

Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats

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ABSTRACT

Objective: The present study aimed to validate homeostasis model assessment of insulin resistance (HOMA-IR) in relation to the insulin tolerance test (ITT) in a model of insulin-resistance in Wistar rats induced by a 19-week high-fat diet. **Materials and methods:** A total of 30 male Wistar rats weighing 200-300 g were allocated into a high-fat diet group (HFD) (55% fat-enriched chow, *ad lib*, n = 15) and a standard-diet group (CD) standard chow, *ad lib*, n = 15), for 19 weeks. ITT was determined at baseline and in the 19th week. HOMA-IR was determined between the 18-19th week in three different days and the mean was considered for analysis. Area under the curve (AUC-ITT) of the blood glucose excursion along 120 minutes after intra-peritoneal insulin injection was determined and correlated with the corresponding fasting values for HOMA-IR. **Results:** AUC-ITT and HOMA-IR were significantly greater after 19th week in HFD compared to CD (p < 0.001 for both). AUC-OGTT was also higher in HFD rats (p = 0.003). HOMA-IR was strongly correlated (Pearson's) with AUC-ITT r = 0.637; p < 0.0001. ROC curves of HOMA-IR and AUC-ITT showed similar sensitivity and specificity. **Conclusion:** HOMA-IR is a valid measure to determine insulin-resistance in Wistar rats. Arch Endocrinol Metab. 2016;60(2):138-42

Keywords

HOMA-IR; insulin-resistance; insulin tolerance test

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INTRODUCTION

Although the hyperinsulinemic euglycemic glucose clamp (HEGC) (1) is the gold-standard method to evaluate insulin sensitivity and resistance in research, important issues such as high-cost, need for pump-infusion equipment, considerable expertise and length greatly limit its clinical applicability (2). Thus, a simpler, less expensive, and less time-consuming alternative method is desirable to evaluate insulin-sensitivity in both clinical practice and experimental research.

Among surrogate methods available to evaluate insulin-sensitivity, the homeostasis model assessment of insulin resistance (HOMA-IR) (3) is the most popular for epidemiological studies, and has been largely validated against the HEGC (4-7). HOMA-IR was developed in 1985, by Matthews (3), as a mathematical model that includes interactions between fasting plasma insu-

lin and fasting plasma glucose concentrations, with a strong correlation with HEGC. HOMA-IR is a simple and particularly helpful tool in the assessment of insulin resistance in epidemiological studies, including subjects with both glucose intolerance, mild to moderate diabetes, and in other insulin-resistance conditions (4-6). The applicability of HOMA-IR in experimental research, is questioned because of lack of data for validation in most animal species (7). The possibility of evaluating insulin sensitivity in animals using a simpler and less traumatic method is, thus, highly interesting for experimental research. In the present study, we examine the applicability of HOMA-IR in experimental research, validating it against the classical insulin tolerance test (ITT), a well-validated method to determine insulin-sensitivity (8-13), in a set of Wistar rats that were made insulin-resistant by a high-fat diet.

MATERIALS AND METHODS

Design

We conducted a controlled experiment in which 15 rats were submitted to a high-fat diet (HFD) for 19 weeks, and 15 rats were maintained in standard chow (CD) *ad libitum*. The HFD diet was composed of a mix of 45% of standard chow and 55% of swine lard. The objective was to induce insulin-resistance in some animals to optimize a study of association between ITT and HOMA-IR. Baseline ITT was performed in all rats. Between the 18th and the 19th week, animals were submitted to 5 tests in separate days along the same week. These tests included a second ITT determination, an oral glucose tolerance test (OGTT), and 3 subsequent fasting HOMA-IR determinations in 3 different days, in sequence.

Animals

A total of 30 male Wistar rats weighing 200-300 g were included. Animals were housed in pairs at controlled room temperature in a 12h light-dark cycle with food and water *ad libitum*.

Insulin Tolerance Test (ITT)

ITT was performed at the beginning of the study and after the 19th week. After a 12-h overnight fast, animals were weighted and blood samples collected from the tail for serial blood glucose determinations. Regular human insulin 0.5 IU/kg (HumulinTM Eli Lilly, São Paulo, Brazil) was injected by intra-peritoneal route, and blood samples were collected at baseline and after 15, 30, 45, 60, 90 and 120 minutes. The area under curve (AUC-ITT) of blood glucose levels between the baseline and 60 minutes, corresponding to the lowest glucose value (nadir), was considered for calculation.

Oral Glucose Tolerance Test (OGTT)

OGTT was obtained only in the day after the ITT, in the 18th to 19th week. After a 12-h overnight fast and baseline sampling, a 2 g/kg glucose solution was administered orally by gavage, followed by 30, 60, 90 and 120 minutes collections of blood samples from the tail for blood glucose determination. All procedures were performed following institutional Animal Welfare Guidelines and were approved by the Ethics Committee in Animal Research at Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil. The area under the curve determined by glucose

levels at baseline and 120 minutes after glucose overload was considered for calculation of AUC-OGTT.

HOMA-IR determination

In the 3 following days, 12h fasting blood samples were obtained for serum insulin and plasma glucose determinations in order to calculate the HOMA-IR. Blood samples for insulin were collected from the retro-orbital artery using a glass cannula and placed directly into an Eppendorf tube. It was then immediately placed in an ice-bath and centrifuged at 1000 x g for 10 minutes at room temperature, according to the manufacturer's instructions. Serum was stored at -80°C until the assay. Rat insulin assay determinations were done by the luminescence method (LuminexTM) using kits purchased from Linco ResearchTM (MilliporeTM, Billerica, MA), with detection limit 28 pg/mL.

Blood samples for glucose were obtained from arterial blood collected from the tail. Blood glucose was determined by the glucose oxidase method, using glucose strips (MedisenseTM Optium Xceed Meter).

The whole procedure lasted for a maximum of 2-hours for all 30 rats, which were tested for the same experiment in a random initial sequence. The sequence was randomly defined for the following days in order to minimize the effect of fasting time. HOMA-IR was determined by the formula (5):

$$HOMA\ IR = \frac{\text{serum insulin (mmol/L)} * (\text{blood glucose (mmol/L)})}{22.5 \cdot \text{Insulin assay}}$$

Statistical analysis

AUC-ITT and AUC-OGTT were determined by the trapezoidal method with NCSSTM 2007 software (NCSS, LLC, Kaysville, UT). Pearson's correlation test and Simple Linear Regression was used to determine association between HOMA-IR and AUC-ITT. We considered the mean of the three determinations of HOMA-IR and analyzed it in two ways: 1: considering all values and 2: ruling out the outlier value when adequate. In order to compare HOMA-IR sensitivity and specificity in relation to AUC-ITT, we compared ROC curves for mean HOMA-IR and AUC-ITT, at different cut-off points, and a kappa value for the comparison was obtained. Student T-test was performed to compare the mean of body weight, FPG, 2hPG, AUC-OGTT, and HOMA-IR between groups. Non-parametric Mann-Whitney Test for independent samples was performed to compare the median of AUC ITT and

fasting insulin variables between groups at baseline and in the 19th week. We used the SPSS™ software version 21 (IBM Cooperation, Somers, NY) for the statistical analyses. Significance was set at *p* value of 0.05.

RESULTS

At baseline, control diet (CD) and high-fat diet (HFD) groups did not differ in relation to AUC-ITT, fasting glucose, and body weight. At the 19th week, body weight was similar between groups, however, AUC-ITT, fasting insulin and HOMA-IR were significantly greater in HFD than in CD group (Table 1) (*p* < 0.001). