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Hepatic and Extrahepatic Insulin Clearance Are Differentially Regulated: Results From a Novel Model-Based Analysis of Intravenous Glucose Tolerance Data

Insulin clearance is a highly variable and important factor that affects circulating insulin concentrations. We developed a novel model-based method to estimate both hepatic and extrahepatic insulin clearance using plasma insulin and C-peptide profiles obtained from the insulin-modified frequently sampled intravenous glucose tolerance test. Data from 100 African immigrants without diabetes (mean age 38 years, body weight 81.7 kg, fasting plasma glucose concentration 83 mg/dL, and fasting insulin concentration 37 pmol/L) were used. Endogenous insulin secretion (calculated by C-peptide deconvolution) and insulin infusion rates were used as inputs to a new two-compartment model of insulin kinetics and hepatic and extrahepatic clearance parameters were estimated. Good agreement between modeled and measured plasma insulin profiles was observed (mean normalized root mean square error 6.8%), and considerable intersubject variability in parameters of insulin clearance among individuals was identified (the mean [interquartile range] for hepatic extraction was 25.8% [32.7%], and for extrahepatic insulin clearance was 20.7 mL/kg/min [11.7 mL/kg/min]). Parameters of insulin clearance were correlated with measures of insulin sensitivity and acute insulin response to glucose. The method described appears promising for future research aimed at characterizing variability in insulin clearance and the mechanisms involved in the regulation of insulin clearance.

Because of the central importance of insulin in regulating many aspects of metabolism and the decline in β-cell function that contributes to diabetes pathogenesis and progression (1), extensive work has been done to estimate insulin secretion and β-cell function in humans. Numerous experimental procedures to measure β-cell function have been developed, including hyperglycemic clamps, frequently sampled intravenous glucose tolerance tests (FSIGTs), graded glucose infusions, arginine and glucagon challenges, and mixed-meal and oral glucose tolerance tests (OGTTs). Commonly reported measures of insulin secretion include first- and second-phase insulin secretion during hyperglycemic clamps (2), acute insulin response to glucose (AIR$_g$) during the FSIGT (3), and acute insulin response to arginine and maximal insulin release from arginine challenges before and after glucose infusions (4).

There has been insufficient emphasis on the fact that the circulating concentrations of insulin are determined by a balance between the secretion rate of insulin from pancreatic β-cells and insulin degradation ("clearance"), which occurs in the liver, kidney, and other tissues, including skeletal muscle. Thus, measured changes in plasma insulin levels can be due to alterations in insulin degradation and/or insulin secretion. It has become increasingly clear that insulin degradation is a highly regulated process that impacts plasma insulin levels and calculated indices of insulin response (5–7). Insulin clearance is genetically coded independently from insulin secretion itself (8).

Consistent with its importance in determining plasma insulin levels, insulin clearance is highly variable, in part due to large and variable first-pass hepatic insulin extraction,

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which occurs with environmental changes, including a high-fat diet (6,9). It has been proposed that hyperinsulinemia is a harbinger of type 2 diabetes that may be reflective of an incremental decline in insulin clearance (10). Yet the extent to which environmentally induced changes in plasma insulin levels are due to changes in clearance, per se, and to what extent such changes in clearance may reflect hepatic versus extrahepatic clearance is unknown. Prior modeling of data from FSIGT (11) and OGTT protocols (12) enabled first-pass hepatic extraction of newly secreted insulin to be estimated, but the subsequent clearance of circulating insulin was combined into a single clearance parameter that included both hepatic and extrahepatic degradation. To address questions related to hepatic versus extrahepatic clearance, we have developed a novel model-based method for using insulin-modified FSIGT data to provide individual-subject estimates of both hepatic and extrahepatic insulin clearance. Here we apply the model to determine the variability in parameters of insulin clearance in a population of African immigrants without diabetes.

This population was chosen because previous studies (13) have suggested that individuals of African descent have reduced hepatic insulin clearance compared with Western subjects. Similarly, FSIGT data from two groups (14,15) showed that African American women had much higher plasma insulin concentrations than European American women during periods of elevated endogenous secretion but not after intravenous insulin infusion, also suggesting reduced hepatic, but not extrahepatic, insulin clearance in African American subjects. Thus, this population was of special interest for applying a model that could quantify both hepatic and extrahepatic insulin clearance.

RESEARCH DESIGN AND METHODS

Subjects
The participants were 101 African immigrants enrolled in the Africans in America cohort (16,17). All participants were born in equatorial Africa (50% in western Africa, 23% in central Africa, and 27% in eastern Africa) and presently live in the Washington, DC, metropolitan area. The study was approved by the National Institute of Diabetes and Digestive Kidney Diseases Institutional Review Board (ClinicalTrials.gov identifier: NCT00001853). Prior to participation, enrollees gave informed written consent. A 41-year-old man with a fasting insulin concentration of >600 pmol/L was excluded from the analysis because no acceptable model fit could be obtained; the final cohort available for analyses was 100.

