

# How to measure insulin sensitivity

Ele Ferrannini<sup>a</sup> and Andrea Mari<sup>b</sup>

Insulin resistance is common in the general population and tends to cluster with glucose intolerance, dyslipidaemia and high blood pressure. The importance of the insulin-resistant phenotype for the assessment of cardiovascular risk and response to intervention is increasingly being recognized. Therefore, there is a need for an accurate and reproducible method for measuring insulin resistance *in vivo*. The euglycaemic insulin clamp is currently the best available standard technique. It provides steady-state measures of insulin action and is easily combined with a number of other investigative methods (tracer dilution, limb catheterization, indirect calorimetry, positron emission tomography and nuclear magnetic resonance scans). Whereas homeostatic model assessment uses fasting plasma glucose and insulin concentrations to derive indices of insulin sensitivity and secretion from a mathematical model, other techniques are based on the exogenous infusion of glucose or insulin, or both, either under steady-state (the insulin suppression test) or under dynamic conditions (insulin tolerance test, intravenous glucose-tolerance test with minimal model analysis, and constant infusion of glucose with model assessment).

## Premises

Insulin is a phylogenetically ancient hormone that has a variety of effects on cells of many types. Its anabolic actions on glucose, lipid and protein metabolism are essential for life, for lack of insulin leads to extreme hyperglycaemia and hyperlipaemia, protein wasting and, ultimately, keto-acidosis and death. Although insulin is central for all of intermediary metabolism, its chief control is exerted over the glucose system. In fact, plasma glucose concentration, unlike those of lipids and amino acids, is a strongly homeostatic variable, whose excursions are confined to a very narrow range (3–8 mmol·l<sup>-1</sup>) under conditions of everyday life. Post-prandial surges and inter-prandial declines of insulin level are tightly coupled to glucose availability and prevent both hypoglycaemia and hyperglycaemia, both of which are intolerable to body tissues. The ability of pancreatic  $\beta$ -cells to react to glycaemic changes by promptly increasing or decreasing insulin release is key to glucose control.

For any amount of insulin secreted by the pancreas, the biological response of a given effector is dependent on its insulin sensitivity. Although there can be as many resistances as there are effectors, insulin resistance is custom-

This article recalls the principles of insulin action, with special reference to the concept of clearance and the equivalence of different approaches to estimating this function. Merits and disadvantages of the various techniques are then concisely reviewed, with emphasis on their relative feasibilities and reliabilities. Recent developments and future trends are mentioned. Criteria for choice and some reference data are given to aid the clinical investigator. *J Hypertens* 16:895–906 © 1998 Lippincott-Raven Publishers.

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<sup>a</sup>Metabolism Unit of the CNR Institute of Clinical Physiology and Department of Internal Medicine, University of Pisa and <sup>b</sup>CNR Institute of Systems Science and Biomedical Engineering, Padova, Italy

Correspondence and requests for reprints to Dr E. Ferrannini, CNR Institute of Clinical Physiology, Via Savi, 8, 56100 Pisa, Italy.

Tel: +39 50 500087; fax: +39 50 663236; e-mail: ferranni@nsifc.ific.pi.cnr.it

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arily referred to glucose metabolism. Insofar as the various insulin effectors are partially independent of one another, resistance of the glucose pathway usually reinforces the insulin signal to other pathways. In fact, when glucose metabolism is impeded, minute increases in plasma glucose concentrations will feed back to the  $\beta$ -cells and stimulate insulin secretion. The resulting hyperinsulinaemia, which is compensatory in origin, will act unopposed on unaffected pathways (e.g. protein turnover). The clinical implication of this phenomenon is that any abnormality in insulin-resistant states can be due to the insulin resistance itself or to the chronic effects of compensatory hyperinsulinaemia [1].

For most hormones, regulation is provided mainly by changes in secretory rates; except under conditions of organ (liver or kidney) failure, degradation is constant at a value typical for each hormone. Regulation through sensitivity of target tissues to hormone action is unusual, however. Thus, resistance to thyroid hormones or sex steroids is known but rare. Insulin stands out as an exception: insulin resistance is a widespread phenomenon both in physiology and in pathophysiology. For example, puberty and pregnancy offer paradigms of physiological

impairment of insulin's effect on glucose disposal; obesity and diabetes, on the other hand, are diseases that are characteristically associated with insulin resistance.

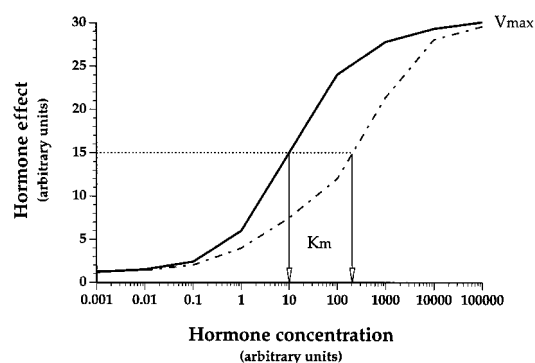
Much is known about the interaction of insulin with its specific cell membrane receptors, their phosphorylation after the binding step and the triggering of intracellular signalling pathways eventually acting upon the various effectors [2]. Insulin action *in vitro* is defined in terms of the dose–response function linking insulin concentrations in the medium with the level of activation of an effector system. As schematized in Figure 1 (and by analogy to enzyme kinetics), any such function has an approximately sigmoidal shape, indicating that it obeys saturation kinetics. According to the Michaelis–Menten approximation, the function can be summarized in terms of two parameters: the maximal response ( $V_{\max}$ ) and the sensitivity ( $K_m$ ), or response at half-maximal hormone concentration. Strictly speaking, insulin sensitivity is the ratio  $V_{\max}/K_m$ . When two curves have the same  $V_{\max}$ , their  $K_m$  measure the respective sensitivities exactly (for example, the two curves in Figure 1 have 20-fold different  $K_m$ ). Within the physiological range of plasma insulin concentrations (i.e. 30–1000 pmol · l<sup>-1</sup>), rate of glucose disposal is roughly proportional to plasma insulin level; therefore, when the maximal response is not determined, the slope of the response function within this range can be used as an index of insulin sensitivity.

At the whole-body level, any insulin effect estimated from measurements based on peripheral blood sampling is a compound of various tissues responses. In addition, it is hardly practical to obtain full dose–response curves in *in vivo* studies. Because of these limitations, several techniques to measure insulin sensitivity *in vivo* have been devised. Their widespread application has generated an amount of information on the significance of insulin resistance that is unprecedented in comparison with that for any other hormone. This article is an overview of the rationales, methodologies and relative merits of some of the more widely used techniques for the measurement of insulin sensitivity *in vivo*; detailed descriptions of the experimental protocols can be found in several previous treatises [3–5].

