Insulin sensitivity and glucose effectiveness from three minimal models: effects of energy restriction and body fat in adult male rhesus monkeys

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The minimal model of glucose disappearance (MINMOD version 3; MM3) and both the one-compartment (1CMM) and the two-compartment (2CMM) minimal models were used to analyze stable isotope-labeled intravenous glucose tolerance test (IVGTT) data from year 10 of a study of the effect of dietary restriction (DR) in male rhesus monkeys. Adult monkeys were energy restricted (R; n = 12) on a semipurified diet to ~70% of control (C) intake (ad libitum-fed monkeys; n = 12). Under ketamine anesthesia, fasting insulin levels were greater among C monkeys. Insulin sensitivity estimates from all models were greater in R than C monkeys, whereas glucose effectiveness estimates were not consistently greater in R monkeys. Fasting plasma glucose as well as hepatic glucose production and clearance rates did not differ between groups. Body fat, in part, statistically mediated the effect of DR to enhance insulin sensitivity indexes. Precision of estimation and intermodel relationships among insulin sensitivity and glucose effectiveness estimates were in the ranges of those reported previously for humans and dogs, suggesting that the models may provide valid estimates for rhesus monkeys as well. The observed insulin sensitivity indexes from all models, elevated among R vs. C monkeys, may be explained, at least in part, by the difference in body fat content between these groups after chronic DR.

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duction profile during the test remained physiologically implausible (17). A more complex two-compartment minimal model (2CMM) was introduced subsequently (58) that 1) described the system in terms of both rapidly and slowly equilibrating glucose compartments, 2) accounted for both a constant glucose uptake component and one that is proportional to plasma glucose concentration, and 3) allowed for the segregation of the effects of uptake from production for both $S_1$ and $S_2$ parameters.

Therefore, in year 10 of this longitudinal study of the effect of DR and aging, to assess the validity of our previous results from version 3 of the minimal model of glucose disappearance (MINMOD v. 3; MM3), we modeled unlabeled IVGTT glucose data with the use of the MM3 and stable isotope-labeled IVGTT (described below). Plasma insulin and total glucose concentrations were analyzed using the MM3 (provided by R. N. Bergman) (10, 12). The plasma insulin, glucose, and [6,6-2H2]glucose concentrations were also analyzed by use of the labeled 1CMM (4, 22) and 2CMM (58) minimal models in Simulation Analysis and Modeling software (SAAM II, v. 1.1.1 for Macintosh; Resource Facility for Kinetic Analysis, SAAM Institute, Seattle, WA).

**IVGTT Protocol**

A stock solution of tracer ([6,6-2H2]glucose, 98% enrichment as a powder; Aldrich, Milwaukee, WI) was prepared by dissolving tracer powder in deionized distilled water and filtering it through a 0.22-μm Millipore filter (Bedford, MA) into sterile vials that were sealed until use. On the morning of the IVGTT, the labeled dosage was prepared at 10% of the total glucose administered by mixing the stock tracer solution with a 50% dextrose solution (Abbott Laboratories, North Chicago, IL). 

After an overnight fast, each monkey was anesthetized with ketamine- HCl (15 mg/kg body wt, im) and weighed. Diazepam (1.25 mg/kg body wt im) was administered before the IVGTT for muscular relaxation, and anesthesia was maintained as necessary with additional injections of ketamine-HCl (5 mg/kg body wt) as needed during the procedure. A catheter was introduced into the saphenous vein and threaded to the inferior vena cava for both glucose administration and blood sampling. At -15, -5, and -1 min before glucose administration, 2.5-ml blood samples were collected. At time 0, glucose (0.3 g/kg body wt, 10% of which was [6,6-2H2]glucose) was infused over ~45 s. Thereafter, 2.5-ml blood samples were drawn at 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 35, 40, 60, 80, 100, 120, 140, 180, 210, and 240 min; an equivalent amount of sterile saline solution (Plasmalyte; Baxter HealthCare, Deerfield, IL) was used to flush the catheter line. The catheter was maintained patent with a saline drip. The tubes were centrifuged, and plasma was stored at ~20°C in three aliquots for analysis of insulin and glucose concentrations and the [6,6-2H2]glucose peak isotope ratios.

**Determination of Plasma Insulin and Glucose Concentrations and Preparation for Mass Spectrometry**

Plasma insulin was measured in duplicate by double-antibody radioimmunoassay (Linco Human Insulin Specific RIA kit; Linco Research, St. Charles, MO). Interassay and intra-assay coefficients of variation (CVs) were 6.39 and 3.29%, respectively. Total glucose was measured in duplicate with an automated analyzer by use of the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, Ohio); the CV for glucose determination was ±2% over the range of glucose values analyzed. Total glycated hemoglobin was measured using a Glyco-Teck affinity column method (Helena Labs, Beaumont, TX). Serum triglycerides were measured using a spectrophotometric assay from a fasted blood sample taken on a separate day during the same assessment period. [6,6-2H2]glucose was analyzed as a aldonoitrile penta-acetate derivative in triplicate after isolation from deproteinized plasma by ion-exchange chromatography. Briefly, a 100-μl
sample of plasma was mixed with 400 μl of 6% trichloroacetic acid (Aldrich) and centrifuged for 10 min. The supernatant was passed through an ion-exchange column containing a mixed-bed anion and cation resin (Sigma, St. Louis, MO), and the column was washed with 5 × 400 μl of deionized distilled water. The eluant was gently dried with forced air, and the tubes were capped and stored with a desiccant in sealed containers. Just before gas chromatography-mass spectrometry (GC-MS) analysis, the labeled glucose in the residue was derivatized by adding 75 μl of each acetic anhydride (Supelco, Bellefonte, PA) and pyridine (Aldrich) and heating the tubes for 30 min at 100°C. Excess derivatizing reagents were gently evaporated in a hood under a stream of nitrogen. Ethyl acetate (Fisher Scientific, Hanoverpark, IL) was used to take up the derivative for GC-MS analysis.

**GC-MS Analyses**

A Hewlett-Packard 5890 gas chromatograph (GC) coupled to a Hewlett-Packard 5988 mass spectrometer (MS; Hewlett-Packard, Palo Alto, CA) was used for isotope analysis. The GC was equipped with a 30-m SE-30 capillary column (J&W Scientific, Folsom, CA). Glucose penta-acetate ions were analyzed by electron ionization under selective ion monitoring at mass-to-charge ratios of 242 (m0), 243 (m1), and 244 (m2). Helium was the carrier gas. The injector temperature was set at 220°C, and the transfer line between the GC and MS was set at 220°C. The initial oven temperature was set at 150°C for 1 min and ramped at a rate of 35°C/min to a maximum of 220°C, where it was held for 0.5 min. The GC-MS instrument was linked to a personal computer by Vector/Two software (Technivent, Madison, WI) for initial analysis of integrated glucose peak areas. Files were downloaded to a second computer for calculating peak isotope ratios as previously described (26).

**Calculations for Plasma Insulin and Glucose and the Glucose Peak Isotope Ratios**

Fasting plasma glucose (\(G_0\)) and insulin (\(I_0\)) levels were calculated as the means of the sample values obtained before the glucose injection. Integrated first phase (0–10 min of the IVGTT) plasma insulin first (0–10 min, acute insulin response to glucose; \(|AIRG|\)) and second (10–180 min; \(|PI2|\)) areas under the curve (AUCs) were calculated by use of the trapezoid rule. Peak isotope ratios were used to calculate the tracer glucose concentration used in the data files for modeling (see **Appendix A**). The measurement error associated with tracer glucose concentration was estimated by error propagation (21). Weighted residuals were calculated as the weighted error between the observed and predicted value at each sample time (21).