Metabolic Tests
Insulin-modified FSIGT procedures were performed at the National Institutes of Health Clinical Research Center in Bethesda, MD. Participants arrived at the site after a 12-h overnight fast. Intravenous catheters were placed in both antecubital veins. After baseline blood samples were obtained, dextrose (0.3 g/kg) was administered intravenously at $t = 0$. Insulin was infused for 5 min starting at

20 min (4 mU/kg/min). Blood samples for measurement of glucose, insulin, and C-peptide levels were obtained at −10, −1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min.

Analytic Measures
Glucose concentration was determined by the glucose oxidase method (Yellow Springs Instrument, Yellow Springs, OH). For the first 36 participants, insulin was measured with a solid-phase, two-site, chemoluminescent sandwich immunometric assay (Immulite 2500; Siemens) (the mean [SD] coefficient of variation [CV] was 11.5% for low concentration [6.2] and 8.9% for high concentration [29]), and C-peptide was measured with a solid-phase, two-site competitive sandwich immunometric assay linked to a chemiluminescent marker (Immulite 2500; Siemens) (CV 7.7% for low concentration [1.12] and 8.3% for high concentration [5.94]). For the remaining 65 participants, insulin and C-peptide levels were measured by electrochemiluminescence immune assay (cobas 6000 analyzer; Roche Diagnostics). For insulin, the CV was 6.0% for low concentration (23) and 2.5% for high concentration (82), and for C-peptide the CV was 2.6% for low concentration (1.83) and 2.8% for high concentration (9.51).

Mathematical Model to Assess Hepatic and Extrahepatic Insulin Clearance
Hepatic and peripheral insulin clearances were estimated using a new model describing insulin kinetics during the FSIGT (Fig. 1 and Eqs. 1 and 2). The main assumptions in the model are:

1. Endogenously secreted insulin enters the portal circulation, whence it travels to the liver before reaching the systemic circulation. The insulin secretion rate (ISR) is calculated by deconvolution using C-peptide kinetic parameters (18). The calculated ISR and the known insulin infusion rate are used as inputs to the model.
2. The rate of delivery of insulin from the systemic circulation to the liver (in picomoles per minute) is equal to the plasma insulin concentration (in picomoles per liter) times the assumed hepatic plasma flow (HPF) rate (0.576 L/min/m^2 [19]).
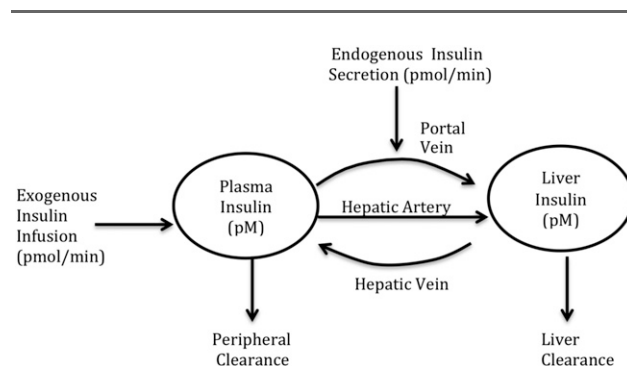
Figure 1—Graphical representation of the mathematical model described by Eqs. 1 and 2. The HPF rate used in the equations is the combined plasma flow to the liver from the portal vein and the hepatic artery.

3. Insulin clearance occurs in both the liver and in extrahepatic (peripheral) tissues, which includes kidney, muscle, and adipose tissue. Extrahepatic clearance is assumed to be proportional to the plasma concentration.

4. Hepatic clearance is modeled using either a linear or a saturable function. Both functions are tested in each subject, and the one providing the best fit is retained. The reason for considering both is that some previous studies (9) have suggested linear clearance, whereas others (11,19,20) have suggested nonlinear and/or time-dependent clearance. In many participants in this study, the data are well described by the linear clearance model, whereas in others there is apparent saturation of clearance during the acute insulin response. No time-dependent changes in parameter values were modeled.

Given these assumptions, the resulting model requires estimating either three (linear model) or four (saturable) parameters from the measured plasma insulin profiles and calculated ISR. Further details describing the parameter estimation and the relative insensitivity of the results to the assumed HPF value are provided in the Supplementary Data.

The equations and parameters corresponding with these assumptions are as follows:

Insulin delivery to liver (pmol/min)

$$\text{Delivery} = ISR + HPF \cdot P$$

Hepatic insulin degradation (pmol/min)

$$\text{Linear model} = FE_L \cdot \text{Delivery}$$

$$\text{Saturable model} = \frac{V_{max} \cdot \text{Delivery}}{K_m + \text{Delivery}}$$

Extrahepatic insulin degradation (pmol/min) $= CL_P \cdot P$

where P is plasma insulin (in picomoles per liter), ISR is reported in picomoles per minute, HPF and CL_P are in liters per minute, FE_L is hepatic fractional extraction (dimensionless), V_{max} is the maximal hepatic degradation rate (in picomoles per minute), and K_m is the hepatic insulin delivery rate at which 50% of maximal degradation occurs (in picomoles per minute).

