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

Diabetes Care 2021;44:2115–2123 | https://doi.org/10.2337/dc21-0545

Roberto Bizzotto,[1] Domenico Tricò,[2] Andrea Natali,[3] Amalia Gastaldelli,[4] Elza Muscelli,[3] Ralph A. De Fronzo,[5] Silva Arslanian,[6] Ele Ferrannini,[4] and Andrea Mari[1]

PATHOPHYSIOLOGY/COMPLICATIONS

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.

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

[1]*CNR Institute of Neuroscience, Padua, Italy*
[2]*Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy*
[3]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy*
[4]*CNR Institute of Clinical Physiology, Padua, Italy*
[5]*Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, TX*
[6]*Center for Pediatric Research in Obesity and Metabolism, UPMC Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA*

Corresponding author: Roberto Bizzotto, roberto.bizzotto@cnr.it

Received 10 March 2021 and accepted 14 June 2021

This article contains supplementary material online at https://doi.org/10.2337/figshare.14785062.

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

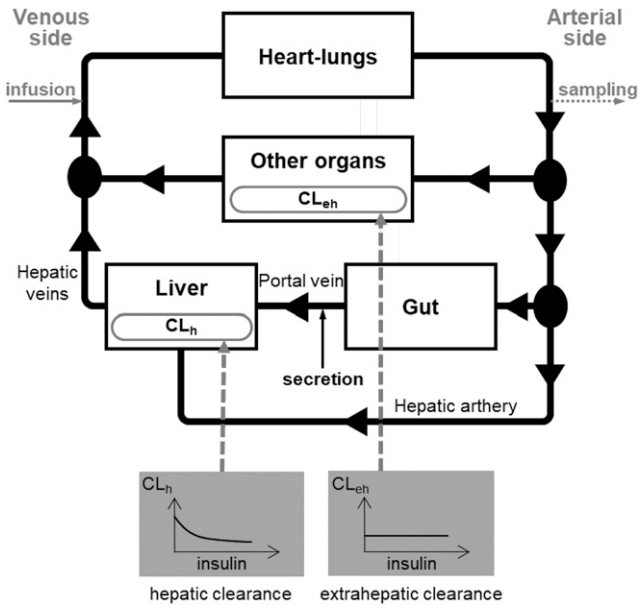

Figure 1—Schematic representation of the mathematical model of insulin kinetics. White rectangles represent lumped organs, black arrows depict fluxes of insulin between organs, and gray rectangles exemplify the relationships between prehepatic insulin concentration and hepatic clearance (CL_h) (left) 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 2*A–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 2*E* 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. 2*E* 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. 3*A*. 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).

