Insulin Effectiveness, a Measure Consisting of Insulin Sensitivity, Secretion, and Clearance, Captures Differences Between Diabetic and Nondiabetic States

Short running title: Insulin effectiveness: a new index of diabetes

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OBJECTIVE

Insulin clearance is implicated in regulation of glucose homeostasis independently of insulin sensitivity and insulin secretion. To understand the relation between blood glucose levels and insulin sensitivity, insulin secretion, and insulin clearance, we performed a mathematical analysis.

RESEARCH DESIGN AND METHODS

We performed a hyperglycemic clamp, a hyperinsulinemic-euglycemic clamp, and an oral glucose tolerance test in 47, 16, and 49 subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes mellitus (T2DM), respectively.

RESULTS

The disposition index (DI), defined as the product of insulin sensitivity and insulin secretion and regarded as a measure of the capacity for glucose handling, did not well explain blood glucose levels at the early stage of glucose intolerance. However, an equation relating DI, insulin clearance, and blood glucose levels was well conserved regardless of the extent of glucose intolerance. As a measure of the effect of insulin, we developed an index, designated "insulin effectiveness," that is based on this equation and corresponds to DI divided by the square of insulin clearance. Insulin effectiveness was not impaired in IGT compared with NGT, possibly as a result of a decrease in insulin clearance in response to a reduction in DI, whereas it was impaired in T2DM relative to IGT.

CONCLUSIONS

Insulin effectiveness, which likely reflects insulin action in the body, well represents changes in glycemia and is impaired during the transition from IGT to T2DM. It may serve as a new indicator for the trajectory of changes in glucose tolerance.

The blood glucose concentration is maintained within a narrow range by feedback-compensatory responses in the body. Insulin sensitivity and insulin secretion are linked by such a relation, with insulin secretion increasing to maintain normal glucose homeostasis as insulin sensitivity declines (1). This compensatory response has been regarded as "moving up" the hyperbolic curve of the product of insulin sensitivity and insulin secretion, and the failure of this response, regarded as "falling off" the hyperbolic curve, is associated with the transition from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM) (1). The disposition index (DI), generally defined as the product of insulin sensitivity and insulin secretion, has therefore been adopted as a measure of glucose handling in the body (1,2).

Several models that explain this moving up–falling off phenomenon as an interplay among insulin sensitivity, insulin secretion, and pancreatic β-cell mass have been described (3–6). Such models account for the physiological features and pathological trajectory of glucose tolerance—including NGT, IGT, and T2DM—to a certain extent, but their conformity to clinical or experimental data is not high (6). Moreover, the moving up and falling off the curve of the product of insulin sensitivity and insulin secretion appear not to adequately represent the relation between blood glucose and insulin levels. For example, comparison of individuals with NGT at extremes of insulin sensitivity revealed that an approximately twofold increase in blood insulin levels was associated with an increase in insulin secretion of <50% (7,8), indicating that the increase in insulin secretion may not fully explain the increase in circulating insulin levels in individuals with insulin resistance (8).

Insulin clearance is implicated as a determinant of circulating insulin levels that is independent of insulin secretion (8–16). Insulin clearance likely contributes to the compensatory responses of the body, with a reduction in insulin clearance in the insulin-resistant state resulting in an increase in circulating insulin levels and a lessening of the

demand for insulin secretion by β-cells (11). In some instances, changes in the circulating insulin levels do not reflect changes in insulin secretion, instead being primarily influenced by insulin clearance (17). Furthermore, changes in insulin clearance have been implicated in the pathogenesis of glucose intolerance (18–21). Whereas these various findings suggest the importance of insulin clearance in the physiology and pathology of glucose homeostasis, it is difficult to directly evaluate this parameter from clinical data (22).

Mathematical models provide insight into phenomena in which various factors mutually interact and for which effects of such factors cannot be measured directly (22–25). We previously developed a mathematical model based on temporal changes in circulating glucose and insulin levels in human subjects during consecutive hyperglycemic and hyperinsulinemic-euglycemic clamps (23). Using this model, we estimated insulin clearance and characterized its role in the regulation of glucose homeostasis, and we identified a square-law relation between insulin clearance and the product of insulin sensitivity and insulin secretion that was conserved among individuals with NGT, IGT, or T2DM (23).

We have now further examined the relation among insulin sensitivity, insulin secretion, and insulin clearance, and we here propose a new index, "insulin effectiveness," that consists of DI corrected by insulin clearance. We have characterized insulin effectiveness in subjects with various levels of glucose tolerance, and we provide evidence that this new index can serve as an indicator of the effect of insulin on glucose homeostasis and can capture the transition from IGT to T2DM.

RESEARCH DESIGN AND METHODS

Subjects and Measurements

We analyzed a previously reported data set (23) in the present study. In brief, a hyperglycemic clamp was performed by intravenous infusion first of a bolus of glucose over

15 min and then of a variable amount of glucose to maintain the plasma glucose level at 200 mg/dL. Ten minutes after the end of the hyperglycemic clamp, a hyperinsulinemic-euglycemic clamp was performed by intravenous infusion of insulin with a target plasma glucose level of 90 mg/dL or of the fasting level in the case of NGT and IGT subjects whose fasting plasma glucose level was <90 mg/dL. These clamp analyses and a 75-g oral glucose tolerance test (OGTT) were performed within a period of 10 days.

The indices k_4, k_5, and k_7, which correspond to insulin sensitivity, secretion, and clearance, respectively, were calculated with a mathematical model described in our previous study (23). The insulin sensitivity index (ISI) and incremental area under the serum insulin concentration curve from 0 to 10 min during the hyperglycemic clamp (AUC_IRI) were also calculated as described previously (23,26,27).

Fifty NGT, 18 IGT, and 53 T2DM subjects were initially used for parameter estimation. As we previously described (23), three NGT, two IGT, and three T2DM individuals were subsequently eliminated as outliers. In addition, one T2DM individual was eliminated because of missing values, with the remaining 47 NGT, 16 IGT, and 49 T2DM subjects being analyzed.