**Minimal Model Analysis of the IVGTT Data**

The insulin and glucose (unlabeled and labeled) data from this assessment period were analyzed using three mathematical models of glucose disappearance. The equations for these models are detailed in **Appendix A**, and a glossary of model parameters and definitions is shown in **Appendix B** (see Table B1). First, the insulin and unlabeled glucose concentrations were analyzed in the minimal model of glucose disappearance (MM3), which assumes a one-compartment glucose pool (8) and does not utilize tracer glucose data. Parameters from the models are estimated from glucose concentration data by weighted nonlinear least squares. The MM3 provides estimates for a number of parameters, including the \(S_2\) index, which is a fractional glucose uptake rate per unit of plasma insulin. This index represents the ability of insulin both to enhance glucose uptake from plasma and to inhibit glucose release into plasma. The MM3 also provides an estimate of \(S_G\) (fractional glucose uptake), which reflects the effect of glucose to enhance its own disappearance from plasma and to inhibit the release of glucose into plasma independently of a rise of plasma insulin above basal level. We used the MM3 for the first nine years of this study in conjunction with the tolbutamide-modified IVGTT protocol (35, 42, 43).

The other models (1CMM and 2CMM) also provided estimates of \(S_1\) and \(S_2\), but the use of a stable isotope-labeled glucose molecule ([6,6-2H2]glucose) as a tracer allows estimation of only the glucose disappearance component of each of these indexes. That is, \(S_1\) and \(S_2\) from the 1CMM and \(S_2^*\) (the superscript 2 denotes the 2nd glucose compartment) and \(S_2^\dagger\) from the 2CMM reflect only the effects of insulin and glucose, respectively, to enhance glucose uptake from plasma. As its name suggests, the 1CMM (4) describes a one-compartment system wherein glucose equilibrates rapidly and from which insulin-dependent glucose uptake occurs; this model assumes that uptake is proportional to plasma glucose concentration. Glucose values in the 2- through 6-min samples do not contribute to the model fit to account for the glucose equilibration that occurs in the single glucose compartment. The data are fit using weighted nonlinear least squares (21). The isotope ratio measurement error variance was used to generate variance of the error associated with tracer measurements by error propagation (21).

Likewise, the 2CMM describes a two-compartment glucose system, with one compartment (accessible) representing rapidly exchanging tissues and the other representing more slowly exchanging tissues (58). Interstitial fluid likely represents the slowly equilibrating (inaccessible) compartment (9) in which insulin-dependent glucose uptake is assumed to occur. Insulin-independent glucose uptake, three times the rate of insulin-dependent glucose uptake in the basal state (24), is assumed to occur in the accessible compartment. Unlike the 1CMM, the 2CMM model assumes that glucose uptake is the sum of two components, one constant and one proportional to glucose concentration. Also, unlike the 1CMM, the 2CMM uses glucose values of the entire test (2–240 min).

Because we had not used the 1CMM and 2CMM previously with data from rhesus monkeys, we used the initial parameter settings from human data in the 1CMM. We used the means of the final parameter estimates provided by that model for the starting parameters of each group (C and R) in the 2CMM. Means were calculated for these after several data sets were run. These were used, in turn, as the initial settings for running the remaining data sets in this model. When the model did not fit the data well, given the original starting parameter values, we allowed some flexibility in the modeling process by treating some parameters (e.g., \(p_2\), \(k_{2,1}\)) as Bayesian terms, i.e., we provided a mean (based on parameter values that data sets of other animals ran well with) and a broad standard deviation to allow the model to fit the data within these constraints. All but one data set ran with acceptable precision (CVs generally <50%) in the 1CMM, and all data sets ran well in the 2CMM. The assumption of the original 2CMM (17), that glucose uptake at time 0, \(R_{0,0}\) (or \(k_{0,1}\)), = 1 mg · kg \(^{-1}\) · min \(^{-1}\), led to an unreliable negative value of the parameter \(k_0\), a proportionality constant used to calculate glucose disappearance from the accessible compartment, \(k_0\) (and, in turn, \(S_2^\dagger\)). As a result of modifying this value with the use of the revised model (and based on previous studies using data from 14...
human subjects; unpublished data), $k_w$ was always a positive value in the revised model results.

**Segregation of Control Group Animals**

Over the previous several years of the study, some C monkeys exhibited either chronic or episodic fasting hyperinsulinemia based on our criteria previously reported (35). Briefly, if for two or more consecutive assessment periods the fasting plasma insulin level was above the 90th percentile ($\sim 55$ mU/ml) of its distribution at the 5-yr assessment period, we considered that animal hyperinsulinemic (hyperinsulinemic controls; HIC). There were four C animals in this subgroup. For some of the regression analyses, we segregated the data of these C monkeys to allow comparison of R monkeys with the normoinsulinemic controls (NIC).

**Statistical Methods**

Comparison of treatment groups at year 10 was made by analysis of variance (ANOVA) using JMP (v. 3.2.2; SAS Institute, Cary, NC). ANOVA was carried out, first, with all C vs. R monkeys and, second, when the C group was split into the HIC and remaining NIC monkeys. When the overall model $F$ statistic for the latter ANOVA was significant ($P < 0.05$), indicating that the treatment group differences varied significantly, NIC vs. R, HIC vs. R, and NIC vs. HIC treatment group comparisons were tested individually by Fisher’s protected least significant difference procedure, which is an acceptable procedure when there are only three groups compared (51).

Because $S_1$ differed between C and R monkeys, we examined the contribution of various measures of body fat (which we will refer to as $Z$) as a mediator to the relationship between DR ($X$) and the various $S_1$ measures ($Y$) from all three minimal models. We tested for the mediation effect by adapting the approach of Baron and Kenny (5). DR was treated as a dichotomous variable. Briefly, first we examined correlations of all possible DR-SI ($X$-$Y$), DR-fat ($X$-$Z$), and $S_1$-fat ($Y$-$Z$) combinations. For each $S_1$-fat pair, only if all correlations were significant ($P < 0.05$), we compared the slopes of two equations: $I$ the slope of the regression of $S_0$ on the energy intake ($Y$ on $X$ and $Z$) the slope of the same regression when the body fat variable was added as a second independent variable ($Y$ on $X$ + $Z$). We used a bootstrap analysis performed in S-PLUS (v. 3.4; Insightful, Seattle, WA) to estimate the variance of the differences in these slopes from 10,000 simulated data sets for each $Y$-$Z$ pair, with data randomly selected with replacement. Finally, we performed a $t$-test to test whether the mean of these 10,000 values was different from zero. A $P$ value < 0.05 provided evidence that the fat variable altered the slope of the original $Y = X$ regression and thus mediated the effect of DR on $S_1$.

The percent change in slope with and without the fat variable was calculated to quantitate an index of the effect of fat on this relationship.

The differences in pre-IVGTT and late-IVGTT glucose and insulin concentrations between C and R groups were tested with the Wilcoxon signed-rank test, a paired, nonparametric test. Intermodel differences in $S_1$ and $S_0$ parameters were also tested within each treatment group with the Wilcoxon signed-rank test. The Spearman rho (nonparametric) correlation coefficients are reported (see Tables 4 and 5). Wilcoxon and Spearman rho analyses were performed using JMP.

**RESULTS**

Characteristics of the subjects at the 10-yr assessment period are summarized in Table 1. Intake of R monkeys was $70\%$ of intake of C monkeys at 10 yr; it was correlated with body weight only among C monkeys (see Table 4). R monkeys weighed less ($8$–$13$ kg vs. $10$–$20$ kg), carried less body fat ($10$–$24\%$ vs. $17$–$44\%$), and exhibited lower serum triglyceride and plasma insulin levels than C monkeys. R monkeys, however, had $G_b$, rate of disappearance of glucose from plasma ($K_g$), and glycated hemoglobin levels similar to those of C monkeys. In addition, differences between the C and R groups could often be attributed to the contribution of a number of chronically HIC animals as described previously (35). Furthermore, when the HIC data were segregated from those of the remaining NIC animals, the differences between NIC and R groups often remained. The HIC animals also exhibited serum triglyceride levels that were elevated above not only R but also NIC monkeys.

