How to measure hepatic insulin resistance?

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Abstract

The liver plays a pivotal role in energy metabolism. Under the control of hormones, especially insulin, the liver stores or releases glucose as needed by the body’s systems. It is also responsible for an important part of non-esterified fatty-acid and aminoacid metabolism. Assessing hepatic insulin resistance is almost always synonymous with measuring hepatic glucose production (HGP) and calculating indices of hepatic insulin resistance. The most frequently used method to this end is the isotope dilution technique using a tracer. Among tracers, stable isotope-labelled glucose molecules are particularly advantageous over radioactive isotope-labelled glucose and are, therefore, the tracers of choice. The tracer is infused either on its own after an overnight fast to evaluate fasting HGP, or with some among the usual insulin-sensitivity tests to assess HGP suppression by insulin and/or glucose. In a fasting state, HGP is easily calculated whereas, during insulin or glucose infusion, some formula are needed to correct for the non-steady-state condition. The hepatic insulin-resistance index is the product of HGP and the corresponding plasma insulin concentration. Although subject to error, the isotope dilution method nevertheless remains an irreplaceable tool for assessing hepatic insulin resistance in clinical research. From a practical point of view, some easily obtainable indices and clinical or biochemical parameters can serve as surrogates or markers of hepatic insulin resistance in clinical practice. Finally, drugs such as metformin or glitazones can improve hepatic insulin resistance, hence their use in hepatic insulin-resistant states such as type 2 diabetes and non-alcoholic fatty liver disease.

Résumé

Comment mesurer la résistance hépatique à l’insuline ?

Le foie joue un rôle central dans le métabolisme énergétique. Sous le contrôle des hormones, notamment l’insuline, il met en réserve ou produit du glucose en fonction des besoins de l’organisme. Il est aussi responsable d’une part importante du métabolisme des acides gras libres et des acides aminés. Évaluer l’insulinorésistance hépatique est presque toujours synonyme de mesurer la production hépatique de glucose (PHG) suivie du calcul des indices d’insulinorésistance hépatique. La méthode la plus fréquemment utilisée à cette fin est la technique de dilution isotopique utilisant un traceur. Le glucose marqué par un isotope stable est plus avantageux que celui marqué à l’isotope radioactif, et constitue le traceur de choix. Le traceur est perfusé soit isolément et à jeun pour évaluer la PHG de base, soit au cours de certains tests usuels de mesure de la sensibilité à l’insuline pour mesurer l’effet suppressif de l’insuline et/ou du glucose sur la PHG. À jeun, la PHG est facilement calculée, mais lorsque du glucose ou de l’insuline est perfusé, des équations sont nécessaires pour tenir compte de l’état de non-équilibre créé. L’index de résistance hépatique à l’insuline est le produit de la PHG et de l’insulinémie correspondante. Malgré les possibilités d’erreurs, la méthode de dilution isotopique reste un outil irremplaçable pour évaluer l’insulinorésistance hépatique en recherche clinique. D’un point de vue pratique, certains indices ou paramètres cliniques ou biochimiques facilement mesurables peuvent servir de substituts ou de marqueurs de l’insulinorésistance hépatique en pratique clinique. Enfin, certains médicaments comme la metformine et les glitazones améliorent l’insulinorésistance hépatique, d’où leur utilisation dans le diabète de type 2 ou la stéatose hépatique non alcoolique.

Keywords: Hepatic insulin resistance; Hepatic glucose production; Tracer; Isotope; Review.

Mots clés : Insulinorésistance hépatique ; Production hépatique de glucose ; Traceur ; Isotope ; Revue.

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1. Introduction

Insulin resistance is considered the primary defect underlying the development of type 2 diabetes [1,2] and associated diabetes subtypes [3]. It is a multisite dysfunction that involves the liver, skeletal muscle and adipose tissue, which are the body’s three main insulin-sensitive tissues [3-5]. Hepatic insulin resistance is of particular interest because it is a major determinant of fasting hyperglycemia and is consequently the major dysfunction in impaired fasting glucose, a prediabetic state [6].