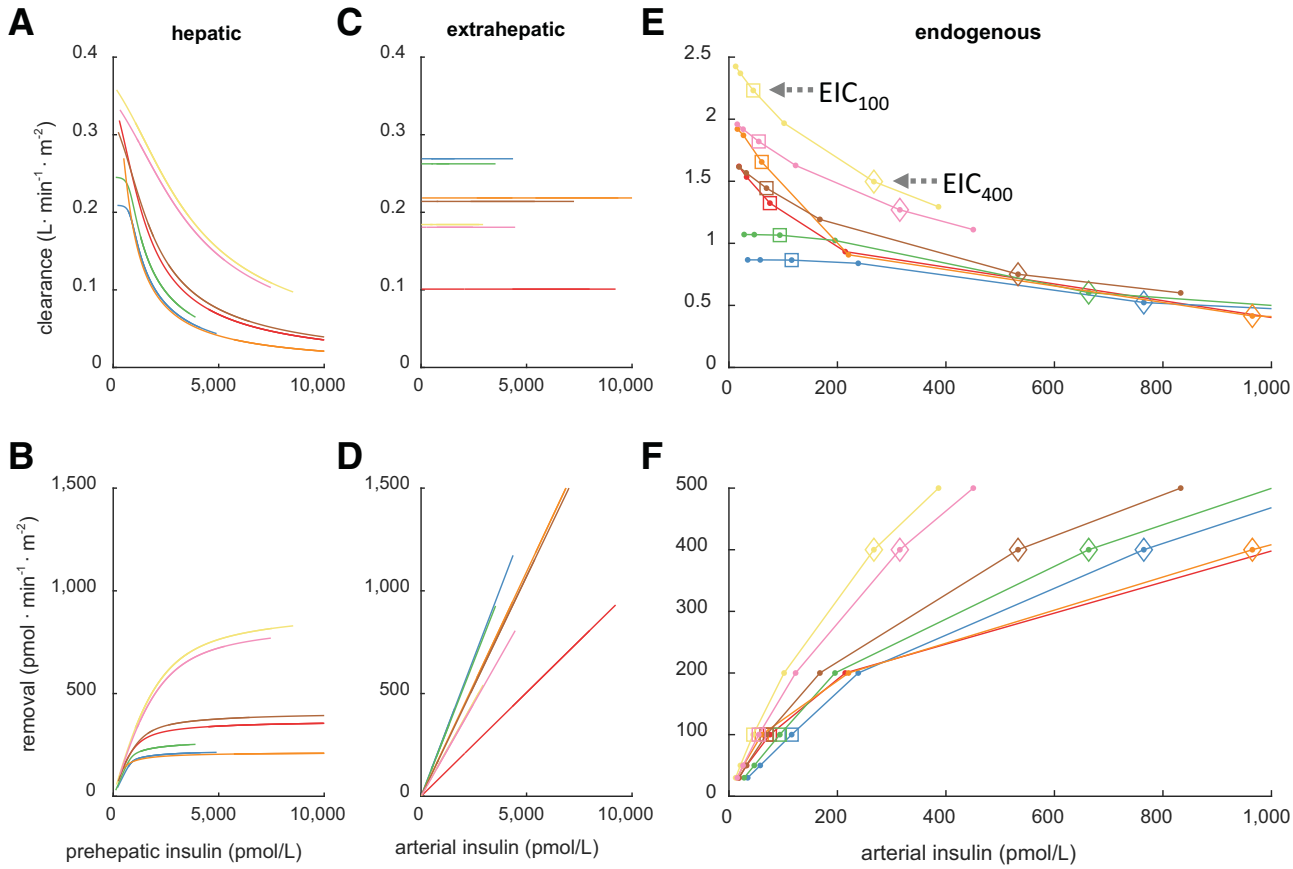

Figure 2—Estimated individual steady-state relationship between insulin concentration and insulin clearance (top) or removal (bottom) in the 3HGclamp study. The panels show hepatic (*A* and *B*), extrahepatic (*C* and *D*), and endogenous (*E* and *F*) insulin clearance and removal against prehepatic 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 · min^{-1} · 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 · min^{-1} · 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 · min^{-1} · 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 · min^{-1} · 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