### The glucose clamp technique

There is general agreement that the glucose clamp technique, particularly in its euglycaemic version, is the best available standard for the measurement of insulin action. The glucose clamp methodology, initially devised by Andres *et al.* [6] by analogy with the voltage clamp method used in the neurosciences, has been developed and widely studied by DeFronzo *et al.* [7]. With the euglycaemic (or insulin) clamp technique, exogenous insulin is administered as a prime followed by a constant infusion at a rate designed to maintain a pre-set hyperinsulinaemic plateau; simultaneously, the plasma glucose concentration

Fig. 1



Ideal dose–response curve. Note the sigmoidal shape of the function (saturation kinetics).

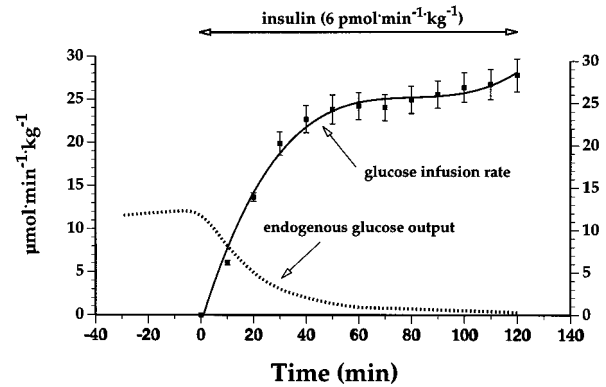
is clamped at the normal fasting ( $\sim 5$  mmol · l<sup>-1</sup>, euglycaemic) or any pre-existing (isoglycaemic) level by means of an exogenous infusion of glucose. By doing this, insulin action is measured *in vivo* under comparable conditions of stimulus (the plasma insulin concentration) and substrate (the plasma glucose concentration). When a steady state is attained, the exogenous glucose infusion rate equals the amount of glucose disposed of by all the tissues in the body and thus provides a quantitation of overall insulin sensitivity.

Technically, there are three major requirements for successfully clamping plasma glucose level. First, two intravenous lines must be kept patent throughout the study: one for the infusion of insulin and glucose, the other for frequent blood sampling. The latter, if arterial catheterization is not feasible, must drain arterialized blood; this is commonly accomplished by retrograde cannulation of a wrist or hand vein while heating the hand at 60–70°C. The reason for this is that, because tissue glucose uptake is stimulated by insulin, the arterio-venous difference in plasma glucose level increases considerably (typically, from 0.1–0.2 to 1.0–2.0 mmol · l<sup>-1</sup>). Thus, if one used venous glucose readings to adjust the exogenous glucose infusion rate, the corresponding arterial glucose levels would rise as insulinization proceeds. Exogenous glucose would be overinfused, the clamp would be variably hyperglycaemic rather than euglycaemic and insulin sensitivity would consequently be overestimated by an unpredictable amount. Moreover, the ensuing hyperglycaemia might stimulate endogenous insulin secretion, thereby falling short of clamping insulin levels. Second, well-calibrated pumps must be used for the infusion of glucose and insulin in order to achieve the desired hyperinsulinaemic plateau and to calculate glucose infusion rates accurately. The glucose pump, in particular, must have sufficiently fine gears for one to adjust the infusion rate by small fractions (0.05–0.1 ml · min<sup>-1</sup>). Third, concentrations of glucose in the arterialized blood samples must be

measured on line (i.e. within 30–45 s) in order to make the necessary adjustments to the exogenous glucose infusion rate. The glucose analyser apparatus must be frequently calibrated, both before and during the clamp study, against a well-preserved standard. Although computerized algorithms to perform the clamp are available [7], the glucose infusion rate can be adjusted empirically, a dozen or so experiments usually being sufficient to train an operator. In most people's experience, when the above requirements are satisfied, the clamp is a relatively easy procedure, which can be performed at the bed side. Producing a paper plot of the glucose concentrations and infusion rates while the clamp is being performed is a useful means of informing patients and winning their compliance.

The time course of glucose infusion rates during euglycaemic clamping of non-diabetic subjects exhibits a quick rise within about 40 min of starting the insulin infusion and then a gentle upwards trend (Fig. 2). This pattern is sensitive to the insulin dose; thus, a higher insulin clamp will produce a steeper initial ascent than will a lower insulin infusion rate. Even though in strict terms the glucose infusion rate never reaches a steady state, its average value during the final 60 min or, better, 40 min of a 2 h study is a satisfactory insulin-sensitivity index for ordinary purposes. It must be noted that the exogenous glucose infusion rate equals whole-body glucose disposal only when endogenous glucose output is nil; otherwise, total glucose disposal is the sum of endogenous and exogenous glucose entry. After insulin administration, endogenous glucose release is effectively suppressed (Fig. 2), so that, after 30–50 min, essentially all the glucose metabolized is of exogenous origin. Inhibition of endogenous glucose output is, however, insulin-dependent.

Fig. 2



Time courses of exogenous glucose infusion and endogenous glucose output for 30 non-diabetic subjects (lean and obese) during a standard euglycaemic insulin clamp.

Therefore, with low insulin infusion rates ( $< 2.5 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) or in insulin-resistant states, endogenous glucose release should be separately estimated with the use of tracer glucose and the indicator dilution technique.

For purposes of comparison, clamp data must be standardized. First, the insulin infusion should be administered per unit of body surface area to avoid over-insulinization of obese individuals (those with body mass indices  $> 30 \text{ kg} \cdot \text{m}^{-2}$ ). Thus,  $6 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  ( $1 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) insulin (i.e. the most common clamp dose), should rather be calculated as  $0.24 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$  ( $40 \text{ mU} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ ) for general application. Next, insulin sensitivity can be indexed in a number of different ways, all of which can be found in the literature. Total glucose uptake can be normalized per kg body weight or fat-free

