New Insights on the Interactions Between Insulin Clearance and the Main Glucose Homeostasis Mechanisms

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OBJECTIVE

Endogenous insulin clearance (EIC) is physiologically reduced at increasing insulin secretion rate (ISR). Computing EIC at the prevailing ISR does not distinguish the effects of hypersecretion from those of other mechanisms of glucose homeostasis. We aimed to measure EIC in standardized ISR conditions (i.e., at fixed ISR levels) and to analyze its associations with relevant physiologic factors.

RESEARCH DESIGN AND METHODS

We estimated standardized EIC (EIC$_{ISR}$) by mathematical modeling in nine different studies with insulin and glucose infusions ($N = 2,067$). EIC$_{ISR}$ association with various traits was analyzed by stepwise multivariable regression in studies with both euglycemic clamp and oral glucose tolerance test (OGTT) ($N = 1,410$). We also tested whether oral glucose ingestion, as opposed to intravenous infusion, has an independent effect on EIC ($N = 1,555$).

RESULTS

Insulin sensitivity (as M/I from the euglycemic clamp) is the strongest determinant of EIC$_{ISR}$, approximately four times more influential than insulin resistance–related hypersecretion. EIC$_{ISR}$ independently associates positively with M/I, fasting and mean OGTT glucose or type 2 diabetes, and β-cell glucose sensitivity and negatively with African American or Hispanic race, female sex, and female age. With oral glucose ingestion, an ISR-independent ~10% EIC reduction is necessary to explain the observed insulin concentration profiles.

CONCLUSIONS

Based on EIC$_{ISR}$, we posit the existence of two adaptive processes involving insulin clearance: the first reduces EIC$_{ISR}$ with insulin resistance (not with higher BMI per se) and is more relevant than the concomitant hypersecretion; the second reduces EIC$_{ISR}$ with β-cell dysfunction. These processes are dysregulated in type 2 diabetes. Finally, oral glucose ingestion per se reduces insulin clearance.

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Insulin clearance is a complex and regulated process, and its role in glucose homeostasis and pathogenesis of prediabetes and diabetes is still unclear. One of the major reasons for this uncertainty is saturation of hepatic insulin removal, which implies that even nonsupraphysiological increments in insulin concentration determine a physiological reduction in insulin clearance (1). If this phenomenon is

not accurately taken into account, comparison of insulin clearance assessed at different insulin concentrations or secretion rates may not reflect intrinsic differences in insulin clearance. In particular, without appropriate analysis, it is not possible to establish to which extent the reduction of insulin clearance observed in obese insulin-resistant subjects is associated with an intrinsic effect of obesity or insulin resistance or due to the concomitant insulin hypersecretion. Similarly, it is problematic to establish whether insulin clearance is affected by oral glucose ingestion, as in this condition, the increase in insulin secretion and concentration may per se induce a reduction in clearance.

We undertook this study to address this problem directly and thoroughly, both in terms of analysis methods and of investigated data. We analyzed a large set of individuals with a wide range of age, obesity, race, glucose tolerance, insulin sensitivity, and β-cell function. Our aims were to: *1*) describe quantitatively the mechanisms of insulin clearance under variable levels of portal and arterial insulin concentrations using a physiology-based representation of insulin kinetics; *2*) derive a standardized estimate of endogenous insulin clearance (EIC$_{ISR}$) (i.e., at fixed insulin secretion levels); *3*) discover the clinical and metabolic traits that independently associate with EIC$_{ISR}$, with a focus on the major metabolic mechanisms involved in glucose homeostasis; and *4*) assess whether oral glucose ingestion as opposed to intravenous infusion modulates insulin clearance.

RESEARCH DESIGN AND METHODS
Study Subjects
This analysis uses data from nine previous human studies in which insulin was infused intravenously or insulin secretion was stimulated by infusion of intravenous glucose or both. Some studies included also oral glucose tolerance tests (OGTTs) or mixed-meal tolerance tests (MMTTs). The studies used the following protocols:

- three-step hyperglycemic clamp followed by an arginine bolus and subsequent insulin infusion (3HGclamp study [2]; $N = 7$);
- hyperglycemic clamp followed by an arginine bolus (HGclamp study [3]; $N = 24$, 4 subjects with 2 repeated tests);
- intravenous glucose infusion producing a plasma glucose ramp (RAMP study [4]; $N = 23$);
- paired 75-g OGTT or MMTT containing 75 g glucose and isoglycemic intravenous glucose infusion (IIGI) mimicking the OGTT or MMTT glucose concentration profile (IIGI-OGTT study [5] with OGTT and IIGI-MMTT study [6] with MMTT; $N = 51$ and 56, respectively);
- isoglycemic hyperinsulinemic clamp with insulin infusion at two rates within the same test (2ISOclamp study [7]; $N = 8$);
- euglycemic-hyperinsulinemic clamp with insulin infusion at one or two rates within the same test (2EUclamp study [8]; $N = 375$);
- euglycemic-hyperinsulinemic clamp with paired 75-g OGTT (EUclamp study [9,10]; $N = 1,257$ with both tests and $N = 16$ with clamp alone); and
- hyperglycemic clamp, euglycemic-hyperinsulinemic clamp, and 1.75 g/kg OGTT in the same subject (HGclamp/EUclamp study [11,12]; $N = 250$).