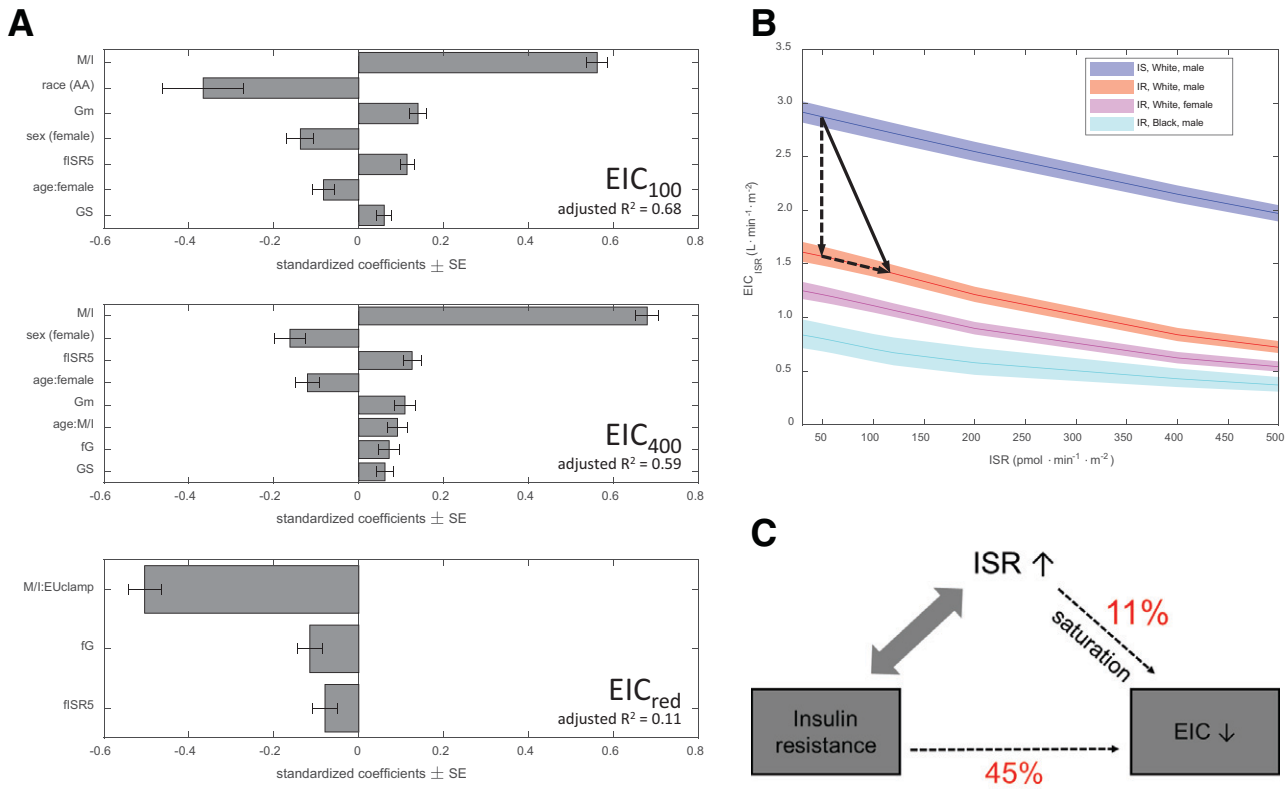

Figure 3—*A*: Standardized coefficients from the stepwise multivariate linear regression analyses of EIC_{100}, EIC_{400}, and EIC_{red} in the EUclamp and the HGclamp/EUclamp studies (see *Research Design and Methods* for the description of the studies). The coefficients of the categorical variables 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.

Acknowledgments. The authors thank Toni Giorgino, PhD, for the technical support in using the software adopted for the analysis and acknowledge the CINECA award under the Italian SuperComputing Resource Allocation initiative for the availability of high-performance computing resources and support.

Funding. This research has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement 115156, the resources of which are composed of financial contributions from the European Union's Seventh Framework Programme (FP7/2007–2013) and European Federation of Pharmaceutical Industries and Associations companies' in-kind contribution. The Drug Disease Model Resources (DDMoRe) project is also financially supported by contributions from academic and small and medium-sized enterprise partners.