The liver is the first organ to pick up nutrients that enter the body from the intestines after a meal and, therefore, plays a pivotal role in energy storage. Its major metabolic function is to maintain plasma glucose levels by storing exogenous carbohydrates after a meal and, later, by releasing glucose [7]. It also has an important role in protein metabolism as amino-acid catabolism occurs mainly in the liver. Indeed, it is the only organ capable of synthesizing urea to eliminate amino-acid nitrogen. Although lipids from a meal bypass the liver as they enter the circulation, the liver takes up non-esterified fatty acids from plasma, which are oxidized or esterified to form triacylglycerol. Glucose is, however, considered to be more important than fats as an energy substrate because it is the only energy source for the cells of tissues found in the brain, retina, blood and germinal epithelium of the gonads [8].

Discussions of hepatic insulin sensitivity are usually restricted to carbohydrate metabolism whereas insulin is also involved in fat and amino-acid metabolism. This should be borne in mind when considering that, in some insulin-resistant states such as non-alcoholic fatty liver disease (NAFLD), the liver may be resistant to insulin with respect to glucose production, but very insulin-sensitive for the synthesis of fatty acids and triacylglycerol from glucose [9].

This report reviews the hepatic metabolic pathways while paying special attention to those that depend on insulin control. Also, among the in vivo tests that are used to assess insulin sensitivity, we discuss those that allow hepatic insulin sensitivity to be explored while addressing some practical aspects. Finally, we briefly discuss the involvement of hepatic insulin in various diseases and its ensuing practical implications.

2. Role of the liver in energy metabolism

Carbohydrates and lipids are the major substrates of energy production. Hepatocytes constitute a large chemically reactive pool with a high rate of metabolism, sharing substrates and energy from one metabolic system to another [8]. Thus, the liver is a central organ for carbohydrate, lipid and amino-acid metabolism. As for carbohydrate metabolism, the liver plays the role of a ‘glucose-buffering system’ [8] in that it takes up glucose and stores it in the form of glycogen when blood glucose concentration rises, and releases it back into the blood when blood glucose concentration falls. Non-esterified fatty acids (NEFA) are the main form of lipids taken up by the liver and oxidized or esterified into triglycerides which, in turn, are either used to synthesize very low-density lipoproteins (VLDL) or are transiently stored within hepatocytes [7,10]. The liver is also the main site of protein synthesis and amino-acid catabolism. The role of insulin in the regulation of different steps of carbohydrate and NEFA metabolism in the liver is far more important and better understood than it is in protein metabolism [10].

2.1. Carbohydrate metabolism in the liver and its control by insulin

The liver performs two major functions that are reciprocally regulated by insulin and glucagon: (1) glucose storage into glycogen; and (2) glucose production by glycogenolysis and gluconeogenesis, essential processes for maintaining plasma glucose during fasting. Numerous other functions, such as the conversion of galactose and fructose to glucose, and the conversion of excess glucose into fatty acids when glycogen-storing capacity is overtaken, also take place in the liver [8]. In addition, as with many other cell types in the body, glycolysis takes place in hepatocytes to provide energy.

2.1.1. Glucose storage

When glucose concentration outside the liver rises—for instance, during or after a meal—glucose is rapidly taken up into hepatocytes, especially the periportal cells, via GLUT2 [10,11]. GLUT2 transporters have a high $K_m$ and are not sensitive to insulin. In the hepatocyte, glucose is temporarily ‘trapped’ by phosphorylation to glucose-6-phosphate, a reaction catalyzed by hepatic glucokinase, which also has a high $K_m$ (around 12 mmol/L) and a high capacity, and is unaffected by insulin—at least in the short term [10]. Thus, the process of glucose uptake and phosphorylation by the hepatocyte depends on the glucose concentration outside of the cell. The role of insulin is crucial in the subsequent steps of the storage process. It stimulates glycogen synthase, the rate-controlling enzyme responsible for polymerization of glucose to glycogen [12]. Insulin also inhibits glycogen phosphorylase, which catalyzes glycogen breakdown, although glucose is the most potent inhibitor of glycogen phosphorylase [12,13]. The liver can then store up to the equivalent of 5-6% of its weight in glycogen, which is about 100 g [8]. When the quantity of glucose entering the liver exceeds the hepatocyte glycogen-storing capacity, insulin promotes the conversion of all the excess glucose into fatty acids that are subsequently packaged as triglycerides [8,14]. This process, known as $de$
**2.1.2. Hepatic glucose production**