Figure 1 shows mean plasma glucose, plasma insulin, and isotope ratio during the labeled IVGTT for R and C groups. Plasma glucose rose in C monkeys to $342 \pm 22$ mg/dl and in R monkeys to $291 \pm 11$ mg/dl after glucose injection. Plasma glucose reached baseline levels at $\sim 42$ min for R and $\sim 61$ min for C monkeys. Distinct first- and second-phase insulin responses were obvious for both groups, and insulin concentration reached baseline levels at $\sim 60$ min for R and $\sim 110$ min for C monkeys. R monkeys exhibited lower plasma insulin responses during first (AIRG) and second (Phig) phases (AIRG for R: $773 \pm 149$ (mU/ml)-min; all C: $1,718 \pm 417$ (mU/ml)-min, $P = 0.0118$; Phig for R: $525 \pm 431$ (mU/ml)-min; all C: $6,089 \pm 2,080$ (mU/ml)-min, $P = 0.0129$). Elevated responses among C monkeys were due, in part, to the relatively HIC

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**Table 1. Characteristics of animals at 10-yr assessment**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, yr</th>
<th>Energy Intake, kcal/day</th>
<th>Body Wt, kg</th>
<th>Body Fat, %</th>
<th>Abdominal Circumference, cm</th>
<th>Serum Triglycerides, mg/dl</th>
<th>Glycated Hemoglobin, %</th>
<th>$K_g$, %/min</th>
<th>$G_b$, mg/dl</th>
<th>$I_a$, mU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>All C</td>
<td>12</td>
<td>19.4 ± 0.5</td>
<td>698 ± 28</td>
<td>14.2 ± 0.71</td>
<td>31 ± 2</td>
<td>64 ± 3</td>
<td>195 ± 49</td>
<td>8.4 ± 0.4</td>
<td>3.62 ± 0.60</td>
<td>63 ± 2</td>
<td>43 ± 12</td>
</tr>
<tr>
<td>NIC</td>
<td>5</td>
<td>19.2 ± 0.4</td>
<td>671 ± 36</td>
<td>13.8 ± 1.06</td>
<td>30 ± 3</td>
<td>63 ± 4</td>
<td>122 ± 75</td>
<td>8.3 ± 0.6</td>
<td>4.39 ± 0.75</td>
<td>63 ± 3</td>
<td>25 ± 44</td>
</tr>
<tr>
<td>HIC</td>
<td>4</td>
<td>19.9 ± 1.3</td>
<td>751 ± 36</td>
<td>15.1 ± 0.31</td>
<td>34 ± 1</td>
<td>66 ± 2</td>
<td>342 ± 133</td>
<td>8.6 ± 0.6</td>
<td>2.08 ± 0.25</td>
<td>63 ± 3</td>
<td>80 ± 28</td>
</tr>
<tr>
<td>R</td>
<td>12</td>
<td>19.2 ± 0.5</td>
<td>493 ± 18</td>
<td>9.8 ± 0.4</td>
<td>15 ± 1</td>
<td>44 ± 1</td>
<td>95 ± 19</td>
<td>7.9 ± 0.3</td>
<td>4.13 ± 0.66</td>
<td>59 ± 3</td>
<td>18 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. Control group (C) was split into fasting hyperinsulinemic (HIC) and normoinsulinemic (NIC) animals, based on continuous or episodic fasting insulin levels over a no. of assessment periods. Restricted (R) group had energy intake limited to 70% of baseline intake for 10 yr. $K_g$, rate of disappearance of glucose from plasma; $G_b$, basal plasma glucose concentration; $I_a$, basal plasma insulin concentration. ($^P < 0.01$ vs. R, $^{2P} < 0.05$ vs. R, $^{3P} < 0.09$, and $^{4P} < 0.05$ vs. HIC.
monkeys in the group, however, even the NIC animals exhibited greater AIRG and Phi2 AUCs vs. R [AIRG for NIC: 1.227 ± 215 (µU/ml)·min, P = 0.0447 vs. R; Phi2 for NIC: 3.748 ± 1.441 (µU/ml)·min, P = 0.0670 vs. R].

Model Fit, SI and SG Estimates, and Intermodel Comparisons

Weighted residuals of both the 1CMM and 2CMM were evenly distributed about zero and showed no systematic deviations, reflecting excellent model fit (not shown). The MM3 provides only the residual sum of squares (C 109 ± 19 vs. R 95 ± 17) as a measure of model fit for each data set as well as CVs of parameter estimation (Table 2).

Estimates and CVs for SI and SG parameters from the MM3 are shown in Table 2. For the MM3, SI was significantly greater among R vs. C monkeys (P = 0.0178). SI ranged from 0.24 to 6.70 × 10⁻⁴·min⁻¹ (µU/ml)⁻¹ for C monkeys and from 1.65 to 10.75 × 10⁻⁴·min⁻¹ (µU/ml)⁻¹ for R monkeys; SG ranged from 1.13 to 6.48 × 10⁻²·min⁻¹ for C monkeys and from 2.43 to 6.02 × 10⁻²/min for R monkeys and differed marginally (P = 0.0556). SI was estimated with a range of precision (CV) of 4–53% (R) and 2–9% (C). CV ranges for SG were 6–57% (R) and 4–67% (C).

Estimates and CVs for the 1CMM and 2CMM SI and SG parameters are also shown in Table 2 (1CMM as SI and SG, and 2CMM as SI* and SG*). Likewise, R monkeys had greater SI* (P = 0.0062) (CV range for R: 3–23%; for C: 1–8%) and SI* values (P = 0.0028) (CV range for R: 3–45%; for C: 2–70%) than C monkeys. R monkeys also exhibited greater SI* (P = 0.0241) (CV range for R: 2–20%; for C: 5–16%) but not SG* values (P = 0.1490) (CV range for R: 3–20%; for C: 3–11%).

Because the SI and SG parameter units differ between 1CMM and 2CMM, multiplying the 1CMM SI* and SG* values by the glucose distribution volume (V) of the single glucose compartment allows one to make intermodel comparisons (58). Likewise, dividing the 2CMM basal plasma glucose clearance rate (PCR) value by the total volume of distribution (VD) from that model provides a fractional glucose clearance rate with units equivalent to the 1CMM SG* (min⁻¹).

Intermodel relationships for SI and SG parameters are shown in Table 3 along with the P values for the paired statistical tests used. Among the C and R groups as well as the subset of NIC monkeys, the MM3 SG parameter is overestimated compared with the 1CMM SG, and the MM3 SI parameter is not different compared with the labeled model values. When the 1CMM and 2CMM parameters were converted to values with equivalent units for comparison, only a trend for a difference between SI* and SG* values among R monkeys was evident. Neither the 1CMM basal hepatic glucose release (HGRb) nor the 2CMM HGRb, PCR, and V and Vp values differed between groups: 1CMM HGRb for R animals (1.47 ± 0.23 mg·kg⁻¹·min⁻¹).

Table 2. Parameters of insulin sensitivity and glucose effectiveness for all minimal models

<table>
<thead>
<tr>
<th></th>
<th>MM3</th>
<th>1CMM</th>
<th>2CMM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SI</td>
<td>SG</td>
<td>SI*</td>
</tr>
<tr>
<td>All C (n = 12)</td>
<td>3.04 ± 0.63(6)</td>
<td>3.09 ± 0.48(17)</td>
<td>2.46 ± 0.43(3)</td>
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<tr>
<td>NIC (n = 8)</td>
<td>3.70 ± 0.75(5)</td>
<td>3.18 ± 0.62(15)</td>
<td>2.96 ± 0.47(2.8)</td>
</tr>
<tr>
<td>HIC (n = 4)</td>
<td>1.72 ± 0.90(7)</td>
<td>2.91 ± 0.85(22)</td>
<td>0.88 ± 0.42(5)</td>
</tr>
<tr>
<td>R (n = 12)</td>
<td>5.90 ± 0.92(11)</td>
<td>4.13 ± 0.40(23)</td>
<td>5.75 ± 0.93(4)</td>
</tr>
</tbody>
</table>

Values are means ± SE, with coefficients of variation (CVs) in parentheses; n = no. of animals. Minimal models are unlabeled minimal model (MM3), labeled 1-compartment minimal model (1CMM), and 2-compartment minimal model (2CMM). Group was split into fasting HIC and remaining NIC animals on the basis of continuous or episodic fasting insulin levels. R group had energy intake restricted to 70% of baseline intake for 10 yr. SI and SG, cold minimal model insulin sensitivity and glucose effectiveness, respectively; SI and SG, insulin sensitivity and glucose effectiveness from the labeled 1CMM, respectively; SI* and SG*, insulin sensitivity and glucose effectiveness from the 2CMM, respectively. V, volume of distribution. Units are as follows: SI and SI* value × 10⁻¹·min⁻¹·(µU/ml)⁻¹; SI and SG, value × 10⁻²·min⁻¹·(µU/ml)⁻¹; SI* and SG*, value × 10⁻³·min⁻¹·(µU/ml)⁻¹. Both SI and SI* refer to the same volume of distribution, which, in the 1CMM, is composed of both the accessible compartment and the total volume of distribution. *P < 0.01 vs. R, **P < 0.05 vs. R, ***P < 0.005 vs. HIC, and ****P < 0.005 vs. HIC.
Correlation Analyses

Tables 4 (C monkeys) and 5 (R monkeys) show the Spearman rho correlation coefficients and P values for model estimates, energy intake, and body composition variables. For C monkeys, MM3 SI correlated with 1CMM SI but not 2CMM S'I, and the 1CMM SI'V and (the 1CMM S'I) estimates were each correlated with the 2CMM S'I'. For R monkeys, all SI parameters were significantly correlated among themselves. In contrast, S0 parameters were not correlated among themselves, with the exception of the marginal association between 1CMM S'O'V and 2CMM S'O' and the significant association between 1CMM S'O and 2CMM S'O for C monkeys.

Among C monkeys only, percent body fat and abdominal circumference, but not body weight, were strongly associated with the 2CMM S'O parameter. The positive correlations between body fat indexes and body weight were expected in both groups, as were the associations among S'O, HGRb, and PCR, since the latter two are calculated from S'O.

Body Fat as a Mediator

Because the SI parameter in all models differed between C and R groups, we assessed the role of percent body fat, abdominal circumference, and total body fat as mediators (independent variables, Z) in the relationship between DR (independent variable, X) and SI parameters (dependent variable, Y) (Table 6). Although all three body fat variables tested appeared to have a marginal effect, percent body fat appeared the most consistent of these variables to act or tend to act as a mediator in the relationships between DR and the three SI indexes. The strongest effects of the fat variables appeared to occur with the MM3 S'I and 1CMM S'I parameters, primarily due to the large variability in the bootstrap means for the 2CMM S'I.

On the basis of values in Table 2, differences within C and R groups were tested by use of the Wilcoxon signed-rank test for paired data.

DISCUSSION

Our analysis of stable isotope-labeled IVGTT data in the 1CMM and 2CMM from the 10-yr assessment period of this ongoing study confirms our findings of approximately nine years using the unlabeled minimal model (MM3) with data from the tolbutamide-modified IVGTT (35). R monkeys, restricted to ~70% of their baseline food intake, exhibited greater insulin sensitivity levels and lower fasting and glucose-stimulated insulin levels than C monkeys. In addition, because the R monkeys weighed less and were leaner than C monkeys, we tested to what extent the relationship between energy intake and insulin sensitivity was mediated by body fat. Our findings support the hypothesis that body fat may explain at least some of the effect of restricted energy intake on insulin sensitivity. Fasting plasma glucose levels did not differ between groups at this assessment period, although they (and total glycated hemoglobin levels) have differed in the past. In light of this, it is not surprising that neither the 2CMM basal hepatic glucose production rate (HGRb) nor the basal glucose PCR differed between groups. Glucose disappearance rate also did not differ between groups at this assessment period. Taken together, these data suggest that moderate DR in nonhuman primates does not appear to alter glucose-related measures to the same extent that it alters insulin-related measures as the animals age, and that enhanced insulin sensitivity and reduced insulin levels may be mediated, in part, by differences in body fat through a reduction in energy intake. An intermodel comparison of the various insulin sensitivity and glucose effectiveness parameters using the same data set also confirmed many of the relationships among these parameters previously reported by others in dogs and humans, providing a validation for use of the minimal models for evaluating rhesus monkey data under the conditions of this study.

Intermodel Values and Relationships

Insulin Sensitivity. The insulin sensitivity index estimated with the MM3 from unlabeled IVGTT data is defined as the ability of insulin to both enhance glucose uptake and inhibit glucose production. The 1CMM and 2CMM provide insulin sensitivity indexes that reflect the ability of insulin to enhance glucose uptake only. Insulin sensitivity index values of all three models (SI, S'I, and S'I') from rhesus monkeys were within the ranges reported for humans and dogs (18, 19, 22, 58). Specifically, 2CMM S'I values for the R monkeys [mean 12.5 × 10⁻² ml·kg⁻¹·min⁻¹·(μU/ml⁻¹)] were comparable to those of young adult humans [13.8 × 10⁻² ml·kg⁻¹·min⁻¹·(μU/ml⁻¹)] reported by Vicini et al. (58). Rhesus monkey insulin sensitivity values were

Table 3. Intermodel relationships among insulin sensitivity and glucose effectiveness parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C (n = 11)</th>
<th>P Value</th>
<th>R (n = 12)</th>
<th>P Value</th>
<th>NIC (n = 8)</th>
<th>P Value</th>
<th>HIC (n = 3)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S'I vs. S'I</td>
<td>0.320</td>
<td>0.0320</td>
<td>0.375</td>
<td>0.850</td>
<td>0.083</td>
<td>0.383</td>
<td>0.850</td>
<td>0.250</td>
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<tr>
<td>S'O vs. S'O</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.008</td>
<td>&lt;0.0001</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.250</td>
</tr>
<tr>
<td>S'I vs. S'I vs. S'O</td>
<td>0.700</td>
<td>0.207</td>
<td>0.641</td>
<td>0.080</td>
<td>0.250</td>
<td>0.080</td>
<td>0.700</td>
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<tr>
<td>S'O vs. S'O vs. S'O</td>
<td>0.520</td>
<td>0.092</td>
<td>0.039</td>
<td>0.003</td>
<td>0.250</td>
<td>0.003</td>
<td>0.250</td>
<td>0.003</td>
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<tr>
<td>PCR/VG vs. S'O</td>
<td>0.002</td>
<td>0.034</td>
<td>0.002</td>
<td>0.034</td>
<td>0.250</td>
<td>0.002</td>
<td>0.250</td>
<td>0.002</td>
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</table>

PCR, plasma glucose clearance rate; V, glucose distribution volume; VG, total volume of distribution.
Table 4. C monkey Spearman rho coefficients and P values for energy intake, select minimal model parameters, and body composition variables