Statistical Analysis

Comparisons among NGT, IGT, and T2DM subjects were performed by ANOVA followed by Tukey's honestly significant difference test. A *P* value of <0.05 was considered statistically significant. For examination of the relations among insulin sensitivity, insulin secretion, insulin clearance, and plasma glucose levels, the residual sum of the square (RSS) between variables was minimized with the use of a nonlinear least squares technique, as described previously (23). We also tested the relations among the variables by standardized

major axis regression (28). The association between indices was evaluated with Spearman's correlation test.

Data and Resource Availability

The data set analyzed in the present study was described in our previous study (23).

RESULTS

Derivation of a Conserved Equation for NGT, IGT, and T2DM from the Relations Among Insulin Sensitivity, Insulin Secretion, Insulin Clearance, and Blood Glucose Levels

We first attempted to derive a relation for the regulation of blood glucose levels that is conserved across various levels of glucose tolerance. We previously analyzed the relations among insulin sensitivity, insulin secretion, insulin clearance, and blood glucose levels using serum insulin and plasma glucose data obtained during consecutive hyperglycemic and hyperinsulinemic-euglycemic clamps in NGT, IGT, and T2DM individuals (23), and we identified a law regarding the three insulin-related parameters (23). This law, a square-law relation between insulin clearance and the product of insulin sensitivity and insulin secretion, was given by the following equation (23):

$$\log k_4 k_5 = 1.98 \log k_7 + \log 0.0945 \qquad \text{Eq (1)}$$

The indices k_4, k_5, and k_7, which correspond to insulin sensitivity, secretion, and clearance, respectively, were derived from the previous study (23).

We reevaluated the fitness of the law in individuals with each category of glucose tolerance. The coefficient of determination (R^2) of Eq (1) was estimated to be 0.26 for all subjects (Fig. S1A) and to be 0.28, 0.29, and –2.3 for NGT, IGT, and T2DM groups, respectively (Fig. S1B), indicating that the fitness of Eq (1) was poor, especially for the

T2DM group. Given that a high concentration of blood glucose is a characteristic of this latter group, it is possible that Eq (1) needs to be modified by blood glucose level.

The DI (product of insulin secretion and insulin sensitivity) has been generally considered to reflect glucose disposal ability (1,2). The relation between DI and the blood glucose level can be represented as follows (29):

$$\log k_4 k_5 = a \log \mathrm{PG120} + b \qquad \text{Eq (2)}$$

where the parameters a and b were estimated to minimize RSS between $\log k_4 k_5$ and $a \log \mathrm{PG120} + b$, and $\mathrm{PG120}$ indicates the plasma glucose concentration at 120 min during the OGTT, an important parameter for evaluation of glucose tolerance. The parameters a and b estimated for all subjects are represented as a_a and b_a, respectively. The R^2 value of Eq (2) estimated for all subjects was 0.66 (Fig. 1A), which is better than that of Eq (1) (Fig. S1A). However, R^2 of Eq (2) for the NGT, IGT, and T2DM groups was 0.17, 0.00, and 0.22, respectively (Fig. 1B), indicating that the goodness-of-fit was particularly poor for IGT. The parameters a_n and b_n, a_i and b_i, and a_t and b_t were estimated to minimize RSS between $\log k_4 k_5$ and $a \log \mathrm{PG120} + b$ in NGT, IGT, and T2DM individuals, respectively. The parameters a_n, a_i, and a_t estimated from Eq (2) were –2.5, –0.24, and –1.8, respectively, and the parameters b_n, b_i, and b_t were 3.2, –1.7, and 1.2, respectively (Fig. 1B), suggesting that the relation between DI and blood glucose levels changes with the progression of glucose intolerance. Given that insulin clearance is likely a determinant of circulating insulin levels that is independent of insulin secretion (8–11), we hypothesized that the addition of insulin clearance might improve the representation of blood glucose, in particular for individuals with IGT.

Given that Eq (1) likely needs to be corrected for blood glucose level and that Eq (2) might be improved by adding insulin clearance, we modified these equations by integrating them as follows:

$$\log k_4 k_5 = 2\log k_7 + a\log \mathrm{PG120} + b \qquad \text{Eq (3)}$$

The parameters a and b were estimated to minimize RSS between $\log k_4 k_5$ and $2\log k_7 + a\log \mathrm{PG120} + b$. The R^2 value of Eq (3) estimated for all subjects was 0.76 (Fig. 1C), which is better than that of Eq (1) or Eq (2) (Fig. S1A and Fig. 1A). It is also better than that of another equation that connects insulin clearance and the product of insulin sensitivity and insulin secretion (Fig. S1C). The R^2 value of Eq (3) for the NGT, IGT, and T2DM groups was 0.35, 0.36, and 0.43, respectively (Fig. 1D). The parameters a_n, a_i, and a_t estimated from Eq (3) were –1.4, –1.2, and –1.5, respectively, and the parameters b_n, b_i, and b_t were 1.7, 1.5, and 1.7, respectively (Fig. 1D). Differences in R^2 and in the parameters of Eq (3) among NGT, IGT, and T2DM groups were smaller compared with those for Eq (1) and Eq (2). These results indicated that Eq (3) in general provides a better representation of blood glucose levels than does Eq (2), especially for IGT. We further examined the characteristics of Eq (3) by different methods, with the results confirming the validity of this equation (Appendix 1, Fig. S2, Fig. S3, and Fig. S4).