The main characteristics of the subjects ($N = 2,067$ in total) are reported in Table 1. The description of study protocols, analytical procedures, and data exclusion criteria are provided in the Supplementary Material. Each study has been approved by local or institutional ethics committees, and informed consent was obtained from the participants, as described in the original publications.

Mathematical Model of Insulin Kinetics
Model Description
Plasma insulin kinetics was described by means of a circulatory model (13), which gives an essential physical description of the processes involved in insulin distribution and removal (Fig. 1). Insulin removal was assumed to be negligible in heart, lungs, and gut, to be dependent on prehepatic insulin concentration via a saturative function in the liver, and to be proportional to arterial insulin concentration in the other organs (referred to as extrahepatic and including the kidneys). Consequently, hepatic insulin clearance decreases with increasing levels of prehepatic insulin concentration, while extrahepatic insulin clearance is constant at varying arterial insulin concentration. This mathematical description of insulin kinetics implies that, at steady state, EIC (i.e., the ratio between insulin secretion rate [ISR] and arterial insulin concentration) decreases with increasing ISR or insulin concentration. In the circulatory model, organ volumes and blood flows were derived from the literature. Model details are provided in the Supplementary Material.

In the tests stimulating insulin secretion, ISR was obtained via C-peptide deconvolution using the model by Van Cauter et al. (14) of C-peptide kinetics. In the euglycemic and isoglycemic clamps, in which C-peptide changes are limited, ISR was modeled as a function of time representing a transition between ISR at fasting and during hyperinsulinemia, estimated from C-peptide, when available.

Parameter Estimation
The individual model parameters were identified using the insulin and C-peptide data from the tests described above, with exclusion of the OGTTs, via nonlinear mixed-effect modeling (see Supplementary Material for details). This allowed determination of the individual curves quantifying hepatic clearance, extrahepatic clearance, EIC, and insulin removal as functions of prehepatic or arterial insulin concentration.

Standardized EIC
The individual parameters of the insulin kinetics model were used to derive individual EIC at two fixed steady-state levels of ISR, namely 100 and 400 pmol · min^{-1} · m^{-2}, representing fasting ISR and average ISR during a glucose challenge, respectively. These EIC values were named "standardized EIC" (EIC$_{100}$ and EIC$_{400}$, or, collectively, EIC$_{ISR}$). EIC$_{ISR}$ values allow comparison of EIC between groups without the confounding effect of different secretory levels. The quantity EIC$_{red}$ = (EIC$_{100}$ − EIC$_{400}$)/EIC$_{100}$ represents the relative reduction in EIC due to ISR increase and consequent saturation of hepatic insulin removal.

Independent Correlates of EIC$_{ISR}$
Clinical and Metabolic Traits
Race, sex, age, BMI, fasting glucose, and diabetic status (without diabetes [ND]

Table 1—Characteristics of the study subjects

Study[a]	N	Glucose tolerance[b]	Sex[c]	Age[d] (years)	BMI[d] (kg/m^2)	Race[e]	M/I[d,f] (μmol · min^{-1} · kg^{-1} · nmol^{-1} L)
3HGclamp	7	NFG	6 M + 1 F	39 (38–57)	31.0 (27.5–41.4)	7 C	NA
HGclamp	24	NFG	24 M	40 (33–44)	26.7 (24.3–29.8)	24 C	NA
RAMP	23	13 NGT + 10 T2D	23 M	30 (26–60)	27.1 (23.3–29.9)	23 C	NA
IIGI-OGTT	51	24 NGT + 17 IGT + 10 T2D	24 M + 27 F	47 (33–54)	32.0 (27.0–40.0)	51 C	NA
IIGI-MMTT	56	7 NFG + 49 T2D	38 M + 18 F	57 (51–63)	29.4 (26.2–32.3)	56 C	NA
2ISOclamp	8	6 NFG + 2 IFG	8 M	44 (36–50)	29.1 (25.1–35.2)	8 C	NA
2EUclamp	375	1 ND + 136 NGT + 40 IGT + 198 T2D	3 NA + 154 M + 218 F	47 (36–55)	29.4 (26.0–34.0)	13 AA + 4 AS + 94 C + 264 H	45.7 (30.5–74.0)
EUclamp	1,273	12 ND + 1,096 NGT + 41 IFG + 105 IGT + 9 IFG and IGT + 10 T2D	566 M + 707 F	43 (37–50)	24.9 (22.7–27.8)	1,273 C	128.6 (92.1–178.9)
HGclamp/ EUclamp	250	169 NGT + 48 IGT + 33 T2D	101 M + 149 F	15 (13–16)	34.7 (31.0–39.4)	119 AA + 7 BI + 124 C	31.7 (19.6–44.7)
All	2,067	13 ND + 1,482 NGR + 262 IGR + 310 T2D	3 NA + 944 M + 1,120 F	42 (34–51)	26.7 (23.7–31.1)	132 AF + 4 AS + 7 BI + 1,660 C + 264 H	NA

[a]See *Research Design and Methods* for expansions of study abbreviations. [b]American Diabetes Association 1997 criteria: IFG, impaired fasting glucose; IGR, impaired glucose regulation (IFG or IGT); IGT, impaired glucose tolerance; NFG, normal fasting glucose; NGR, normal glucose regulation (non-T2D and non-IFG and/or non-IGT); NGT, normal glucose tolerance. [c]F, females; M, males. [d]Median (IQR). [e]AA, African American; AS, Asian; BI, biracial African Caucasian; C, Caucasian; H, Hispanic. [f]M/I computed per kilogram of fat-free mass. NA, not available.

vs. with type 2 diabetes [T2D]) were assessed in all studies.