<table>
<thead>
<tr>
<th>MM3 S0</th>
<th>1CMM Sf</th>
<th>1CMM Sf</th>
<th>1CMM Sf V</th>
<th>1CMM Sf V</th>
<th>2CMM Sf</th>
<th>2CMM Sf</th>
<th>Energy Intake</th>
<th>Body Wt</th>
<th>% Body Fat</th>
<th>Abdom Circ</th>
<th>Basal HGP</th>
<th>Basal PCR</th>
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<tbody>
<tr>
<td>r = 0.09;</td>
<td>r = 0.65;</td>
<td>r = 0.01;</td>
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<td>r = 0.43;</td>
<td>r = 0.38;</td>
<td>r = -0.52;</td>
<td>r = -0.49;</td>
<td>r = -0.32;</td>
<td>r = -0.31;</td>
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<td>r = 0.38;</td>
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<tr>
<td>P = 0.7787</td>
<td>P = 0.0299</td>
<td>P = 0.9788</td>
<td>P = 0.0021</td>
<td>P = 0.7495</td>
<td>P = 0.1667</td>
<td>P = 0.2170</td>
<td>P = 0.0849</td>
<td>P = 0.1063</td>
<td>P = 0.3079</td>
<td>P = 0.306</td>
<td>P = 0.1591</td>
<td>P = 0.2170</td>
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<td>r = 0.31;</td>
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<td>r = 0.01;</td>
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<tr>
<td>P = 0.3614</td>
<td>P = 0.4841</td>
<td>P = 0.3550</td>
<td>P = 0.8529</td>
<td>P = 0.9656</td>
<td>P = 0.3663</td>
<td>P = 0.1309</td>
<td>P = 0.1993</td>
<td>P = 0.4168</td>
<td>P = 0.4299</td>
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<td>P = 0.3663</td>
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<td>r = -0.22;</td>
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<td>r = 0.01;</td>
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<tr>
<td>P = 0.2515</td>
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<td>P = 0.0182</td>
<td>P = 0.3319</td>
<td>P = 0.5183</td>
<td>P = 0.8002</td>
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<td>P = 0.4171</td>
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<td>P = 0.9155</td>
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<td>P = 0.3118</td>
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<td>r = 0.012</td>
<td>r = 0.0010</td>
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<td>P = 0.7904</td>
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<td>P = 0.4171</td>
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<td>P = 0.0604</td>
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<td>P = 0.1591</td>
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<td>P = 0.0051</td>
<td>P = 0.0001</td>
<td>P = 0.0001</td>
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<td>r = 0.41;</td>
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<td>P = 0.0000</td>
<td>P = 0.0000</td>
<td>P = 0.0000</td>
<td>P = 0.0000</td>
<td>P = 0.0000</td>
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<td>P = 0.0000</td>
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<td>P = 0.0000</td>
<td></td>
</tr>
</tbody>
</table>

Abdom circ, abdominal circumference; basal HGP and PCR, basal hepatic glucose production rate and basal plasma glucose clearance rate, respectively, from the 2CMM.
Table 5. *R* monkey Spearman rho correlation coefficients and *P* values for energy intake, select minimal model parameters, and body composition variables.

<table>
<thead>
<tr>
<th></th>
<th>MM3 S₀</th>
<th>1CMM S₁</th>
<th>1CMM S₀*V</th>
<th>1CMM S₀</th>
<th>2CMM S₀</th>
<th>Energy Intake</th>
<th>Body Wt</th>
<th>% Body Fat</th>
<th>Abdom Circ</th>
<th>Basal HGP</th>
<th>Basal PCR</th>
</tr>
</thead>
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<td>MM3 S₁</td>
<td>r = 0.39; P = 0.2081</td>
<td>r = 0.84; P = 0.0006</td>
<td>r = -0.08</td>
<td>r = 0.80</td>
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<td>r = 0.30</td>
<td>r = -0.37</td>
<td>r = -0.22</td>
<td>r = -0.59</td>
<td>r = -0.41</td>
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<td>r = -0.32; r = 0.95</td>
<td>r = -0.35; r = 0.71</td>
<td>r = -0.17; r = 0.15</td>
<td>r = -0.23</td>
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<td>r = 0.66;</td>
<td>r = 0.29</td>
<td>r = -0.18</td>
<td>r = 0.05</td>
<td>r = -0.34</td>
<td>r = -0.12</td>
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<td>r = 0.09</td>
<td>r = -0.22;</td>
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<td></td>
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<td>r = -0.13</td>
<td>r = 0.31; r = -0.20;</td>
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<td>r = -0.10</td>
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</tr>
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<td>r = 0.34;</td>
<td>r = 0.18;</td>
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<tr>
<td>Energy intake</td>
<td>r = 0.5419</td>
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<tr>
<td>%Body fat</td>
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<td>r = 0.0001</td>
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<td>r = 0.0001</td>
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</tr>
<tr>
<td>Abdom circ</td>
<td>r = 0.5717</td>
<td>r = 0.6951;</td>
<td>r = 0.5717</td>
<td>r = 0.6951</td>
<td>r = 0.5717</td>
<td>r = 0.6951</td>
<td>r = 0.5717</td>
<td>r = 0.6021</td>
<td>r = 0.6806</td>
<td>r = 0.6806</td>
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</tr>
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<td>Basal HGP</td>
<td>r = 0.6951</td>
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<td>r = 0.6951</td>
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<td>r = 0.6876</td>
<td>r = 0.6876</td>
<td>r = 0.6876</td>
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</tr>
</tbody>
</table>
also comparable to our previously reported values for these animals after 8.5 yr of DR, despite a somewhat different IVGTT protocol (35). One would expect insulin sensitivity estimates from the MM3 to be greater than 1CMM estimates, because the MM3 parameter reflects both uptake and production components and the 1CMM parameter reflects uptake alone. S_I is reported to be underestimated compared with the insulin sensitivity values of the other models (18, 19) and of insulin-stimulated glucose disposal, as measured by the glucose clamp technique (55), the current “gold standard” for this measurement in vivo. Because the single compartment assumption results in the overestimation of S_G and S_G after insulin has returned to the basal level, there may be a compensatory underestimation of insulin action and insulin sensitivity (19). In addition, there is undermodeling (i.e., inadequate modeling resulting in underestimation) of S_I in the labeled 1CMM because of the single-compartment assumption, but it is less pronounced than in the MM3 because this parameter does not also account for the effect of insulin on hepatic glucose production. We found that MM3 S_I and 1CMM S_I were not different; this similarity may result from effects on S_I that essentially cancel each other out: the combined effects of uptake and production may be preferable to the 2CMM because S_I is estimated with poorer precision.

With regard to the correlation data within each group, only among R monkeys were estimates of insulin sensitivity (from MM3 and 2CMM) negatively associated with body fat. A threshold effect may explain the absence of this relationship with C monkey data. Correlations among the insulin sensitivity estimates of the three models are consistent with what others have reported (58).

Glucose effectiveness. The MM3 S_G parameter provides a combined estimate of the effect of glucose to enhance its uptake and to inhibit its production at basal insulin, and its value should be larger than the 1CMM parameter S_G, which reflects only the uptake component (22). In dogs, S_G was 4.5-fold greater than S_G (22). Our data show not only similar absolute values for each parameter, but the magnitude of the difference between parameters was also similar (3.5- to 4.5-fold).

The data suggest that the component of S_G relating to inhibition of glucose production may be much larger than the uptake component. When the contribution of each component to the S_G value was examined with the use of a stepped hyperglycemic clamp procedure in dogs, however, the uptake component was found to compose ~70% of the total S_G value (2).

MM3 S_G is estimated between ~8 and 20 min after glucose administration (23). In contrast, the labeled S_G and S_G parameters are estimated using the end-test basal glucose level to better separate the effects of insulin and glucose on glucose uptake (19), thereby improving precision of the estimates. It is only in the latter part of the IVGTT, when the glucose disappearance curve is a function of the kinetics of the inaccessible compartment, that S_G is overestimated (19). Before this time, the interpretation of S_G must be made with caution in the face of differing insulin secretion levels (32). The C monkeys exhibited elevated insulin responses during the early part of the IVGTT, suggesting perhaps that their S_G levels were greater; however, we did not observe a significant association between AIR_G and S_G levels in either C or R groups (data not shown). Furthermore, because insulin levels reached baseline in ~60 (R) vs. ~110 min (C) and glucose levels reached baseline levels in ~40 (R) vs. ~60 min (C), there was a greater amount of time in the later IVGTTs

Table 6. Effect of fat as a mediator

<table>
<thead>
<tr>
<th>Minimal Model Parameter (Y)</th>
<th>Fat Variable (Z)</th>
<th>%Change in Slope</th>
<th>P Value for Z (Y=X+Z)</th>
<th>Bootstrap Mean</th>
<th>Bootstrap SE</th>
<th>Bootstrap P Value</th>
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<tr>
<td>MM3 S_I</td>
<td>%Body fat</td>
<td>92</td>
<td>0.1077</td>
<td>-2.906</td>
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<td>Abdom circ</td>
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<td>Total fat</td>
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<td>%Body fat</td>
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<tr>
<td>2CMM S_I</td>
<td>Abdom circ</td>
<td>42</td>
<td>0.3204</td>
<td>-3.059</td>
<td>3.047</td>
<td>0.3154</td>
</tr>
<tr>
<td>2CMM S_I</td>
<td>Total fat</td>
<td>49</td>
<td>0.2547</td>
<td>-3.875</td>
<td>3.077</td>
<td>0.2080</td>
</tr>
</tbody>
</table>

Effect of fat as mediator (Z) in the relationship between dietary restriction (DR; X) and insulin sensitivity (Y) parameters from all minimal models, using bootstrap analysis for an estimate of variance. Significant correlations (P < 0.05) among all DR, body fat variables, and insulin sensitivity parameters were a prerequisite for bootstrap analysis. %Body fat, abdom circ, or total body fat (referred to as Z) was added to the insulin sensitivity (Y) and DR (X), as a dichotomous variable; regression equation. %Change in slope is the relative reduction in slopes from Y = X to Y = X + Z. P value for X (not shown) for all pairs of DR and insulin sensitivity parameters did not achieve significance. P value for Z is for the effect of fat in the Y = X + Z regression equation. Bootstrap mean and SE are derived from 10,000 simulation data sets. Bootstrap P value reflects whether the difference in slopes is significant.