"Insulin Effectiveness" as a Glucose Homeostatic Law–Derived Index

Equation (3), which well describes the relation between the three insulin-related indices and blood glucose levels among NGT, IGT, and T2DM individuals, can be transformed as follows:

$$\mathrm{PG120} = 10^{-\frac{b}{a}} \left(\frac{k_4 k_5}{{k_7}^2}\right)^{\frac{1}{a}} \qquad \text{Eq (4)}$$

This transformation separates blood glucose concentration and the insulin-related indices onto the left and right sides. We designated this combination of the insulin-related indices on the right side (insulin sensitivity multiplied by insulin secretion and divided by the square of insulin clearance) as "insulin effectiveness." The product of k_4 and k_5, which corresponds to

DI, was significantly lower in individuals with IGT or T2DM than in those with NGT (23), whereas insulin effectiveness was significantly lower in individuals with T2DM than in those with NGT or IGT but was similar in the latter two groups (Fig. 2A). These results were validated by the values of insulin sensitivity, secretion, and clearance estimated from the serum C-peptide concentration (Appendix 1 and Fig. S5). Moreover, insulin effectiveness can be approximated as the extension of the basal insulin effect (30) to the insulin effect after glucose administration (Appendix 2). In addition, the sum of insulin effectiveness and glucose effectiveness determines blood glucose levels after glucose administration (Appendix 3), suggesting that insulin effectiveness can literally be considered as the overall effect of insulin on blood glucose levels.

Given that DI is the product of insulin secretion and insulin sensitivity, it is generally represented as a hyperbolic curve with these two insulin-related parameters as the axes of coordinates. The hyperbolic curves corresponding to the mean values of DI in NGT, IGT, and T2DM subjects are shown in Figure 2B. In general, as insulin sensitivity decreases, insulin secretion increases to maintain normal glucose homeostasis (the phenomenon of moving up the hyperbolic curve) (2). On the other hand, insulin effectiveness can be represented as a hyperbolic curve with DI and 1/insulin clearance^2 as the axes of coordinates, given that insulin effectiveness is the product of these two parameters. The hyperbolic curves corresponding to the mean values of insulin effectiveness in NGT, IGT, and T2DM subjects are shown in Figure 2C. The relation between DI and 1/insulin clearance^2 can also be interpreted as moving up the curve, with 1/insulin clearance^2 increasing as DI decreases. Of note, we previously found that k_7 (parameter for insulin clearance) was significantly smaller in individuals with IGT or T2DM than in those with NGT—that is, 1/insulin clearance^2 increased with the progression of glucose intolerance (23).

We next investigated the relation between DI and insulin effectiveness. We therefore represented insulin effectiveness as a hyperbolic surface of insulin sensitivity, insulin secretion, and 1/insulin clearance^2 (Fig. 2D and E). The mean values of insulin effectiveness in NGT, IGT, and T2DM subjects that are shown as hyperbolic curves in Figure 2C are thus represented as hyperbolic surfaces in Figure 2D. Figure 2E shows the relation between Figure 2B and Figure 2D (that is, the relation between DI and insulin effectiveness), with DI corresponding to the intersection of the surface representing insulin effectiveness and the plane representing the mean value of 1/insulin clearance^2. The DI for IGT subjects was significantly lower than that for NGT subjects (23), which can be interpreted as falling off the hyperbolic curve (Fig. 2B). Insulin effectiveness was similar for NGT and IGT (Fig. 2A), however, with the result that the corresponding hyperbolic curves (Fig. 2C) and hyperbolic surfaces (Fig. 2D) were almost identical. The difference in DI between NGT and IGT is reflected as a change in the intersection of the surface corresponding to insulin effectiveness and the plane representing the mean value of 1/insulin clearance^2 (Fig. 2E).

Of note, the phenomenon of moving up the curve of insulin effectiveness for IGT represents almost the same phenomenon as predicted in a previous modeling study (19), indicating a pathway to failure of glucose homeostasis in which a change in insulin clearance may alter glucose dynamics even when the normal glucose set point is maintained (Appendix 4).

Modified Mathematical Model of the Feedback Loop Linking Glucose and Insulin

We determined insulin effectiveness by performing a hyperglycemic clamp and a hyperinsulinemic-euglycemic clamp to measure insulin secretion and sensitivity together with clearance (22,23,31), respectively. The performance of two clamp tests is laborious, however, and so we attempted to estimate insulin effectiveness from a single clamp.

Given that we were not able to estimate insulin effectiveness with a single clamp analysis using our previous model (23) (Appendix 5, Fig. S6, and Fig. S7A), we modified the model by changing the positions of influx I and influx G as shown in Figure 3A (Appendix 6). We simulated the time courses of blood glucose and insulin concentrations in the hyperinsulinemic-euglycemic clamp analysis, with one example for an NGT subject being shown in Figure 3B. To confirm that the modified model appropriately captured the characteristics of the time-series data, we evaluated the consistency between the ISI calculated from the actual measurements and that calculated from the simulation data. The coefficient of determination (R^2) between measured ISI and simulated ISI was 0.96 (Fig. 3C), indicating that the model was reasonably able to capture the essential characteristics of the time-series data.

Prediction of insulin effectiveness from only a hyperinsulinemic-euglycemic clamp

Some of the estimated parameters—including k_{m7}_ins, corresponding to insulin clearance estimated from the modified model for the hyperinsulinemic-euglycemic clamp alone—showed a tendency to diverge (Fig. 4A). However, $k_{\mathrm{m5}}/{k_{\mathrm{m7}}}^2$_ins was significantly correlated with $k_5/{k_7}^2$_full ($r = 0.36, P = 1.2 \times 10^{-4}$), and $k_{\mathrm{m4}}k_{\mathrm{m5}}/{k_{\mathrm{m7}}}^2$_ins was significantly correlated with $k_4k_5/{k_7}^2$_full ($r = 0.52, P = 5.9 \times 10^{-9}$) (Fig. 4B and Fig. S7B). Of note, insulin sensitivity, insulin secretion, and insulin clearance estimated from the modified model for both the hyperglycemic clamp and the hyperinsulinemic-euglycemic clamp (k_{m4}_full, k_{m5}_full, and k_{m7}_full, respectively) were significantly correlated with those estimated from the original model (k_4_full, k_5_full, and k_7_full, respectively) (Fig. S8), indicating that both models can be used to estimate the insulin-related parameters when both the hyperglycemic and hyperinsulinemic-euglycemic clamps are performed.

