Insulin is an essential peptide hormone whose metabolic actions maintain whole body glucose homeostasis and promote efficient glucose utilization (3). Insulin stimulates increased glucose disposal in skeletal muscle and adipose tissue, whereas it inhibits gluconeogenesis in liver to help regulate glucose homeostasis. In addition to these classical insulin target tissues, there are many other important physiological targets of insulin, including the brain, pancreatic β-cells, heart, and vascular endothelium, that help to coordinate and couple metabolic and cardiovascular homeostasis under healthy conditions (3, 54, 72, 79). Insulin has concentration-dependent saturable actions to increase whole body glucose disposal. The maximal effect of insulin defines “insulin responsiveness,” whereas the insulin concentration required for a half-maximal response defines “insulin sensitivity” (Fig. 1A).

Insulin resistance is typically defined as decreased sensitivity or responsiveness to metabolic actions of insulin, such as insulin-mediated glucose disposal and inhibition of hepatic glucose production (HGP). The concept of insulin resistance was proposed as early as 1936 (52) to describe diabetic patients requiring high doses of insulin. Insulin resistance plays a major pathophysiological role in type 2 diabetes and is tightly associated with major public health problems, including obesity, hypertension, coronary artery disease, dyslipidemias, and a cluster of metabolic and cardiovascular abnormalities that define the metabolic syndrome (32, 76, 86). A global epidemic of obesity is driving the increased incidence and prevalence of type 2 diabetes and its cardiovascular complications (77). Therefore, it is of great importance to develop tools for quantifying insulin sensitivity/resistance in humans and animal models that may be used to appropriately investigate the epidemiology, pathophysiological mechanisms, outcomes of therapeutic interventions, and clinical courses of patients with insulin resistance.
DIRECT MEASURES OF INSULIN SENSITIVITY

Hyperinsulinemic Euglycemic Glucose Clamp

Procedure and concept. The glucose clamp technique, originally developed by DeFronzo et al. (33), is widely accepted as the reference standard for directly determining metabolic insulin sensitivity in humans. After an overnight fast, insulin is infused intravenously at a constant rate that may range from 5 to 120 mU·m⁻²·min⁻¹ (dose per body surface area per minute). This constant insulin infusion results in a new steady-state insulin level that is above the fasting level (hyperinsulinemic). As a consequence, glucose disposal in skeletal muscle and adipose tissue is increased, whereas HGP is suppressed. Under these conditions, a bedside glucose analyzer is used to frequently monitor blood glucose levels at 5- to 10-min intervals while 20% dextrose is given intravenously at a variable rate to “clamp” blood glucose concentrations in the normal range (euglycemic). An infusion of potassium phosphate is also given to prevent hypokalemia resulting from hyperinsulinemia and increased glucose disposal. After several hours of constant insulin infusion, steady-state conditions can typically be achieved for plasma insulin, blood glucose, and the glucose infusion rate (GIR). Assuming that the hyperinsulinemic state is sufficient to completely suppress HGP, and since there is no net change in blood glucose concentrations under steady-state clamp conditions, the GIR must be equal to the glucose disposal rate (M). Thus, whole body glucose disposal at a given level of hyperinsulinemia can be determined directly. M is typically normalized to body weight or fat-free mass to generate an estimate of insulin sensitivity. Alternatively, an insulin sensitivity index (SI) derived from clamp data can be defined as $\text{SI}_{\text{clamp}} = M/(G \times \Delta I)$, where M is normalized for G (steady-state blood glucose concentration) and $\Delta I$ (difference between fasting and steady-state plasma insulin concentrations) (56).

Assumptions and considerations. The validity of glucose clamp measurements of insulin sensitivity depends on achieving steady-state conditions. It is often assumed that data from an arbitrary time point at the end of the clamp study (e.g., 2 h) represent steady-state values. However, defining “steady state” as a period $>30$ min ($\geq 1$ h after initiation of insulin infusion), during which the coefficient of variation for blood glucose, plasma insulin, and GIR is $<5\%$, is a more rigorous approach that may determine more accurate values for M and $\text{SI}_{\text{clamp}}$ (22, 56).

Another important assumption of the glucose clamp method is that HGP is completely suppressed by steady-state hyperinsulinemia. For subjects with normal insulin sensitivity, this may be achieved with an insulin infusion rate in the range of 40–60 mU·m⁻²·min⁻¹ (16, 87). However, there may be incomplete suppression of HGP at lower insulin infusion rates or in insulin-resistant populations. To address this issue, it is possible to use radiolabeled glucose tracers under clamp conditions to estimate HGP so that appropriate corrections can be made to M (38, 68, 85, 87). An alternative approach is to choose an insulin infusion rate that is sufficiently high to completely suppress HGP according to the insulin sensitivity/resistance of the population to be studied.

A critical consideration for interpretation of glucose clamp data is that M is routinely obtained at only a single insulin infusion rate. The choice of this rate determines a level of hyperinsulinemia that is roughly comparable among all subjects studied. Comparisons between M or $\text{SI}_{\text{clamp}}$ among different subjects is valid only if the same insulin infusion rate is used for all subjects. Importantly, the use of a single insulin infusion rate for comparisons of insulin sensitivity/resistance assumes that the steady-state insulin level achieved is in the range where M can vary according to differences in insulin sensitivity. If the insulin infusion rate is not appropriately matched to the insulin sensitivity/resistance of the population being studied, it is possible that erroneous conclusions may be
drawn. For example, in an insulin-resistant population, a higher insulin infusion rate is required to avoid missing potential differences in insulin sensitivities among subjects (Fig. 1B, curve c). Similarly, for an insulin-sensitive population, a lower insulin infusion rate is more appropriate (Fig. 1B, curve a). To address these issues, the ideal approach is to generate a full insulin dose-response curve by performing the glucose clamp procedure at multiple insulin infusion rates in a stepwise fashion (16, 87). However, this is rarely done, since feasibility issues arising from this more complete approach are daunting even in sophisticated research settings.

