

Model-Based Assessment of Hepatic and Extrahepatic Insulin Clearance from Short Insulin-Modified IVGTT in Women with a History of Gestational Diabetes

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Abstract— Insulin clearance is an integral component of insulin metabolism. Yet, little is known about separate contribution of hepatic and extrahepatic insulin clearance in type 2 diabetes and in high-risk populations, such as women who experienced gestational diabetes mellitus (pGDM). A model-based method was recently proposed to assess both contributions from 3-hour insulin-modified intravenous glucose tolerance test (IM-IVGTT); the aim of this study was to assess the reliability of short (1 hour) IM-IVGTT in the application of such model-based method and to evaluate the role of the two contributions in determining insulin clearance in pGDM. A total of 115 pGDM women and 41 who remained healthy during pregnancy (CNT) were analyzed early postpartum and underwent a 3-hour IM-IVGTT. Peripheral insulin clearance (CL_p), hepatic fractional extraction (FE_L) and extrahepatic distribution volume (V_p) were estimated by performing a best-fit procedure on insulin IM-IVGTT data considering firstly the overall 3-hour duration and then limiting data to 1 hour. Results showed no significant difference in parameter values between the 3-hour and the 1-hour IM-IVGTT. Comparison between pGDM and CNT (1-hour) showed no significant difference in CL_p (0.23 [0.29] vs. 0.27 [0.43] $L \cdot \text{min}^{-1}$; $p=0.64$), FE_L (50.2 [15.1] vs. 50.9 [11.7] %; $p=0.63$) and V_p (2.01 [2.99] vs. 2.70 [4.00] L; $p=0.92$). In conclusion, short IM-IVGTT provides a reliable assessment of hepatic and extrahepatic insulin clearance through such model-based method. Its application to the study of pGDM women showed no alteration in hepatic and extrahepatic contributions with respect to women who had a healthy pregnancy.

Clinical Relevance— This study proves the reliability of short (1 hour) IM-IVGTT to assess hepatic and extrahepatic insulin clearance in women who experienced gestational diabetes.

I. INTRODUCTION

Insulin clearance is the removal of insulin from the blood in the entire organism and represents an integral component of insulin metabolism since it regulates the cellular response to the hormone by modulating plasma insulin concentration [1]. The primary site of insulin clearance is the liver, with hepatic insulin clearance accounting for approximately 80% of total

insulin clearance; extrahepatic insulin clearance accounts for the remaining part and takes place in several organs, among which kidneys and skeletal muscles [2].

Insulin clearance has been shown to have a key role in type 2 diabetes (T2D) pathophysiology since it was found altered in subjects with T2D [3]; moreover, hepatic and extrahepatic insulin clearance are differentially regulated [4] and, to date, little is known about their separate contribution in T2D pathogenesis although some hypotheses have been formulated [5]. These observations suggest the importance of a reliable but - at the same time - simple insulin clearance assessment, segregating hepatic and extrahepatic contributions, in pathological and/or high-risk populations.

Women who experienced gestational diabetes mellitus (pGDM) - defined as any degree of glucose intolerance with onset or first recognition during pregnancy [6] - are classified as subjects at high risk of developing T2D. If observed shortly after pregnancy, insulin clearance was found not altered in pGDM compared to healthy women [7,8]. Instead, insulin clearance was found altered in pGDM women progressing with respect to those who did not progress towards T2D [9]. However, in these studies analysis was limited to the assessment of insulin clearance without distinguishing between hepatic and extrahepatic contributions.

A model-based method has been recently proposed [4] to estimate hepatic and extrahepatic contribution to insulin clearance using data obtained from the insulin-modified intravenous glucose tolerance test (IM-IVGTT) and consisting of plasma concentrations of insulin and C-peptide. However, the standard time frame of IM-IVGTT used by this method is 3 hours, too long for routinely application. Thus, the aim of this study was twofold: on one side, to assess the reliability of a short (1 hour) IM-IVGTT for the assessment of hepatic and extrahepatic insulin clearance using the previously mentioned model-based method and, on the other side, to evaluate the role of the two contributions in determining insulin clearance in pGDM.