We also attempted to estimate insulin effectiveness using a simple analytically computable method. We hypothesized that insulin effectiveness can be analytically estimated as follows (Appendix 7):

$$\text{Insulin effectiveness} = \frac{k_{\text{sen}} k_{\text{sec}}}{{k_{\text{cle}}}^2} \sim \frac{f_{\text{g}} I_0 (I_{\text{SS}} - I_0)}{f_{\text{i}} G_{\text{SS}} G_0 I_{\text{SS}}}$$

where k_{sen}, k_{sec}, and k_{cle} represent insulin sensitivity, secretion, and clearance, respectively, and G_{SS}, G_0, I_{SS}, I_0, f_{g}, and f_{i} represent blood glucose level at the steady state during insulin administration, blood glucose level in the fasting state, blood insulin level at the steady state during insulin administration, blood insulin level in the fasting state, glucose infusion rate at the steady state, and insulin infusion rate at the steady state, respectively (Fig. 4C). This analytically calculated insulin effectiveness ($k_{\text{sen}} k_{\text{sec}} / {k_{\text{cle}}}^2$) was significantly correlated with $k_4 k_5 / {k_7}^2$_full ($r = 0.67, P = 5.8 \times 10^{-16}$) (Fig. 4D), indicating that insulin effectiveness can be analytically estimated from a hyperinsulinemic-euglycemic clamp alone. Of note, insulin effectiveness can also be analytically estimated from a hyperinsulinemic-euglycemic clamp alone by using the serum C-peptide concentration ($r = 0.71, P = 3.9 \times 10^{-18}$), OGTT ($r = 0.43, P = 4.2 \times 10^{-6}$), or a fasting blood test ($r = 0.54, P = 1.1 \times 10^{-9}$) (Appendix 1, Appendix 8, Fig. S9 and Fig. S10).

CONCLUSIONS

We have here derived an equation relating insulin sensitivity, secretion, clearance, and blood glucose levels [Eq (3)] that was well conserved among individuals with various levels of glucose tolerance, and we showed that this equation provides a better fit, particularly for individuals with IGT, than does an equation relating only insulin sensitivity, insulin secretion, and blood glucose levels [Eq (2)]. Insulin clearance is implicated in the regulation of blood glucose concentration (8), and our present data also suggest that, together with insulin sensitivity and secretion, insulin clearance is important for such regulation, especially in

individuals with IGT. Our present results also suggest that insulin effectiveness, an index that corrects DI for insulin clearance, reflects glucose disposal ability, possibly by representing the effect of insulin on blood glucose concentration.

Insulin effectiveness is represented as a hyperbolic curve of DI and 1/insulin clearance[2] or as a hyperbolic surface of insulin sensitivity, insulin secretion, and 1/insulin clearance[2]. The deterioration of glucose tolerance is sometimes explained by the failure of insulin secretion to increase sufficiently in compensation for a decline in insulin sensitivity, with this failure being referred to as falling off the hyperbolic curve of DI (1,2). Our data now suggest that, even when DI decreases, insulin effectiveness is preserved if insulin clearance is also reduced. In general, moving up the hyperbolic curve of DI eventually positions individuals at unstable extreme points of the curve where the risk of disease onset increases (1), a phenomenon that has been referred to as canalization and decanalization (1,2) or as glucose allostasis (32). Given that individuals with IGT are at high risk of developing T2DM, the progression from NGT to IGT and T2DM can be referred to as canalization and decanalization of the hyperbolic surface of insulin effectiveness.

Our data also indicate that IGT encompasses the condition of decreased insulin clearance with a relatively well preserved insulin effectiveness and glucose set point (Appendix 4), and that T2DM encompasses that of decreased insulin effectiveness with a disrupted glucose set point. A modeling study with simulated data indicated that changes in insulin clearance may affect glucose dynamics even when the normal glucose set point is maintained, and can constitute a pathway for progression of glucose intolerance (19). Our study based on mathematical models and observational data now indicates that the phenomenon predicted by this previous model can occur in IGT. Our findings are also consistent with those of a previous study indicating that an increase in insulin secretion is

followed by a decrease in first-pass hepatic insulin clearance when insulin resistance is induced (15).

Despite the apparent analogy of the moving up–falling off phenomenon, whether the body possesses the ability to reduce insulin clearance in response to a decline in DI and the potential physiological mechanism underlying such a response remain unknown. However, evidence suggests that changes in insulin clearance can be a determinant of glucose intolerance (21,33). Whereas the direction of causation of decreased insulin clearance and glucose intolerance remains unclear, a classification based on insulin effectiveness may provide new insight into the trajectories of glucose intolerance and T2DM.

Whereas we showed that insulin effectiveness can be estimated from a hyperinsulinemic-euglycemic clamp test alone, performance of even a single clamp is labor-intensive. Moreover, the correlation between insulin effectiveness estimated from the two clamps and that estimated from the single clamp was only 0.52, 0.67 or 0.71. It will therefore be important to develop more accurate and convenient methods for estimation of insulin effectiveness in order to validate its physiological relevance and clinical usefulness.

The current study has several limitations. For simplicity, we considered only insulin sensitivity, insulin secretion, and insulin clearance. However, insulin secretion has basal, first-phase, and second-phase components (34), and both insulin sensitivity and insulin clearance have hepatic and peripheral components (3,22). Saturation of or time-dependent changes in insulin clearance (6,22) were not considered. Whereas it is necessary to correct DI by insulin clearance for accurate assessment of the effect of insulin on blood glucose levels, individuals with the same insulin effectiveness can have different blood glucose levels depending on the input (Appendix 4). Several better approaches to evaluation of the hyperbolic law have also been presented, and a power function describing the relation between insulin sensitivity and insulin secretion gives a better representation (35–37). Given

that insulin sensitivity and insulin clearance are correlated (38), the plane representing 1/insulin clearance^2 is not necessarily horizontal (Fig. 2E), and the curve corresponding to DI also departs from a hyperbola when the plane departs from the horizontal. Future research on the coefficients of insulin sensitivity, secretion, and clearance in the formula defining the effect of insulin on blood glucose levels is therefore warranted. In addition, the capacity for glucose disposal is affected not only by insulin, but also by other hormones including glucagon as well as by glucose (39,40). It is possible that differences in such factors may explain the glucose dynamics of glucose tolerance. Further studies are also warranted to develop extended equations that include glucose and glucagon to represent mechanisms of blood glucose regulation.