When subjects are nondiabetic, their fasting glucose level is within the normal range. Some investigators will clamp blood glucose at fasting levels (isoglycemic), whereas others will clamp blood glucose at an arbitrary predetermined level of glycemica within the normal range (euglycemic). In the case of diabetic subjects, the difference between euglycemia and isoglycemia may be large. There are pros and cons to choosing either approach for diabetic subjects and nondiabetic subjects. The rationale for performing isoglycemic clamps in diabetic subjects is that large acute changes in glycemica may alter insulin sensitivity. For diabetic subjects under isoglycemic conditions, it may be necessary to correct M for urinary losses of glucose. In addition, even in nondiabetic subjects there is variability in fasting glucose levels. Thus, for isoglycemic clamps, SI_{Clamp} is more appropriate to use than M because SI_{Clamp} normalizes for differences in fasting glucose levels (as well as for differences between fasting and steady-state clamp insulin levels). With euglycemic clamps in nondiabetic subjects, either M or SI_{Clamp} is appropriate to use (although M does not correct for differences between fasting and steady-state clamp insulin levels).

Differences between arterial and venous blood glucose increase in proportion to insulin sensitivity and dose. Therefore, adjusting glucose infusion rates on the basis of venous sampling of glucose may lead to overestimation of insulin sensitivity. To minimize this issue, the hand used for blood sampling may be cannulated in retrograde fashion and warmed with a heating pad (opening arteriovenous anastomoses) to “arterialize” the venous blood (71).

Computer-based algorithms have been developed to help control the GIR in response to frequently measured blood glucose levels (33, 41). However, efficient regulation of GIR may be empirically achieved with an experienced, proficient operator.

Advantages and appropriate usage. The main advantage of using the glucose clamp to estimate insulin sensitivity/resistance in humans is that it directly measures whole body glucose disposal at a given level of insulinemia under steady-state conditions. Conceptually, the approach is straightforward, and there are a limited number of assumptions that are clearly defined. In addition, the glucose clamp has excellent test characteristics. For example, M typically has a coefficient of variation of 0.10 and a discriminant ratio of ~6 (a measurement of both reproducibility and the ability to distinguish individual results) (65). When radiolabeled glucose tracers are used under clamp conditions it is possible to simultaneously quantify HGP and whole body glucose disposal (18, 87). Under these conditions, one can study and distinguish hepatic and peripheral (predominantly skeletal muscle) insulin sensitivity/resistance. Similarly, radiolabeled tracers of glycerol or amino acids may be used during the glucose clamp to assess insulin sensitivity with respect to lipolysis or protein metabolism (42, 44). In addition, ^31P magnetic resonance spectroscopy may be used in conjunction with the glucose clamp to assess rates of insulin-stimulated muscle mitochondrial ATP synthase flux and insulin-stimulated increases in concentrations of intramyocellular inorganic phosphate (75). Doppler and contrast ultrasound imaging have also been used in conjunction with the clamp to study insulin sensitivity with respect to vascular actions of insulin (24). In research settings where assessing insulin sensitivity/resistance is of primary interest and feasibility is not an issue (e.g., study population <100) it is appropriate and important to use the reference standard glucose clamp technique.

Limitations. The main limitations of the glucose clamp approach are that it is time consuming, labor intensive, expensive, and requires an experienced operator to manage the technical difficulties. Thus, for epidemiological studies, large clinical investigations, or routine clinical applications (e.g., following changes in insulin resistance after therapeutic intervention in individual patients) the glucose clamp is not appropriate. In addition, if measuring insulin sensitivity/resistance is not a primary study outcome, then the cost/benefit ratio for the glucose clamp may not be favorable. Another limitation is that the clamp utilizes steady-state insulin levels that may be supraphysiological. This results in a reversal of the normal portal to peripheral insulin gradient. Thus, the glucose clamp may not accurately reflect insulin action and glucose dynamics under physiological conditions that a dynamic test such as an oral meal or oral glucose load may determine.

Insulin Suppression Test

Procedure and concept. The insulin suppression test (IST), another method that directly measures metabolic insulin sensitivity/resistance, was introduced by Shen et. al. (90) in 1970 and subsequently modified by Harano et. al. (50). After an overnight fast, somatostatin (250 μg/h) or the somatostatin analog octreotide (25 μg bolus, followed by 0.5 μg/min) (74) is intravenously infused to suppress endogenous secretion of insulin and glucagon. Simultaneously, insulin (25 mU·m⁻²·min⁻¹) and glucose (240 mg·m⁻²·min⁻¹) are infused into the same antecubital vein for 3 h. From the contralateral arm, blood samples for glucose and insulin determinations are taken every 30 min for 2.5 h and then at 10-min intervals from 150 to 180 min of the IST. The constant infusions of insulin and glucose will determine steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations. The steady-state period is assumed to be from 150 to 180 min after initiation of the IST. SSPI concentrations are generally similar among subjects. Therefore, the SSPG concentration will be higher in insulin-resistant subjects and lower in insulin-sensitive subjects; i.e., SSPG values are inversely related to insulin sensitivity. The IST provides a direct measure (SSPG) of the ability of exogenous insulin to mediate disposal of an intravenous glucose load under steady-state conditions where endogenous insulin secretion is suppressed.

Assumptions and considerations. As with the glucose clamp, the validity of the IST depends on achieving steady-state conditions. However, this is not routinely verified. Moreover, it is assumed that combined effects of hyperinsulinemia, hyper-
glycemia, and hypoglucagonemia during the IST are sufficient to completely suppress HGP. It is also assumed that the somatostatin infusion is sufficient to completely suppress endogenous insulin and glucagon secretion. The same insulin infusion rate is universally applied in the IST. However, this rate may not be ideal for every population being studied. Thus, for the IST, issues regarding erroneous determinations of insulin sensitivity/resistance in populations with different degrees of insulin resistance are similar to those described above for the glucose clamp. Another consideration is that the insulin infusion rate universally applied in the IST does not result in the same SSPI for all subjects (due to differences in insulin clearance, volume of distribution, etc.). Since SSPG is not corrected for SSPI, this may introduce some error.

