsymbol{\alpha}, t)$ after 2, 3, or 4 hours in contrast to results for other populations [31]. Specifically, previous work describing implementations of OMM with 2- or 3- hour OGTT protocols has imposed an exponential decay in $Ra_{meal}(\boldsymbol{\alpha}, t)$ to account for the time course of glucose absorption in 2- or 3-hour OGTT protocols when glucose concentrations have not returned to baseline levels by the 120 min or 180 min time point, respectively. This method was developed in relatively healthy adults and successfully described glucose dynamics in these participants [30]. However, this assumption did not represent the glucose dynamics observed in most participants in this study even when time constants of decay were estimated as a parameter of the model. This mismatch was particularly pronounced in the glucose profiles of participants with the lowest S_I estimates whose

glucose concentrations tended to remain high beyond 120 minutes. Furthermore, the assumption of exponential decay in the 4-hour exponential model adversely affected reliability of S_I estimates, although this effect was minimized for participants with 4-hour glucose concentrations near or below baseline glucose levels where this assumption minimally affected glucose dynamics. These results highlight the challenges of applying OMM in populations with diverse metabolic phenotypes such as adolescents with increased insulin secretion rates, women and girls with PCOS, and individuals with other metabolic diseases.

Our results demonstrating that estimates of S_I may depend on protocol duration suggest that preliminary assessment of the typical features of the glucose-insulin dynamics of the study population is necessary to select a protocol duration that is sufficiently long to obtain a reliable estimate of S_I. Specifically, shorter OGTT protocols may be sufficient for OMM-based estimates of S_I when the return to baseline following peak glucose is rapid but may not be sufficient for populations with atypical glucose-insulin dynamics. Thus, the requirements for the precision of S_I estimates should be considered when designing OGTT protocols for OMM-based measures of S_I in IR populations. Furthermore, care should be taken when comparing S_I values estimated using OMM implementations with different protocol durations. Future work using data assimilation techniques may allow for more robust identification of optimal protocol durations for assessing S_I using the OMM in patient populations with diverse metabolic phenotypes.

Author Contributions

K.B. researched data, performed modeling and statistical analysis, and wrote the manuscript; A.M.C. researched data, performed modeling and co-wrote the manuscript; D.X. performed calculations and edited the manuscript; Y.G.R. researched data and edited the manuscript; H.R. researched data and edited the manuscript; LP contributed to statistical analysis and edited the manuscript; M.C.G. and K.J.N. designed the study, researched data, contributed to discussion and edited the manuscript; C.D.B. contributed to study design, performed modeling and statistical analysis, and wrote the manuscript.

Declaration of Competing Interests

The authors declare no competing financial interests.

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FIGURE LEGENDS

Fig. 1. Glucose and insulin concentrations. Glucose (A) and insulin (B) concentration ranges for all study participants (n=68, all female) for six-hour OGTT. Mean glucose and insulin concentrations (black line), ± max/min values (gray shading) indicate range of variability across cohort.

Fig. 2. Simulated glucose dynamics based on different OMM time models. Glucose and insulin (**A, B, C**, **D**) profiles for representative participants with high (**A**, **B**) or low (**C**, **D**) insulin sensitivity as assessed by 6h S_I. The simulated glucose dynamics obtained using the 2h Ex, 3h Ex, 4h Tr, 4h Ex, and 6h implementations of OMM are plotted with the glucose data for four participants. For truncated models (4h Tr and 6h), there are no assumptions of glucose dynamics beyond the specified time breakpoints. For the exponential models (2h Ex, 3h Ex and 4h Ex), exponential decay of the rate of appearance of glucose is assumed after 120 min., 180 min., and 240 min., respectively.

Fig. 3. Estimated rate of appearance of drink glucose by OMM model duration. The rate of appearance of exogenous glucose (Ra) estimated for each model for the 4 representative participants reported in Fig. 2. Onset of exponential decay of 2h Ex, 3h Ex and 4h Ex OMM models is initiated after 120, 180, and 240 min, respectively.

Fig. 4. Correlations between S_I estimated with the 6-hour OMM as a reference and OMM implemented with shorter durations (n = 68, all female). Scatter plots showing the correlations of the 2-hour exponential **(A)**, the 3-hour exponential **(B)**, the 4-hour truncated **(C)**, and the 4-hour exponential (**D**) OMM S_I estimates with the 6-hour OMM S_I estimates. The grey line is the least squares fit to the data, and the black line indicates equality of estimates obtained using different models.

Fig. 5. Bland-Altman plot showing the bias of the S_I estimates computed with the 6-hour OMM and with shorter OMM implementations (n=68, all female). The bias (solid line), the confidence interval of the bias (shaded region), and the 95% limits of agreement (dashed line) are reported. The 2-hour exponential model **(A)**, 3-hour exponential model **(B)**, and 4-hour exponential model **(C)**, all showed a small positive bias indicating that these models underestimate S_I. The 4-hour truncated model **(D)** had a small negative bias indicating this model overestimated S_I.

Fig. 6. Strength of correlation of S_I estimates using 4- and 6-hour OMM protocols varies for subgroups specified by participants' 4-hour glucose concentrations relative to their baseline glucose concentrations. S_I estimates calculated with the 4-hour exponential OMM were strongly correlated with 6-hour OMM S_I estimates for participants who were 10 mg/dL within (n=24, all female, $p < 0.001$) and below (n=37, all female, $p < 0.001$)

their baseline glucose concentrations after the first 4 hours of the OGTT protocol but not for participants who were 10 mg/dL above (n=7, all female, p=0.06) (**A**). S_I estimates calculated with the 4-hour truncated OMM were correlated with 6-hour OMM S_I estimates for participants who were 10 mg/dL above (n=7, all female, p=0.03), within (n=24, all female, $p < 0.001$), and below (n=37, all female, $p < 0.001$) their baseline glucose concentrations after the first 4 hours of the OGTT protocol (**B**).

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TABLES

Table 1: Participant Characteristics. Data shown are mean ± standard deviation of the mean unless otherwise noted.

Age (years)	15.8 ± 1.2
Race/Ethnicity (N) Non-Hispanic White Hispanic White Black Asian	 23 35 8 2
Age at Menarche (Years)	11.6 ± 1.5
Family history of T2D (%)	76%
Weight (kg)	94.0 ± 16.5
Height (cm)	164 ± 7
BMI (kg/m^2)	35.4 ± 5.6
BMI percentile	97.6 ± 2.0
BMI Z-score	2.08 ± 0.34
Waist:Hip Ratio	0.89 ± 0.07
Hemoglobin A1c (%)	5.4 ± 0.3
Fasting glucose (mg/dL)	90 ± 9
2 hour glucose (mg/dL)	136 ± 24
Fasting Insulin (mIU/L)	28 ± 16
2 hour insulin (mIU/L)	276 ± 200

Table 2. Average S_I estimates, precision of S_I estimates and MSE. Values are mean ± SD. Reported is the average precision of S_I estimates is given as the coefficient of variation (CV %) and the mean squared error (MSE) of S_I estimates from shorter OMM implementations to estimates of S_I from the 6-hour.

OMM Implementation	S_I, 10^{-4} dl/kg/min per μU/ml	Precision, CV %	MSE, 10^{-9}
2-hour Ex	2.86 ± 3.31	24.27 ± 5	16.1
3-hour Ex	2.55 ± 2.62	19.35 ± 7	10.3
4-hour Ex	2.81 ± 2.59	16.25 ± 6	6.79
4-hour Tr	3.13 ± 3.14	18.60 ± 6	5.97
6-hour	3.06 ± 2.85	12.77 ± 6	