HOW CAN WE MEASURE INSULIN SENSITIVITY?

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Abstract

Insulin resistance is common in general population and prevalent in patients with obesity and Type 2 diabetes. Insulin sensitivity, reciprocal to insulin resistance, can be measured with a variety of experimental methods ranging from the "gold" standard glucose clamp to the simple HOMA assessment. Each method has its merit and is applicable under different circumstances. Adoption of glucose tracers in the experimental protocols and more specifically in glucose clamp and minimal model allows hepatic vs. peripheral insulin sensitivity to be discriminated and estimated separately. The objective of this review is to give an account of the minimal modelling approach and provide summary information about other measurement methods together with information about reproducibility of the most popular methods, the minimal model and the glucose clamp techniques.

1. INTRODUCTION

In a normal human subject insulin is present in the circulation at all times. Throughout life it exerts an influence over the metabolism of every tissue either directly via its own receptor, or indirectly via its effects on substrate concentrations. Any change in glucose concentration from a narrow normal range results in an insulin response appropriate to restore the homeostasis. In parallel, any change in insulin concentration also aims at restoring homeostasis. Hand in hand, the presence of these two feedback mechanisms maintain and control glucose concentration.

In disease and notably in diabetes, these feedback mechanisms do not operate effectively or break down completely. Insulin resistance represents the inability of tissues to respond to insulin stimulation. Insulin resistance is an inclusive term, which describes the impairments at various levels including intracellular level or extracellular level.

At the whole body level, insulin resistance or its reciprocal quantity, insulin sensitivity, has been of interest since the very discovery of insulin. Early observations indicated that same amount of insulin cause variable reductions in glucose among subjects. With increasing demands on accuracy and relevance, experimental procedures to estimate insulin sensitivity moved from the insulin tolerance test to glucose clamp techniques and minimal model analysis [1,2]. The addition of tracer methodologies and in combination with sophisticated mathematical modelling approaches, insulin sensitivity can now be estimated with high accuracy and physiological relevance. In particular, it is now possible to discriminate between peripheral and hepatic resistance, estimated metabolic fluxes during dynamic conditions, and perform population studies.

The minimal model of glucose kinetics has contributed greatly to the understanding and quantification of insulin resistance in disease and in health. It is now used by an increasing number of researchers and further research ensures that "hot" issues are investigated. Tracer methodologies added considerable knowledge although they still await their full exploitation despite the methodological building blocks being well in place.

3. METHODS TO MEASURE INSULIN SENSITIVITY

3.1. Minimal model analysis

The minimal model of glucose disappearance [3] has been developed to measure parameters of carbohydrate metabolism using a simpler experimental design---an intravenous glucose tolerance test (IVGTT) -while employing a more sophisticated data processing than the glucose clamp technique [4]. The minimal model provides two parameters, which describe carbohydrate metabolism: insulin sensitivity, which quantifies the sensitivity to insulin of tissue glucose utilisation, and glucose effectiveness, which quantifies the effect of glucose levels per se on its disappearance. The need to employ a model-based approach to analyse IVGTT data is due to (i) the dynamic nature of the glucose and insulin data and (ii) the delay in insulin action.

Although employed in numerous studies [5-9], it has been argued that the original minimal model approach has been used far less than might be expected from its potential value [10]. Elaborating on the original minimal model, two different approaches have been suggested to advance its performance. First, modified IVGTT protocols have been developed using either tolbutamide

injection [11] or insulin infusion [7,8] to enhance the resolution of the insulin effect on glucose disposal. Second, the labelled minimal model using either a radioactive tracer [12] or more recently a stable-isotope tracer [10] have been developed. The former methods improve the precision of parameter estimates [13] and allow the parameters of the minimal model to be estimated in subjects without endogenous insulin secretion (the insulin-modified IVGTT protocol) but do not separate glucose disposal from glucose production. The latter methods separate the two processes and improve substantially the precision of parameter estimates [10,12]. A good correlation was obtained between insulin sensitivity estimates obtained by the minimal model and glucose clamp [14]. Simulation studies have been used to evaluate minimal model performance. It appears that minimal model insulin sensitivity underestimates true insulin sensitivity and that glucose effectiveness overestimates true fractional clearance rate [15].