Advantages and appropriate usage. The SSPG is a highly reproducible direct measurement of metabolic actions of insulin that is less labor intensive and less technically demanding than the glucose clamp. Indeed, since there are no variable infusions with the IST, steady-state conditions are more easily achieved with the IST than with the glucose clamp. Estimates of insulin sensitivity determined by SSPG correlate well with reference standard glucose clamp estimates in normal subjects \((r = 0.93)\) and in patients with type 2 diabetes mellitus \((r = 0.91)\) \((43, 69)\). Indeed, SSPG has positive predictive power for cardiovascular disease events and onset of type 2 diabetes \((36, 103)\). In research settings where assessing insulin sensitivity/resistance is of primary interest and feasibility is not an issue, it is appropriate to use the IST. Moreover, the IST can be used for larger populations that may pose difficulties for application of the glucose clamp \((102)\).

Limitations. Many of the limitations of the IST are similar to those described above for the glucose clamp (with the exception that the IST is less technically demanding). Thus, it is impractical to apply the IST in large epidemiological studies or in the clinical care setting. In exquisitely insulin-sensitive individuals, it is possible that subjects may become hypoglycemic during the IST. In individuals with type 2 diabetes, hyperglycemia may lead to glycosuria and underestimation of insulin resistance by SSPG. The infusion of somatostatin during the IST may independently modulate splanchnic blood flow and peripheral glucose clearance \((12, 70, 99)\). This may potentially affect estimates of insulin resistance determined by SSPG. Finally, SSPG under ideal conditions determines primarily skeletal muscle insulin sensitivity and is not designed to reflect hepatic insulin sensitivity.

**INDIRECT MEASURES OF INSULIN SENSITIVITY**

**Minimal Model Analysis of Frequently Sampled Intravenous Glucose Tolerance Test**

Procedure and concept. The minimal model, developed by Bergman et al. \((14)\) in 1979, provides an indirect measurement of metabolic insulin sensitivity/resistance on the basis of glucose and insulin data obtained during a frequently sampled intravenous glucose tolerance test (FSIVGTT). After an overnight fast, an intravenous bolus of glucose \((0.3 \text{ g/kg body wt})\) is infused over 2 min starting at time \(0\). Currently, a modified FSIVGTT is used where exogenous insulin \((4 \text{ mU·kg}^{-1} \text{ min}^{-1})\) is also infused over 5 min beginning 20 min after the intravenous glucose bolus \((39, 81, 89)\). Some studies use tolbutamide instead of insulin in the modified FSIVGTT to stimulate endogenous insulin secretion at this time \((9, 15, 89, 101)\). Blood samples are taken for plasma glucose and insulin measurements at \(-10, -1, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 160,\) and 180 min. These data are then subjected to minimal model analysis using the computer program MINMOD to generate an index of insulin sensitivity \((S_I)\).

The minimal model is defined by two coupled differential equations with four model parameters (Fig. 2). The first equation describes plasma glucose dynamics in a single compartment. The second equation describes insulin dynamics in a “remote compartment.” The structure of the minimal model allows MINMOD to uniquely identify model parameters that determine a best fit to glucose disappearance during the modified FSIVGTT. \(S_I\) is calculated from two of these model parameters and is defined as fractional glucose disappearance per insulin concentration unit.

Assumptions and considerations. Unlike the glucose clamp and IST, which depend on steady-state conditions, the minimal model approach uses dynamic data. Valid minimal model analysis of the modified FSIVGTT requires several important assumptions. First, instantaneous distribution of the glucose bolus in a monocompartmental space is assumed to occur.

**Fig. 2. Schematic equations and parameters for the minimal model of glucose metabolism. Differential equations describing glucose dynamics \(G(t)\) in a monocompartmental “glucose space” and insulin dynamics in a “remote compartment” \(X(t)\) are shown at the top. Glucose leaves or enters its space at a rate proportional to the difference between plasma glucose level, \(G(t)\), and the basal fasting level, \(G_0\). In addition, glucose also disappears from its compartment at a rate proportional to insulin levels in the “remote” compartment \(X(t)\). In this model, \(r = time; G(t) = plasma\) glucose at time \(t; I(t) = plasma\) insulin concentration at time \(t; X(t) = insulin\) concentration in “remote” compartment at time \(t; G_0 = basal\) plasma glucose concentration; \(I_0 = basal\) plasma insulin concentration; \(G(0) = G_0\) (assuming instantaneous mixing of the iv glucose load); \(p_1, p_2, p_3\), and \(G_0\) = unknown parameters in the model that are uniquely identifiable from frequently sampled intravenous glucose tolerance test; glucose effectiveness \(= p_1\), and insulin sensitivity \(= p_2/p_3\).**
Second, glucose disappearance in response to glucose and/or insulin is assumed to occur at a monoexponential rate. Third, the glucose concentration at the end of the FSIVGTT is assumed to be identical to the beginning concentration. Fourth, insulin is assumed to act from a “remote compartment” (extravascular) to promote glucose disappearance. This “remote compartment” may represent an interstitial or extracellular space where insulin directly exerts its metabolic actions (11). Fifth, the minimal model lumps together effects of insulin to promote glucose disposal in skeletal muscle and suppress HGP. Finally, to obtain a valid estimate of $S_I$ the minimal model assumes that total insulin secretion (endogenous plus exogenous) during the FSIVGTT is above a certain threshold (10).

In addition to $S_I$, other minimal model parameters may be used to estimate a “glucose effectiveness” index ($S_G$). $S_G$ is defined as the ability of glucose per se to promote its own disposal and inhibit HGP in the absence of an incremental insulin effect (i.e., when insulin is at basal levels). The minimal model was originally developed in dogs that have a delayed insulin secretory response to the FSIVGTT when compared with humans (14). By contrast, in humans, the peak insulin secretion induced by the FSIVGTT temporally overlaps with the period in which high glucose concentrations are the primary determinant of glucose disappearance. Therefore, to increase the accuracy of both $S_I$ and $S_G$ in humans, a modified FSIVGTT was developed where exogenous insulin (or tolbutamide) is given at a time where glucose levels have decreased to near-normal levels. This allows the minimal model to more effectively distinguish between glucose effectiveness and insulin sensitivity (15, 101). Finally, because the FSIVGTT is a dynamic test, information about $\beta$-cell function can also be derived from minimal model analysis (11, 13, 46).