The differential equations for the linear (Eq. 1) and saturable (Eq. 2) assumptions are as follows:

$$V_P \frac{dP}{dt} = \text{Infusion Rate} + (1 - FE_L) \cdot ISR$$
$$- (HPF \cdot FE_L + CL_P) \cdot P \qquad \text{(Eq. 1)}$$

$$V_P \frac{dP}{dt} = \text{Infusion Rate} + ISR - CL_P \cdot P - \frac{V_{max} \cdot \text{Delivery}}{K_m + \text{Delivery}}$$
$$\text{(Eq. 2)}$$

where V_P is the extrahepatic distribution volume for insulin (in liters). Model-identified parameters were normalized by body weight (BW) for comparison across subjects. For the saturable clearance model, fractional hepatic extraction varies with changes in ISR and P; FE_L values were calculated in the basal state when compiling parameter summaries.

To provide measures of clearance that are related to analogous measures obtained by other experimental methods, Eqs. 1 and 2 were used to calculate measures that would be estimated from steady-state insulin infusions administered either intravenously (as in hyperinsulinemic clamps) or in the portal vein (as in endogenous secretion). The corresponding clearance values from the model for an intravenous infusion (CL_{IV}) or for a portal infusion (CL_{portal}) can be calculated by dividing the insulin infusion rate by the steady-state plasma insulin concentration. For the linear model, this gives

$$CL_{IV} = CL_P + HPF \cdot FE_L$$

$$CL_{portal} = \frac{CL_{IV}}{1 - FE_L}$$

For the saturable clearance model, the effective clearance parameters vary with the insulin infusion rate, and values were calculated assuming an infusion rate of 240 pmol/min/m^2 (a commonly used infusion rate in hyperinsulinemic clamps). The proportion of total insulin degradation occurring in hepatic versus extrahepatic tissues was calculated by integrating the degradation rates over the 180-min interval. The insulin sensitivity index (S_I) was determined from the FSIGT using MINMOD Millenium version 6.02.

Statistical Analysis

For each participant, the measures used to quantify the differences between modeled and measured concentrations were the coefficients of determination ($r^2 = 1 -$ sum of squares of residuals/total sum of squares) and the normalized root mean square error (NRMSE = 100 × RMSE/[$I_{max} - I_{min}$], where I_{max} and I_{min} are the maximum and minimum plasma insulin concentrations and RMSE $= \sqrt{(\text{sum of squares of residuals})/n}$, where n is the number of measured plasma insulin concentrations). Descriptive statistics for model parameters are provided using mean and interquartile range (IQR). Relationships between demographic factors and clearance parameters were assessed using linear regression analysis for continuous variables and the Wilcoxon rank sum test for categorical variables; values of $P < 0.05$ were considered statistically significant. All statistical calculations were performed using Matlab, version 8.4.

RESULTS

Population Characteristics

A total of 100 participants was included in the analysis (Table 1). Seventy-two of the participants were male; the

Table 1—Demographic information for participants in the study

Demographic information	Values
N	100
Male sex (%)	72
Age (years)	38 (10)
Participants with prediabetes (%)	28
BW (kg)	81.7 (12.9)
BMI (kg/m^2)	27.7 (4.3)
Waist circumference (cm)	90 (10)
Visceral adipose tissue (cm^3), *n* = 99	99.4 (70)
Body fat (%), *n* = 85	26.9 (9.2)
Fasting glucose (mg/dL)	83 (7)
Fasting insulin (pmol/L)	37 (30)
Fasting C-peptide (ng/mL)	1.6 (0.6)
S_I (10^{-4}/[pmol/L · min])	0.64 (0.40)
AIR_g (pmol/L · min)	4,187 (2,716)

Values are shown as the mean (SD), unless stated otherwise.