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II. MATERIALS AND METHODS

A. Study Population

This study is a secondary analysis of existing data [10]. The considered population included a total of 156 women, divided in those with a previous history of GDM (n=115, pGDM) and those who had a healthy pregnancy (n=41, CNT). All women were analyzed early postpartum (4–6 months after delivery). Data collection was performed in agreement with the Declaration of Helsinki and received approval of the respective local ethics committee.

B. Insulin-Modified Intravenous Glucose Tolerance Test

All the subjects underwent a 3-hour IM-IVGTT; glucose was injected at time 0–0.5 min (0.3 g/kg) and insulin (0.03 U/kg, Humulin R; Eli Lilly, Indianapolis, IN) was infused intravenously at time 20 for 5 min [10]. Blood samples for measurement of glucose (mmol·L⁻¹), insulin (pmol·L⁻¹) and C-peptide (pmol·L⁻¹) levels were obtained at 0, 3, 4, 5, 6, 8, 10, 14, 19, 22, 27, 30, 35, 40, 50, 70, 100, 140, and 180 min. For each subject, mean glucose, insulin and C-peptide concentrations were evaluated as the ratio between the related area under the curve and the test duration.

C. Model-based assessment of hepatic and extrahepatic insulin clearance

Assessment of hepatic and extrahepatic insulin clearance is based on the mathematical modelling approach proposed by Polidori et al. [4] and summarized in Fig. 1. According to this, changes in plasma insulin concentration (I(t), pmol·L⁻¹) are regulated by the following differential equation:

$$V_P \frac{dI(t)}{dt} = IR(t) + (1 - FE_L) \cdot ISR(t) - (HPF \cdot FE_L + CL_P) \cdot I(t) \quad (1)$$

where IR(t) (pmol·min⁻¹) is the known infusion rate used during the IM-IVGTT, ISR(t) (pmol·min⁻¹) is the prehepatic insulin secretion rate, V_P (L) is the extrahepatic distribution volume, CL_P (L·min⁻¹) is the extrahepatic insulin clearance, FE_L (%) is the hepatic fractional extraction and HPF (L·min⁻¹) is the hepatic plasma flow rate. In relation to (1), the hepatic insulin clearance rate (HICR(t), pmol·min⁻¹) and the extrahepatic insulin clearance rate (EICR(t), pmol·min⁻¹) are:

$$HICR = FE_L \cdot (ISR(t) + HPF \cdot I(t)) \quad (2)$$

$$EICR = CL_P \cdot I(t) \quad (3)$$

ISR(t) and IR(t) represent the inputs to the model. For each subject, ISR(t) has been computed according to Van Cauter et al. [11] by computing individualized (depending on subject' anthropometric characteristics) C-peptide kinetic parameters and performing deconvolution from plasma C-peptide concentration. In order to run this model, it is necessary to assume a constant hepatic plasma flow, while it is known that during a glucose test this flow is variable. Therefore, we must assume a HPF of 0.576 L/min/m².

D. Parameter Estimation

Model parameter vector $p = [CL_P, FE_L, V_P]$ related to 3-hour and 1-hour IM-IVGTT, respectively, was estimated in each subject by performing a best-fit procedure on plasma insulin concentration data, considering firstly the overall 3-hour data and then limiting the data to 1 hour. MATLAB R2019b environment was used for model implementation. The

model discrete time response was obtained by means of ltitr built-in function. Best-fit procedure was accomplished through the lsqnonlin function by solving the following nonlinear least-square curve fitting problem:

$$\min_p \|f(p)\|_2^2 = \min_p \left\| \text{Residuals} + w_1 \cdot \frac{1}{FE_L} + w_2 \cdot \frac{1}{CL_P} \right\|_2^2 \quad (4)$$

where the first term represents the differences between model insulin response and measured insulin data, whereas the second and the third terms are constraints in the form of regularization terms added to enhance information for minimization problem solution and facilitate a posteriori identifiability. The latter two terms have been added in the consideration that FE_L and CL_P cannot assume values near to zero. Minimization problem has been solved through the trust-region-reflective algorithm by considering (0; +∞) as lower and upper bounds for the parameters. Function and step-size tolerances have been set to 10⁻¹⁵.