Advantages and appropriate usage. Minimal model analysis of the modified FSIVGTT is easier than the glucose clamp method because it is slightly less labor intensive, steady-state conditions are not required, and there are no intravenous infusions that require constant adjustment. Unlike the glucose clamp or IST, information about insulin sensitivity, glucose effectiveness, and $\beta$-cell function can be derived from a single dynamic test. The minimal model generates excellent predictions of glucose disappearance during the FSIVGTT. The coefficient of variation for $S_I$ is comparable to glucose clamp estimates of insulin sensitivity (37, 93). Reasonable correlations between estimates of insulin sensitivity derived from minimal model analysis and the reference glucose clamp method have been demonstrated in healthy subjects (correlations are weaker in insulin-resistant populations) (9, 15, 88). $S_I$ is a strong predictor of the development of diabetes in a prospective study of children of diabetic parents (64). Moreover, the insulin-modified FSIVGTT may be used in relatively large-scale population studies. This is evident from the Insulin Resistance Atherosclerosis Study (53), a large ($n = 1,624$), prospective epidemiological study examining associations between $S_I$ and atherosclerosis or other risk factors for cardiovascular disease. Therefore, in research settings where assessing insulin sensitivity along with glucose effectiveness and $\beta$-cell function is of interest, minimal model analysis of the insulin-modified FSIVGTT may be appropriate. However, as discussed below, caveats regarding limitations of the minimal model and the availability of simpler, more accurate methods for assessing insulin sensitivity should be noted.

Limitations. The minimal model approach is simpler than direct methods for determining insulin sensitivity. Nevertheless, it still involves intravenous infusions with multiple blood sampling over a 3-h period that are nearly as labor intensive as the glucose clamp or IST. In addition, many limitations of minimal model analysis stem from the fact that the model oversimplifies the physiology of glucose homeostasis. For example, monocompartmental representation of glucose dynamics results in systematic overestimation of $S_G$ and simultaneous underestimation of $S_I$ (25, 26, 82). Indeed, two-compartment representation of glucose dynamics improves the results of minimal model analysis. Consequently, better estimates of $S_I$ and $S_G$ are obtained by using radiolabeled glucose during the FSIVGTT (“hot” FSIVGTT) that simultaneously incorporate a second compartment for glucose into the minimal model (6, 19, 20, 97). Alternatively, it is possible to use a Bayesian approach (using prior knowledge during model identification routines) with addition of a nonaccessible compartment to the original “cold” model (26).

Another oversimplification of the minimal model involves lumping together effects of insulin to promote peripheral glucose utilization and suppress HGP. As insulin sensitivity/resistance varies, the relative contribution of HGP to $S_I$ may vary significantly. Since the minimal model relies on a dynamic test to evaluate insulin sensitivity, estimates of $S_I$ are much less reliable in individuals with impaired insulin secretion and/or significant insulin resistance (when compared with healthy subjects). Under these conditions, the minimal model may overestimate $S_G$ to accurately predict the disappearance of glucose during the FSIVGTT. Indeed, estimates of $S_G$ are spuriously affected by differences in insulin secretory capacity (28, 40, 82). Moreover, for similar reasons, minimal model analysis often generates nonsensical negative values for $S_I$ in a substantial proportion of subjects with diabetes who have minimal insulin secretory capacity and significant insulin resistance (40, 56, 88). These nonsystematic errors inherent in the minimal model approach are highlighted by calibration model analysis demonstrating that some simple surrogate indexes of insulin sensitivity have better absolute accuracy for predicting $S_{I\text{Clamp}}$ than the minimal model-derived $S_I$ (21).

Oral Glucose Tolerance Test/Meal Tolerance Test

The oral glucose tolerance test (OGTT) is a simple test widely used in clinical practice to diagnose glucose intolerance and type 2 diabetes (4). After overnight fast, blood samples for determinations of glucose and insulin concentrations are taken at 0, 30, 60, and 120 min following a standard oral glucose load (75 g) or a standard meal (4, 30). Oral glucose tolerance reflects the efficiency of the body to dispose of glucose after an oral glucose load or meal. The OGTT or meal tolerance test mimics the glucose and insulin dynamics of physiological conditions more closely than conditions of the glucose clamp, IST, or FSIVGTT. However, it is important to recognize that glucose tolerance and insulin sensitivity are not equivalent concepts. In addition to metabolic actions of insulin, insulin secretion, incretin effects, and other factors contribute importantly to glucose tolerance. Thus, the OGTT and meal tolerance tests provide useful information about glucose tolerance but
not insulin sensitivity/resistance per se. Nevertheless, as discussed in the next section, a number of surrogate indexes of insulin sensitivity/resistance incorporate the results of the OGGT or meal tolerance test.

**SIMPLE SURROGATE INDEXES FOR INSULIN SENSITIVITY/RESISTANCE**

**Surrogates Derived from Fasting Steady-State Conditions**

**Procedure and concept.** After an overnight fast, a single blood sample is taken for determination of blood glucose and plasma insulin. In healthy humans, the fasting condition represents a basal steady state where glucose is homeostatically maintained in the normal range such that insulin levels are not significantly changing and HGP is constant; i.e., basal insulin secretion by pancreatic β-cells determines a relatively constant level of insulinemia that will be lower or higher in accordance with insulin sensitivity/resistance such that HGP matches whole body glucose disposal under fasting conditions.

**Assumptions and considerations.** A critical condition and assumption of simple surrogate indexes is that subjects are strictly fasting and in a basal steady-state condition with respect to glycemia, insulinemia, and HGP. Surrogate indexes based on fasting glucose and insulin concentrations reflect primarily hepatic insulin sensitivity/resistance. However, under most conditions, hepatic and skeletal muscle insulin sensitivity/resistance are proportional to each other. In the diabetic state with fasting hyperglycemia, fasting insulin levels are inappropriately low and insufficient to maintain euglycemia. Therefore, definitions of the more useful surrogate indexes take these considerations into account. Due to lack of a standardized insulin assay, it is not possible to use surrogate indexes to define universal cutoff points for insulin resistance.