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R1348 MINIMAL MODELING IN ADULT RHESUS MONKEYS
of R monkeys when \( S_G \) could have been overestimated. That may explain why the R monkeys exhibited marginally greater \( S_G \) values. \( S_G \) from the 1CMM is similarly underestimated but, again, not to the same extent as MM3 \( S_G \), since hepatic glucose production is not a component of this value (19).

\( S_G \) values of the R and C monkeys in the present study did not differ and were somewhat higher (1.5–2.0 \( \times 10^{-2} \)min) than those reported by Vicini et al. (58) for humans (0.85 \( \times 10^{-2} \)min). The kinetic parameters describing the exchange between accessible and inaccessible glucose compartments and the loss of glucose from the inaccessible compartment were twofold or greater than those reported for humans, providing a reasonable explanation for the monkey vs. human difference.

Because \( S_G \) is greater among monkeys than humans, PCR values in monkeys are also greater (PCR is calculated from \( S_G \)). Neither PCR nor HGR\(_b\) from the 2CMM differed between groups, and this was expected given that fasting glucose itself did not differ between groups. During many, but not all, previous assessment periods, fasting plasma glucose has been observed to be lower in R vs. C monkeys (35). As Vicini et al. (58) showed, PCR is greater than either glucose effectiveness parameter from the labeled models (\( S_G \) or \( S_G^2 \)). The data in the present study follow this same trend, suggesting that the monocompartimental assumption of 1CMM underestimates PCR in both humans and rhesus monkeys.

With respect to correlation data, only among C monkeys were the 1CMM and 2CMM glucose effectiveness parameters associated, again mirroring what Vicini et al. (58) have reported. It is not clear why this same relationship was not observed among R monkeys. The 2CMM glucose effectiveness parameters (\( S_G^2 \) and PCR) and HGR\(_b\) were negatively associated with body fat and abdominal circumference only among C monkeys. Although causation cannot be concluded from correlation analysis, it is not surprising that elevated body fat and, by extension, plasma fatty acids (34) may influence glucose uptake or production. Randle et al. (54) proposed that when fatty acid oxidation is enhanced, the resulting elevated levels of acetyl-CoA inhibit glucose utilization (54). In addition, intracellular triglyceride levels are associated with reduced insulin-stimulated glucose disposal in skeletal muscle (38), and they may also affect non-insulin-stimulated pathways. The reason for the inverse association between body fat and basal glucose production, however, is not clear, but it may be indicative of some level of dysfunction of the liver. Under dynamic conditions of the IVGTT, inhibition of lipolysis by insulin normally reduces circulating fatty acids, and their reduced concentration in plasma is proposed as a peripheral signal leading to the inhibition of glucose production (1).

**Fat as mediator.** The literature is replete with reports of body fat content or its distribution and its relationship to insulin sensitivity (e.g., see Refs. 16, 28, and 44) as well as what contribution a DR-induced reduction in body fat makes to the effects of chronic DR on glucose regulation (6). In general, lower body fat is associated with tighter regulation of glucose. Given that DR induces both a loss of body fat and improved insulin sensitivity, how independent, if at all, are these effects? From one perspective, a number of studies of energy restriction have reported changes in plasma insulin or glucose levels before detectable changes in body composition in humans (39, 48, 60). In addition, it is well known that a modest reduction of body weight (fat) can often reduce or eliminate the requirement for a pharmacological therapy for type 2 diabetes. Recently, it has been shown that a diet and physical activity intervention program in middle-aged obese individuals with impaired glucose tolerance could result in moderate weight loss and a reduced risk of developing of type 2 diabetes (57). When development of spontaneous obesity, as often observed among middle-aged rhesus monkeys (40, 41), is prevented by maintaining body weight at 10–12 kg throughout adulthood, development of type 2 diabetes is also postponed or prevented (36). Furthermore, short-term DR initiated in older rhesus monkeys resulted in reduction of triglyceride levels, as well as reduced fasting and peak insulin response levels, before a detectable reduction in body fat (47). In the present study, HIC monkeys were not more overweight or more insulin resistant compared with other controls, but R monkeys had much less body fat and were highly insulin sensitive. Neither body fat nor abdominal circumference was strongly correlated with insulin sensitivity parameters in either group, however. Because insulin resistance can be present in nonobese individuals (3), the question remains as to whether and/or to what extent the effect of DR to enhance insulin sensitivity is influenced by the loss of, or maintenance of lower levels of, body fat in these animals.

We have attempted to address this question by examining body fat as a potential mediator of the effect of DR on insulin sensitivity. We tested whether addition of the body fat variable (percent body fat, abdominal circumference, or total body fat) to the regression of insulin sensitivity on DR (i.e., \( Y = X + Z \)) significantly alters the original slope of \( Y \) on \( X \). For each body fat-insulin sensitivity pair, the main effect of DR on insulin sensitivity in the regression analyses was no longer significant after the fat variable was added, suggesting that the effect of DR to enhance insulin sensitivity was not independent of body fat. However, the main effect of the fat variable also did not achieve significance or remained only borderline significant when added to the \( X-Y \) regression. To quantitate the extent to which body fat mediated the DR-insulin sensitivity relationship, we calculated the relative reduction in slope after the fat variable was added. Several of the bootstrap \( P \) values for the regression analyses were either significant or marginally so, suggesting that body fat may explain at least some of the effect DR has on insulin sensitivity. In particular, for the 1CMM \( S_G^2 \) parameter, percent body fat and total fat reflected a
change in slope of up to two-thirds or more of the effect of DR to enhance insulin sensitivity, leaving a portion of this effect unaccounted for. The fact that abdominal circumference seemed to elicit a more marginal effect in this analysis suggests that centrally deposited fat may not be as important to peripheral insulin sensitivity as total body fat in these animals. We have not, however, differentiated between subcutaneous and visceral depots here, and this is likely an important distinction (30).

Furthermore, because rhesus model values have compared well with human values derived from not only simulation analyses (20, 25, 59) but also direct measurements (22) and the glucose clamp technique (7, 13, 15, 32), it is reasonable to suggest that the three models have been, in part, indirectly validated for use with rhesus monkey data. It may not be necessary to validate the model with each new condition, but one must consider the nature of the variation, for example, the dynamics of glucose and insulin in plasma after a glucose bolus in humans vs. monkeys (31).

**Perspectives**

This report compares insulin sensitivity and glucose effectiveness parameters estimated from three minimal models using data from the same set of IVGTT's performed in adult male rhesus monkeys, one-half of which had been energy restricted for 10 yr. We observed values, levels of precision of estimation, and intermodel relationships among insulin sensitivity and glucose effectiveness parameters to be in the ranges of those reported previously for humans and dogs, suggesting that the models may provide valid estimates of these parameters for rhesus monkeys as well. The similarity of values and relationships among the glucose effectiveness model parameters suggests that the undermodeling that leads to inaccurate or imprecise estimates in the MM3 for humans and dogs affects data of rhesus monkeys in a similar fashion. Likewise, it also suggests that the estimation of some parameters can be improved by using the combination of tracer data and a model that more accurately reflects the dynamic physiological system.