impaired glucose tolerance [IGT] [2-h glucose concentration between 140 and 199 mg/dL], 3 participants with isolated impaired fasting glucose [IFG] [fasting glucose concentration between 100 and 125 mg/dL], and 3 participants with both IFG and IGT concentrations).
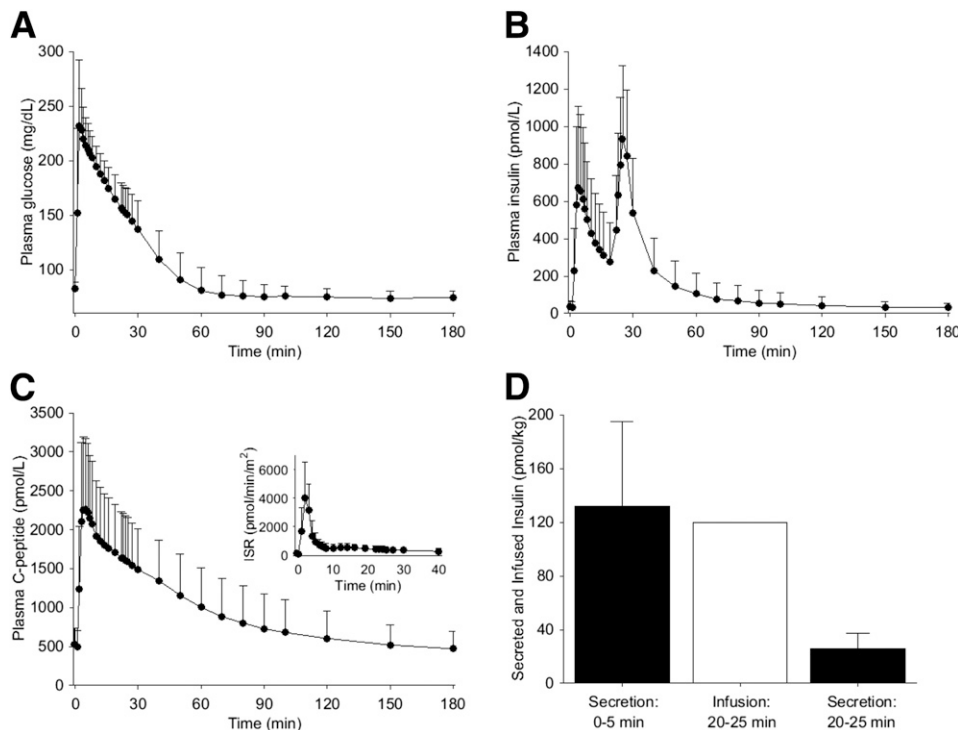
Plasma Profiles
The mean plasma glucose, insulin, and C-peptide profiles and the calculated ISRs are shown in Fig. 2*A*–*C*. Figure 2*D* provides a comparison of the amount of insulin infused with the amount of insulin secreted over the first 5 min after the glucose infusion and over the 5-min interval when insulin is infused (*t* = 20–25 min); of note, the amount of infused insulin was nearly equal to the mean amount of insulin secretion over the first 5 min.
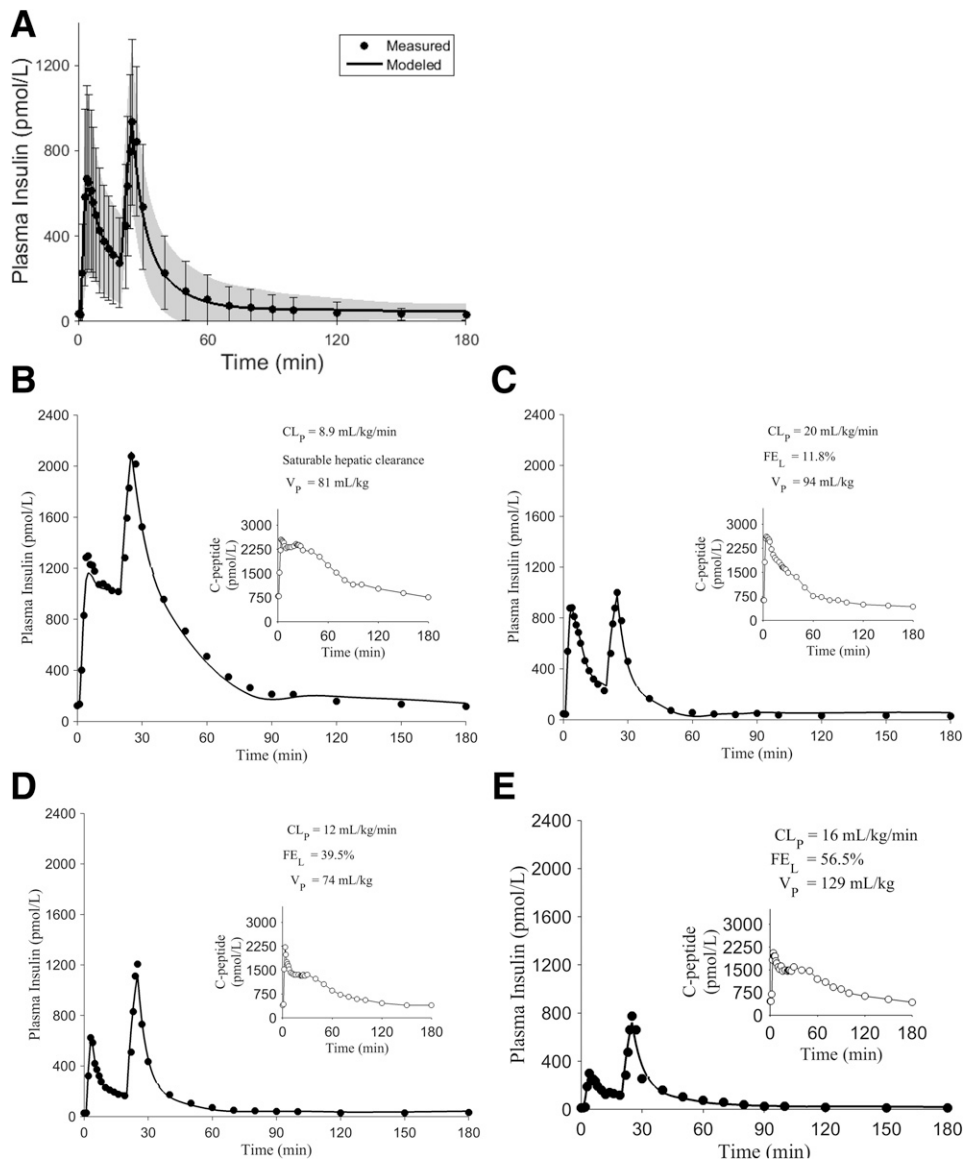
Modeling of Plasma Insulin Profiles
The model is able to accurately capture the dynamic profile observed after both the intravenous glucose bolus and the intravenous insulin infusion (Fig. 3*A*). Across all participants, mean (SD) r^2 = 0.94 (0.05) and NRMSE = 6.8% (2.8%). Further details on the residuals and parameter estimation are provided in the Supplementary Data.