**General advantages and appropriate usage.** Simple surrogate indexes of insulin sensitivity/resistance are inexpensive quantitative tools that can be easily applied in almost every setting, including epidemiological studies, large clinical trials, clinical research investigations, and clinical practice. If a direct measurement of insulin sensitivity is not required, not feasible to obtain, or if insulin sensitivity is of secondary interest, it may be appropriate to use a surrogate index. The relative merits and limitations of individual surrogate indexes are discussed below.

1/(Fasting insulin). In healthy subjects, elevations in fasting insulin levels (with normal fasting glucose levels) correspond to increased insulin resistance. Indeed, in nondiabetic subjects, 1/(fasting insulin) is a well-known proxy for insulin sensitivity that decreases as subjects become more insulin resistant (and fasting insulin levels rise) (59). However, insulin concentrations are not normally distributed. Thus, linear correlations between 1/(fasting insulin) and estimates of insulin sensitivity from the glucose clamp are not that strong. In addition, this index does not take into account the inappropriately low insulin secretion in the face of hyperglycemia seen in diabetic subjects or glucose-intolerant subjects. Consequently, using 1/(fasting insulin) as a measurement of insulin sensitivity/resistance in patients with glucose intolerance or type 2 diabetes who have diminished pancreatic reserve leads to erroneous results.

**Glucose/insulin ratio.** A number of studies (60, 91, 98) have used the fasting glucose/insulin ratio (G/I ratio) as an index of insulin resistance (particularly in patients with polycystic ovarian syndrome). In the case of nondiabetic subjects, the G/I ratio is essentially functionally equivalent to 1/(fasting insulin) since fasting glucose levels are all in the normal range. However, the G/I ratio does not appropriately reflect the physiology underlying the determinants of insulin sensitivity (80). For example, given the same level of relative fasting hyperinsulinemia in a diabetic and a nondiabetic insulin-resistant subject, 1/(fasting insulin) remains unchanged. However, under these same conditions, the G/I ratio paradoxically and erroneously increases in the diabetic subject. Therefore, the fasting G/I ratio is a conceptually flawed index of insulin sensitivity.

**Homeostasis model assessment.** Homeostasis model assessment (HOMA), developed in 1985 (67), is a model of interactions between glucose and insulin dynamics that is then used to predict fasting steady-state glucose and insulin concentrations for a wide range of possible combinations of insulin resistance and β-cell function. Both the original HOMA and the updated HOMA2 assume a feedback loop between the liver and β-cell (61, 67, 100); i.e., glucose concentrations are regulated by insulin-dependent HGP, whereas insulin levels depend on the pancreatic β-cell response to glucose concentrations. Thus, deficient β-cell function reflects a diminished response of β-cell to glucose-stimulated insulin secretion. Likewise, insulin resistance is reflected by diminished suppressive effect of insulin on HGP. HOMA describes this glucose-insulin homeostasis by a set of empirically derived nonlinear equations. The model predicts fasting steady-state levels of plasma glucose and insulin for any given combination of pancreatic β-cell function and insulin sensitivity. Computer simulations have been used to generate a normogram from which mathematical transformations of fasting glucose and insulin data from individual subjects determine unique combinations of S_I (HOMA%S) and β-cell function (HOMA%B) from steady-state conditions. The updated HOMA2 accommodates assessment of HOMA%S and HOMA%B in subjects with glucose levels ≲25 mM, accounts for renal glucose losses, assumes reduced suppression of HGP and increased insulin secretion in response to glucose levels >10 mM, and allows for the use of total or specific insulin assays. An important caveat for HOMA%B (determined from fasting glucose and insulin concentrations) is that it imputes a dynamic β-cell function (i.e., glucose-stimulated insulin secretion) from fasting steady-state data. In the absence of dynamic data, it is difficult, if not impossible, to determine the true dynamic function of β-cell insulin secretion. In practical terms, most studies using HOMA employ an approximation described by a simple equation to determine a surrogate index of insulin resistance. This is defined by the product of the fasting glucose and fasting insulin divided by a constant. Thus, homeostasis model of insulin resistance (HOMA-IR) = [(fasting insulin (μU/ml)) × [fasting glucose (mmol/l)]]/22.5. The denominator of 22.5 is a normalizing factor; i.e., the product of normal fasting plasma insulin of 5 μU/ml and normal fasting plasma glucose of 4.5 mmol/l typical of a “normal” healthy individual = 22.5. Therefore, for an individual with “normal” insulin sensitivity, HOMA-IR = 1. HOMA-IR has a reasonable linear correlation with glucose clamp and minimal model estimates of insulin sensitivity/resistance in several studies of distinct populations (84, 100). The coefficient of variation for HOMA-IR varies considerably depending upon the number of fasting samples obtained and the type of insulin assay used (17, 35, 67, 100).
It is important to note that, over wide ranges of insulin sensitivity/resistance, log (HOMA-IR) transforms the skewed distribution of fasting insulin values to determine a much stronger linear correlation with glucose clamp estimates of insulin sensitivity (56). Log (HOMA-IR) is useful for evaluation of insulin resistance in individuals with glucose intolerance, mild to moderate diabetes, and other insulin-resistant conditions. However, in subjects with severely impaired or absent β-cell function, HOMA-IR may not give appropriate results. HOMA or log (HOMA) is used extensively in large epidemiological studies, prospective clinical trials, and clinical research studies (29, 47, 100). In research settings where assessing insulin sensitivity/resistance is of secondary interest or feasibility issues preclude the use of direct measurements by glucose clamp, it may be appropriate to use log (HOMA-IR). However, as discussed below, other surrogate indexes have certain advantages over HOMA or log (HOMA) in some circumstances.