Duality of Interest. A.N. has received financial support for clinical research from Boehringer Ingelheim and Eli Lilly and Company. S.A. has participated in advisory boards for Eli Lilly and Company, Novo Nordisk, and Boehringer Ingelheim; has received research funding from Eli Lilly and Company and Novo Nordisk; and is on the Data and Safety Monitoring Board for AstraZeneca. E.F. has participated in advisory boards for Boehringer Ingelheim, Eli Lilly and Company, and Sanofi; has received research funding from Boehringer Ingelheim and AstraZeneca; and has received honoraria for speaking engagements from AstraZeneca, Sanofi, Boehringer Ingelheim, Eli Lilly and Company, and Merck Sharp & Dohme. A.M. has received financial support from Eli Lilly and Company and is a consultant for Eli Lilly and Company. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. R.B. and A.M. designed the analysis, analyzed the data, and wrote the manuscript. A.N., A.G., E.M., R.A.D.F., S.A., and E.F. provided useful data for the analysis. A.M. supervised the analysis. D.T., A.N., A.G., S.A., and E.F. reviewed the manuscript. R.B. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Ferrannini E, Cobelli C. The kinetics of insulin in man. II. Role of the liver. Diabetes Metab Rev 1987;3:365–397
2. Toschi E, Camastra S, Sironi AM, et al. Effect of acute hyperglycemia on insulin secretion in humans. Diabetes 2002;51(Suppl. 1):S130–S133
3. Natali A, Ribeiro R, Baldi S, et al. Systemic inhibition of nitric oxide synthesis in non-diabetic individuals produces a significant deterioration in glucose tolerance by increasing insulin clearance and inhibiting insulin secretion. Diabetologia 2013;56:1183–1191
4. Seghieri M, Rebelos E, Astiarraga BD, Baldi S, Mari A, Ferrannini E. Impact of a mild decrease in fasting plasma glucose on β-cell function in healthy subjects and patients with type 2 diabetes. Am J Physiol Endocrinol Metab. 2016;310:E919–E924
5. Muscelli E, Mari A, Casolaro A, et al. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes 2008;57:1340–1348
6. Muscelli E, Casolaro A, Gastaldelli A, et al. Mechanisms for the antihyperglycemic effect of sitagliptin in patients with type 2 diabetes. J Clin Endocrinol Metab 2012;97:2818–2826
7. Natali A, Gastaldelli A, Camastra S, et al. Dose-response characteristics of insulin action on glucose metabolism: a non-steady-state approach. Am J Physiol Endocrinol Metab 2000;278:E794–E801
8. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab 2005;90:493–500
9. Ferrannini E, Balkau B, Coppack SW, et al.; RISC Investigators. Insulin resistance, insulin response, and obesity as indicators of metabolic risk. J Clin Endocrinol Metab 2007;92:2885–2892
10. Hills SA, Balkau B, Coppack SW, et al.; Report prepared on behalf of the EGIR-RISC Study Group. The EGIR-RISC STUDY (The European group for the study of insulin resistance: relationship between insulin sensitivity and cardiovascular disease risk): I. Methodology and objectives. Diabetologia 2004;47:566–570
11. Arslanian S, Kim JY, Nasr A, et al. Insulin sensitivity across the lifespan from obese adolescents to obese adults with impaired glucose tolerance: Who is worse off? Pediatr Diabetes 2018;19:205–211
12. Michaliszyn SF, Mari A, Lee S, et al. β-cell function, incretin effect, and incretin hormones in obese youth along the span of glucose tolerance from normal to prediabetes to type 2 diabetes. Diabetes 2014;63:3846–3855
13. Mari A. Circulatory models of intact-body kinetics and their relationship with compartmental and non-compartmental analysis. J Theor Biol 1993;160:509–531
14. 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
15. Mari A, Tura A, Gastaldelli A, Ferrannini E. Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation. Diabetes 2002;51(Suppl. 1):S221–S226
16. 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
17. Lorenzo C, Hanley AJG, Wagenknecht LE, et al. Relationship of insulin sensitivity, insulin secretion, and adiposity with insulin clearance in a multiethnic population: the insulin Resistance Atherosclerosis study. Diabetes Care 2013;36:101–103
18. Galderisi A, Polidori D, Weiss R, et al. Lower insulin clearance parallels a reduced insulin sensitivity in obese youths and is associated with a decline in β-cell function over time. Diabetes 2019;68:2074–2084
19. Kim MK, Reaven GM, Kim SH. Dissecting the relationship between obesity and hyperinsulinemia: role of insulin secretion and insulin clearance. Obesity (Silver Spring) 2017;25:378–383
20. Marini MA, Frontoni S, Succurro E, et al. Differences in insulin clearance between metabolically healthy and unhealthy obese subjects. Acta Diabetol 2014;51:257–261
21. van Vliet S, Koh HE, Patterson BW, et al. Obesity is associated with increased basal and postprandial β-cell insulin secretion even in the absence of insulin resistance. Diabetes 2020;69:2112–2119
22. Gastaldelli A, Abdul Ghani M, DeFronzo RA. Adaptation of insulin clearance to metabolic demand is a key determinant of glucose tolerance. Diabetes 2021;70:377–385
23. Jensen MD, Nielsen S, Gupta N, Basu R, Rizza RA. Insulin clearance is different in men and women. Metabolism 2012;61:525–530