To further illustrate the modeling and parameter estimation, data from four subjects are shown in Fig. 3*B*–*E*. These subjects were chosen for illustration because their plasma insulin profiles have different characteristics that are indicative of differences in insulin clearance, and these differences are captured and quantified using the

mean (SD) age was 38 years (10 years), BW was 81.7 kg (12.9 kg), fasting plasma glucose concentration was 83 mg/dL (7 mg/dL), and fasting insulin concentration was 37 pmol/L (30 pmol/L). Twenty-eight of the participants had prediabetes (22 participants with isolated

Figure 2—Measured profiles for plasma glucose (*A*), insulin (*B*), and C-peptide (*C*) concentrations. The calculated ISR is shown in the inset of *C*; only the first 40 min are shown to highlight the region where insulin secretion is highest. In panel *D*, the total amount of insulin secreted and infused over the intervals 0–5 min and 20–25 min are shown. Values shown are the mean ± SD.

Figure 3—Comparison of modeled and measured insulin profiles. *A*: Mean ± SD for measured (dots and error bars) and modeled (line and shaded region) values. *B–E*: Profiles for four representative participants having different plasma insulin profiles and different parameter estimates for hepatic and peripheral clearance.

model-derived parameters. The subject in Fig. 3*A* has high plasma insulin concentrations after both the intravenous glucose bolus and the insulin infusion, which is consistent with their low CL_P values and their low hepatic clearance, which was best described by the saturable clearance model. The linear hepatic clearance model was preferred for the other three subjects. The subjects in Fig. 3*C* and *E* have lower plasma insulin concentrations after the intravenous insulin infusion than the other two subjects, which is consistent with the relatively high CL values in these subjects. The subjects in Fig. 3*D* and *E* both have insulin concentrations after intravenous glucose infusion that are well below the concentrations after intravenous insulin infusion, which is consistent with the relatively high FE_L values estimated for these subjects.

Distribution of Parameter Values

The model-estimated parameters generally appear to be approximately log-normally distributed (Fig. 4). For extrahepatic clearance and distribution volume, the mean (IQR) values are CL_P = 20.7 mL/kg/min (11.7 mL/kg/min) and V_P = 141 mL/kg (68 mL/kg). The linear hepatic clearance model provided the preferred fit in 63 participants; in these subjects, fractional hepatic extraction was highly variable with the mean (IQR) FE_L = 19.9% (31.0%). In the 37 subjects where the saturable hepatic clearance model was preferred, the mean (IQR) V_{max} = 240 pmol/min (164 pmol/min) and K_m = 403 pmol/L (319 pmol/L). There were no apparent differences in demographic parameters (age, BW, BMI, fasting insulin concentration, or C-peptide concentration) between the subjects modeled
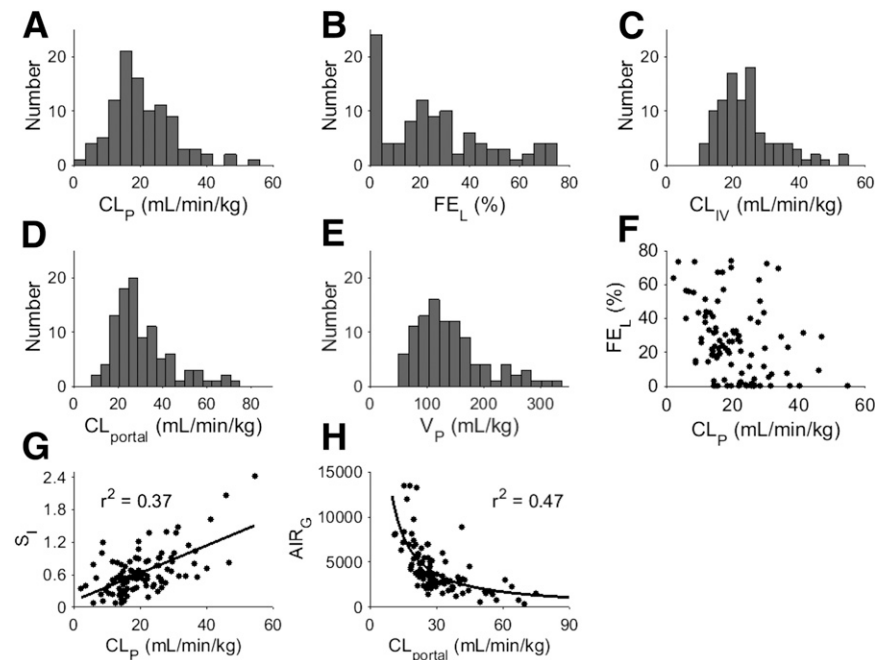
Figure 4—Distribution of parameter values and the relationship between the clearance for endogenously secreted insulin (CL_{portal}) and measures of insulin sensitivity and release. Panels A–E show the distribution of values for the various model parameters, with the y-axis showing the number of subjects in each range of parameter values. Panel F shows the relationship between FE_L and CL_P in the population. Panels G and H show the relationships between CL_P and S_I (panel G) and CL_{portal} and AIR_g (panel H); units for S_I are 10^{-4}/(pmol/L)/min and units for AIR_g are pmol/L · min. Regression relationships were obtained using either linear regression (panel G) or nonlinear regression with a power function ($AIR_g = \beta_1 \cdot CL_{portal}^{\beta_2}$).