Table 1 Insulin sensitivity measured by the clamp technique

	Men (n = 389)		Women (n = 219)	
Fasting plasma glucose ( $\text{mmol} \cdot \text{l}^{-1}$ )	5.0 [10]	(4.0–6.0)	4.9 [9]	(4.1–5.8)
Steady-state plasma glucose ( $\text{mmol} \cdot \text{l}^{-1}$ )	5.0 [8]	(4.2–5.8)	5.0 [10]	(4.0–6.0)
Fasting plasma insulin ( $\text{pmol} \cdot \text{l}^{-1}$ )	52 [43]	(7–95)	53 [45]	(5–101)
Steady-state plasma insulin ( $\text{pmol} \cdot \text{l}^{-1}$ )	457 [25]	(231–683)	448 [23]	(244–652)
$M$ ( $\text{mmol} \cdot \text{min}^{-1}$ )	2.8 [32]	(1.0–4.6)	2.3 [34]	(0.7–3.8)
$M_{\text{bw}}$ ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )	39 [30]	(15–63)	37 [32]	(13–61)
$M_{\text{fwm}}$ ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )	53 [30]	(20–84)	53 [31]	(19–87)
$M_{\text{ree}}$ ( $\text{mmol} \cdot \text{kJ}^{-1}$ )	0.60 [31]	(0.23–0.97)	0.54 [24]	(0.28–0.80)
$M/I$ ( $\text{mmol} \cdot \text{min}^{-1} \cdot \text{nmol}^{-1}$ )	6.5 [42]	(1.1–11.9)	5.2 [38]	(1.2–9.1)
$M_{\text{bw}}/I$ ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \cdot \text{nmol}^{-1}$ )	93 [42]	(15–171)	86 [37]	(22–150)
$M_{\text{fwm}}/I$ ( $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \cdot \text{nmol}^{-1}$ )	122 [41]	(22–222)	121 [37]	(31–211)
$M_{\text{ree}}/I$ ( $\mu\text{mol} \cdot \text{kJ}^{-1} \cdot \text{nmol}^{-1}$ )	1.4 [44]	(0.2–2.6)	1.4 [36]	(0.4–2.3)
$\text{MCR}$ ( $\text{l} \cdot \text{min}^{-1}$ )	0.57 [33]	(0.20–0.95)	0.46 [35]	(0.14–0.79)
$\text{MCR}_{\text{bw}}$ ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )	8.0 [31]	(3.0–13.1)	7.5 [34]	(2.5–12.5)
$\text{MCR}_{\text{fwm}}$ ( $\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ )	10.6 [32]	(3.9–17.3)	10.7 [33]	(3.7–17.7)
$\text{MCR}_{\text{ree}}$ ( $\text{ml} \cdot \text{kJ}^{-1}$ )	119 [32]	(41–197)	107 [24]	(57–157)

Values are expressed as means [percentage coefficients of variation] ( $\pm 2\text{SD}$ ). Data were obtained for non-diabetic, normotensive Caucasian subjects with body mass indices  $\leq 25 \text{ kg} \cdot \text{m}^{-2}$  receiving a  $6 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  euglycaemic insulin clamp for 2 h (calculated from data presented in reference [25]).  $M$ ,  $M_{\text{bw}}$ ,  $M_{\text{fwm}}$  and  $M_{\text{ree}}$ , total body glucose metabolism in absolute units and values normalized per kg body weight, fat-free mass and resting energy expenditure, respectively;  $M/I$ ,  $M_{\text{bw}}/I$ ,  $M_{\text{fwm}}/I$ , and  $M_{\text{ree}}/I$ , the same indices divided by the steady-state plasma insulin concentration ( $I$ );  $\text{MCR}$ ,  $\text{MCR}_{\text{bw}}$ ,  $\text{MCR}_{\text{fwm}}$  and  $\text{MCR}_{\text{ree}}$ , metabolic clearance rates, obtained by dividing the respective  $M$  values by the steady-state plasma glucose concentrations.

mass. Given that glucose uptake can occur only in lean tissues (cell mass), steady-state total body glucose metabolism (or the  $M$  value) is best normalized by the fat-free mass ( $M_{\text{ffm}}$ ). Because the resting energy expenditure rate ( $\text{kJ} \cdot \text{min}^{-1}$ ) is a quantitative measure of the total metabolically active cell mass [9],  $M$  can be normalized by resting rate of expenditure of energy ( $M_{\text{rec}}$ ) whenever the latter can be determined in the same session of the clamp study. Using the uncorrected  $M$  value leads, for example, to overestimation of insulin sensitivity of men compared with that of women (Table 1), whereas normalizing  $M$  by body weight ( $M_{\text{bw}}$ ) exaggerates the insulin resistance of the obese [10]. However it is expressed, the  $M$  value can be further corrected by the steady-state plasma insulin concentration ( $I$ ) to account for minor differences in clamp insulin levels. Because the increase in glucose disposal rate is proportional to plasma insulin level within a concentration range including the physiological values (e.g. Fig. 1), all expressions for  $M$  can be divided through the insulin plateau (thereby yielding  $M/I$ ,  $M_{\text{bw}}/I$ ,  $M_{\text{ffm}}/I$  and  $M_{\text{rec}}/I$ ). Finally, any glucose uptake can be divided by the steady-state plasma glucose concentration, thereby calculating the metabolic clearance rate of plasma glucose (MCR). Glucose clearance is a primary concept, formally defined as follows:

$$\text{MCR} = E \times \text{flow rate} = kV \quad (1)$$

where  $k$  is the average disappearance rate constant,  $V$  is the glucose distribution volume, and  $E$  (extraction ratio) is given by

$$E = (C_A - C_V)/C_A \quad (2)$$

where  $C_A$  and  $C_V$  are the arterial and venous glucose concentrations, respectively. Then we have

$$\frac{\text{glucose}}{\text{disposal rate}} = \text{MCR} \times C_A = (C_A - C_V) \times \text{flow rate} \quad (3)$$

In words, for any given set of plasma glucose and insulin concentrations, glucose clearance is the fraction of incoming blood flow that is completely cleared of glucose by virtue of the specific ability of the tissue to extract the substrate from the arterial side, thereby creating an arterio-venous gradient ( $C_A - C_V$ ) [equations (1) and (2)]. Equation (3) shows that glucose disposal is equivalently the product of the clearance rate and the arterial glucose level or the product of blood flow rate and the arterio-venous gradient (Fick's principle). At the whole-body level, the flow rate is cardiac output and the arterio-venous gradient is that between arterial and central venous blood (e.g. that in the right atrium), a mix of all venous returns from body tissues. It is therefore clear that the clamp method is a non-invasive short-cut for the Fick principle, which itself would require central venous catheterization and measurement of cardiac output. With the clamp, what

is actually measured is the glucose disposal rate, while glucose clearance can be calculated according to equation (3). When using a glucose tracer, on the other hand, glucose clearance is calculated directly from the tracer's disappearance function. Glucose clearance is dependent not only on plasma insulin level but also on plasma glucose levels in a complex fashion [8]. Therefore, comparison of insulin sensitivity between subjects clamped at very different plasma glucose concentrations should be done cautiously, particularly for insulin-sensitive individuals [9].