In the studies with an OGTT, β-cell function was evaluated by means of five parameters, derived by mathematical modeling and with clear physiological interpretation (15): among all, glucose sensitivity (GS) (i.e., the slope of the relationship between glucose concentration and ISR) and ISR at 5 mmol/L glucose in fasting conditions (fISR5). Mean glucose during the OGTT was also evaluated.

In the euglycemic clamp studies, insulin sensitivity was calculated as the M/I index, computed as the ratio between the steady-state glucose infusion rate and insulin concentration. The index was computed at 240 pmol · min^{-1} · m^{-2} insulin infusion in the EUclamp and 2EUclamp studies and at 480 pmol · min^{-1} · m^{-2} insulin infusion in the HGclamp/EUclamp study.

Statistical Analysis

To investigate the traits associated with standardized insulin clearance and its reduction with increasing ISR, we performed three separate stepwise multivariable linear regression analyses, with EIC$_{100}$, EIC$_{400}$, and EIC$_{red}$ as dependent variables. In each stepwise analysis, we included the subjects who underwent both a euglycemic clamp and an OGTT and with available fasting C-peptide concentration during the intravenous tests: $N = 1,254$ from the EUclamp study and $N = 156$ from the HGclamp/EUclamp study ($N = 1,410$ in total). In this subject group, β-cell function and glucose tolerance parameters could be included in the regression analysis. As age of participants and clamp insulin dose were different between the EUclamp and HGclamp/EUclamp studies, we analyzed the interactions of the independent variables chosen via stepwise analysis with age and the interactions of M/I with study.

The independent variables selected at each step and their interactions were included in the regression models when their effects had a P value <0.01. All analyses were adjusted for the study. Regression coefficients were standardized in order to allow comparison of the effect size of variables with different interindividual variability and units. Both untransformed and log-transformed values were considered for the continuous independent variables. EIC$_{100}$ and EIC$_{400}$ were log-transformed, and EIC$_{red}$ was logit-transformed.

In three secondary nonstepwise analyses, we added the participants of the 2EUclamp study with M/I index and fasting C-peptide concentration available ($N = 1,602$ in total): in this study, the OGTT was not available, but many adult patients with T2D and Hispanic participants were involved (Table 1). We included in three new multivariable linear regression models the effects of diabetic status (in place of mean OGTT glucose) and Hispanic race, together with the effects of the independent variables found, in the stepwise analyses, to have significant independent associations with EIC$_{100}$, EIC$_{400}$, or EIC$_{red}$; the effects of β-cell function parameters were not available and therefore excluded.

Insulin Kinetics After Glucose Ingestion

The insulin kinetics model was deliberately developed from intravenous tests only, as it is not clear whether oral ingestion of nutrients could modify the mechanisms of insulin clearance. To
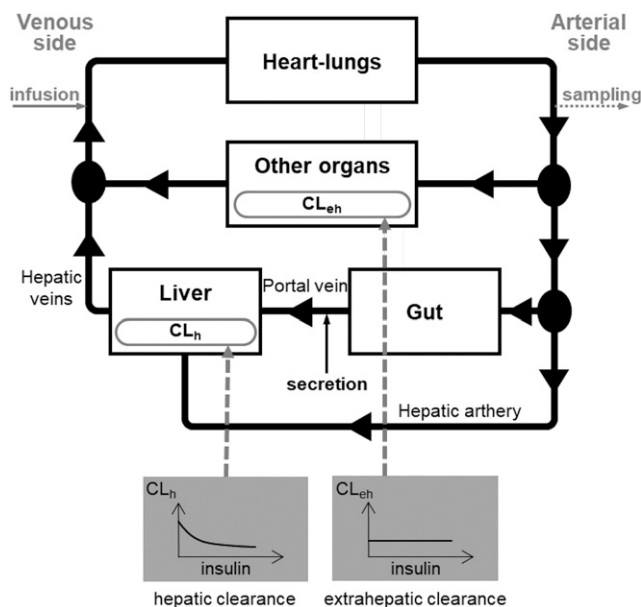
Figure 1—Schematic representation of the mathematical model of insulin kinetics. White rectangles represent lumped organs, black arrows depict fluxes of insulin between organs, and gray rectangles exemplify the relationships between prehepatic insulin concentration and hepatic clearance (CL_h) (left) and between arterial insulin concentration and extrahepatic clearance (CL_{eh}) (right).