In conclusion, we have here shown that insulin effectiveness, a new index that corrects DI by insulin clearance, better reflects changes in glycemia compared with DI. By analogy with falling off the hyperbolic curve of DI, the progression of glucose intolerance may be considered as falling off the hyperbolic surface of insulin effectiveness. In theory, insulin effectiveness represents the effect of insulin on blood glucose levels, and it may serve as an indicator for the trajectory of changes in glucose tolerance. Further study is warranted to understand the physiological and pathological relevance of this new index.

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Conflict of Interest. The authors declare no competing interests.

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Figures

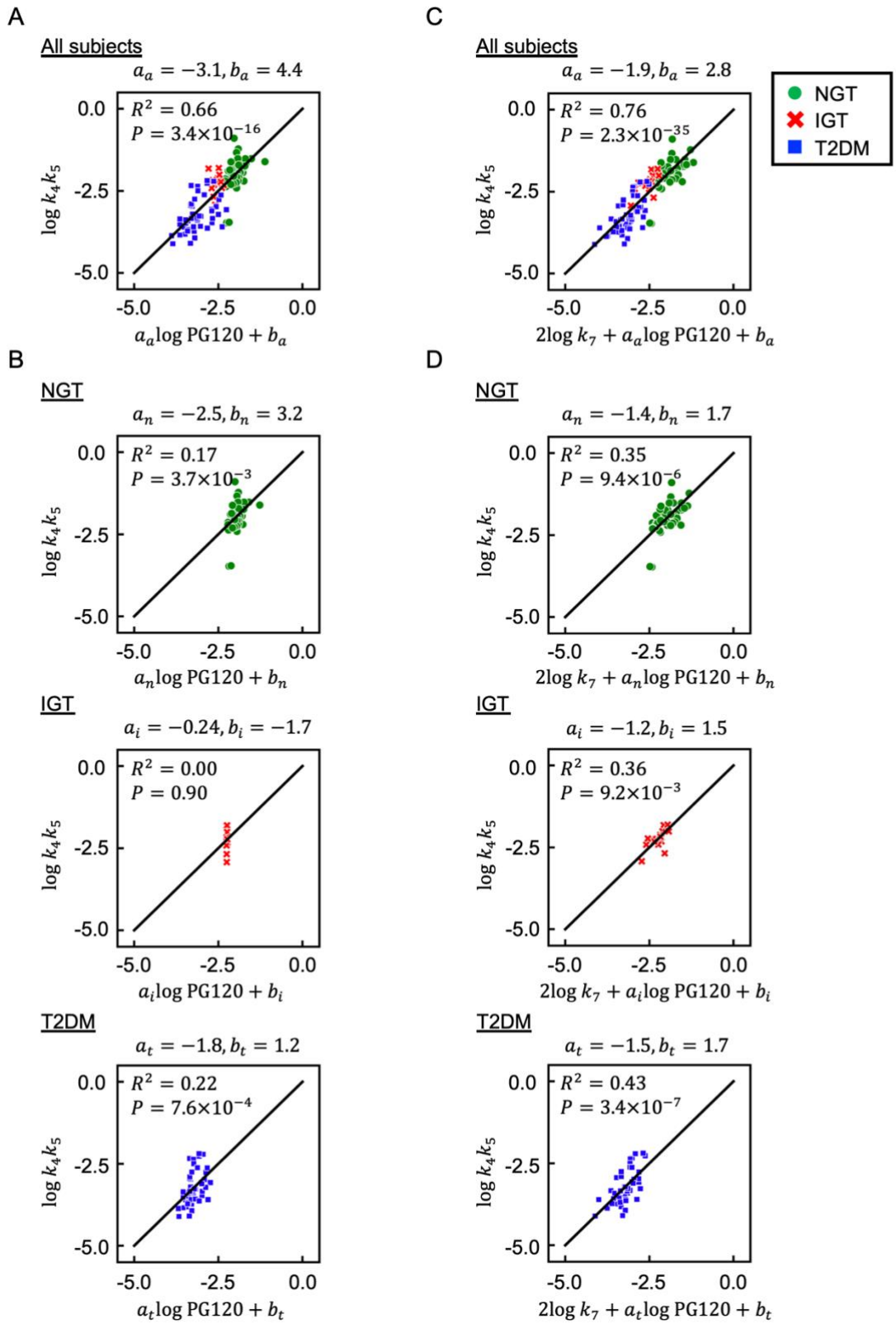

Figure 1. Relation between insulin-related parameters and blood glucose levels. (A) Scatter plot for $a_a \log \mathrm{PG120} + b_a$ versus $\log k_4k_5$ (Eq (2)). Each point corresponds to the values for

a single subject. Green circles, red crosses, and blue squares indicate NGT, IGT, and T2DM subjects, respectively. R^2 is the coefficient of determination, and the P value is for testing the hypothesis of no correlation. The parameters a_a and b_a were estimated to minimize RSS between $a_a \log \mathrm{PG120} + b_a$ and $\log k_4 k_5$ in all subjects. (B) Scatter plots for $a \log \mathrm{PG120} + b$ versus $\log k_4 k_5$ in NGT, IGT, and T2DM subjects (Eq (2)). The parameters a_n and b_n, a_i and b_i, and a_t and b_t were estimated to minimize RSS between $a \log \mathrm{PG120} + b$ and $\log k_4 k_5$ in NGT, IGT, and T2DM subjects, respectively. (C) Scatter plot for $2\log k_7 + a_a \log \mathrm{PG120} + b_a$ versus $\log k_4 k_5$ (Eq (3)). The parameters a_a and b_a were estimated to minimize RSS between $2\log k_7 + a_a \log \mathrm{PG120} + b_a$ and $\log k_4 k_5$ in all subjects. (D) Scatter plots for $2\log k_7 + a \log \mathrm{PG120} + b$ versus $\log k_4 k_5$ in NGT, IGT, and T2DM subjects (Eq (3)). The parameters a_n and b_n, a_i and b_i, and a_t and b_t were estimated to minimize RSS between $2\log k_7 + a \log \mathrm{PG120} + b$ and $\log k_4 k_5$ in NGT, IGT, and T2DM subjects, respectively. The indices k_4, k_5, and k_7, which correspond to insulin sensitivity, insulin secretion, and insulin clearance, respectively, were calculated by a previously described method (23), and PG120 is the plasma glucose concentration at 120 min during an OGTT.