with linear or saturable hepatic insulin clearance, although there did tend to be a greater proportion of females among the subjects modeled with saturable clearance (41% female) compared with those modeled with linear clearance (21% female; $P = 0.04$ for comparison using the Fisher exact test).

To provide measures of hepatic clearance that could be compared across all subjects, fractional hepatic insulin extraction was calculated at basal insulin concentrations and basal insulin secretion in all subjects, and CL_{portal} was calculated for a portal insulin infusion of 240 pmol/min/m^2 (with no endogenous secretion). Mean (IQR) values for these parameters (calculated in all 100 subjects) are $FE_L = 25.8\%$ (32.7%) and $CL_{portal} = 31.2$ mL/min/kg (14.7 mL/min/kg). The calculated clearance for an intravenous insulin infusion of 240 pmol/min/m^2 is $CL_{IV} = 24.3$ mL/min/kg (9.6 mL/min/kg). Over the entire 180-min period, the proportion of total insulin degradation occurring in the liver was 27.1% (32.6%), with 72.9% (32.6%) occurring in extrahepatic tissues.

Hepatic and peripheral insulin clearance values appear to be independently regulated, with many subjects tending to have relatively high values for one of the parameters with relatively low values for the other (Fig. 4F). None of the clearance parameters were significantly correlated with age, and none of the parameters were significantly different between males and females. There was also no correlation between BW and clearance parameters when expressed in liters per minute, and there were only modest negative correlations between BW and clearance

parameters when expressed in milliliters per minute per kilogram ($r^2 < 0.12$ for all). Participants with IFG and/or IGT had reduced extrahepatic insulin clearance compared with those with normal glucose levels (mean [IQR] $CL_P = 16.0$ mL/kg/min [9.6 mL/kg/min] in participants with IFG/IGT vs. 22.4 mL/kg/min [11.0 mL/kg/min] in participants with normal glucose concentrations, $P < 0.001$ for comparison), whereas hepatic extraction at basal insulin levels was higher in participants with IFG/IGT than in participants with normal glucose concentrations ($FE_L = 38.5\%$ [33.0%] in participants with IFG/IGT vs. 21.1% [27.6%] in participants with normal glucose concentrations).

Relationship Between Insulin Clearance Parameters and Insulin Sensitivity and Secretion Measures

Both insulin sensitivity and the AIR_g were correlated with measures of insulin clearance. As shown in Fig. 4, insulin sensitivity tends to be higher in subjects with higher extrahepatic insulin clearance, and AIR_g values tend to be lower in subjects with higher clearance for portally delivered insulin.

DISCUSSION

The insulin-modified FSIGT results, in which an intravenous bolus of glucose is injected at $t = 0$ followed by a short intravenous infusion of insulin beginning at $t = 20$ min, provide a unique experimental protocol that enables estimation of both hepatic and extrahepatic insulin clearance. During the first 20 min, all of the plasma insulin is derived

from endogenous secretion (which can be estimated by deconvolution of measured C-peptide concentrations) and is thus subject to first-pass hepatic extraction. Then, at $t = 20$ min, a known amount of insulin is infused intravenously, leading to a rapid increase in plasma insulin concentrations. The observation of time periods wherein all insulin is secreted into the portal vein (0–20 min) with periods where most insulin is delivered intravenously can be used to provide accurate information regarding the relative contributions of hepatic versus extrahepatic insulin clearance.

The model-based method for estimating hepatic and extrahepatic insulin clearance used in the article has advantages over previously reported model-independent methods including 1) dividing the insulin infusion rate during hyperinsulinemic clamps by the resulting plasma insulin concentration (21) and 2) calculating the area under the curve (AUC) of plasma insulin during the FSIGT divided by the insulin dose (8). Although both of these methods provide in vivo insulin clearance estimates, the administered insulin appears first in the peripheral circulation, making it difficult to separate hepatic from extrahepatic degradation. Another method for estimating insulin clearance is to measure plasma C-peptide and insulin concentrations after a meal tolerance test and to divide the AUC of the calculated ISR by the plasma insulin AUC. Although this method gives an estimate of whole-body insulin clearance for endogenously secreted insulin, it provides no information about the relative contributions of hepatic and extrahepatic clearance. It is likely that these different components of insulin degradation can be independently regulated.