assess the effects of an OGTT or MMTT, we have simulated the insulin profiles during these tests using the model and the individual insulin kinetics parameters estimated from the intravenous tests and the time course of ISR separately calculated via deconvolution of C-peptide concentration during the oral test. The simulated insulin profiles were compared with the observed insulin concentration in the subjects with available fasting C-peptide concentration during the intravenous tests ($N = 51, 56, 1,254,$ and 194 in the IIGI-OGTT, IIGI-MMTT, EUclamp, and HGclamp/EUclamp studies, respectively). Assessment of the concordance was based on the individual incremental (i.e., above fasting value) areas under the insulin concentration curves, computed from the data ($iAUC_{data}$) and the simulations ($iAUC_{sim}$). We also calculated the relative difference between the two quantities as ($iAUC_{data} - iAUC_{sim}$)/$iAUC_{data}$. A positive relative difference indicates that insulin clearance is reduced more with an orally induced increment of ISR than with an intravenous stimulus.

RESULTS

Performance of the Mathematical Model of Insulin Kinetics
The insulin kinetics model was able to accurately describe the measured insulin data in all tests. The model residuals (i.e., the difference between the measured and the predicted insulin or C-peptide concentration) were distributed around zero, and their median coefficients of variations were 5.7% and 4.9%, respectively (Supplementary Fig. 1). These percentages are of the same magnitude as the expected assay errors.

Insulin Clearance and Removal
Figure 2A–D exemplifies, for the 3HGclamp study, the steady-state relationship between insulin concentration and insulin clearance or removal, both hepatic and extrahepatic. In the liver, clearance decreases as removal approaches saturation with increasing prehepatic insulin concentration; in the other organs (including kidneys), clearance is assumed to be constant, and removal increases proportionally with arterial insulin concentration. On average, hepatic fractional extraction was 0.65 ± 0.09, 0.59 ± 0.10, 0.33 ± 0.10, and 0.18 ± 0.07 (mean ± SD) at prehepatic insulin concentrations of 180, 720, 3,600, and 7,800 pmol/L, respectively. These values substantially overlap with those reported by Ferrannini and Cobelli (1) with the use of hepatic vein catheterization (16). Extrahepatic fractional extraction was 0.24 ± 0.11. The individual curves representing suppression of hepatic insulin clearance at

increasing prehepatic insulin concentration and the values of extrahepatic insulin clearance were homogeneous among the different studies (Supplementary Figs. 2 and 3).

Standardized EIC
Figure 2E and F show EIC and extraction in relation to arterial insulin levels in the 3HGclamp study. Within the same subject (same color), EIC decreases with increasing insulin concentration (or ISR), and endogenous insulin extraction increases less than proportionally. At a given reference ISR level (Fig. 2E and F: diamonds for ISR = 100 pmol · min^{-1} · m^{-2} and squares for ISR = 400 pmol · min^{-1} · m^{-2}), the corresponding value of EIC_{ISR} varies among subjects; in those with higher EIC_{ISR}, the resulting steady-state insulin concentration is lower. Considering all studies together, the median value of EIC_{100} was 1.64 L · min^{-1} · m^{-2}, with an interquartile range (IQR) of 0.98–2.20; the median value of EIC_{400} was 1.14 L · min^{-1} · m^{-2}, with an IQR of 0.62–1.66. The median value of EIC_{red} (i.e., of the relative reduction from EIC_{100} to EIC_{400}) was 24%, with an IQR of 21–33%.