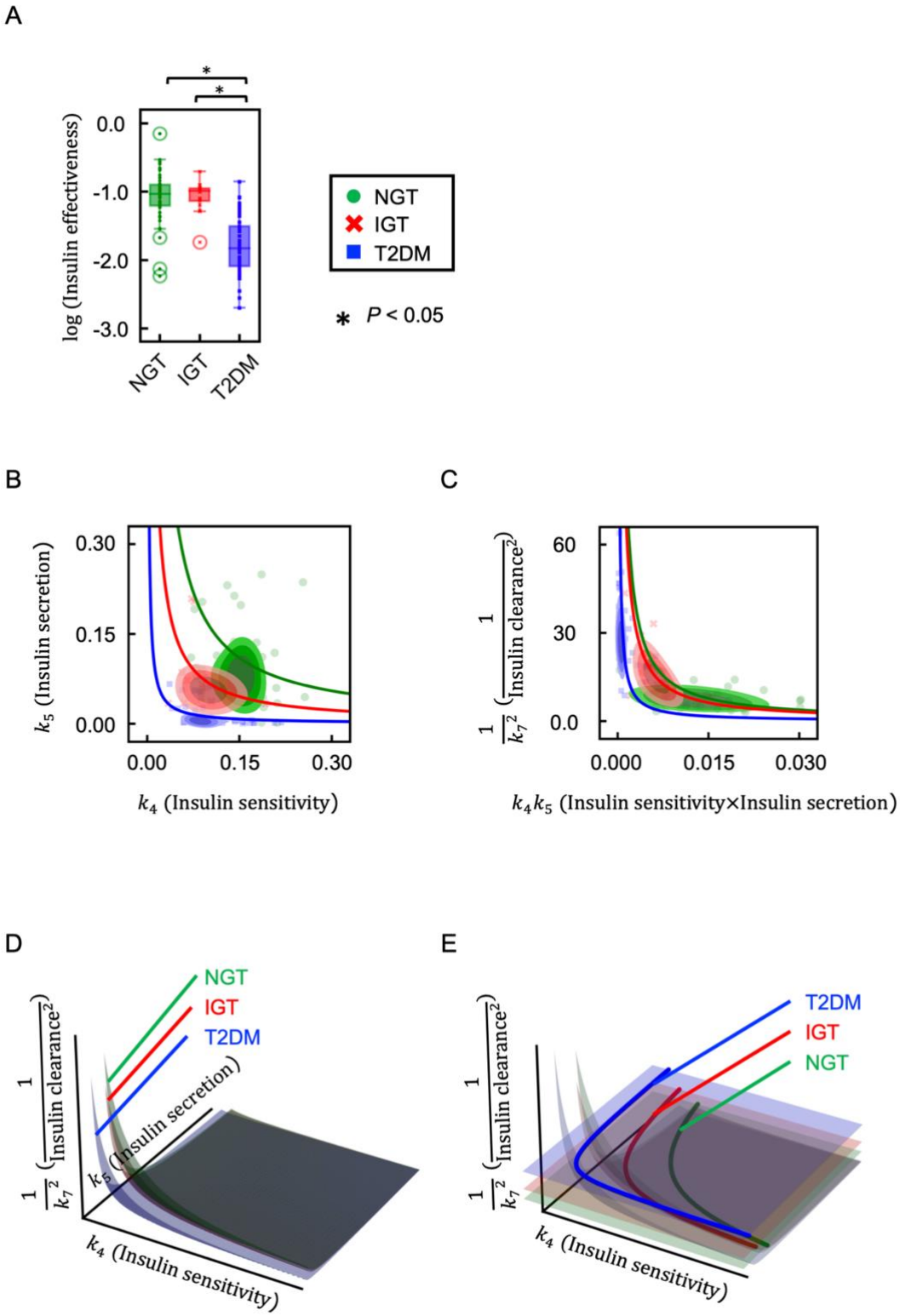

Figure 2. Relation between DI and insulin effectiveness. (A) Box plots of log (insulin effectiveness), corresponding to the logarithm of $k_4k_5/{k_7}^2$, in NGT, IGT, and T2DM subjects. Each point corresponds to the value for a single subject, with green circles,

red crosses, and blue squares indicating NGT, IGT, and T2DM subjects, respectively. The boxes denote the median and upper and lower quartiles. *P* values were determined by one-way ANOVA and Tukey's post hoc test. (B) Scatter plots for k_4 and k_5 fitted with hyperbolic curves (based on the function $xy = \text{constant}$), and kernel density plots. The hyperbolic curves correspond to the mean value of DI for NGT, IGT, or T2DM subjects. Each point corresponds to the values for a single subject, with green, red, and blue indicating NGT, IGT, and T2DM, respectively. The bandwidth of each kernel is based on the rule-of-thumb bandwidth. (C) Scatter plots for $k_4 k_5$ and $1/{k_7}^2$ fitted with hyperbolic curves (based on the function $xy = \text{constant}$), and kernel density plots. The hyperbolic curves correspond to the mean value of insulin effectiveness in NGT, IGT, or T2DM subjects. Each point corresponds to the values for a single subject, with green, red, and blue indicating NGT, IGT, and T2DM, respectively. The bandwidth of each kernel is based on the rule-of-thumb bandwidth. (D) Hyperbolic surfaces (based on the function $xyz = \text{constant}$) corresponding to the mean value of insulin effectiveness in NGT, IGT, or T2DM subjects. (E) Hyperbolic surfaces corresponding to the mean value of insulin effectiveness as well as planes corresponding to the mean value of 1/insulin clearance^2 in NGT, IGT, or T2DM subjects. The axes correspond to k_4, k_5, and ${1/k_7}^2$ as in (D), but the label for k_5 was omitted for clarity. The intersections (solid lines) of the surfaces representing insulin effectiveness and the planes representing the mean value of 1/insulin clearance^2 correspond to the hyperbolic curves in (B).