E. Statistical Analysis

To evaluate the hypothesis that each variable had a normal distribution with unspecified mean and variance, the Lilliefors test was used. Normally and skewed distributed variables were presented as mean ± standard deviation (SD) and median [interquartile range, IQR], respectively. Unpaired Student's t-test was used to test differences in mean values of variables between groups; paired Student's t-test was used to test differences in mean values of variables between the 3-hour and 1-hour IM-IVGTT. A Two One-Sided Test for equivalence (TOST) was performed to test the minimum equivalence margin (epsilon parameter) between 3-hour and 1-hour IM-IVGTT estimated parameters. Log-transformed values were used in case of skewed distributed variables. The two-sided significance level was set at $p < 0.05$.

III. RESULTS

Demographic information for the study population is reported in Table I. The model was able to accurately capture the dynamic plasma insulin profile in the 3-hour and 1-hour IM-IVGTT (Fig. 2).

TABLE I.

DEMOGRAPHIC INFORMATION FOR THE STUDY POPULATION.

Demographic information	CNT	pGDM
Age (years)	31.6 ± 0.8	33.4 ± 0.4
BW (kg)	67.5 ± 11.9	75.5 ± 16.7 *
H (m)	1.66 ± 0.05	1.65 ± 0.08
BSA (m ²)	1.76 ± 0.15	1.85 ± 0.22 *
G _b (mmol·L ⁻¹)	4.56 [0.39]	4.94 [0.78] *
I _b (pmol·L ⁻¹)	46 [28]	47 [30]
CP _b (pmol·L ⁻¹)	150 [50]	166 [93]
G _{mean} (mmol·L ⁻¹)	4.63 [0.68]	5.39 [1.01] *
I _{mean} (pmol·L ⁻¹)	197 [113]	218 [116]
CP _{mean} (pmol·L ⁻¹)	178 [91]	218 [156] *

CNT: healthy pregnancy; pGDM: previous history of gestational diabetes; BW: Body Weight; H: Height; BSA: Body Surface Area; G_b, I_b, CP_b: Basal glucose, insulin, and C-peptide concentration; G_{mean}, I_{mean}, CP_{mean}: Mean glucose, insulin, and C-peptide concentration during the test. Values are expressed as mean ± standard deviation or as median [interquartile range]; * p<0.05.

Values for model parameters (CL_P , FE_L and V_P) obtained considering the 3-hour IM-IVGTT and limiting the IM-IVGTT data to 1 hour are reported in Table II. No significant difference was found in mean values between 3-hour and 1-hour IM-IVGTT. According to the TOST procedure, all the parameters estimated from the 1-hour IM-IVGTT data were shown to be equivalent to those estimated from the 3-hour IM-IVGTT data, with small equivalence margins: epsilon was in fact not higher than 0.1 standard deviation (SD) of the estimated parameter value from the 3-hour IM-IVGTT. No significant difference was also detected in model parameters (CL_P , FE_L and V_P) between CNT and pGDM groups, neither with 3-hour IM-IVGTT nor with 1-hour IM-IVGTT (Table II).

TABLE II.

MODEL PARAMETERS AND INSULIN CLEARANCE VALUES FOR 3-HOUR AND 1-HOUR IM-IVGTT FOR CNT AND pGDM GROUPS.