Importantly, we observed insulin sensitivity indexes from all models to be consistently elevated among R vs. C monkeys, in accord with our previous reports relating to this group of monkeys subjected to chronic DR. Our data provide some evidence, however, that the effect of long-term, moderate DR to enhance insulin sensitivity may not be entirely independent of the reduction in body fat that accompanies chronic DR. This does not preclude the possibility that alterations in glucoregulatory endpoints initiated by short-term energy restriction occur by the same mechanism(s) of action as those observed with long-term DR. Alternatively, it is possible that very subtle alterations in body fat or its distribution after initiation of DR, undetectable by conventional methods, may be central to or may work in tandem with other factors that together contribute to the mechanism(s) by which DR enhances insulin sensitivity. Changes in insulin- and glucose-related variables have often been observed before alterations in body composition can be detected, shortly after initiation of DR in several species (29, 39, 47, 48), suggesting that DR may alter these values, at least in part, independently of a reduction in fat mass. Perhaps early DR-induced alterations in levels of cytokines or other adipose tissue secretory products (11, 33, 52, 56) may be important to the mechanism by which DR exerts its effects on insulin sensitivity (6).

Future studies combining modeling for a whole body perspective and molecular-level work should provide greater insight into the mechanism(s) of action of DR. In addition, the use of the euglycemic hyperinsulinemic clamp in conjunction with modeling will further validate the use of these models in rhesus monkeys.

**APPENDIX A**

**Calculations of Tracer Glucose Levels and Measurement Error Estimation**

After derivatization of the plasma samples as described in RESEARCH DESIGN AND METHODS, samples for each animal were analyzed by GC-MS in triplicate. Six replicates of each of the glucose standards (100% unlabeled, 2% labeled, 50% labeled, and 100% labeled) were analyzed before and after each animal's plasma samples. In addition, the 100% unlabeled standard was run in duplicate between every three or four plasma samples. Six replicates of each baseline plasma sample (i.e., at -15, -5, and -1 min before glucose administration) were also analyzed, and isotope ratios (as defined below) were calculated. Ten infusate solutions (the glucose bolus composed of unlabeled and labeled glucose; 10% of total dose) were prepared as for each IVGTT and analyzed by GC-MS. The mean isotope ratios from these solutions were used to estimate the actual infusate isotope ratios for use in the subsequent calculations.

The peak isotope ratio (pr), i.e., the ratio between the labeled and unlabeled isotopes from each of the plasma and infusate samples, was used to calculate the tracer (exogenous glucose) concentration in each plasma sample. The [6,6-2H2]glucose tracer used in this study does not recycle (37). There are three isotope species that we measured: 1) the [6,6-2H2]glucose molecule containing two atoms of deuterium (“m2”), 2) the incompletely labeled [6,6-1H2-2H1]glucose molecule containing a single deuterium atom (“m1”), and 3) the unlabeled [6,6-1H2]glucose molecule (“m0”).

From these isotope species we calculated peak ratios pr and pr, in the infusate solutions (subscript i), baseline plasma samples (subscript b), and other plasma samples

\[
pr = \frac{[6,6-2H_2]glucose}{[6,6-2H_2]glucose} \quad \text{or} \quad m2/m0
\]

\[
pr = \frac{[6,6-1H_2-2H_1]glucose}{[6,6-1H_2]glucose} \quad \text{or} \quad m1/m0
\]

For each plasma sample taken after the glucose bolus, we calculated Z, the ratio of tracer (exogenous) glucose to tracee (endogenous) glucose

\[
Z(t) = \frac{[pr - pr]([1 + r_i + r_i^2])}{[pr^2 - pr^2(t)][1 + r_i + r_i^2]}
\]
where \( r_1^{-1} \) and \( r_1^{+2} \) are the infusate isotope ratios

\[
\begin{align*}
  r_1^{-1} &= r_1^{+1} + pr_1^{-1} - pr_1^{+1} \\
  r_1^{+2} &= r_1^{+2} - (pr_1^{-1} - r_1^{+1})(pr_1^{+1} - pr_1^{-1}) + pr_1^{+2} - pr_1^{-2}
\end{align*}
\]

and where \( r_1^{+1} \) and \( r_1^{-1} \) are isotope ratios based on knowledge of natural abundance; \( r_1^{-1} = 0.0003 \) and \( r_1^{+2} = 0 \) (negligible). The tracer glucose concentration (\( G^* \)) was then calculated as

\[
G^*(t) = \frac{G(t)Z(t)}{1 + Z(t)}
\]

where \( G(t) \) is the total plasma glucose concentration measured with the automated glucose analyzer.

**Calculation of Measurement Error by Error Propagation**

Error propagation was used to estimate the error associated with the measurement of both labeled (tracer) and unlabeled (tracce) glucose. The CV for total glucose concentration was calculated from duplicate measurements on the automated analyzer. All values were \( \leq 2 \% \). For labeled glucose, the standard deviation (SD) of the triplicate isotope ratios (\( r \)) for each plasma sample was calculated. Linear regression was used to determine the relationship between \( r \) and SD values for each animal. The SD of the tracer-to-tracer ratio [\( Z(t) \)] was calculated using error propagation (21)

\[
SD[Z(t)] = SD[r(t)] \cdot \frac{(r_1 - r_2)(1 - r_1)}{[r_1 - r(t)]^2(1 + r_1)}
\]

The measurement error variance \( (\text{Var}) \) associated with labeled glucose was also found using error propagation

\[
\text{Var}[G^*(t)] = [\alpha G^*(t)]^2 \frac{[SD[Z(t)]G^*(t)]^2}{[1 + Z(t)]^4}
\]

where \( \alpha \) is the CV/100 of the duplicate unlabeled glucose measurements, in this case, \( \alpha = 0.02 \).

**APPENDIX B**

**Glossary of Minimal Model Terms**

Table B1 shows a glossary of minimal model parameters from the unlabeled MM3 and the labeled 1CMM and 2CMM.

**APPENDIX C**

**Minimal Model Equations and Calculation of \( S_I \) and \( S_G \)**

MM3. This one-compartment model is identified on insulin and unlabeled glucose data from a standard IVGTT through 180 min using MM3 software from R. Bergman. The equations for this (and other minimal models) relate to the effect

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Definition</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold MM (no Tol)</td>
<td>( S_I )</td>
<td>Effect of insulin to enhance glucose uptake from plasma and to inhibit glucose production.</td>
<td>( \cdot 10^{-4} \text{ min}^{-1}(\mu\text{U/ml})^{-1} )</td>
</tr>
<tr>
<td>Cold MM (no Tol)</td>
<td>( S_G )</td>
<td>Effect of glucose to enhance its uptake from plasma and to inhibit its production, independent of a rise in insulin above basal.</td>
<td>( \cdot \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 1CMM</td>
<td>( S_{G}^{*} )</td>
<td>Effect of insulin to enhance glucose uptake from plasma. *Use of tracer data in 1CMM model to distinguish it from ( S_I ) of MM3 using unlabeled glucose.</td>
<td>( \cdot 10^{-4} \text{ min}^{-1}(\mu\text{U/ml})^{-1} )</td>
</tr>
<tr>
<td>Labeled 1CMM</td>
<td>( HGR_{b} )</td>
<td>Hepatic glucose release to plasma in basal state.</td>
<td>( \cdot \text{min}^{-1} )</td>
</tr>
<tr>
<td>From 1CMM, for equiv to 2CMM</td>
<td>( S_{G}^{*} )</td>
<td>Effect of insulin to enhance glucose uptake from plasma, multiplied by ( V_I ) to make units compatible for comparison with 2CMM ( S_{G}^{*} ).</td>
<td>( \cdot 10^{-2} \text{ ml}^{-1} \text{kg}^{-1} \text{min}^{-1}(\mu\text{U/ml})^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( S_{G} )</td>
<td>Effect of glucose to enhance its uptake from plasma, independent of a rise in insulin above basal.</td>
<td>( \cdot \text{ml}^{-1} \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( HGR_{b} )</td>
<td>Hepatic glucose release to plasma in basal state.</td>
<td>( \cdot \text{mg}^{-1} \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( PCR )</td>
<td>Basal state plasma glucose clearance rate.</td>
<td>( \cdot \text{ml}^{-1} \text{kg}^{-1} \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( V_I )</td>
<td>Volume of distribution of rapidly equilibrating compartment.</td>
<td>( \cdot \text{ml}^{-1} \text{kg}^{-1} \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( k_{12} )</td>
<td>Glucose kinetic parameter reflecting the fractional rate of transfer of glucose from the slowly equilibrating pool to the quickly equilibrating pool.</td>
<td>( \cdot \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( k_{21} )</td>
<td>Glucose kinetic parameter reflecting the fractional rate of transfer of glucose from the quickly equilibrating pool to the slowly equilibrating pool.</td>
<td>( \cdot )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( k_{02} )</td>
<td>Glucose kinetic parameter reflecting the irreversible fractional rate of loss of glucose from the slowly equilibrating pool.</td>
<td>( \cdot \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( k_p )</td>
<td>Proportionality constant.</td>
<td>( \cdot \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( s_k )</td>
<td>Insulin sensitivity parameter of tissues represented by the slowly exchanging glucose (inaccessible) compartment, in which utilization is directly controlled by insulin.</td>
<td>( \cdot \text{min}^{-1} \text{kg}^{-1} \text{min}^{-1} \text{ml}^{-1} \text{kg}^{-1} \text{min}^{-1} )</td>
</tr>
<tr>
<td>Labeled 2CMM</td>
<td>( V_D )</td>
<td>Calculated, not an estimated parameter. Total volume of glucose distribution.</td>
<td>( \cdot \text{ml}^{-1} \text{kg}^{-1} \text{min}^{-1} )</td>
</tr>
</tbody>
</table>