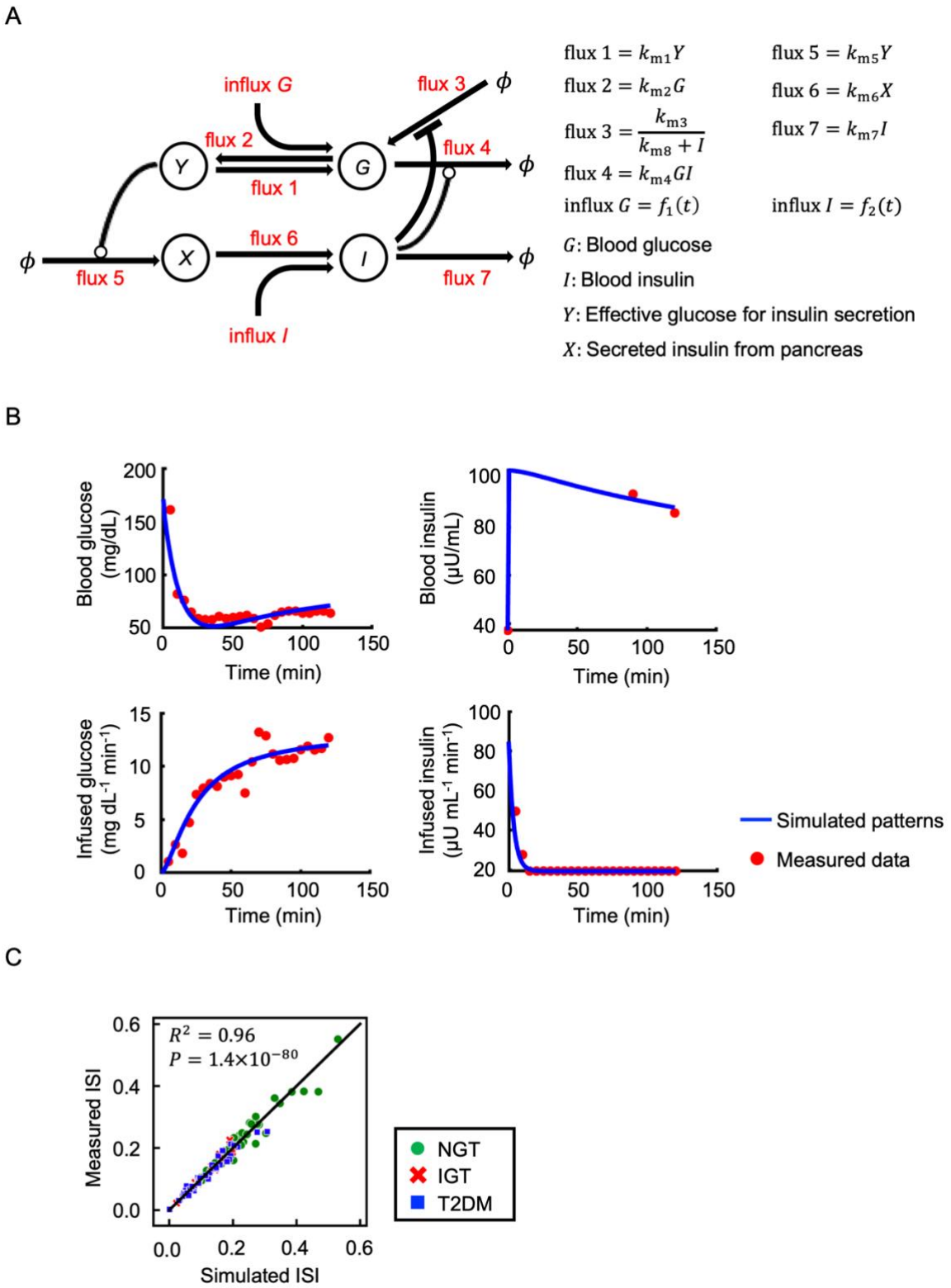

Figure 3. Modified mathematical model of the feedback loop that links glucose and insulin. (A) Model diagram. The letters within circles indicate the variables of the model, the arrows denote the fluxes, and the lines ending in circles or bars represent activation or inactivation,

respectively. ϕ indicates a fixed value. The variables G and I represent blood glucose and insulin concentrations, respectively. The variable Y corresponds to the effective glucose concentration for induction of variable X, which represents insulin secreted from pancreatic β-cells. (B) Time courses of blood glucose, blood insulin, infused glucose, and infused insulin during hyperinsulinemic-euglycemic clamp analysis for an NGT subject. The blue lines indicate the simulated patterns, and the red circles indicate the measured time course data. The simulated values of each variable were rescaled to absolute concentration and plotted. (C) Scatter plot for simulated ISI versus measured ISI. Each point corresponds to the values for a single subject, with green circles, red crosses, and blue squares indicating NGT, IGT, and T2DM subjects, respectively. The measured and simulated ISI values were calculated from the normalized time course and plotted. R^2 is the coefficient of determination, and the P value is for testing the hypothesis of no correlation.