		3-hour IM-IVGTT	1-hour IM-IVGTT	p-value
CL_P ($L \cdot \text{min}^{-1}$)	CNT	0.27 [0.43]	0.27 [0.43]	0.83
	pGDM	0.23 [0.30]	0.23 [0.29]	0.17
	p-value	0.63	0.64	
FE_L (%)	CNT	50.9 [11.6]	50.9 [11.7]	0.23
	pGDM	49.7 [14.3]	50.2 [15.1]	0.57
	p-value	0.62	0.63	
V_P (L)	CNT	2.70 [4.00]	2.70 [4.00]	0.18
	pGDM	2.01 [2.99]	2.01 [2.99]	0.19
	p-value	0.91	0.92	

CL_P : peripheral insulin clearance; FE_L : hepatic fractional extraction; V_P : extrahepatic distribution volume; CNT: healthy pregnancy; pGDM: previous history of gestational diabetes. Values are expressed as median [interquartile range].

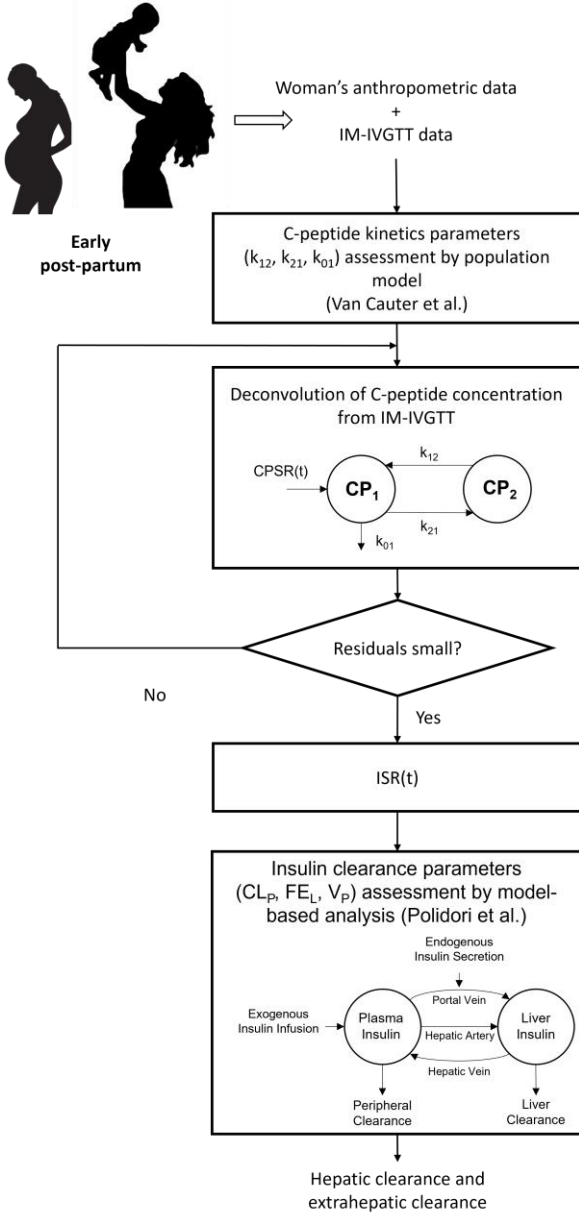


Figure 1. Model-based method for the assessment of insulin clearance from Insulin-Modified Intravenous Glucose Tolerance Test (IM-IVGTT) data. $ISR(t)$ is insulin secretion rate; CL_P is the peripheral insulin clearance; FE_L is the hepatic fractional extraction; V_P is the extrahepatic distribution volume.