Recently, a new stable-isotope tracer (stable-label) *two compartment* model of glucose disappearance has been suggested and evaluated [16,17]. The central compartment represents glucose in the accessible (measurable) pool, the peripheral compartment represents glucose in the non-accessible pool. Unlike the (one compartment) minimal model, the two compartment model provides plausible estimates of hepatic glucose output during IVGTT due to the adoption of a more realistic structure of glucose kinetics [18]. It also provides estimates of insulin sensitivity and plasma clearance expressed in the same units as indices from glucose clamp studies. However, compared to the minimal model, limited information is available about the performance of the two compartment model [15].

3.2. Glucose clamp

Glucose clamp is the gold standard method to measure insulin sensitivity developed by Andres *et al* [19] and further developed by DeFronzo *et al* [4].

During the glucose clamp, plasma insulin concentration is raised by a constant (primed) insulin infusion while glucose concentration is maintained at euglycaemia (euglycaemic clamp), ambient glucose level (isoglycaemic clamp), or at hyperglycaemia (hyperglycaemic clamp) by a variable exogenous glucose infusion. After reaching the (quasi) steady-state usually within 90 to 180 minutes, the rate of glucose infusion (M-value), which maintains the desired glucose level, is taken as an index of insulin sensitivity.

Early assumptions were made that exogenous insulin fully suppresses hepatic glucose production and thus that M-value is identical to peripheral glucose uptake. This assumptions has been shown invalid at least in subjects with Type 2 diabetes [20] and doubts prevail about the insulin levels needed to suppress HGP.

The use of isotope dilution methodology is able to determine HGP during the clamp. This allows the peripheral glucose uptake R_d to be correctly estimated. The calculations can be carried out with a variety of models ranging from the original Steele's one compartment model [21] to Mari's two compartment model [22]. The latter approach provides more accurate estimates due to the adoption of a more realistic model of glucose kinetics. However, the calculation process can be made model-independent with the adoption of specific activity clamp (or tracer-to-tracee clamp in case of stable isotopes).

Although the gold standard technique, the glucose clamp is the most experimentally demanding procedure. The successful performance of the clamp depends on number of factors including cannulation of both hands for the delivery of infusates and for regular sampling (including real-time measurement of plasma glucose), the use of an algorithm to calculate exogenous infusion rate, sampling of arterialised blood (due avoid the effect of A-V glucose difference), the use of well calibrated infusion pumps.

The major advantage of glucose clamp is that indices of insulin sensitivity have unequivocal physiological interpretation. Glucose uptake can be measured, in principle, at any combination glucose and insulin levels and the whole dose-response curve generated. The opponents of the procedure argue on the grounds of the non-physiological nature of the experiment, which requires infusion of considerable amount of substrates (glucose and insulin).

3.3. Insulin suppression test

The insulin suppression test is a reverse clamp. An exogenous glucose infusion is kept constant while glucose concentration is allowed to vary during a constant insulin infusion. The steady-state concentration of glucose is then taken as an index of insulin resistance.

Insulin suppression test normally induces hyperglycaemia, which stimulates endogenous insulin secretion. Thus, a concomitant infusion of somatostatin is usually delivered to suppress the secretion. There are other possible suppressors such as adrenaline, which can be used instead of somatostatin. The choice is dictated by practical issues and although by the need to avoid a side effect on glucose clearance.

The insulin suppression test is not a widely used technique. The "sensitivity" index, i.e. the resulting hyperglycaemia, is difficult to interpret from the physiological point of view. There is also a possibility that glucose concentration does not reach its plateau within the preset experimental time. In insulin sensitive subjects, glucose can decrease below basal level. On the other hand, in insulin insensitive subjects, glucose can increase above the renal threshold and lead to glucosuria.