Quantitative insulin sensitivity check index. Quantitative insulin sensitivity check index (QUICKI) is an empirically derived mathematical transformation of fasting blood glucose and plasma insulin concentrations that provides a reliable, reproducible, and accurate index of insulin sensitivity with excellent positive predictive power (21, 22, 48, 56, 65). During development of QUICKI, sensitivity analysis of data from the first 20 min of an FSIVGTT revealed that physiological fasting steady-state values of plasma insulin and glucose contain critical information about insulin sensitivity as determined by the reference standard glucose clamp (SIClamp) (56). Since fasting insulin levels have a nonnormal skewed distribution, log transformation improves its linear correlation with SIClamp. However, as with 1/(fasting insulin) and the G/I ratio, this correlation is not maintained in diabetic subjects with fasting hyperglycemia and impaired β-cell function that is insufficient to maintain euglycemia. To accommodate these clinically important circumstances where fasting glucose is inappropriately high and insulin is inappropriately low, addition of log (fasting glucose) to log (fasting insulin) provides a reasonable correction such that the linear correlation with SIClamp is maintained in both diabetic and nondiabetic subjects. The reciprocal of this sum results in further transformation of the data, generating an insulin sensitivity index that has a positive correlation with SIClamp. Thus, QUICKI = 1/[log (fasting insulin, μU/ml) + log (fasting glucose, mg/dl)]. Over a wide range of insulin sensitivity/resistance, QUICKI has a substantially better linear correlation with SIClamp (r = 0.8–0.9) than SI derived from the minimal model or HOMA-IR (22, 56, 65). Log (HOMA) is roughly comparable to QUICKI in this regard. QUICKI and HOMA were derived in a completely different conceptual fashion. Nevertheless, these two surrogate indexes are mathematically related; i.e. QUICKI is proportional to 1/log (HOMA-IR). Multiple independent studies find excellent linear correlations between QUICKI and glucose clamp estimates (either GIR or SIClamp) in healthy subjects, obesity, diabetes, hypertension, and many other insulin-resistant states (8, 55, 65, 83, 92, 96, 104). QUICKI performs best in insulin-resistant subjects, whereas SI from the minimal model performs best in healthy, insulin-sensitive subjects. Test characteristics examining repeatability and reliability of QUICKI (including coefficient of variation and discriminant ratio) demonstrate that QUICKI is superior to 1/(fasting insulin), log (insulin), and HOMA-IR and comparable with log (HOMA) and GIR from the glucose clamp. Evaluation of absolute predictive accuracy is also important in the validation of a surrogate index. Calibration model analysis has been used to evaluate the ability of QUICKI and other surrogates to accurately predict the reference standard SIClamp. In this regard, error functions for QUICKI and log (HOMA-IR) are both excellent and significantly smaller than for HOMA-IR, 1/HOMA-IR, fasting insulin, and SI derived from the minimal model (21). Moreover, changes in QUICKI after therapeutic interventions are significantly correlated with changes in SIClamp (22, 55). Under similar conditions, changes in SI derived from the minimal model do not significantly correlate with changes in SIClamp. A large meta-analysis of insulin-resistant subjects demonstrates that QUICKI is the simple surrogate index with the best positive predictive power for determining development of diabetes (48). Taking all of the factors discussed above, it is evident that validation studies for QUICKI and other simple surrogates should use direct estimates obtained from the reference standard glucose clamp for comparisons rather than the indirect SI derived from minimal model analysis. QUICKI is among the most thoroughly evaluated and validated surrogate index for insulin sensitivity. As a simple, useful, inexpensive, and minimally invasive surrogate for glucose clamp-derived measurements of insulin sensitivity, QUICKI is appropriate and effective for use in large epidemiological or clinical research studies, to follow changes after therapeutic interventions, and for use in studies where evaluation of insulin sensitivity is not of primary interest.

Surrogates Derived from Dynamic Tests

Procedure and concept. Surrogate indexes of insulin sensitivity that use information derived from dynamic tests such as OGTT, meal tolerance tests, and IVGTT have been developed. Procedures for these tests have been described in Oral Glucose Tolerance Test/Meal Tolerance Test. Specific indexes, including Matsuda index (66), Stumvoll index (94), Avignon index (5), oral glucose insulin sensitivity index (63), Gutt index (45), and Belfiore index (10), use particular sampling protocols during the OGTT or the meal. In addition, minimal model approaches have been used to model plasma glucose and insulin dynamics during an OGTT or a meal to determine insulin sensitivity/resistance (27). Glucose disposal after an oral glucose load or a meal is mediated by a complex dynamic process that includes absorption, glucose effectiveness, neurohormonal actions, incretin actions, insulin secretion, and metabolic actions of insulin that primarily determine the balance between peripheral glucose utilization and HGP. Surrogate indexes that depend on dynamic testing take into account both fasting steady-state and dynamic postglucose load plasma glucose and insulin levels.

Assumptions and considerations. The oral route of glucose delivery is more physiological than intravenous glucose infusion. However, poor reproducibility of the OGTT and meal tolerance test due to variable glucose absorption, splanchnic glucose uptake, and additional incretin effects needs to be considered. Thus, distinguishing direct metabolic actions of insulin following oral ingestion of glucose or a mixed meal is more problematic than after FSIVGTT. In addition, as with many other measurements of insulin sensitivity, surrogates

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derived from dynamic testing generally incorporate both peripheral and hepatic insulin sensitivity. Although OGTT involves considerably less work than FSIVGTT, dynamic testing in general requires more effort and cost than fasting blood sampling.

**General advantages and appropriate usage.** Many surrogate measures derived from dynamic data correlate reasonably well with glucose clamp estimates of SI (45, 63, 66). Estimates of insulin sensitivity derived from OGTT predict the development of type 2 diabetes in epidemiologic studies (2, 48, 49). Furthermore, insulin sensitivity (SI_{Oral}) estimated from minimal model analysis of OGTT or meal tolerance tests correlates well with glucose clamp measures (27, 31). The advantage of surrogates based on dynamic testing is that information about insulin secretion can be obtained at the same time as information about insulin action. However, if one is interested only in estimating insulin sensitivity/resistance, fasting surrogates may be preferable to dynamic surrogates because they are simpler to obtain.

**Insulin sensitivity index-Matsuda.** Originally proposed by Matsuda and DeFronzo (66), insulin sensitivity index-Matsuda [ISI_{Matsuda}] is an insulin sensitivity index that reflects a composite estimate of hepatic and muscle insulin sensitivity determined from OGTT data. ISI_{Matsuda} = 10,000\sqrt{[\text{G}_{\text{fasting}} \times \text{I}_{\text{fasting}}] \times (\text{G}_{\text{OGTT mean}} \times \text{I}_{\text{OGTT mean}})}], where fasting glucose and insulin data are taken from time 0 of the OGTT and mean data represent the average glucose and insulin values obtained during the entire OGTT. The square root is used to correct for nonlinear distribution of insulin, and 10,000 is a scaling factor in the equation. ISI_{Matsuda} correlates reasonably well with estimates of whole body insulin sensitivity determined by the glucose clamp. The fasting component reflects hepatic insulin sensitivity, whereas the mean of the dynamic data primarily represents skeletal muscle insulin sensitivity. This partitioning concept has recently been validated using glucose clamp studies (1). The insulin secretion/insulin resistance (disposition) index calculated as the product of insulin secretion measured with \((\Delta G_{0\rightarrow30}/\Delta G_{0\rightarrow30}) or \Delta G_{0\rightarrow120}/\Delta G_{0\rightarrow120}) and ISI_{Matsuda} [or modified ISI_{Matsuda}] using plasma glucose and insulin concentrations at 30 min during the OGTT] had excellent power to predict onset of type 2 diabetes (2).

**Gutt index: ISI (0,120).** The ISI (0,120) was adapted from the Cederholm index by omitting the constant terms and using the plasma glucose and insulin concentration from fasting (0 min) and 120-min samples from the OGTT (45). The ISI (0,120) index is defined as: MCR/log MSI =\text{m/MPG/log MSI}. The glucose uptake rate in peripheral tissues, \(m\) (mg/min), is obtained by the formula: \(m = [75,000 \text{ mg} + (\text{Glucose}_0 - \text{Glucose}_{120}) \times 0.19 \times \text{BW}] / 120 \text{ min} \), where the term (0.19 \times \text{BW}) denotes glucose space, and \text{BW} is body weight (kg). The metabolic clearance rate (MCR) is obtained by MCR = \text{m/MPG, mean of the 0- and 120-min glucose values from the OGTT}. The mean serum insulin (MSI, mU/l) is the mean plasma insulin concentrations obtained from the 0- and 120-min samples of the OGTT. The index correlates well with direct estimates of insulin sensitivity obtained from the glucose clamp study (r = 0.63) (45). In a large prospective study, this index was the best at predicting onset of type 2 diabetes when compared with other surrogate indexes derived from dynamic tests (including Avignon, Belfiore, and Stumvoll) (48).

### ASSESSING INSULIN SENSITIVITY/RESISTANCE IN ANIMALS

Animal models of insulin resistance are helpful for understanding pathophysiology and for developing and evaluating therapeutic agents used for treatment and/or prevention of insulin resistance and its associated diseases. As in humans, the hyperinsulinemic euglycemic glucose clamp is considered the reference standard for measuring insulin sensitivity in animals (7). In addition, various dynamic tests, including IVGTT, OGTT, and intraperitoneal insulin tolerance test (ITT), have been used (23, 34, 73). Several studies (51, 62, 78) also use surrogates derived from fasting conditions, such as fasting insulin levels, HOMA-IR, and QUICKI.

#### Hyperinsulinemic Euglycemic Glucose Clamp

**Procedure and concept.** Conceptually, the glucose clamp in animals is the same as in humans. Briefly, after an overnight fast (~15 h), constant hyperinsulinemia is achieved by a primed, continuous infusion of insulin (15 pmol·kg^{-1}·min^{-1}), and a variable infusion of 20% glucose is used to maintain euglycemia. Basal and insulin-stimulated whole body glucose turnover is estimated with a continuous infusion of [^{3}H]glucose 2 h prior to and throughout the clamp study. As in humans, the GIR provides a measurement of insulin sensitivity. Rates of HGP and insulin-stimulated whole body glucose turnover are determined as the ratio of the [^{3}H]glucose infusion rate (disintegrations/min) to the specific activity of plasma glucose (dpm/μmol) at the end of the basal period and during the final 30 min of the clamp procedure, respectively. HGP during hyperinsulinemic clamp conditions is determined by subtracting the GIR from whole body glucose turnover.

**Limitations and considerations.** Regarding the glucose clamp in animals, additional considerations are needed while conducting and interpreting these studies, especially for rodents (7). Unlike humans, rodents have no true physiological fasting state. Imposing a nonphysiological fasting period leads to significant decrease in body weight, depletion of hepatic glycogen content, and improved insulin sensitivity. Therefore, under these nonphysiological fasting conditions, plasma glucose and insulin levels may not attain steady-state conditions. Furthermore, it is likely that the glucose clamp technique in rodents is not as accurate and reliable as in humans. Rodents have very small blood volumes (~2 ml in mice). Therefore, limited sampling ability makes it difficult to achieve and rigorously verify steady-state clamp conditions (especially with respect to insulin levels). In addition, the glucose clamp is stressful in mice (even if done under anesthetized conditions).

**Advantages and appropriate usage.** The glucose clamp can be used to measure tissue-specific insulin action and glucose metabolism such as 1) basal and insulin-stimulated HGP, 2) insulin-stimulated whole body glucose uptake, glycolysis, and glycogen synthesis, and 3) insulin-stimulated glucose uptake, glycolysis, and glycogen synthesis in individual tissues (e.g., skeletal muscle, adipose tissue, and heart) (58). Additionally, biochemical/molecular assays may be performed to assess tissue-specific insulin-signaling activities and tissuespecific triglyceride contents (57). When assessing insulin sensitivity is a primary objective and feasibility is not an issue, the glucose clamp study is the method of choice in animals.
**GTTs and ITTs**

Procedure and concept. After an overnight fast, glucose (~2 g/kg body wt) is administered orally (OGTT), intraperitoneally (IPGTT), or intravenously (IVGTT) into awake rats or mice. During an ITT, animals are fasted for 5 h, and insulin (~0.5 U/kg body wt ip or iv) is administered. Blood samples are taken at 10, 20, 30, 60, 90, and 120 min following administration of glucose or insulin for measurement of plasma glucose and insulin concentrations. Blood samples are usually obtained from the tail or from an indwelling vascular cannula.

Assumptions and considerations. As mentioned previously, the OGTT and IPGTT provide useful information about glucose tolerance but not insulin sensitivity/resistance per se. Impaired glucose tolerance is reflected in a larger incremental area under the curve (AUC) of the plasma glucose disappearance curve. For the ITT, the rate and magnitude of decline of plasma glucose concentration in response to an injection of insulin provide an estimate of insulin sensitivity. These tests are frequently used to assess glucose tolerance and insulin sensitivity in mouse models of diabetes or insulin resistance with isolated and specific genetic changes.

Advantages, limitations, and appropriate usage. ITTs and GTTs can be conducted with more ease than the glucose clamp. However, unlike the clamp, these methods do not provide precise estimates of insulin sensitivity or tissue-specific glucose disposal. IPGTT has an advantage over OGTT in that it excludes incretin effects. In the case of ITT, the possibility of hypoglycemia and ensuing counterregulatory homeostatic mechanisms have the potential to confound estimates of insulin sensitivity. All of these tests can be stressful to the animals. In addition, as discussed before, imposing a nonphysiological fasting state in rodents may complicate interpretation of results. If assessing insulin sensitivity is of secondary interest in the metabolic profiling of animals or if feasibility issues preclude the use of the glucose clamp, ITT and GTT may be appropriate to use. However, investigators should be cognizant of the differences between the concepts of glucose tolerance and insulin sensitivity.

**Surrogates Derived from Fasting Steady-State Conditions**

Fasting insulin levels, HOMA-IR, and QUICKI have been used as surrogate measurements of insulin sensitivity in animals. However, validation of these surrogates against glucose clamp measurements has not been extensively conducted in animals. All surrogate indexes rely on the assumption that fasting glucose and insulin levels represent a basal steady-state condition. Thus, surrogate measures obtained from “fasting” states in rodents may not be as accurate as in humans. This is evident from modest correlations between surrogates and clamp estimates of insulin sensitivity in rats (95). In animals, where determination of insulin sensitivity is of secondary interest, surrogate indexes may be useful. However, further rigorous validation studies of these surrogate indexes are needed in animals.

Table 1. Current approaches for assessing insulin sensitivity and resistance in vivo

<table>
<thead>
<tr>
<th>Method</th>
<th>Measurement of Insulin Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct measurements</strong></td>
<td></td>
</tr>
<tr>
<td>Hyperinsulinemic euglycemic glucose clamp</td>
<td>Steady-state GIR = M. SI_Clamp = M/(G × ΔI), where M is normalized for G (steady-state blood glucose concentration) and ΔI (difference between fasting and steady-state plasma insulin concentrations)</td>
</tr>
<tr>
<td>IST</td>
<td>SSPG concentration during constant infusions of insulin and glucose with suppressed endogenous insulin secretion</td>
</tr>
<tr>
<td><strong>Indirect measurements</strong></td>
<td></td>
</tr>
<tr>
<td>Minimal model analysis of FSIVGTT</td>
<td>Minimal model uniquely identifies model parameters that determine a best fit to glucose disappearance during the modified FSIVGTT. Sr: fractional glucose disappearance per insulin concentration unit; So: ability of glucose per se to promote its own disposal and inhibit HGP in the absence of an incremental insulin effect (i.e., when insulin is at basal levels)</td>
</tr>
<tr>
<td><strong>Simple surrogate indexes</strong></td>
<td></td>
</tr>
<tr>
<td>Surrogates derived from fasting steady-state conditions</td>
<td>Reciprocal of fasting plasma insulin concentration, μU/ml</td>
</tr>
<tr>
<td>GIR ratio</td>
<td>Ratio of fasting plasma glucose (mg/dl) and insulin (μU/ml) concentration</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>HOMA-IR = [(fasting insulin (μU/ml) × [fasting glucose (mmol/l)])/22.5]</td>
</tr>
<tr>
<td>QUICKI</td>
<td>QUICKI = 1/Log (fasting insulin, μU/ml) + Log (fasting glucose, mg/dl)</td>
</tr>
<tr>
<td>Surrogates derived from dynamic tests (OGTT)</td>
<td></td>
</tr>
<tr>
<td>Matsuda index</td>
<td>SI(Matsuda) = 10,000/[ [(Gfasting (mg/dl) × Ifasting (μU/ml)) × (Gmean × Imean)]</td>
</tr>
<tr>
<td>Gut index: SI1(120, 120), mg·l2·mmol−1·mIU−1·min−1</td>
<td>SI(0, 120) = 75,000 + (G0–G120) × (Gmean)/0.19 × BW/120 × I120 × VD</td>
</tr>
<tr>
<td>Avignon index, SiM</td>
<td>SIM = [10.137 × Sib] + S12 h/2, where Sib = 106/(I0 (μU/ml) × G0 (mmol/dl) × VD)</td>
</tr>
<tr>
<td>Stumvoll index</td>
<td>SIStumvoll = 0.156–0.0000459 × I120 (pmol/dl) × 0.00321 × I0 (pmol/dl) × VD</td>
</tr>
</tbody>
</table>

GIR, glucose infusion rate; M, glucose disposal rate; IST, insulin suppression test; SSPG, steady-state plasma glucose; FSIVGTT, frequently sampled intravenous glucose tolerance test; Si, insulin sensitivity index; Sib, glucose effectiveness index; HGP, hepatic glucose production; GIR ratio, glucose/insulin ratio; HOMA, homeostasis model assessment; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; OGTT, oral glucose tolerance test; Gmean, mean plasma glucose concentration during OGTT; Imean, mean insulin concentration during OGTT; G120, plasma glucose concentration at 120 min; BW, body weight; I0, plasma insulin concentration during fasting; I120, plasma insulin concentration at 120 min; VD, glucose distribution volume (150 ml/kg BW).
CONCLUSIONS

This Frontiers article has examined a wide variety of methods currently available for estimating insulin sensitivity/resistance (but is by no means an exhaustive review). These range from complex, time-consuming, labor-intensive, invasive procedures to simple tests involving a single fasting blood sample (Table 1). It is important to understand the concepts underlying each method so that relative merits and limitations are appropriately matched to proposed applications. The glucose clamp method is the reference standard for direct measurement of insulin sensitivity. Regarding simple surrogates, QUICKI and log (HOMA) are among the best and most extensively validated. Dynamic tests are useful if information about both insulin secretion and insulin action are needed. As with all measurement techniques, correct interpretation of data from different methods for measuring insulin sensitivity requires a complete understanding of the technique.

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