In the postabsorptive state, the liver is responsible for at least 75% of the total endogenous glucose production. In humans, the hepatic glucose production (HGP) rate is around 2 mg/kg body weight/min [11]. HGP originates from two mechanisms: glycogenolysis and gluconeogenesis. Glycogenolysis—glycogen breakdown to release glucose—depends on two key enzymes: glycogen phosphorylase and glucose-6-phosphatase. Glycogen phosphorylase breaks down glycogen into glucose-1-phosphate which, in turn, is converted in a reversible reaction to glucose-6-phosphate (G6P) by phosphoglucomutase, and glucose-6-phosphatase then dephosphorylates to produce glucose, which diffuses out of hepatocytes [8,10]. Gluconeogenesis is glucose synthesis *de novo* from non-carbohydrate precursors—namely, lactate, amino acids (especially alanine) and glycerol. If fasting is prolonged, the relative contribution of gluconeogenesis in relation to total HGP increases. When labelled nuclear magnetic resonance (MR) spectroscopy was used to measure glycogenolysis rate, the average relative contribution of gluconeogenesis rate (obtained by subtraction of glycogenolysis from HGP) was 64% during the first 22 hours of fasting, 82% after 22-46 hours and 96% after 46-64 hours of fasting [18]. The key enzyme of gluconeogenesis is phosphoenolpyruvate carboxykinase (PEPCK). Glycogen phosphorylase, glucose-6-phosphatase and PEPCK are inhibited by insulin and activated by glucagon.

**2.1.3 Effects of insulin on hepatic glucose production**

Insulin inhibits HGP through direct and indirect effects. Insulin binds to receptors on the hepatocyte membrane and, in the course of its signalling pathway, it also inhibits the enzymes involved in gluconeogenesis and glycogenolysis, the two mechanisms of HGP [19,20]. These direct effects have been well demonstrated in dogs and have been recently shown to dominate the indirect effects [21]. SREBP-1c mediates the inhibitory effect of insulin on the PEPCK gene, the central enzyme of gluconeogenesis [22]. Indirect inhibitory effects of insulin on HGP include: (1) inhibition of glucagon secretion by acting on islet alpha cells; (2) inhibition of lipolysis and proteolysis in muscle, and of lipolysis in adipose tissue, thereby decreasing the availability of gluconeogenic precursors; and (3) central action through the hypothalamus [19].

**2.2. Fatty acid metabolism**

NEFA taken up by the liver have two major fates: β-oxidation or esterification [10]. β-oxidation splits NEFA into acetyl coenzyme A (acetyl-CoA). Acetyl-CoA either enters the tricarboxylic acid cycle in hepatocyte mitochondria to produce energy—used, in particular, for energy-demanding pathways such as gluconeogenesis—or is condensed into ketone bodies (acetoacetate and hydroxybutyric acids) and exported in the circulation to other cells [8,10]. In these cells, ketone bodies are converted back to acetyl-CoA and used in the tricarboxylic acid cycle to provide energy. Esterification of NEFA forms triglycerides, which are stored in the hepatocyte and used for VLDL formation. The balance between β-oxidation and esterification is controlled by insulin and glucagon [10]. Insulin promotes storage either directly, by activating triglyceride synthesis, or indirectly by its ‘fat-sparing’ effects (insulin does not favor the use of NEFA as energy substrates), whereas glucagon promotes oxidation.

**2.3. Amino-acid metabolism**

Amino acids are not direct energy substrates *per se*. The major pathways of amino-acid metabolism in the liver (such as protein synthesis, deamination, interconversion of amino acids and formation of urea for removal of ammonia) are not intended to produce energy [8]. However, some amino acids, such as alanine, are substrates for gluconeogenesis and, therefore, contribute indirectly to energy production. Insulin promotes protein synthesis, and inhibits amino-acid uptake and use as gluconeogenic substrates.