For reference purposes, in Table 1 we list the  $M$  values obtained for a large number of lean, non-diabetic subjects undergoing a standard ( $6 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ ) euglycaemic insulin clamp. It can be appreciated that insulin sensitivity spans a wide range even among healthy individuals, that all indices of insulin sensitivity have essentially similar ( $\sim 30\%$ ) coefficients of variation (but normalization by insulin level augments inter-individual dispersion) and that sex differences in insulin sensitivity disappear when indices are normalized by the fat-free mass.

The insulin clamp has distinct merits. First, any combination of plasma glucose and insulin concentrations can be realized, thereby allowing the experimenter to investigate various domains of the glucose-insulin system. For example, the clamp has been employed to construct full dose-response curves [10] and to investigate hypoglycaemic counter-regulation [11]. Second, the clamp can be combined with a number of other procedures to enhance the information content of the study (Table 2). Thus, if tracer glucose is simultaneously used, the inhibitory effect of insulin on endogenous glucose release can be quantitated. By infusing tracers of non-esterified fatty acids (or glycerol) and amino acids, one can assess the influence of insulin *per se* on lipolysis and protein degradation, respectively. When a deep vein of the forearm is also cannulated and forearm blood flow is separately measured, the effect of insulin on glucose extraction [equation (2)] and uptake [equation (1)] by the forearm tissues can be determined concurrently with total body glucose disposal. Similarly, if a post-hepatic vein is catheterized and splanchnic blood flow is measured, the effect of insulin on liver glucose metabolism can be studied. When indirect calorimetry is performed during a clamp, the effect of insulin on the pattern of substrate oxidation and the stimulatory action of the hormone on thermogenesis can be measured. In combination with the clamp, positron emission tomography with [ $^{18}\text{F}$ ]-deoxy-glucose will quantitate regional (e.g. myocardial) insulin-stimulated glucose uptake while brilliantly imaging body structures. Nuclear magnetic resonance spectroscopy with [ $^{13}\text{C}$ ]-glucose has been used to measure insulin-stimulated glycogen accumulation. Finally, more recently discovered actions of insulin, such as the ability of the hormone to induce peripheral vasodilatation, to activate the sympathetic nervous

**Table 2** Techniques used in combination with the glucose clamp

Technique	Measure	Reference
Tracer glucose	Endogenous glucose output	[10]
Multiple tracers	Muscle glucose transport	[12]
Forearm (or femoral) vein catheterization	Skeletal muscle glucose uptake	[13]
Post-hepatic vein catheterization	Splanchnic glucose exchange	[10]
Positron emission tomography	Regional glucose uptake	[14]
Nuclear magnetic resonance spectroscopy	Glycogen synthesis	[15]
Indirect calorimetry	Substrate oxidation	[16]
Tracer non-esterified fatty acids or glycerol	Lipolysis	[17]
Tracer amino acids	Protein turnover	[18]
Dye-dilution or thermo-dilution, plethysmography	Insulin-induced vasodilatation	[19–21]
Microneurography, spectral analysis	Sympathetic activation	[22,23]

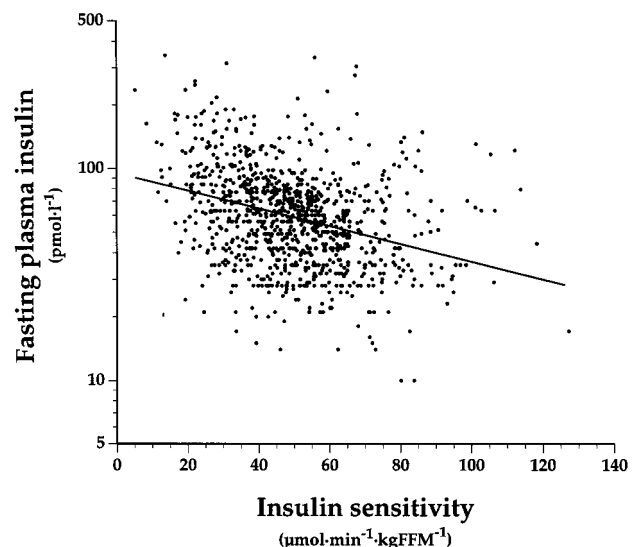
system and to affect the baroreflex control of heart rate, are being investigated by taking advantage of the standardized, stationary conditions created by the euglycaemic clamp.

In its hyperglycaemic version, the glucose clamp is a reliable test of insulin secretion. When  $\beta$ -cells are exposed to a square wave of hyperglycaemia, they respond with a typical biphasic pattern of insulin release, in which a prompt initial surge (lasting approximately 10 min) is followed by a progressively increasing insulin secretory phase. Early and late insulin responses have been used to characterize pre-diabetic states and to study the pancreatic effects of hormones and drugs. Finer aspects of insulin secretory dynamics, such as glucose priming and potentiation, can be studied by creating hyperglycaemic ramps [24]. It should be mentioned that an  $M/I$  index can be calculated from a hyperglycaemic clamp by dividing the glucose infusion rate by the average insulin concentration during the test [5,7]. Such  $M/I$  values obviously reflect insulin sensitivity, but cannot be used to compare insulin action between subjects or groups with very different endogenous plasma insulin responses.

The disadvantages of the clamp method are its requirements (two intravenous lines, calibrated pumps and on-line plasma glucose level determination) and the need for trained personnel. At the end of a clamp study, particularly with a high insulin dose, the subject's plasma glucose level must be monitored for some time because the hypoglycaemic effect extends beyond the return of plasma insulin level to its baseline value. In its standard version, the clamp experiment measures the response to only one level of hyperinsulinaemia (i.e. one point only on the dose–response curve; Fig. 1). Finally, even though the interruption of the normal feedback connection between insulin and glucose levels is the very rationale of the clamp method, it has been argued that the conditions created with the clamp are non-physiological. This is obviously the case, although the same objection can be raised against many investigative techniques. Nevertheless, the euglycaemic clamp has generated a large amount of valuable, reproducible information and has been employed successfully at the population level [25].