24. Yki-Järvinen H, Koivisto VA, Karonen S-L. Influence of body composition on insulin clearance. Clin Physiol 1985;5:45–52
25. 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
26. Fosam A, Sikder S, Abel BS, et al. Reduced insulin clearance and insulin-degrading enzyme activity contribute to hyperinsulinemia in African Americans. J Clin Endocrinol Metab 2020;105:e1835–e1846
27. Haffner SM, Stern MP, Watanabe RM, Bergman RN. Relationship of insulin clearance and secretion to insulin sensitivity in non-diabetic Mexican Americans. Eur J Clin Invest 1992;22:147–153
28. 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
29. Osei K, Schuster DP, Owusu SK, Amoah AGB. 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
30. Tricò D, Galderisi A, Mari A, et al. Intrahepatic fat, irrespective of ethnicity, is associated with reduced endogenous insulin clearance and hepatic insulin resistance in obese youths: a cross-sectional and longitudinal study from the Yale Pediatric NAFLD cohort. Diabetes Obes Metab 2020;22:1628–1638
31. Ahrén B, Pacini G. Age-related reduction in glucose elimination is accompanied by reduced glucose effectiveness and increased hepatic insulin extraction in man. J Clin Endocrinol Metab 1998;83:3350–3356
32. Basu R, Breda E, Oberg AL, et al. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. Diabetes 2003;52:1738–1748
33. Ehrhardt N, Cui J, Dagdeviren S, et al. Adiposity-independent effects of aging on insulin sensitivity and clearance in mice and humans. Obesity (Silver Spring) 2019;27:434–443
34. Minaker KL, Rowe JW, Tonino R, Pallotta JA. Influence of age on clearance of insulin in man. Diabetes 1982;31:851–855
35. Pivovarova O, Bernigau W, Bobbert T, et al. Hepatic insulin clearance is closely related to metabolic syndrome components. Diabetes Care 2013;36:3779–3785
36. Bizzotto R, Jennison C, Jones AG, et al.; IMI DIRECT consortium. Processes underlying glycemic deterioration in type 2 diabetes: an IMI DIRECT study. Diabetes Care 2021;44:511–518
37. Tillil H, Shapiro ET, Miller MA, et al. Dose-dependent effects of oral and intra-venous glucose on insulin secretion and clearance in normal humans. Am J Physiol 1988;254:E349–E357
38. Meier JJ, Holst JJ, Schmidt WE, Nauck MA. Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans. Am J Physiol Endocrinol Metab 2007;293:E849–E856
39. Mari A, Tura A, Grespan E, Bizzotto R. Mathematical modeling for the physiological and clinical investigation of glucose homeostasis and diabetes. Front Physiol 2020;11:575789
40. Polidori DC, Bergman RN, Chung ST, Sumner AE. Hepatic and extrahepatic insulin clearance are differentially regulated: results from a novel model-based analysis of intravenous glucose tolerance data. Diabetes 2016;65:1556–1564
41. Najjar SM, Perdomo G. Hepatic insulin clearance: mechanism and physiology. Physiology (Bethesda) 2019;34:198–215