**3. Overview of methods used to assess insulin sensitivity in humans**

Liver, skeletal muscle and adipose tissue are the three major insulin-sensitive organs involved in glucose homeostasis. Some common methods and indices used to evaluate insulin sensitivity in terms of glucose metabolism are:

- clamps (euglycemic-hyperinsulinemic, and hyperglycemic);
- frequently sampled intravenous glucose tolerance test with minimal modelling;
- indices calculated from oral glucose tolerance test;
- indices computed from fasting plasma insulin and glucose, such as HOMA;
- insulin-sensitivity test;
4. Methods to measure hepatic insulin resistance

Before choosing which test to use for hepatic insulin-resistance assessment, the first question should be: Which pathway of insulin-sensitive hepatic metabolism (glucose or fatty-acid metabolism) is being explored? When it comes to glucose metabolism itself, another question raised is: Which fate of glucose (glycogen synthesis or fatty-acid synthesis) is being studied? Most techniques focus on carbohydrate metabolism by measuring HGP rate. Although HGP is clinically the most useful indicator of hepatic insulin resistance because of the spectrum of diseases in which its study can be applied, it should be remembered that, in some ‘insulin-resistant’ states such as NAFLD, the resistance of insulin to suppress HGP is reflected by the sensitivity of insulin to stimulate NEFA synthesis from glucose. Apart of direct methods that can be used to measure HGP or NEFA uptake and reesterification by the liver, metabolic indices and liver imaging features may be used as surrogates or correlates, and clinical and biological parameters may be used as markers of hepatic insulin resistance.

4.1. Direct measurement of HGP, and NEFA uptake and reesterification

Measurement of HGP is by far the most commonly used method of assessing hepatic insulin resistance. HGP itself is measured as part of glucose turnover. Three direct techniques can be used: (1) the arteriovenous-difference technique; (2) the isotope dilution technique; and (3) labelled nuclear MR spectroscopy.

4.1.1. The arteriovenous-difference technique

Also known as Fick’s principle, this consists of the simultaneous measurement of liver blood flow, and the difference between arterial and venous glucose concentrations [27]. HGP is then calculated as the product of the two parameters [28]. Although it measures the net hepatic glucose output, Fick’s principle is invasive, as it requires venous and arterial catheterization. Moreover, the liver incoming blood flow originates from two sources, the portal vein and the hepatic artery. These reasons preclude its practical application.

4.1.2. The isotope dilution technique

This is the most widely used technique. Depending on the tracer used, it can study hepatic insulin sensitivity through glucose metabolism (measurement or HGP) or NEFA metabolism (measurement of NEFA uptake and reesterification by the liver).

For glucose metabolism, one or many tracers are infused either alone or during glucose or insulin administration to estimate basal or suppressed HGP. Various ‘indices of hepatic insulin resistance’ are then calculated. Among the methods for assessing insulin sensitivity, the labelled euglycemic–hyperinsulinemic clamp is the most frequently used for measuring hepatic insulin sensitivity in response to glucose and insulin infusions. However, minimal modelling during the labelled intravenous glucose tolerance test can be used as well [29,30]. Multiple (dual- or triple-) tracer approaches can also provide more precise measurements of glucose metabolic pathways. For instance, using labelled gluconeogenic precursors (13C-lactate or 13C-glycerol) or measuring the incorporation of 2H2O (deuterated water) allows estimation of gluconeogenesis and, therefore, its relative contribution to HGP [31]. In addition, labelled mixed meal or oral glucose can also be used in a dual- or triple-tracer approach to evaluate the postprandial or post-load outcome of ingested glucose [32,33].

- 4.1.2.1. Tracers used to study HGP

**Definition:** A tracer is a labelled form of a substance [34]. To label a substance, atoms in the unlabelled form of that substance are replaced by their rare isotopes. Metabolically speaking, a tracer is used to study another substance, usually a naturally available molecule in the organism where the tracer is being introduced. The label makes the tracer detectable by the observer. Its structure and metabolic outcome should be identical to that of the molecule studied and, ideally, it should not interfere with the normal behavior of the system being studied [28,34]. These conditions are achieved by using the tracer in very small quantities. In glucose metabolism, glucose itself is usually labelled and used as a tracer. In labelled glucose, the isotope may be an atom of hydrogen or carbon, it may be stable or radioactive and it may be located at any of the six carbon atoms of the glucose molecule.