### Plasma insulin concentration

The circulating level of insulin has been used widely as a surrogate for insulin sensitivity. In fact, a predicted output of the insulin–glucose feedback is that the worse the insulin resistance the higher the plasma insulin concentration. Both fasting and post-glucose plasma insulin levels have been used; the product of fasting plasma insulin and fasting plasma glucose levels in addition to their ratio have likewise been proposed as proxies for insulin action. When these indices are tested against insulin sensitivity measured by using the euglycaemic insulin clamp, all of them are found to be correlated to insulin sensitivity but can only explain a small fraction (5–25%) of the variability of insulin action (e.g. Fig. 3) no matter what transformation or manipulation of the variables is used. This result clearly arises from the fact that insulin levels, in addition to tissue insulin sensitivity, also depend on secretion, distribution and degradation of insulin. Glucose levels, on the other hand, are controlled by more factors than just

**Fig. 3**

Reciprocal relationship between insulin sensitivity, derived from a standard euglycaemic insulin clamp and the corresponding fasting plasma insulin concentrations (logarithmically transformed) in 1140 non-diabetic subjects (from the EGIR study [25]). FFM, fat-free mass.  $r^2 = 0.14$ ,  $P < 0.0001$ .

insulin (e.g. portal glucagon levels). At the population level, fasting plasma insulin levels behave better than do post-glucose insulin concentrations as correlates and predictors of the components of the insulin-resistance syndrome (i.e. non-insulin-dependent diabetes mellitus, essential hypertension and dyslipidaemia) [26].

### Insulin-tolerance test

The very first method developed to evaluate insulin sensitivity *in vivo* [27] was the insulin-tolerance test, which is based on the measurement of the rate of decay of plasma glucose level after a bolus injection of regular insulin ( $0.1 \text{ U} \cdot \text{kg}^{-1}$  body weight). On plotting plasma glucose concentrations measured every 5 min from 10 to 40 min after the intravenous insulin injection on a semi-logarithmic scale, a reasonably linear decline is observed in most cases. The slope of this line ( $k_{\text{ITT}}$ ) can be calculated simply (0.693 divided by the plasma glucose half-time) and used to rank insulin sensitivity (i.e. the greater the slope the better the insulin sensitivity). The rationale of this method assumes that the glucose system is a single compartment, from which insulin accelerates the net disappearance of the substrate both by promoting its uptake into target tissues and by shutting off endogenous production. Indeed, by also assuming a glucose distribution volume (usually,  $200\text{--}250 \text{ ml} \cdot \text{kg}^{-1}$ ), a clearance rate can be calculated according to equation (1). Not surprisingly, either the  $k_{\text{ITT}}$  index or the glucose clearance thereof is found to be correlated to clamp-derived estimates of insulin sensitivity [28]. It should be noted, however, that the  $k_{\text{ITT}}$  value is dependent on the time interval over which it is calculated, precisely because glucose disappearance is not a mono-exponential but rather a multi-exponential process [29]. Also, the plasma insulin levels achieved with the bolus used in this test are pharmacological (ranging from  $150 \text{ nmol} \cdot \text{l}^{-1}$  soon after injection to  $15 \text{ nmol} \cdot \text{l}^{-1}$  towards the end).

The principal drawback of this test is hypoglycaemia, which, in addition to being unpleasant, can cause neurological and cardiovascular side effects, particularly in diabetic and in elderly subjects with diffuse atherosclerotic disease. Furthermore, hypoglycaemic counter-regulation will antagonize insulin effect, thereby contaminating the insulin sensitivity estimate.

### Insulin-suppression test

The insulin-suppression test is a reverse clamp, by which the exogenous glucose infusion rate during infusion of insulin is kept constant while the plasma glucose concentration is allowed to vary: at steady state, the higher the level of hyperglycaemia attained the worse the insulin sensitivity. Because the experimentally induced hyperglycaemia will stimulate endogenous insulin release, two different approaches to suppress  $\beta$ -cell response have been taken.

In the quadruple-infusion technique devised by Shen *et al* [30], a constant infusion of  $6 \mu\text{g} \cdot \text{min}^{-1}$  adrenaline was used to inhibit insulin secretion. Because adrenaline is a potent stimulus for endogenous glucose production, this effect of the hormone was blocked by administration of a bolus of 5 mg propranolol and then a constant infusion of  $80 \mu\text{g} \cdot \text{min}^{-1}$  propranolol. When  $0.48 \text{ nmol} \cdot \text{min}^{-1}$  insulin and  $33 \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  glucose were added to the infusion cocktail, plasma insulin concentrations would plateau within 3 h at about  $600 \text{ pmol} \cdot \text{l}^{-1}$  (termed steady-state plasma insulin level); plasma glucose level would gradually rise, to stabilize eventually [steady-state plasma glucose level (SSPG)] at a level dependent on individual insulin sensitivity ( $5\text{--}8 \text{ mmol} \cdot \text{l}^{-1}$  for non-diabetic subjects). The main problem of this method is that the biological effects of adrenaline on glucose metabolism (both tissue uptake and liver production) may be incompletely (and unpredictably) blocked by propranolol. More importantly, adrenaline can cause significant disturbances of cardiac rhythm even in the presence of propranolol; insofar as this effect cannot be anticipated on the basis of the resting or exercise electrocardiogram, the use of the quadruple-infusion technique in patients is somewhat hazardous.

A better (and safer) way to suppress endogenous insulin response is to infuse  $250 \mu\text{g} \cdot \text{h}^{-1}$  somatostatin, which reduces the circulating levels of C-peptide and glucagon by about 50% within 1 h. This modification of the insulin-suppression test, first introduced by Harano *et al* [31], has virtually replaced the quadruple-infusion technique. Because endogenous glucose release is effectively inhibited by the combined action of hyperinsulinaemia, hyperglycaemia and hypoglucagonaemia, the SSPG obtained during this test is a faithful reflection of peripheral insulin sensitivity.

Problems with the insulin suppression test are that plasma glucose concentrations may not stabilize satisfactorily over the infusion period and, in very sensitive subjects, may occasionally drop below baseline. Conversely, SSPG in diabetic, insulin-resistant individuals may exceed the renal threshold, thereby leading to glycosuria. Moreover, somatostatin inhibits a multitude of other hormones, gastro-intestinal and pituitary; authors of at least one study [32], found an independent effect of somatostatin on glucose clearance. Finally, one can calculate a glucose clearance by dividing the exogenous glucose infusion rate by SSPG (having first subtracted glycosuria, if it occurs); such a value, however, cannot be equated with the corresponding clamp value because of the different experimental conditions (i.e. hyperglycaemia versus euglycaemia) [8,9].