Independent Correlates of EIC_{ISR}
The traits independently associated with EIC_{100}, EIC_{400}, and EIC_{red}, in the subjects from the EUclamp and HGclamp/EUclamp studies, are shown in Fig. 3A. The values of adjusted R^2 were 0.68, 0.59, and 0.11, respectively. M/I had the strongest independent association, positive with EIC_{100} and EIC_{400} and negative with EIC_{red} in the EUclamp study: one-SD reduction in M/I was associated with a 27% reduction of EIC_{100}, with a 44% reduction of EIC_{400}, and a ~25% increase in EIC_{red}. African American race was associated with lower EIC_{100} in comparison with Caucasian race. β-Cell function, as fISR5 and GS, was associated positively with EIC_{100} and EIC_{400}; fISR5 was also negatively associated with EIC_{red}. Mean OGTT glucose was positively associated with EIC_{100} and EIC_{400}. In the same direction, fasting glucose was positively correlated with EIC_{400} and negatively with EIC_{red}. Female sex was associated with lower EIC_{100} and EIC_{400}, and in females, EIC_{100} and EIC_{400} decreased with age. Older age made the relationship between M/I and EIC_{400} steeper (via a positive interaction between M/I and age in EIC_{400} regression model).
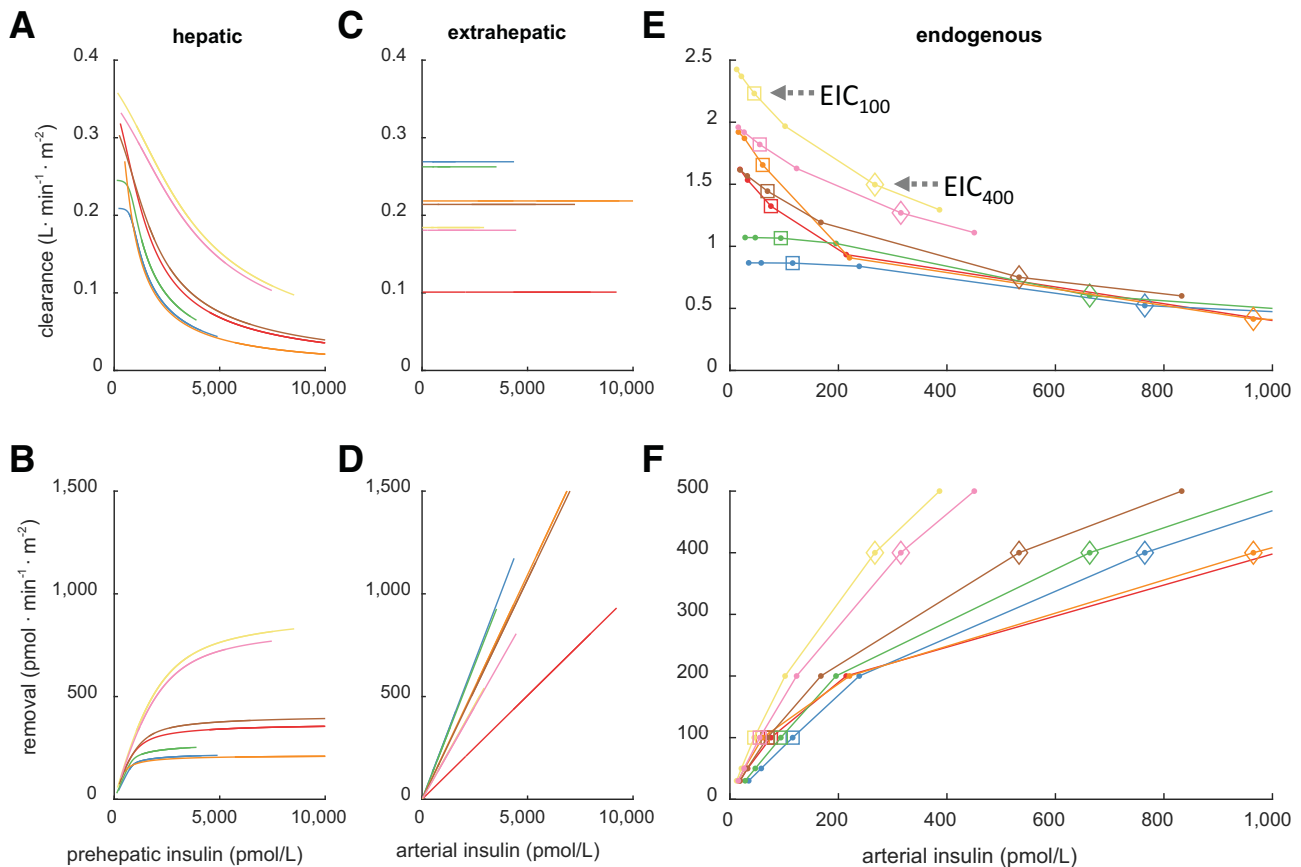
Figure 2—Estimated individual steady-state relationship between insulin concentration and insulin clearance (top) or removal (bottom) in the 3HGclamp study. The panels show hepatic (*A* and *B*), extrahepatic (*C* and *D*), and endogenous (*E* and *F*) insulin clearance and removal against prehepatic insulin concentration (*A* and *B*) and arterial insulin concentration (*C–F*). Each color represents a subject. The model-derived individual relationships between hepatic (*A* and *B*) and extrahepatic (*C* and *D*) clearance or removal and insulin concentration are displayed for the insulin concentration span observed during the tests. Individual EIC and removal (*E* and *F*) are computed at six levels of ISR and linearly interpolated. The symbols on the lines represent standardized EIC and removal at ISR of 100 (diamonds) and 400 (squares) $pmol \cdot min^{-1} \cdot m^{-2}$. Representative EIC_{100} and EIC_{400} are shown in *E*.

Further details are provided in Supplementary Tables 1–6.

The independent effects of the main traits associated with EIC_{ISR} (M/I, sex, and race) can be appreciated in Fig. 3*B*, together with the dependence of EIC on ISR. In this figure, we first computed the average EIC with ISR ranging from 30 to 500 $pmol \cdot min^{-1} \cdot m^{-2}$ in four groups with different combinations of M/I (insulin resistant or insulin sensitive, with M/I around its 10th and 90th percentile, respectively; see the Supplementary Material for details), race, and sex. We then considered EIC_{50} and EIC_{120} as representative of fasting EIC in insulin-sensitive and insulin-resistant subjects, in whom typical fasting ISR values are 50 and 120 $pmol \cdot min^{-1} \cdot m^{-2}$, respectively. The difference in fasting EIC between insulin-sensitive White males and insulin-resistant White males (solid arrow in Fig. 3*B*) could be

decomposed into two components (dashed arrows in Fig. 3*B*): the reduction in EIC_{ISR} associated with insulin resistance per se (i.e., the effect of M/I, vertical dashed arrow in Fig. 3*B*), equal to 45% on average; and the reduction from EIC_{50} to EIC_{120} due to fasting hypersecretion in insulin-resistant subjects (120 vs. 50 $pmol \cdot min^{-1} \cdot m^{-2}$), equal to 11% on average (slanting dashed arrow in Fig. 3*B*). This example shows that insulin resistance per se is a stronger modulator of EIC as compared with the hypersecretion induced by insulin resistance (Fig. 3*C*).