**Choice of tracer:** Hydrogen- vs carbon-labelled glucose: To label the glucose molecule, either hydrogen is replaced by 3H (deuterium) or 4H (tritium), or carbon is replaced by 13C or 14C [28]. In hydrogen-labelled glucose, the major labelled degradation product is deuterated or tritiated water. The reincorporation of labelled water in glucose is unlikely—in other words, when a hydrogen-labelled tracer enters a metabolic pathway, it is totally cleared from the body. Thus, it is called an ‘irreversible’ (non-recycling) tracer, as opposed to a ‘reversible’ (recycling) tracer such
as carbon-labelled glucose [28,31]. When glucose is carbon-labelled, lactate, the major metabolic product of glucose degradation, is labelled and may be reincorporated in glucose through gluconeogenesis. This leads to an underestimation of glucose turnover, and specific methods are needed to take this recycling into account.

Position of the isotope in the glucose molecule: The position of the isotope is a matter only for hydrogen-labelled glucose. In the liver, chemical reactions may occur concomitantly with their opposites, but catalyzed by different enzymes: for example, the first steps of glycolysis occur simultaneously with their opposites, the last steps of gluconeogenesis [28]. In this case, a molecule of glucose may undergo one or two steps of glycolysis and lose the label, then enter the reverse reaction of gluconeogenesis and be released from the liver as a newly synthesized glucose molecule. As the marker has been lost, this will be counted as used glucose molecule whereas no molecule has been used until the energy producing step, thus leading to overestimation of glucose release. Labelling hydrogen atoms can be located on any carbon, but the most common sites are positions 2 (C-2) ([2-2H]glucose or [2-2H]lactate), 3 (3-2H)glucose or [3-2H]lactate) or 6 (6,6-2H2]glucose or [6,6-2H2]lactate). The Figure 1 is based on the assumption that 2H is the isotope and C-2, C-3 and C-6 are all labelled [31]. 2-2H is released precociously in the second step of glycolysis, when glucose-6-phosphate is converted to fructose-1,6-biphosphate; 3-2H is released later, in the fourth step of glycolysis, when glucose-6-phosphate is converted to dihydroxyacetone phosphate; G3-P: glyceraldehyde-3-phosphate; 2-2H, 3-2H, 6-2H: H loss from C-2, C-3, C-6, respectively.

Table 1

Comparison of stable- and radioactive-isotope tracers for metabolic purposes (adapted from Solomon [37]).

<table>
<thead>
<tr>
<th>Radioisotopes</th>
<th>Stable isotopes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety</td>
<td>Some risk, especially for pregnant or lactating women, and children</td>
</tr>
<tr>
<td>As tracers</td>
<td>True tracers, as they are not naturally present</td>
</tr>
<tr>
<td>Study time</td>
<td>Half-life of the radioisotope can affect duration of study</td>
</tr>
<tr>
<td>Combination of tracers in one study</td>
<td>Generally only one radioisotope is given</td>
</tr>
<tr>
<td>Analysis</td>
<td>Sample analysis must be timely, based on the half-life of the isotope; sample preparation minimal; tracer and trace are measured by different techniques</td>
</tr>
</tbody>
</table>

Fig. 1. Level of release of 2H during glycolysis depends on the carbon location in the glucose molecule (adapted from Coggan [31]).

[2H]Glucose, 2H-labelled glucose on C-2, C-3, C-6; Glucose-6-P: glucose–phosphate; Fructose 1,6-biP: fructose 1,6-biphosphate; DHAP: dihydroxyacetone phosphate; G3-P: glyceraldehyde-3-phosphate; 2-2H, 3-2H, 6-2H: H loss from C-2, C-3, C-6, respectively.
• 4.1.2.2. Basic principles and technical procedures

Concepts and terminology: Assumptions: For mathematical purposes, the constituents of a living system can be represented as being located in distinguishable volumes called ‘pools’ or ‘compartments’ [34]. Some basic assumptions govern tracer experiments, but they are not always valid in all circumstances and, thus, there may be a need for appropriate corrections. The first basic assumption is that the tracer element follows its unlabelled isotope faithfully in all biological reactions [38]-in other words, the metabolic behavior of the tracer is the same as that of its unlabelled counterpart [34]. However, an isotope effect is to be expected and has been demonstrated in the rates of certain reactions, particularly those involving $^{13}$C and isotopes of hydrogen. However, for hydrogen-labelled tracers, the isotope effect is usually negligible. The second assumption is that, within a given compartment, the substance being studied is uniformly distributed at all times. This assumption implies instantaneous and homogeneous mixing within the compartment, and is invalid for many physiological conditions [38].