No Tol, absence of tolbutamide during the intravenous glucose tolerance test (IVGTT) procedure at 10 yr (tolbutamide-modified IVGTTs were performed for the first 9 yr of this study); equiv, equivalence.
of glucose (SG) and the effect of insulin (SI) to both promote glucose uptake and inhibit its production

\[
G(t)/t = -[p_1 + X(t)]G(t) + p_2G_0 \quad G(0) = G_0 + DV
\]

\[
X(t)/t = -p_3X(t) + p_4[I(t) - I_0] \quad X(0) = 0
\]

where \(G(t)\) is the plasma glucose concentration, \(I(t)\) is plasma insulin concentration, \(X(t)\) is insulin action from the remote compartment, \(D\) is glucose dose, and \(V\) is glucose distribution volume. Model parameters \(p_1, (k_1 + k_3), p_2, k_4, k_5, k_6, k_7, k_8, k_9, k_{10}\), and \(V\) are estimated from glucose and insulin data using nonlinear least squares. The parameter \(p_3\) is the rate constant of the remote insulin compartment, which relates to the rise and fall of insulin action. \(S_I\) and \(S_G\) are calculated from the estimated parameters

\[
S_I = p_2/p_3 = k_3(k_4 + k_5)\text{ (min}^{-1}\text{)}
\]

\[
S_G = p_1 = k_1 + k_5\text{ (min}\text{ }^{-1}\text{ }\mu\text{U}^{-1}\text{ }\cdot\text{ml})
\]

We used the automatic weighted and initial parameter estimates of the minimal model program software for all data sets. \(G(0)\) is peak glucose at time 0 extrapolated from the data, assuming instantaneous mixing after glucose injection.

**1CMM equations.** This one-compartment glucose model is identified on insulin and labeled glucose data from a standard IVGTT through 240 min using SAAM II software (4, 18, 22). The equations of this model are

\[
g(t) = -[p_1 + x(t)]g(t) \quad g(0) = g_0 + DV
\]

\[
x(t) = -p_2x(t) + p_4[\alpha(t) - I_0] \quad X(0) = 0
\]

where \(g\) is the labeled glucose concentration, \(x\) is insulin action from the remote insulin compartment \(I\), \(V\) is the glucose distribution volume, and \(I_0\) is the end-test insulin (basal) concentration. Parameters \(p_1, p_2, p_3, x\) and \(\alpha\) relate to the rate constants \(k_1, k_2, k_3, k_4\) and \(k_5\) and insulin concentration in the remote compartment \(I\) in \(x(t) = k_4z(t)p_1 = k_1; p_2 = k_3; p_3 = k_4k_5\).

As in the MM3, \(S_I\) and \(S_G\) are calculated from the estimated parameters \(S_I = p_2/p_3\) and \(S_G = p_2/p_4 = k_3k_4k_5\), although they reflect only the effect of glucose and insulin, respectively, to affect glucose disposal.

**2CMM.** This two-compartment model is identified on insulin and labeled glucose data from a standard IVGTT through 240 min using SAAM II software (58). The equations of this model are

\[
q_1(t) = -[k_p + (R_{M,0} \cdot \alpha)q_1(t) + k_{21}q_2(t) + k_{22}q_2(t)] \quad q_1(0) = d
\]

\[
q_2(t) = k_{21}q_1(t) - [k_{22} + x(t) + k_{12}q_2(t)] \quad q_2(0) = 0
\]

\[
x(t) = -p_2x(t) - b_q[I(t) - I_0] \quad X(0) = 0
\]

\[
g(t) = [q_2(t)/V_1]
\]

where \(q_1\) and \(q_2\) are the labeled glucose masses in first (accessible) and second (slowly exchanging) glucose compartments; \(X(t) = k_4t\) is insulin action, where \(\Gamma(t)\) is insulin in the remote insulin compartment; \(I(t)\) is plasma insulin concentration; \(I_0\) is basal (end-test) insulin concentration; \(Q(t)\) is cold glucose mass in accessible pool; \(g(t)\) is labeled plasma glucose concentration; \(d\) is labeled glucose dosage; \(V_1\) is volume of distribution of accessible pool; \(k_p\) is the proportionality constant; \(k_{21}, k_{12}, k_{22}\) and \(k_{12}\) are rate constants relating to glucose kinetics; \(p_2 = k_6\) and \(s_0 = (k_kh_{60})\) are parameters of insulin action in the periphery. Irreversible loss \((k_{20})\) from slowly exchanging pool \((q_2)\) is insulin dependent, and insulin exerts its effect from a remote compartment \((\Gamma(t), k_{12}, k_{21}, k_{20}\) and \(k_{01}\) are glucose kinetic parameters; and \(k_4, k_5, k_6\) and \(k_8\) are kinetic parameters relating to insulin action on glucose disappearance. This 2CMM is a revised version of the one used in Vicini et al. (58).

The earlier version of the 2CMM, which assumed an \(R_{d,0}\) (glucose uptake at time 0) to be 1 mg/kg·min⁻¹ on the basis of earlier studies (14), often resulted in a negative value for the kinetic proportionality parameter, \(k_p\). The revised model used in this study allowed some flexibility for \(R_{d,0}\) to take on values <1 mg/kg·min⁻¹. The modification was to incorporate as a part of the constant uptake component a fixed proportion of the basal glucose disappearance from the accessible compartment. This constant, \(\alpha\) (0.465), was derived in previous studies from the data of 14 normal human subjects (unpublished data). The only model parameters affected by this modification were \(S_I\) and \(S_G\). As a result, \(k_p\) was always a positive value in the revised model results. Equations modified with \(\alpha\) in this model are

\[
k_{20} = k_p + (\alpha \cdot G_0)/[(1 - \alpha) \cdot (G) \cdot I_n + [k_{02}k_{21}]/[k_{02} + k_{12}]]
\]

\[
k_p = (3 - 4 \cdot \alpha) \cdot (k_{21} \cdot k_{02}/[k_{02} + k_{12}])
\]

\[
\text{PCR} = S_I^2/(1 - \alpha)
\]

We are grateful for the hard work of the Wisconsin Primate Research Center (WPRC) animal care, technical, and veterinary staff and for the many helpful suggestions provided by Drs C. Cobelli and G. Toffolo of Padua University on running the minimal models used for this study.

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**DISCLOSURES**

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