The separate nonstepwise analyses of the larger set of EUclamp, HGclamp/EUclamp, and 2EUclamp studies yielded similar explained variance (adjusted R^2 = 0.65, 0.59. and 0.07 for EIC_{100}, EIC_{400}, and EIC_{red}, respectively) and consistent results, with few exceptions (Supplementary Fig.

4). Weaker effects were observed for some variables (fasting glucose for EIC_{400}, the interaction between age and sex for EIC_{100}, and between age and M/I for EIC_{400}). Like African American race, Hispanic race was negatively associated with EIC_{100}. Hispanic race was also negatively associated with EIC_{red}. T2D status was associated with higher EIC_{100} (+17%) and EIC_{400} (+16%), consistently with a high mean OGTT glucose, as reported above.

Insulin Kinetics After Glucose Ingestion

Pearson correlations between the incremental insulin concentration AUCs from an OGTT or MMTT and from the respective model predictions were 0.81, 0.88, 0.63, and 0.91 in IIGI-OGTT, IIGI-MMTT, EUclamp, and HGclamp/EUclamp studies, respectively ($P < 0.001$ in each case). Accordingly, the time course of insulin concentration during the tests was closely
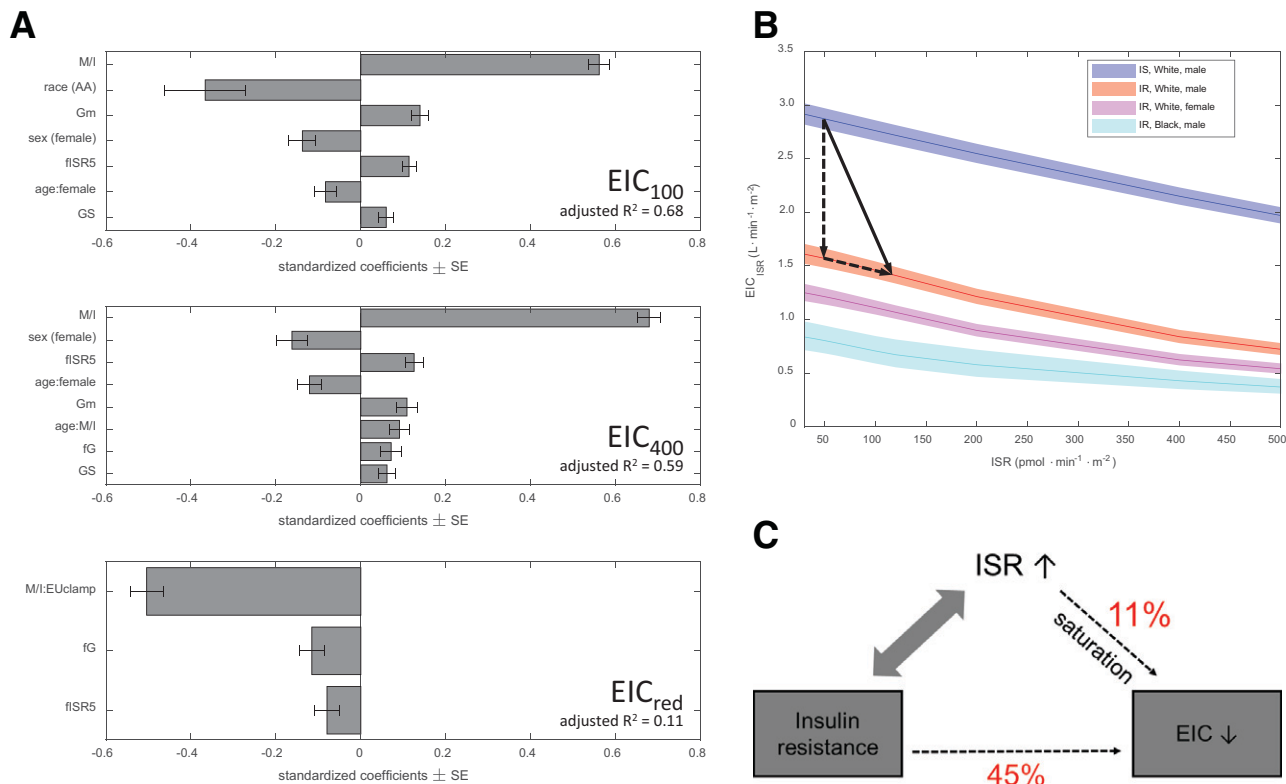
Figure 3—*A*: Standardized coefficients from the stepwise multivariate linear regression analyses of EIC_{100}, EIC_{400}, and EIC_{red} in the EUclamp and the HGclamp/EUclamp studies (see *Research Design and Methods* for the description of the studies). The coefficients of the categorical variables are not standardized. For all variables, $P < 0.01$. M/I, fISR5, fG, EIC_{100}, and EIC_{400} are log-transformed, and EIC_{red} is logit-transformed. *B*: Representation of the main effects shown in *A* (insulin resistance, race, and sex), together with the modulation of EIC_{ISR} by ISR. Curves and shaded areas depict EIC_{ISR} as mean ± SE calculated at ISR levels between 30 and 500 pmol · min^{-1} · m^{-2} in four groups of subjects. The solid arrow represents the difference in EIC_{ISR} between insulin-sensitive and insulin-resistant subjects without diabetes and is decomposed into two components (dashed arrows): the reduction in EIC_{ISR} associated with insulin resistance (i.e., the effect of M/I) and the reduction in EIC_{ISR} due to hypersecretion in insulin-resistant subjects. *C*: Schematic representation of the relationships described in *B* among insulin resistance, hypersecretion, and EIC. AA, African American; fG, fasting glucose; Gm, mean glucose during the OGTT; IR, insulin-resistant; IS, insulin-sensitive.