Terminology: “Steady-state”: This term applies to compartments where the rates of removal of the substances under study are equal to the rates of replacement, so that the concentrations and amounts of the substances are constant during the period of observation. Such a situation is obtained after an overnight fast, when a primed constant infusion of tracer is performed for at least 60 min—the amount of time it takes the tracer to become completely mixed with the body glucose pool. Blood glucose is very constant, and the glucose produced by the liver is assumed to equal glucose uptake by peripheral tissues, especially the brain. “Non-steady-state”: When exogenous insulin or non-labelled glucose is introduced into the system either orally or intravenously, this creates a disequilibrium within the system that is called a ‘non-steady-state’. To account for this, formulas such as Steele’s equation [39] are used to calculate HGP. The non-steady-state is of major importance as it reflects what actually occurs in the physiological state most of the time.

Technical procedures: The model described here includes the use of a stable-isotope tracer. To measure basal (fasting) HGP, the tracer is infused alone after an overnight fast either as a single injection, or as a priming bolus followed immediately by a continuous infusion (primed constant infusion), which is the most suitable method for administering the tracer [40] because it allows a simpler estimation of HGP [41]. With the single bolus injection commonly used in the past, estimation of HGP was more complicated, and tended to produce too-high values for the body’s glucose pool [40]. Blood samples for measuring fasting HGP should be collected after at least 60 min of constant tracer infusion, the time needed for the priming dose to completely mix with the glucose pool. This was described in dogs using $^{14}$C, where the plasma glucose-specific activity reached a plateau and remained stable after 60 min of constant tracer infusion [40]. Sample analysis is performed by mass spectrometry that allows measurement of both the tracer and tracee, and the tracer enrichment is calculated as the plasma tracer concentration divided by the plasma tracee concentration.

Measurement of HGP under suppression (glucose and/or insulin administration) can be performed during a labelled euglycemic–hyperinsulinemic clamp or a labelled frequently sampled intravenous glucose tolerance test (FSIVGTT). Measuring HGP under such conditions gives an idea of the dynamics of liver response to the suppressive effects of glucose and/or insulin. During a euglycemic clamp, for instance, the constant tracer infusion is used at the same rate as during fasting, and is maintained until the end of the test. Blood samples collected during the last 20 or 30 min of the clamp, or from each clamp step in the case of a multistep clamp, are used to measure plasma tracer enrichment by mass spectrometry under non-steady-state conditions.

• 4.1.2.3. Calculation of HGP rate and indices of hepatic insulin resistance

Calculation of HGP rate: Basal HGP rate corresponds to the hepatic response to physiological plasma insulin. To facilitate estimations, a one-compartment model with constant volume is used (Fig. 2). Based on the above-mentioned assumptions, under steady-state conditions, the glucose rate of appearance (Ra) equals its rate of disappearance (Rd); and the ratio of plasma tracer/tracee (C*/C), which corresponds to tracer enrichment ($\varepsilon$), is equal to the ratio of tracer infusion rate/glucose rate of appearance (Ra*/Ra). Thus, $\varepsilon = \frac{Ra*}{Ra}$ (Ra = Ra*/$\varepsilon$). As Ra corresponds to the HGP rate in steady-state, HGP = Ra*$/\varepsilon$.
HGP measured during the clamp is an estimate of hepatic response to supraphysiological insulin concentrations (residual HGP). Under non-steady-state conditions created by glucose and insulin infusion, special models are needed to calculate the residual HGP. The most widely used of these models is Steele’s equation [39], which proposes a monocompartmental model with constant volume. Computation is based on the derivation of tracer and tracee measurements performed in plasma. For more details, readers are referred to Steele et al. [39]. Another, more complex, model was proposed by Radziuk et al. [42], and a more recent model that accounts for the error in Steele’s equation has also been proposed to estimate HGP in non-steady-state conditions [43].

When HGP is measured during labelled FSIGTT, the HGP rate is obtained by deconvolution using minimal modelling [29,44].