### The minimal model

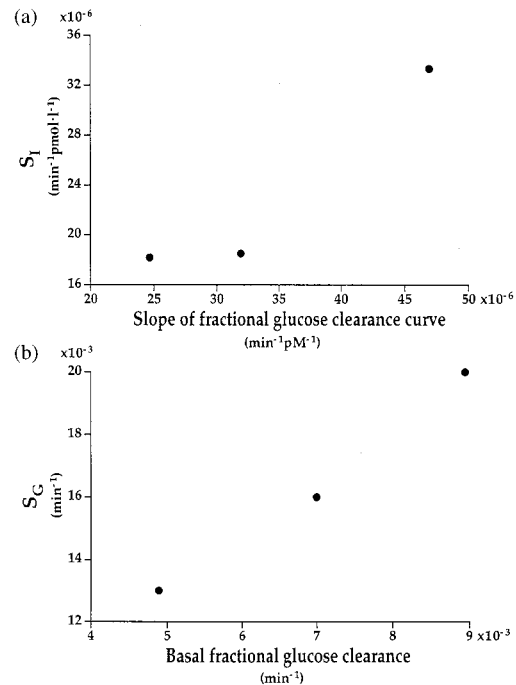
The minimal model is a development of the intravenous glucose-tolerance test (IVGTT), a time-honoured

approach for the assessment of insulin sensitivity. At its earliest stage, before the insulin assay had become available, insulin sensitivity was judged from the slope of the decay curve of plasma glucose concentration measured after an intravenous glucose injection (the so-called glucose disappearance constant or Conard's  $k$ ). Because effects of insulin were not taken into account, this test could not determine whether a difference in glucose disappearance rate was due to a difference in insulin sensitivity or in glucose-induced insulin response. The minimal model proposed by Bergman *et al.* [33] accounts for both insulin and glucose concentrations during the IVGTT by using a simplified mathematical representation of the glucose–insulin relationships. The minimal model describes the glucose disappearance curve with two differential equations. One equation represents glucose kinetics, thus assuming a single-compartment model for glucose distribution. The other equation describes the insulin effect, which is assumed to take place in a compartment remote from plasma (the effect compartment). The insulin-sensitivity index of the minimal model represents the link between insulin levels in the effect compartment and glucose disappearance from the glucose compartment. The fractional disappearance rate ( $\text{min}^{-1}$ ) from the glucose compartment is assumed to be the linear function ( $S_G + S_I Z$ ), where  $Z$  is increment over the basal value of insulin concentration in the effect compartment,  $S_I$  ( $\text{min}^{-1} \cdot \text{pmol} \cdot \text{l}^{-1}$ ) is the insulin-sensitivity index and  $S_G$  ( $\text{min}^{-1}$ ) is the so-called glucose effectiveness. By using the measured insulin concentration as the input to the model, insulin sensitivity and glucose effectiveness are estimated by least-squares fitting of the IVGTT glucose concentration profile (which is sampled at a high frequency for 240 min).

An inherent limitation of the minimal model analysis of the IVGTT is that it requires a discrete insulin response (i.e. insulin concentrations that rise detectably and consistently above basal). As a result, the minimal model analysis of the IVGTT fails for insulin-deficient subjects, for no effect of insulin on glucose disappearance rate is seen. In this situation, the IVGTT protocol is modified to include an intravenous bolus of tolbutamide (to stimulate endogenous insulin secretion) or a brief exogenous insulin infusion, both administered 20 min after the injection of the glucose bolus. Several alternative protocols have been proposed [34–36].

Because of the empirical nature of the minimal model, the physiological interpretation of the indices  $S_I$  and  $S_G$  has not unequivocally been established.  $S_I$  has been considered to represent 'the ability of insulin to enhance total net glucose disappearance from the extracellular fluid, both by diminishing endogenous glucose production and by augmenting glucose utilization' [4].  $S_G$ , on the other hand, has been described as 'a measure of the effect of glucose to enhance its own disappearance at basal

Fig. 4



Characterization of the minimal model indices as descriptors of the relationship between insulin concentration and fractional glucose clearance obtained with a glucose clamp (the dose–response curve). (a) Comparison between insulin sensitivity ( $S_I$ ) and the slope of the dose–response curve.  $S_I$  underestimates the slope. (b) Comparison between glucose effectiveness ( $S_G$ ) and basal fractional glucose clearance.  $S_G$  overestimates the basal fractional glucose clearance. Redrawn from reference [37], data from reference [38].

insulin independent of any increase of insulin' [4]. Although opinions concerning the meaning of  $S_I$  and  $S_G$  differ, both theoretical and experimental results [37,38] indicate that the minimal model indices are biased estimates of two characteristics of the dose–response function depicted in Figure 1.  $S_I$  (expressed in  $\text{min}^{-1} \cdot \text{pmol} \cdot \text{l}^{-1}$ ) is an underestimate of the average slope of the curve at insulin concentrations below the non-linearity threshold (when the hormone's effect is expressed as fractional glucose clearance, i.e. glucose clearance/glucose distribution volume, in  $\text{min}^{-1}$ ; Fig. 4b).  $S_G$  ( $\text{min}^{-1}$ ) is an overestimate of the fractional glucose clearance at basal insulin level (Fig. 4a). Both biases arise from the model simplifications (i.e. from the very 'minimality' of the model).

$S_I$  has been shown to correlate with the analogous estimate obtained from the glucose clamp over a wide range of insulin sensitivity [38]. However, in severe insulin resistance the minimal model often yields negative values for  $S_I$  and the correlation to the clamp can worsen significantly [38]. This problem, which is imputable to the model's simplifications [37], occurs only when the dose of insulin injected is insufficient and can be prevented by an adequate insulin dose [36,39–41]. Thus,



when enough exogenous insulin is used,  $S_I$  becomes a generally valid index quantifying the slope of the insulin dose-response curve.

The usefulness of  $S_G$  as an estimate of basal fractional glucose clearance is less certain, insofar as no comparison between  $S_G$  and tracer-determined basal glucose clearance has been reported. Saad *et al.* [38] were able to detect the impaired basal glucose clearance of insulin-resistant subjects by using  $S_G$ . However, results of a simulation study [42] suggested that modelling errors can introduce substantial variability into the estimation of  $S_G$ . Thus, it is possible that  $S_G$  is correlated to basal glucose clearance only if this spans over a sufficiently wide range. Furthermore, some dependence on the specific experimental circumstances has been demonstrated to occur [43–45]. In any case, it should be understood that  $S_G$ , as an estimate of the basal fractional glucose clearance, is also an index of insulin sensitivity.  $S_G$  does not express a property that is independent of insulin action, as the term glucose effectiveness might suggest. The emphasis that has been given to glucose effectiveness as an important determinant of glucose tolerance [46,47] is well placed. However, it is a re-statement of the well-established concept that, in subjects who are severely insulin-resistant and in those lacking a pancreatic response, the overall ability to remove glucose from plasma is mainly determined by the residual (basal) glucose clearance. Other interpretations of  $S_G$  are inappropriate. Interpreting a decrease in  $S_G$  as a loss of non-insulin-mediated glucose uptake, or as an impairment of the mechanisms that regulate glucose utilization independently of insulin [41,48,49] fails to appreciate that  $S_G$  reflects the glucose clearance at basal insulin level and not an insulin-independent process.