3.4. Insulin tolerance test

The insulin tolerance test was the first method to evaluate insulin sensitivity [23]. The test is based on the measurement of the decay of plasma glucose after a bolus injection insulin (normally 0.1 U/kg body weight).

The decrease during the time 10 to 40 minutes after the bolus evaluated as a rate (slope) on a logarithmic scale gives the insulin sensitivity index. This approach implicitly assumes a single compartment model of glucose and that insulin injection stimulates glucose efflux from the compartment. The index has been found correlated with indices obtained with the glucose clamp technique. However, the value of the index depends on the evaluation period. This can be informally explained by the fact that the index represents an "average" fractional removal of glucose and that the bolus insulin injection results in temporal changes in the fractional clearance rate.

The major problem of the test is hypoglycaemia, which presents both undesired subject's state but also can alter the insulin sensitivity index through the involvement of metabolic processes associated with glucose counter-regulation.

3.5. Homeostatic Model Assessment and Constant Infusion of Glucose Model Assessment

The homeostatic model assessment (HOMA) [24] and constant infusion of glucose model assessment (CIGMA) [25] approaches employ a model-based approach to provide a scaling factor (1 = normal) indicating the relative insulin sensitivity and beta-cell function.

The HOMA method is the simplest method among all methods measuring insulin sensitivity. It calculates insulin resistance from fasting plasma glucose and fasting plasma insulin. Clearly, fasting levels are influenced by a number of factors and therefore the accuracy of the method is limited. However, HOMA can be used as a supportive tool or in large population studies where reduction in the accuracy is compensated by a large subject cohort [26,27].

The CIGMA method processes glucose and insulin concentration after 1 hour intravenous glucose infusion employing a more elaborated model of glucose metabolism. CIGMA is a form of a simplified, shortened hyperglycaemic glucose clamp. Like HOMA, CIGMA provides results which do not have immediate physiological interpretation and are, largely, model dependent.

4. REPRODUCIBILITY

Reproducibility and repeatability are essential characteristics of any usable measurement method. Normally, the reproducibility assessment employs two critical measures. The within subject CV indicates the expected variability when making replicate measurements in one subject. The within subject variation as % of total variation puts the within subject variability within the context of the overall variability [28]. For a variable to be highly reproducible and able to discriminate between subjects, both measures should attain a low value (say < 20%).

Here we comment on reproducibility of the minimal model and glucose clamp, which employ stable-label glucose compounds.

Reproducibility of parameters of the labelled minimal models is better than that of the unlabelled unmodified model. Reproducibility of labelled insulin sensitivity indices is acceptable in healthy subjects (within subject CV 17% vs. 19%) [17].

The reproducibility of insulin sensitivity and glucose effectiveness provided by the *stable-label unmodified* (one compartment) minimal model is comparable to the reproducibility provided by the unlabelled *tolbutamide-modified* (one compartment) minimal model in normal subjects. Within subject CV of 20% for S_I has been reported [13]. This confirms the logical expectation that the modified protocols improve reproducibility of insulin sensitivity (but not reproducibility of glucose effectiveness). Ferrari et al [29] also reported highly reproducible estimates of insulin sensitivity with the tolbutamide-modified IVGTT using the unlabelled (one compartment) minimal model in normal subjects.

The critical question is the performance of these models in impaired glucose tolerance at various diabetes states and especially in Type 2 diabetes. Current evidence suggests that the modification of IVGTT by insulin is essential for a good performance in Type 2 diabetes. The insulin-modified unlabelled IVGTT has been successfully employed in Type 2 diabetes and a good comparison with glucose derived clamp indices has been obtained [14]. Avogaro *et al* [30] documented an improved precision of estimates of insulin sensitivity in Type 2 diabetes with the use of labelled glucose. Whether the improvement justifies the use of labelled glucose is difficult to assess but the ability of the labelled models to discriminate between glucose uptake and production may influence the decision in certain applications, e.g. in the assessment of peripheral sensitivity.