Previous model-based approaches for estimating hepatic fractional insulin extraction from intravenous glucose tolerance test and OGTT protocols have also been developed (11,12). Although these models enabled measures of hepatic insulin extraction to be estimated, the fractional extraction calculation was based only on newly secreted insulin, which represents only a portion of the insulin delivered to the liver (with the rest coming from the recirculation of plasma insulin through the portal vein and hepatic artery, which in many conditions is greater than the endogenous secretion rate). These models also contained an additional systemic insulin clearance rate, which implicitly included both hepatic and extrahepatic clearance. The approach developed here explicitly accounts for the hepatic circulation, and thereby enables the relative contributions of hepatic and extrahepatic clearance to be estimated.

Our findings confirm prior reports of marked variability in hepatic insulin extraction. Specifically, the estimated mean (IQR) fractional extraction (under basal conditions) was 25.8% (32.7%), and individual subject values ranged from negligible to 74%. In addition, extrahepatic insulin clearance was also variable and appeared to be regulated independently of hepatic insulin clearance without a clear relationship observed between the FE_L and CL_P parameters, with several subjects having high values for one parameter and low values for the other.

The model-identified clearance parameters correlated with other measures of insulin secretion and insulin action. An inverse relationship was found between insulin clearance and AIR_g, with participants having the highest insulin clearance tending to have the lowest AIR_g and vice versa. The causality associated with the inverse relationship is not fully understood; although reduced hepatic insulin clearance will certainly tend to increase AIR_g values by decreasing first-pass extraction, it is also possible that high insulin secretion leads to a downregulation in hepatic insulin clearance. In a high-fat–fed dog model followed longitudinally, the initial increase in fasting plasma insulin level was attributed to increased secretion, but, after a longer time period, the fasting hyperinsulinemia was attributed to decreased hepatic insulin clearance rather than increased secretion (9). Insulin clearance is also related to insulin sensitivity, with subjects who have higher insulin clearance tending to have higher insulin sensitivity; this is consistent with findings from studies using other measurements of insulin clearance and may reflect the insulin receptor–mediated uptake and removal of insulin that contributes to insulin degradation (6).

The mean relative contribution of hepatic clearance to the overall clearance of insulin was estimated to be ~27% in the African immigrants in this study. This is considerably lower than previous estimates of the contribution of hepatic clearance in Western populations (22) but is consistent with those from a previous report (13) of reduced hepatic insulin extraction in both Africans and African immigrant subjects compared with Western subjects. The results are also consistent with expectations based on the raw data shown in Fig. 2, in which roughly similar amounts of endogenous insulin secretion during the first 5 min and intravenously infused insulin during the 20- to 25-min period led to roughly similar increases in plasma insulin over these periods; if there was extensive first-pass hepatic insulin extraction, the rise in plasma insulin level during the first 5 min would have been expected to be much smaller than the rise during the 20- to 25-min period. Similarly, FSIGT data from two other studies (14,15) showed that African American women had much higher plasma insulin concentrations than European American women after intravenous glucose infusion (during the time of endogenous insulin secretion), but not after the insulin infusion. Preliminary analysis of data from the study by Ellis et al. (15) using the modeling approach described here gives an estimated hepatic contribution to total insulin clearance in the overnight-fasted state of 29% in the African American women versus 59% in the European American women, providing further evidence that hepatic insulin clearance is considerably reduced in African American subjects and that further research into understanding this is warranted.

There are some important limitations associated with the modeling approach used. The approach requires an experimental protocol wherein there are distinct periods in which most/all of the insulin is coming from

endogenous secretion and other periods in which much of the insulin is coming from a peripheral infusion (as in the FSIGT); in the absence of such distinct periods, the model would not have sufficient information to differentiate hepatic from extrahepatic clearance. Thus, although the approach can be used with FSIGT data, it cannot be readily applied to data from hyperinsulinemic clamp studies or OGTTs. In addition, none of these experimental methods provide direct measures of hepatic and extrahepatic insulin clearance to compare with the model-based estimates. Although it is difficult to obtain these estimates in humans, it can be done in animal models using portal and peripheral insulin infusions (9). Future work is planned to compare the CL_{portal} and CL_{IV} values obtained by the model-based analysis of FSIGT data in dogs with the more directly measured values obtained from continuous infusions.

Other limitations with the modeling work are associated with the assumptions used in analysis. One assumption is that the ISR is estimated from deconvolution using estimated individual subject C-peptide kinetic parameters, and this ISR is used as a model input. The second is that the HPF rates were fixed at specified values. The C-peptide kinetic parameters and/or the HPF estimates may not be accurate in several situations such as renal and/or hepatic impairment. Even in subjects with normal hepatic and renal function, the true endogenous secretion rates will be modestly different than the calculated rates due to intersubject variability in C-peptide kinetics that are not fully captured by C-peptide kinetic parameters. Another limitation of the work described here is that, to date, the analysis has been applied only to a population of African immigrants. We have performed preliminary analyses using data from European American individuals, but further work is required to determine whether the model used is able to accurately describe the data in other populations.