predicted (Supplementary Fig, 5). However, the median relative difference in incremental AUC was positive in all studies, implying a stronger EIC reduction with oral versus intravenous glucose: 12% in IIGI-OGTT, $P = 0.002$ from signed rank test; 25% in IIGI-MMTT, $P < 10^{-3}$; 6% in EUclamp, $P = 0.003$; and 5% in HGclamp/EUclamp, $P = 0.27$. These results suggest that additional factors may reduce insulin clearance upon glucose ingestion.

CONCLUSIONS

Our analysis reemphasizes the relevance of the dependence of EIC on ISR in physiological conditions (1): for instance, EIC decreases by ∼24% when ISR increases from fasting levels to values typically seen after an OGTT. To assess EIC independently of ISR, we used a physiological model of insulin kinetics based on a minimum of assumptions. The model successfully predicted insulin kinetics, including both exogenous and endogenous sources of insulin, in diverse studies involving >2,000 subjects, with wide range of age, race, insulin resistance, obesity, β-cell function, and glucose tolerance. The key model-based measure was the standardized EIC, which was used to explore the independent traits associated with insulin clearance at fixed ISR levels, while avoiding the bias of empirical methods. The model also allowed us to test whether the reduction of insulin clearance during an OGTT could be ascribed to saturation or other factors.

Our analysis was able to determine to what extent the reduction of insulin clearance observed in obese, insulin-resistant subjects is associated with an intrinsic effect of obesity/insulin resistance or due to the concomitant insulin hypersecretion. The results show for the first time that insulin resistance, and not the concomitant hypersecretion, is the most important modulator of EIC (Fig. 3*B* and *C*) and that obesity (as BMI) is not an independent correlate. Per se, fasting hypersecretion does reduce EIC by ∼11%, but the independent reduction associated with insulin resistance is ∼45% (i.e., four times greater).

Lorenzo et al. (17) detected an independent role of BMI in the correlation between insulin resistance and clearance. Other studies including metabolically healthy obese individuals found that insulin-sensitive subjects, either nonobese or obese, had the same insulin clearance (18–21). However, in these studies or in similar ones (e.g., Gastaldelli et al. [22]), insulin clearance was calculated at different levels of insulin secretion. In fact, in the current data BMI is negatively associated with nonstandardized insulin clearance—both fasting and

post-OGTT—but it is not independently associated with standardized EIC_{100}, EIC_{400}, or EIC_{red} (Supplementary Fig. 6).

Besides insulin resistance, the model-based approach and the large data set allowed us to investigate other factors that are independently related with EIC_{ISR}. The known relationships with sex and race are confirmed for EIC_{ISR}, with some novelties. We found that female sex is associated with lower EIC_{ISR}, thereby extending to the multivariable context previous analyses of nonstandardized splanchnic insulin clearance (23). This association may reflect the typical greater fat-to-muscle ratio in females as well as the fact that insulin clearance is directly related to percent muscle and inversely related to percent fat (24). A similar consideration applies to the observed independent reduction of EIC_{ISR} with increasing age in females, as fat content generally increases with age in females.

In a similar manner, we found that African American and Hispanic race are independently associated with lower EIC_{100} and, in Hispanics, with a stronger reduction in insulin clearance with hypersecretion, thereby extending previous analyses using nonstandardized insulin clearance (17,25–30). In particular, we show that the association between African American or Hispanic race and EIC_{ISR} cannot be explained by differences in insulin sensitivity, β-cell function, and BMI, as instead suggested for nonstandardized insulin clearance (17).

Age and EIC_{ISR} were positively associated in pairwise analysis (Supplementary Fig. 7), in agreement with previous observations on hepatic extraction (31,32), but not on exogenous insulin clearance (32–34). This association was, however, lost in multivariable analysis and replaced by an interaction with insulin resistance that makes the relationship between EIC_{400} and insulin resistance steeper as age increases. This implies that the difference in EIC_{400} between insulin-sensitive and insulin-resistant subjects is higher in older than in younger individuals. While the physiology underlying this finding requires further investigation, at least we show that such interaction between age and insulin clearance does not depend on insulin resistance or adiposity.