The clear advantage of the minimal model analysis of the IVGTT is that it derives an index of insulin sensitivity and two indices of insulin secretion (early phase,  $\phi_1$ , and late phase,  $\phi_2$ ) from a single test. The insulin-modified version of the minimal model approach generates a robust index of insulin sensitivity ( $S_I$ ) and is widely applicable [50]. A further advantage is that on-line glucose measurement and clamping are not necessary. Several drawbacks remain. First, the experiment itself is not among the simplest: two venous lines are required, blood must be sampled frequently (22 times in the original protocol) for relatively long periods of time (4 h) and hypoglycaemia might occur late into the test, thereby introducing potentially confounding influences from counter-regulation. Second, giving an injection of exogenous insulin after the glucose bolus improves the estimation of  $S_I$  but modifies late-phase endogenous insulin secretion. Third, the model's oversimplification introduces biases into its estimates, which can vary depending on the test circumstances; comparison with a reference method might be necessary. Finally, as is the case with any modelling analysis, the

minimal model demands the investigator's confidence in a 'black box' of calculations and its output is not immediately accessible to the patient.

### Homeostatic model assessment and constant glucose infusion with model assessment

With the homeostatic model assessment (HOMA) and constant infusion of glucose with model assessment (CIGMA) approaches, insulin sensitivity is determined from the steady (or near-steady) glucose and insulin concentrations measured under basal conditions (HOMA) or after a standardized, 1 h intravenous glucose infusion (CIGMA) [51,52]. Insulin sensitivity is expressed as an index of relative insulin resistance,  $R$  (dimensionless or a percentage), which is calculated as a function of the measured glucose and insulin levels. The HOMA and CIGMA functions for calculating  $R$  are derived from a model of glucose homeostasis. This model accounts for glucose distribution, production and utilization. Glucose uptake and production are assumed to be dependent on glucose and insulin concentrations and on the insulin-resistance index  $R$ . This dependence is represented by two empirical functions that match some reference set of experimental data. The role of  $R$  in the glucose-production and glucose-uptake functions is such that  $R = 1$  corresponds to normal homeostasis, whereas  $R$  values greater than unity indicate the presence of insulin resistance, both hepatic and peripheral. By solving the model equations, the  $R$  value corresponding to a given pair of glucose and insulin concentration values can be calculated. In practice,  $R$  is determined from a contour plot in which glucose and insulin concentrations are reported on the abscissa and ordinate, and each contour line represents a constant  $R$  value obtained from the model. From a pair of glucose and insulin concentration values a unique contour line and thus an  $R$  value is identified. In HOMA, the contour lines are simple hyperbolae, so  $R$  is proportional to the product of glucose and insulin concentrations. In CIGMA, the lines do not have a simple mathematical representation and  $R$  can be obtained only from the plot.

The measure of insulin resistance  $R$  is defined as "the ratio given by the actual level of insulin in a subject divided by the amount of insulin which would achieve the same effect in a non-diabetic 'standard' subject" [51]. This definition makes  $R$  hardly comparable with the clamp and minimal model insulin-sensitivity indices. The reciprocal of  $R$  is an index of insulin sensitivity, but it cannot be expected that  $1/R$  is linearly related to the clamp or minimal model insulin-sensitivity index, as was pointed out in the original articles [51,52]. There is no clear relationship between  $R$  and the dose-response curve of Figure 1, although HOMA refers to the basal point of the curve and CIGMA to conditions of mild hyperinsulinaemia. This makes it difficult to interpret the relationships between  $R$  and other variables, such as, for instance, the body mass index.



In HOMA, the measurement of just basal glucose and insulin concentrations could be sufficient to detect insulin resistance on a qualitative basis. However, in general it cannot be taken for granted that two subjects with the same value of the glucose–insulin concentration product (i.e. the same  $R$ ) have the same insulin sensitivity. Moreover, with HOMA the site of insulin resistance, hepatic versus peripheral, remains undetermined, whereas the standard clamp and the minimal model essentially assess peripheral tissue insulin resistance. CIGMA, being a sort of simplified hyperglycaemic clamp, is more informative. However, the interpretation of  $R$  remains unclear, particularly when the insulin response is insufficient to stimulate glucose uptake, as occurs in insulin-deficient subjects.

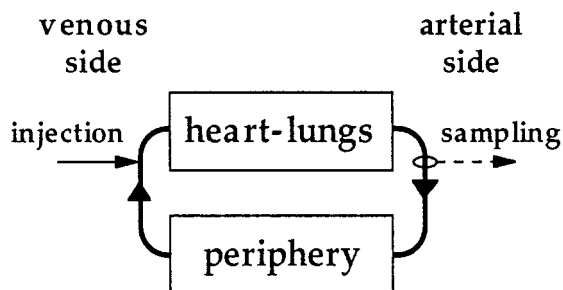
The experiments for HOMA and CIGMA are simple, which is an important advantage. However, the scantiness of primary information necessarily makes the assessment of insulin sensitivity markedly model-dependent.

### Future trends

The direct methods so far reviewed (clamp, insulin-tolerance test and insulin-suppression test) yield limited information on the insulin-sensitivity curve depicted in Figure 1. In fact, all these techniques provide a single index of insulin sensitivity, which reflects predominantly peripheral sensitivity or a mixture of hepatic and peripheral sensitivities at a given plasma insulin concentration. More recent approaches attempt to improve the information on the insulin-sensitivity function along the lines of the minimal model analysis. The strongest of these new approaches are based on the use of a glucose tracer in combination with modelling analysis of the IVGTT. Because the glucose tracer is co-injected with the cold glucose bolus, the complexity of the experimental protocol is comparable to that of the minimal model test, so

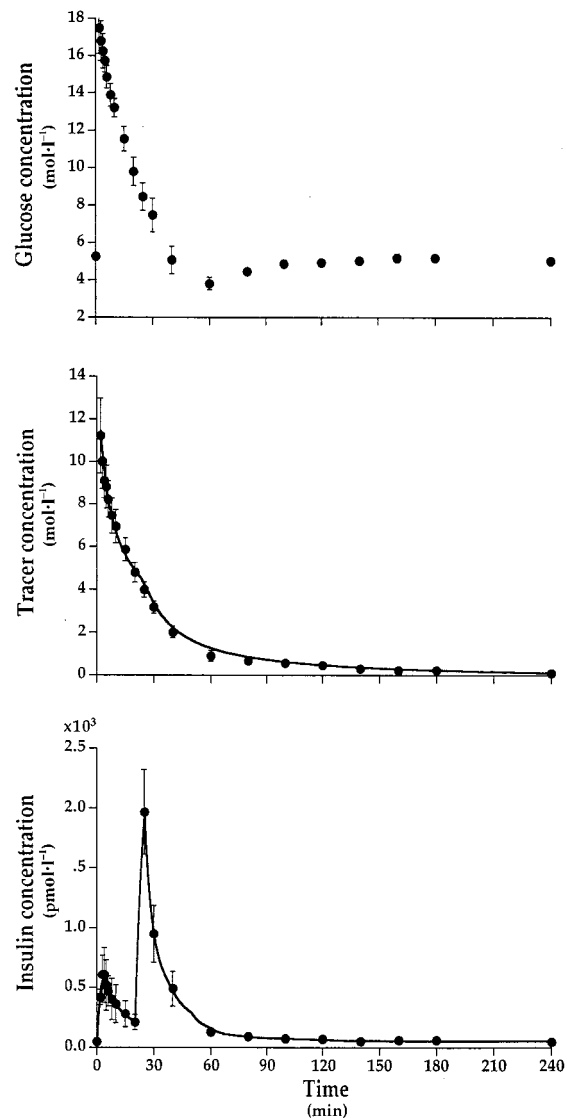
that use in the clinical setting is possible. Various modelling analyses of the tracer data have been proposed. One approach is based on compartmental analysis and either the minimal model [53,54] or a two-compartment model of glucose kinetics [55] has been used. With the minimal model, a somewhat more precise  $S_I$  can be obtained [53], but the problems with the interpretation of  $S_G$  and  $S_I$  are not overcome. With the two-compartment model, the calculated parameters of glucose kinetics (i.e. glucose clearance and glucose production) are directly comparable to those derived from a clamp experiment coupled with

Fig. 5



Circulatory model for the assessment of insulin sensitivity from the intravenous glucose-tolerance test. The heart chambers and the lungs are included in a heart–lungs block, the remaining tissues in the periphery block. The two blocks are regarded as single-inlet–single-outlet organs and mathematically described using techniques derived from the methods developed by Zierler. Blood flow for the organ blocks is the cardiac output.

Fig. 6



Average glucose concentration, tracer concentration and insulin concentration during an intravenous glucose-tolerance test (data from five normal subjects, re-drawn from reference [56]). Glucose ( $300 \text{ mg} \cdot \text{kg}^{-1}$ ) is injected at time 0. After 20 min, insulin is infused for 5 min at a constant rate (total dose:  $30 \text{ mU} \cdot \text{kg}^{-1}$ ). Insulin sensitivity is estimated from the tracer and insulin concentrations using the circulatory model. The solid lines represent the model fit.

tracer infusion [55]. The parameters reported for normal subjects agree with literature values, but a direct comparison with the clamp has not yet been made available.

Another approach is based on a circulatory model [56]. Circulatory models represent the glucose system rather more realistically than compartmental models do (Fig. 5). Glucose kinetics in the organ blocks encompassed by the circulatory loop are mathematically described using techniques derived from the so-called model-independent methods developed by Meier and Zierler [57] and Zierler [58]. Arbitrary assumptions such as those required by multi-compartmental analysis to specify a model configuration are not needed. For example, from the IVGTT data of Figure 6 the circulatory model yields an enriched set of parameters characterizing insulin sensitivity, namely, basal glucose clearance, peripheral insulin sensitivity and basal glucose production rate. The parameters reported for normal subjects and non-insulin-dependent diabetes mellitus patients agree with the respective literature values [56,59], although comparison with more established methods has yet to be made.

These methods, or modifications based on similar principles, are promising in that they make it possible to use a simple test to obtain a complete description of insulin sensitivity. The use of a tracer, however, limits their applicability somewhat.

### Concluding remarks

The choice of a method to measure insulin sensitivity *in vivo* is ample and can accommodate diverse needs. Each technique mentioned above has been validated against the best available standard method, the euglycaemic clamp; each has proven able to reproduce clamp-based information on the presence of insulin resistance in disease states (impaired glucose tolerance, overt diabetes and essential hypertension). Although validation is an inevitable stage for any alternative technique, the goodness of a method is not exclusively the goodness of its correlation with the clamp. The latter correlation critically depends on the size and selection of the sample used for the comparison; various other factors, both controllable and unknown, may affect the comparison. Thus, the absolute goodness of a technique is the validity of its assumptions about the system being studied and the rigour of the experimental set-up. The relative goodness of a technique is the combination of features that best serve the purpose of the study itself.

In our experience, if the goal is to measure peripheral tissue sensitivity to insulin action on glucose uptake, the euglycaemic clamp should be preferred whenever practically feasible. A standard ( $6 \text{ mU} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  insulin) version will shut off endogenous glucose release in the great majority of subjects, thus yielding a direct, clean and fully 'usable' estimate of peripheral insulin sensitivity.

Table 3 Reproducibility of insulin sensitivity measurements

Technique	Coefficient of variation (%)	Reference
Euglycaemic clamp	10	[7]
Minimal model <sup>a</sup>	20–28	[60,61]
Constant infusion of glucose with model assessment	20	[51]
Homeostatic model assessment	30	[52]

<sup>a</sup>Values for the tolbutamide-boosted test, with 30 and 12 blood samples, respectively. Coefficients of variation with the standard intravenous glucose-tolerance test are expected to be higher.

The clamp is also the technique of choice to be combined with other experimental methods in more specific studies (Table 2). According to reported evidence, the clamp has better reproducibility than do other methods (Table 3). When the clamp is not feasible, the next best choice is the somatostatin modification of the insulin-suppression test: it is easy and safe and it can be performed at the bed side with a minimum of training. In epidemiological databases, on the other hand, the fasting plasma insulin concentration will take the concept of insulin resistance as far as it can reach short of obtaining direct measurements; although it has been investigated less thoroughly, HOMA probably can do just as well. Finally, in physiological and pathophysiological investigations aimed at improving the understanding of insulin biology, tracers and modelling are inescapable. The former now employ stable isotopes, which are safe and increasingly cheap; the latter exploits a long-standing tradition of biomathematics, which has generated a formal description of the insulin-glucose system that is of unparalleled sophistication (e.g. the artificial pancreas). It might not be long before a clinically usable test to investigate the complete kinetics of insulin action *in vivo* simultaneously with the dynamics of insulin release in a rigorous and reliable fashion becomes available.

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