The possible difference in insulin degradation by the liver versus extrahepatic tissues is likely important in the pathogenesis of type 2 diabetes. It is possible that the ability of the liver compared with other tissues to modulate insulin degradation is protective of the β-cells and provides an alternative mechanism by which the β-cell is "rested" during temporary states of insulin resistance. If a given group is more dependent upon nonhepatic clearance mechanisms, one may postulate that such mechanisms are less able to compensate for insulin resistance, thus putting the β-cells more at risk for dysfunction in the face of insulin resistance. Further understanding of the mechanisms of insulin clearance are necessary to fully understand the relative importance of hepatic versus extrahepatic insulin clearance and whether therapeutic agents may be developed to address possible defects in the balance between these mechanisms.

In conclusion, using the insulin-modified FSIGT protocol and a novel modeling approach, we have developed a method for estimating both hepatic and extrahepatic insulin clearance using measured plasma insulin and C-peptide profiles only and without requiring invasive sampling

of the hepatic vein. The model, which contains a single differential equation with either three or four parameters, is able to accurately reproduce the full plasma insulin profiles observed during the FSIGT and to identify clear differences in parameter values between individuals. This approach appears to provide a promising method for evaluating changes in insulin clearance mechanisms in future studies.

References

1. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. Diabetologia 2003;46:3–19
2. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223
3. Kahn SE, Prigeon RL, McCulloch DK, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 1993;42:1663–1672
4. Robertson RP, Raymond RH, Lee DS, et al.; Beta Cell Project Team of the Foundation for the NIH Biomarkers Consortium. Arginine is preferred to glucagon for stimulation testing of β-cell function. Am J Physiol Endocrinol Metab 2014; 307:E720–E727
5. Mittelman SD, Van Citters GW, Kim SP, et al. Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced beta-cell response. Diabetes 2000;49:2116–2125
6. Ader M, Stefanovski D, Kim SP, et al. Hepatic insulin clearance is the primary determinant of insulin sensitivity in the normal dog. Obesity (Silver Spring) 2014;22:1238–1245
7. Lee CC, Haffner SM, Wagenknecht LE, et al. Insulin clearance and the incidence of type 2 diabetes in Hispanics and African Americans: the IRAS Family Study. Diabetes Care 2013;36:901–907
8. Goodarzi MO, Langefeld CD, Xiang AH, et al. Insulin sensitivity and insulin clearance are heritable and have strong genetic correlation in Mexican Americans. Obesity (Silver Spring) 2014;22:1157–1164
9. Kim SP, Ellmerer M, Kirkman EL, Bergman RN. Beta-cell "rest" accompanies reduced first-pass hepatic insulin extraction in the insulin-resistant, fat-fed canine model. Am J Physiol Endocrinol Metab 2007;292:E1581–E1589
10. Corkey BE. Diabetes: have we got it all wrong? Insulin hypersecretion and food additives: cause of obesity and diabetes? Diabetes Care 2012;35:2432–2437
11. Toffolo G, Campioni M, Basu R, Rizza RA, Cobelli C. A minimal model of insulin secretion and kinetics to assess hepatic insulin extraction. Am J Physiol Endocrinol Metab 2006;290:E169–E176
12. Campioni M, Toffolo G, Basu R, Rizza RA, Cobelli C. Minimal model assessment of hepatic insulin extraction during an oral test from standard insulin kinetic parameters. Am J Physiol Endocrinol Metab 2009;297:E941–E948

13. Osei K, Schuster DP, Owusu SK, Amoah AG. Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: comparative studies of three populations of West African ancestry and white Americans. Metabolism 1997;46:53–58

14. Chow CC, Periwal V, Csako G, et al. Higher acute insulin response to glucose may determine greater free fatty acid clearance in African-American women. J Clin Endocrinol Metab 2011;96:2456–2463

15. Ellis AC, Alvarez JA, Granger WM, Ovalle F, Gower BA. Ethnic differences in glucose disposal, hepatic insulin sensitivity, and endogenous glucose production among African American and European American women. Metabolism 2012;61:634–640

16. Sumner AE, Thoreson CK, O'Connor MY, et al. Detection of abnormal glucose tolerance in Africans is improved by combining A1C with fasting glucose: the Africans in America Study. Diabetes Care 2015;38:213–219

17. Thoreson CK, Chung ST, Ricks M, et al. Biochemical and clinical deficiency is uncommon in African immigrants despite a high prevalence of low vitamin D: the Africans in America study. Osteoporos Int 2015;26:2607–2615

18. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. Diabetes 1992;41:368–377

19. Caumo A, Florea I, Luzi L. Effect of a variable hepatic insulin clearance on the postprandial insulin profile: insights from a model simulation study. Acta Diabetol 2007;44:23–29

20. Meier JJ, Veldhuis JD, Butler PC. Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. Diabetes 2005;54:1649–1656

21. Arslanian SA, Saad R, Lewy V, Danadian K, Janosky J. Hyperinsulinemia in African-American children: decreased insulin clearance and increased insulin secretion and its relationship to insulin sensitivity. Diabetes 2002;51:3014–3019

22. Ferrannini E, Wahren J, Faber OK, Felig P, Binder C, DeFronzo RA. Splanchnic and renal metabolism of insulin in human subjects: a dose-response study. Am J Physiol 1983;244:E517–E527