With respect to β-cell function, we found that β-cell GS and fISR5 are positively associated with EIC_{ISR}. This result

suggests that reduced insulin clearance may be a way to mitigate not only the effects of insulin resistance, but also those of β-cell dysfunction, a dominant factor in the etiology of T2D. Previous analyses (e.g., Pivovarova et al. [35]) reported instead increased insulin clearance with reduced β-cell function (or insulin secretion). The associations of both fISR5 and GS with EIC_{100}, EIC_{400}, and EIC_{red} disappeared when M/I (or, for GS, mean OGTT glucose) was excluded from our regression models (Supplementary Tables 1–3). Thus, in order to dissect out the role of β-cell function, it was necessary to take into account the nonlinearities of insulin utilization and to adjust for insulin sensitivity and glucose tolerance. The availability of a gold-standard measure of insulin sensitivity and of specific β-cell function parameters (GS and fISR5) likely were other favorable factors.

Our findings concerning the role of glucose levels and glucose tolerance are particularly novel and of considerable interest for T2D pathogenesis. We show that OGTT glucose levels—as a continuous variable or the T2D status—are associated with a relatively increased EIC_{ISR}. In particular, in subjects with T2D in the 2EUclamp study, EIC_{100} was increased by 17%, which is similar to the 26% reduction of EIC_{100} associated with one-SD reduction in M/I. Thus, according to these relationships, in subjects with T2D or who are markedly hyperglycemic, the effects of the compensatory mechanisms that reduce insulin clearance with insulin resistance and relative β-cell dysfunction are partially lost. Although the underlying reasons are unknown, we have observed an analogous phenomenon in T2D progression, as faster progression was observed in subjects lacking the reduction of insulin clearance expected from insulin sensitivity and β-cell function deterioration (36). From our analysis, higher fasting glucose appears to be related specifically to higher EIC_{400} and lower EIC_{red} (i.e., to impaired hypersecretion-induced reduction of insulin clearance). In contrast, elevated mean OGTT glucose or T2D is associated with a general increase in EIC_{ISR}, including the fasting condition (EIC_{100}). To the best of our knowledge, this is the first analysis studying the diabetic condition independently of obesity, insulin

resistance, and variable insulin secretion. Overall, our analysis suggests that in the glucose system, there exist multiple adaptive processes involving insulin clearance, in part described previously (insulin clearance reduction with insulin resistance) and in part emerging from our analysis (insulin clearance reduction with β-cell dysfunction), which become progressively dysregulated as glycemia deteriorates toward T2D.

Using the model and our rich data set, including OGTTs and MMTTs, we could also assess whether the reduction in insulin clearance observed after glucose ingestion originates from saturation of insulin removal or from mechanisms related to the oral route. We found that saturation could almost entirely explain the insulin profiles during OGTTs and MMTTs, although an ~10% clearance decrease was specific to oral glucose. Previously, Tillil et al. (37) and Meier et al. (38) analyzed the differences in insulin clearance after intravenous and oral glucose administration, but did not adjust for different insulin secretion levels. More precise evaluation of the insulin clearance reduction during an OGTT, and whether the incretin hormones are implied (38), requires further investigation.

The use of mathematical models to assess insulin clearance is time-honored, but most models use a compartmental structure that does not represent the physiological interplay of hepatic and extrahepatic clearance correctly (39), with unclear consequences on the accuracy of clearance estimates. Our circulatory representation restores the appropriate correspondence between physiology and mathematics. The model represents hepatic insulin removal as a saturative process consistently in all studies and subjects, a feature often lacking in previous models (39). The impact on glucose homeostasis of insulin clearance modulation by ISR is remarkable, as shown in Supplementary Fig. 8. The adopted mixed-effect modeling approach allowed estimation of the model parameters in all individuals, even in those with limited information on insulin clearance saturation.

The most relevant assumption of our model is the constancy of extrahepatic clearance. This assumption is in substantial agreement with the studies based on splanchnic catheterization, although

performed on healthy subjects only (16). To mitigate the impact of possible changes in extrahepatic clearance, we have deliberately calculated EIC, which is less assumption-dependent (see the section "Role of model assumptions" in the Supplementary Material). In fact, at steady state for a given level of insulin secretion, EIC is simply the ratio between insulin secretion and arterial insulin concentration (i.e., model-independent). For the same reason, we have not used the individual organ-specific clearance estimates, even though our estimated mean values of hepatic fractional extraction substantially agree with previous estimates from splanchnic catheterization (16). Previous modeling studies estimating hepatic and extrahepatic clearance separately (e.g., Polidori et al. [40]) sometimes found implausible individual estimates, a limitation not affecting our model. Thus, in our regression analysis, we have preferred to rely on the more robust estimates of EIC, as the study of the relationships with hepatic and extrahepatic insulin clearance is expected to be less reliable.

An inherent limitation of our study, as of most studies in humans, is that the associations revealed by regression analysis, though biologically plausible, do not establish causality. Some studies in animals suggest that alteration of hepatic insulin clearance (through CEACAM1) may induce changes in insulin sensitivity (41), but whether these mechanisms underlie our observations remains to be determined.

In conclusion, this study describes a new powerful and accurate approach for the study of insulin clearance and highlights the complex interactions between insulin clearance and glucose homeostasis, which have relevant implications for future research on the pathophysiology of T2D.

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