Indices of hepatic insulin resistance: The HGP rate offers an idea of hepatic resistance to the suppressive action of insulin on glucose production: the higher the HGP rate, the higher the hepatic insulin resistance. To provide a more metabolically significant estimate of hepatic insulin resistance, HGP should be related to plasma insulin concentration. Hepatic insulin resistance indices are calculated as the product of HGP rate and the corresponding plasma insulin concentration [6,45] either at baseline (basal HGP × fasting plasma insulin) or during the plateau phase of the clamp.

• 4.1.2.5. Limitations of HGP measurement

It should be noted that, throughout the above-described procedures for measuring HGP, a number of inconsistencies make the isotope dilution method less than perfect, although it nevertheless remains one of the best available tools for studying hepatic glucose metabolism in vivo. Indeed, what we measure using the tracer is, in reality, endogenous glucose production, and it should be borne in mind that the liver is not the only glucose-producing organ during fasting conditions. The kidney cortex produces glucose by gluconeogenesis, and its relative contribution to endogenous glucose production in the postabsorptive state is estimated to range from 5% to 28% [19, 46-48]. Also, as already stated above, the monocompartmental model and the assumptions that constitute the basic principles of tracer methodology may be subject to error. Another inconsistency is the computation of HGP rate using Steele’s equation, which often generates negative values of HGP during the euglycemic clamp perhaps due to an error in the equation itself. These negative values are assumed to correspond to zero—that is, complete suppression of HGP. For instance, in our team’s study of insulin sensitivity in Africans with ketosis-prone diabetes vs controls [3], 22 out of 32 participants had negative HGP values during the high-dose (80 mU.m⁻².min⁻¹) insulin-infusion clamp step.

• 4.1.2.6. Isotope dilution technique to study NEFA metabolism

The principles and procedures for studying NEFA turnover are, in general, the same as for the study of glucose metabolism. To measure hepatic insulin sensitivity to plasma NEFA (released from adipose tissue triglycerides) uptake and reesterification, a labelled fatty acid is infused, and the relative and absolute contributions of labelled triglyceride secreted from the liver measured. The test can also be performed at baseline and in response to insulin. Labelled palmitate ([1-13C]palmitate or [1,2,3,4-13C₄]potassium palmitate) has been used to this end to show that, in patients with NAFLD compared with control subjects, the relative contribution of triglycerides produced by de novo lipogenesis to the total secreted triglycerides released by the liver was increased at the expense of triglyceride synthesized from NEFA reesterification [17,49].

4.1.3. Labelled nuclear magnetic resonance spectroscopy

13C-labelled nuclear MR spectroscopy has been used to measure net hepatic glycogen synthesis and glycogenolysis in humans [13,18]. Net hepatic glycogenolysis is calculated as the slope of glycogen concentration decrements over a period of time, multiplied by liver volume (measured by MR imaging) [18]. When combined with the isotope dilution technique, it allows calculation of the relative contribution of glycogenolysis and gluconeogenesis (obtained by subtraction of glycogenolysis rate from total HGP rate) either during fasting, or in response to insulin or glucagon [50].

MR spectroscopy is also the technique used to measure hepatic triglyceride content (HTGC). An increased HTGC is the hallmark of NAFLD as well as a good correlate of hepatic insulin resistance.

4.2. Surrogates, correlates and clinical markers of hepatic insulin resistance

Many metabolic indices and features of liver imaging may correlate well with the index of hepatic insulin resistance obtained by the isotope dilution method, while some biochemical parameters may be associated with the increased needs of exogenous basal insulin doses in type 2 diabetic patients (supposedly a suppressor of HGP). In addition, anthropometric measurements may be associated with hepatic insulin-resistant states. These indices, features and parameters may therefore, be considered either surrogates, correlates, or clinical markers of hepatic insulin resistance. Their importance in clinical practice is that, as direct methods are reserved for clinical research and cannot be used in routine practice, these markers may therefore serve as screening or diagnostic tools for hepatic insulin-resistant conditions.
HGP is almost the only source of plasma glucose in the fasting state. As HGP is normally inhibited by insulin, fasting plasma insulin or indices of basal (fasting) insulin resistance are good correlates of hepatic insulin resistance and may be used as surrogates. For instance, the HOMA-IR has been shown to correlate well ($r = 0.64$) with the basal hepatic insulin-resistance index [51].