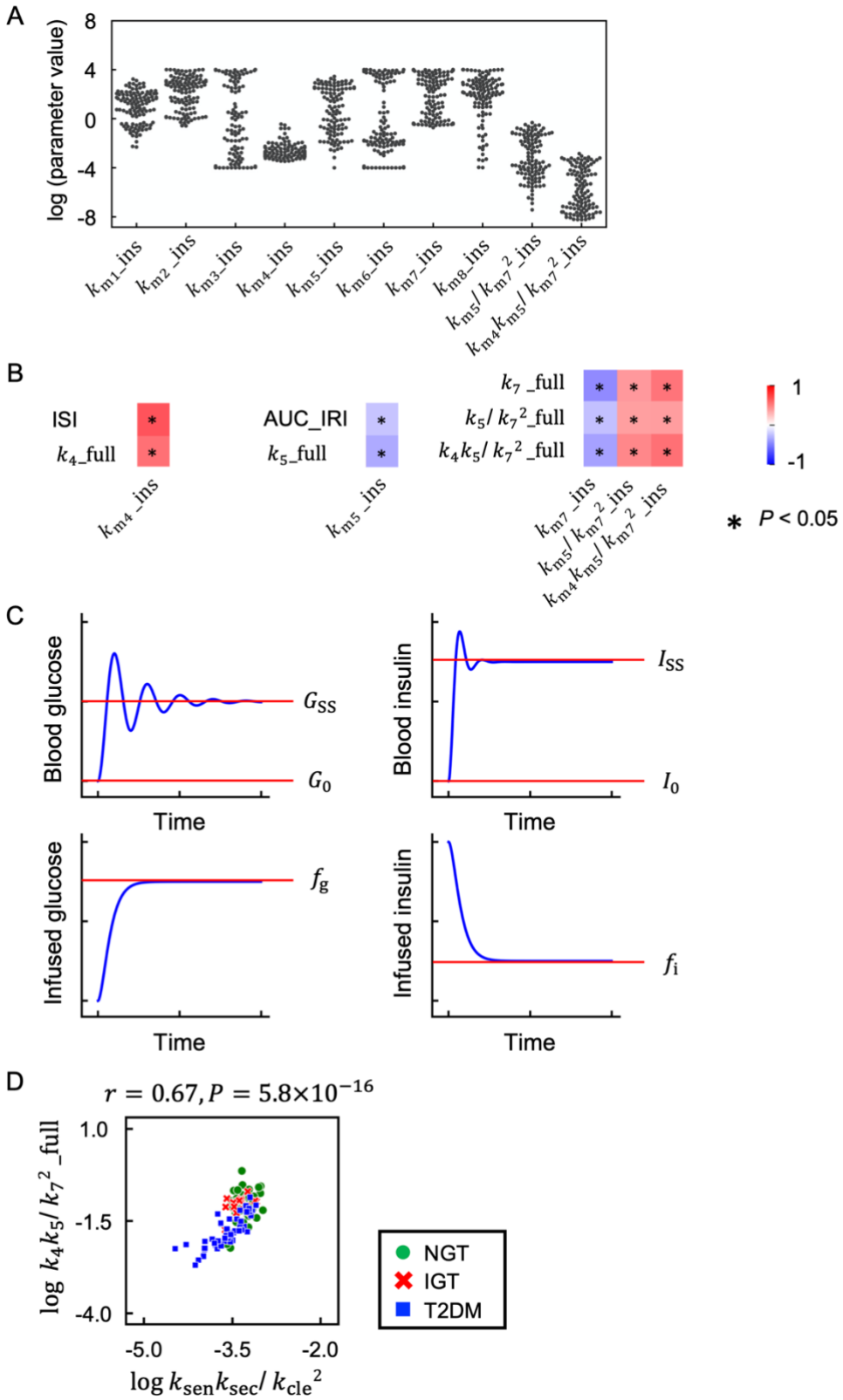

Figure 4. Relation between parameters estimated from a hyperinsulinemic-euglycemic clamp alone and those estimated from both hyperglycemic and hyperinsulinemic-euglycemic clamps. (A) Estimated parameters of the modified model based on a hyperinsulinemic-

euglycemic clamp alone. (B) Heatmap of Spearman's correlation coefficient, with the *P* values corresponding to testing the hypothesis of no correlation. The parameters k_4_full, k_5_full, k_7_full, $k_5/{k_7}^2$_full, and $k_4k_5/{k_7}^2$_full correspond to insulin sensitivity, insulin secretion, insulin clearance, the ratio of insulin secretion to the square of insulin clearance, and insulin effectiveness estimated from both hyperglycemic and hyperinsulinemic-euglycemic clamps by the previously described mathematical model (23) (Fig. S6A), respectively. The parameters k_{m4}_ins, k_{m5}_ins, k_{m7}_ins, $k_{\mathrm{m5}}/{k_{\mathrm{m7}}}^2$_ins, and $k_{\mathrm{m4}}k_{\mathrm{m5}}/{k_{\mathrm{m7}}}^2$_ins correspond to insulin sensitivity, insulin secretion, insulin clearance, the ratio of insulin secretion to the square of insulin clearance, and insulin effectiveness estimated from the hyperinsulinemic-euglycemic clamp alone by the modified mathematical model (Fig. 3A), respectively. The insulin sensitivity index (ISI) and area under the insulin curve during the first 10 min of the hyperglycemic clamp were also calculated. (C) Simulated time courses of blood glucose, blood insulin, infused glucose, and infused insulin during a hyperinsulinemic-euglycemic clamp. G_{SS}, blood glucose level at the steady state during insulin administration; G_0, blood glucose level in the fasting state; I_{SS}, blood insulin level at the steady state during insulin administration; I_0, blood insulin level in the fasting state; f_{g}, glucose infusion rate at the steady state; and f_{i}, insulin infusion rate at the steady state. (D) Scatter plot for log $k_{\mathrm{sen}}k_{\mathrm{sec}}/{k_{\mathrm{cle}}}^2$ versus log $k_4k_5/{k_7}^2$_full. The parameters $k_{\mathrm{sen}}k_{\mathrm{sec}}/{k_{\mathrm{cle}}}^2$ and $k_4k_5/{k_7}^2$_full correspond to insulin effectiveness analytically estimated from the hyperinsulinemic-euglycemic clamp alone (Appendix 7) and that estimated from the hyperglycemic and hyperinsulinemic-euglycemic clamps by the previously described mathematical model (23) (Fig. S6A), respectively. Each point corresponds to the values for a single subject, with green circles, red crosses, and blue squares indicating NGT, IGT, and T2DM subjects, respectively. r is Spearman's correlation coefficient, and the *P* value is for testing the hypothesis of no correlation.