The reproducibility of glucose clamp is comparable to that of the minimal model. Within subject CV of 11% and 19% were reported for M-value and R_d [31]. The within subject variance as % of total variance was higher for the two indices but still acceptable (18% and 30%).

It is also beneficial to compare reproducibility of insulin sensitivity with that of hepatic glucose production. Wolfe *et al* [32] reported intra-subject variability of 4.7% in 12 normal male subjects who underwent the assessment of HGO on three to four separate occasions using 6-3H-glucose. Robert *et al* [33] observed 5% and 7% day-to-day variation in HGO in young and elderly subjects respectively. Similar results were obtained in healthy male subjects [34].

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APPENDIX

This appendix provides further details about the minimal modelling approach. It shows formal treatment of mathematical description of three models, the unlabelled minimal model, the labelled minimal model, and the two compartment labelled model. It also describes the approach to calculate hepatic glucose production.

1. Unlabelled Minimal Model of Glucose Disappearance (One Compartment Model)

The unlabelled minimal model of glucose disappearance is described by two differential equations

$$dg(t)/dt = -[p_1 + x(t)]g(t) + p_1 g_b \qquad g(0) = g_0 \tag{A3}$$

$$dx(t)/dt = -p_2 x(t) + p_3[i(t) - i_b] \qquad x(0) = 0 \tag{A4}$$

where g(t) is plasma concentration of total (labelled and unlabelled) glucose, i(t) is plasma insulin concentration, x(t) is a variable associated with the remote insulin compartment, g_b is basal glucose concentration, i_b is basal insulin concentration, and p_1, p_2, p_3 and g_0 are model parameters. Insulin sensitivity is defined as a ratio $S_I = p_3 / p_2$, glucose effectiveness as $S_G = p_1$.

2. Stable-Label Minimal Model of Glucose Disappearance (One Compartment Model)

The stable-label minimal model of glucose disappearance consists of two differential equations

$$dg^*(t)/dt = -[p_1^* + x(t)]\, g^*(t) \qquad g^*(0) = g_0^* \tag{A5}$$

$$dx(t)/dt = -p_2^*\, x(t) + p_3^*\, [i(t) - i_b] \qquad x(0) = 0 \tag{A6}$$

where g*(t) is plasma concentration of 6,6-^{2}H-glucose, p^*_1, p^*_2, p^*_3 and g^*_0 are model parameters. Insulin sensitivity is defined as a ratio $S_I^* = p_3^* / p_2^*$, glucose effectiveness as $S_G^* = p_1^*$.

3. Stable-Label Two Compartment Model of Glucose Disappearance (Two Compartment Model)

The stable-label two compartment model of glucose disappearance during an IVGTT is described by a set of differential equations

$$dq_1^*(t)/dt = -\left[k_p + F_{01}/V_1/g(t) + k_{21}\right]q_1^*(t) + k_{12}q_2^*(t) \qquad q_1^*(0) = D^* \tag{A7}$$

$$dq_2^*(t)/dt = -\left[k_{02} + x(t) + k_{12}\right]q_2^*(t) + k_{21}q_1^*(t) \qquad q_2^*(0) = 0 \tag{A8}$$

$$dx(t)/dt = -k_b x(t) + k_a[i(t) - i_b] \qquad x(0) = 0 \tag{A9}$$

$$g^*(t) = q_1^*(t)/V_1 \tag{A10}$$

where q^*_1 and q^*_2 are masses of the tracer glucose in the two compartments, V_1 is the volume of the accessible compartment, k_p is the proportional term of glucose disposal, k_{21}, k_{12}, and k_{02} are fractional rate parameters, k_a and k_b have similar meaning as p^*_3 and p^*_2 of the one-compartment stable-label minimal model, F_{01} is the constant component of glucose uptake (fixed at 1mg/kg/min [35]), and D* is the administered dose of the tracer glucose. The proportional term of glucose disposal k_p is constrained to produce insulin-independent utilisation three times higher than the insulin dependent utilisation at the basal glucose concentration (g_b) and the basal insulin concentration (i_b)

$$k_p = 3k_{21}k_{02}/(k_{02} + k_{12}) - F_{01}/(V_1 g_b) \tag{A11}$$

guaranteeing theoretical identifiability of the model [16].

Basal clearance rate PCR, fasting hepatic glucose output HGO_b, and insulin sensitivity index S^*_{Ib} ($S^*_{Ib} = \partial PCR/\partial i \,|i=i_b$) are calculated as

$$PCR = F_{01} / g_b + V_1 k_p + V_1 k_{21} k_{02} / (k_{02} + k_{12}) \quad \text{(A12)}$$

$$HGO_b = g_b PCR \quad \text{(A13)}$$

$$S^*_{Ib} = V_1 k_{21} k_{12} k_a / (k_b (k_{02} + k_{12})^2) \quad \text{(A14)}$$

4. Hepatic Glucose Output

Hepatic glucose output can be estimated using the labelled two compartment model with reconstruction of the input function [17]. The plasma glucose component due to hepatic glucose output ($g_e(t)$, endogenous glucose component) [36,37] is calculated prior to calculating HGO. The total glucose concentration g(t) contains three components, the endogenous glucose component $g_e(t)$, the unlabelled exogenous component $g_x(t)$, and the labelled exogenous component g*(t),

$$g(t) = g_e(t) + g_x(t) + g^*(t). \quad \text{(A15)}$$

The last two components are due to the exogenous glucose injection. They are fixed at a ratio E, E= $g^*(t)/g_x(t)$, which is given by the enrichment of the glucose bolus administered at the start of IVGTT. Solving Eq. (A15) for $g_e(t)$ we obtain that

$$g_e(t) = g(t) - g^*(t)\frac{1+E}{E}. \quad \text{(A16)}$$

The calculation of the endogenous glucose component is model-independent and only an assumption about the isotopic indistinguishability is made [38].

The relation between $g_e(t)$ and hepatic glucose output HGO(t) can be described by the integral equation

$$g_e(t) = \int_{-\infty}^{t} h(t,\tau) HGO(\tau) d\tau \quad \text{(A17)}$$

where $h(t,\tau)$ is the (time-variant) unit impulse response of the unlabelled glucose system.

The impulse response $h(t,\tau)$ is defined as a sum of two exponentials (a function of τ) for t between two sampling points, i.e. a piecewise linearisation at the sampling points of the two compartment model was adopted,

$$h(t,\tau) = h_i(\tau) = A_{1,i} e^{-\lambda_{i,1}\tau} + A_{2,i} e^{-\lambda_{2,i}\tau} \qquad \text{for} \quad t_i \le t < t_{i+1}, \quad \text{(A18)}$$

where constants $A_{i,1}$, $A_{i,2}$, $\lambda_{i,1}$, and $\lambda_{i,1}$ are determined from parameters of the two compartment model, plasma glucose concentration, and predicted remote insulin (Eq. A9). Equations A7 and A8 can be solved analytically for plasma glucose $g(t_i)$ and remote insulin $x(t_i)$ and the unit impulse response of tracer glucose system $g^*(t)$ (Eq A10) expressed as a sum of two exponentials. The impulse response of the tracee glucose system is identical to the impulse response of the tracer glucose system assuming tracer indistinguishability.

The integral Eq. (A17) can be solved using a regularisation method with the regularisation component consisting of the norm of second differences [16,39] to provide (piecewise constant) HGO(t). The regularisation coefficient defines the amount of smoothing adopted by the regularisation method.

PART III: COUNTRY REPORTS