A good correlation ($r = 0.64$) with the basal hepatic insulin-resistance index was also reported recently for an index obtained with the oral glucose tolerance test (OGTT). This index was computed as the product of the total area under the curve (AUC) for glucose and insulin during the first 30 min of the OGTT ($\text{glucose}_{0-30}[\text{AUC}] \times \text{insulin}_{0-30}[\text{AUC}]$) [51].

Hepatic triglyceride content measured by MR spectroscopy to ascertain NAFLD is also well correlated with the hepatic insulin-resistance index, and was recently reported to be a strong predictor of insulin action in liver, skeletal muscle and adipose tissue [52,53].

Alanine aminotransferase (ALT) is an enzyme secreted by hepatocytes. In the LANMET study, comparing insulin glargine with NPH insulin as basal insulin treatment in metformin-treated type 2 diabetics, serum ALT levels were among the positive predictors of basal insulin dose in these patients [54]. For this reason, serum ALT levels may be a rough clinical marker of hepatic insulin resistance.

Furthermore, features of the metabolic syndrome have been shown to be highly prevalent in patients with NAFLD [55], including central obesity (waist circumference $> 102\, \text{cm}$ in men and $> 88\, \text{cm}$ in women) (47%), hypertriglyceridemia $> 2\, \text{mmol/L}$ (47%) and levels of HDL cholesterol $< 1\, \text{mmol/L}$ (43%). These parameters may also be useful markers or predictors of hepatic insulin resistance in clinical practice.

5. Hepatic insulin resistance in diseases

Hepatic insulin resistance is an underlying factor or feature of many non-communicable diseases or syndromes that, in turn, may be related between each other. Non-alcoholic fatty liver disease (NAFLD) is an increasing health problem that is associated with insulin resistance, the metabolic syndrome, type 2 diabetes and other conditions [55,56] (this topic is discussed in depth in the other articles in this issue). In patients with NAFLD, basal HGP rates may be similar [55,57] or higher [58] compared with those of control subjects, but the hepatic insulin-sensitivity index or insulin-mediated HGP suppression is concordantly impaired. More important, intrahepatic triglyceride (IHTG) content, measured by MR spectroscopy to ascertain NAFLD, correlates well with the hepatic insulin-resistance index, and was recently reported to be a strong predictor, independent of body mass index (BMI), of insulin action in liver, skeletal muscle and adipose tissues [52,53]. In patients with NAFLD, pioglitazone, an antidiabetic drug, was shown to decrease hepatic fat content by 54%, and to improve hepatic insulin sensitivity by 47% as well as biochemical markers of hepatic insulin resistance [33]. This study was of special interest as they used a double-tracing technique (intravenous and orally labelled glucose).

In type 2 diabetes, HGP is often increased, and contributes to fasting and postprandial hyperglycemia. The increased HGP is attributed to an increased rate of gluconeogenesis [59,60] and impaired glycogen metabolism [61]. The oral antidiabetic agent metformin decreases HGP through inhibition of hepatic gluconeogenesis [60].

In liver cirrhosis, glucose metabolism is impaired, yet HGP rates are similar to those in normal subjects [62]. Therefore, there is no hepatic insulin resistance per se in cirrhosis; instead, there is a disequilibrium in the glucose-producing mechanisms, with gluconeogenesis being increased at the expense of glycogenolysis [62].

6. Conclusion

Hepatic glucose production and the ensuing index are the best clinically accessible indicators of hepatic insulin resistance which, in turn, is an important pathogenic factor or feature of type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and the metabolic syndrome. Measuring HGP requires methods that use isotope-labelled glucose, but caution should be used in light of the dangers of radioactive isotopes to specific patient populations and to the environment. Although subject to error, the method, in use for more than half a century, nevertheless remains an irreplaceable tool for assessing hepatic insulin resistance in clinical research when used rigorously. From a practical point of view, there are easily obtainable indices and clinical or biochemical parameters that can serve as surrogates or markers of hepatic insulin resistance in clinical practice. Finally, drugs such as metformin or glitazones can improve hepatic insulin resistance and are, therefore, of value in hepatic insulin-resistant conditions such as NAFLD.

Conflicts of interest: The authors have none to declare.

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