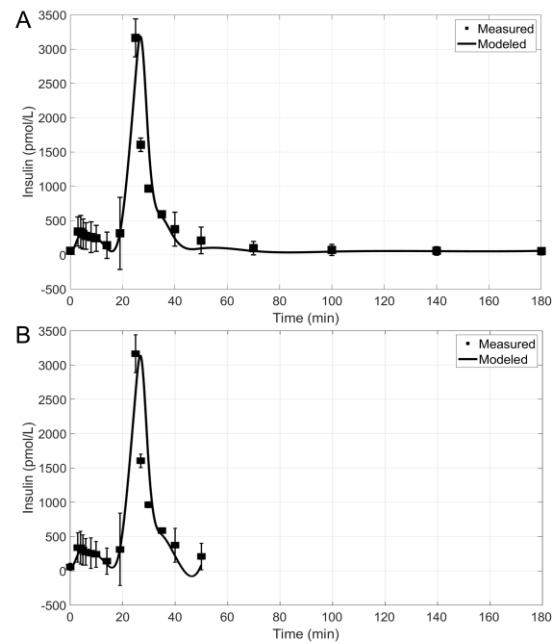


Figure 2. Comparison between mean measured plasma insulin concentrations and model outputs during the 3-hour (panel A) and the 1-hour (panel B) IM-IVGTT. Data are reported as mean \pm standard deviation.

IV. DISCUSSION

This study demonstrated the reliability of short (1 hour) IM-IVGTT to assess hepatic and extrahepatic insulin clearance in women with a history of gestational diabetes. It also showed that, at the time of the study (4-6 months after delivery), neither the hepatic nor the extrahepatic clearance components are different between the pGDM women and the group with healthy pregnancy.

Women who experienced pGDM are known to have high risk to develop T2D [10,12] and early detection of metabolic abnormalities might reduce such risk. For this reason, regular post-partum screenings are suggested, and a simple procedure may increase the adherence to them. The possibility to assess insulin clearance in its hepatic and extrahepatic contributions improves characterization of the metabolic status that can be achieved every time a short IM-IVGTT (that also includes C-peptide measures, besides glucose and insulin) is carried out.

In fact, insulin clearance can be added to those parameters (insulin sensitivity and glucose effectiveness), assessed from short IM-IVGTT [13,14] or short regular IVGTT [13,15], which describe glucose metabolism.

Insulin clearance is partially genetically coded independently from other metabolic variables [16] but may depend on many factors [17]. In addition, further factors have been recently suggested as associated to insulin clearance, though a causal relationship have not been clearly demonstrated yet. An example of these additional factors possibly affecting insulin clearance is glucagon. It was already known that plasma glucagon levels are affected by the action of insulin, as quantified in detail by a mathematical model of glucagon kinetics [18]. However, it has been hypothesized that, on the other hand, glucagon may act on insulin kinetics (i.e., insulin clearance), since associations between glucagon and insulin clearance have in fact been documented in a recent study [19]. The current study, however, was mainly focused on the analysis of the two components of insulin clearance, i.e., hepatic and extrahepatic, thus the analysis of the factors possibly affecting insulin clearance was not performed. This will be considered in future studies, focused on the determinants of insulin clearance possibly specific to the hepatic and the extrahepatic components.

Different mathematical models have been developed for the assessment of insulin clearance, as outlined in a review study [20]. However, model assessing separately the hepatic and extrahepatic components are rare. For the analysis of the oral glucose tolerance test (OGTT), one model was developed based on simple “one-compartment” modelling approach [21,22], which was however acceptable for the OGTT, as explained in [23]. The model by Polidori et al. [4], for some aspects, improves the previous modelling approaches, though on the other hand it is reported to apply only to IM-IVGTT [4]. In addition, we experimented some numerical limitations in the parameter estimation process, and this motivated us to introduce some improvements compared to the original Polidori’s model formulation. In particular, as done in previous studies [18], regularization terms have been added to the minimization problem to facilitate a posteriori identifiability. Moreover, we avoided saturable dynamics for the hepatic clearance, not having physiological evidence. In fact, the model with linear dynamics provided good results in all subjects.

In conclusion, the short (1 hour) IM-IVGTT is a reliable procedure for model-based assessment of hepatic and extrahepatic insulin clearance since it provides results similar to those of the standard 3-hour test. Moreover, application of such methodology showed that both contributions are not altered in women with former gestational diabetes compared to women with normal pregnancy.

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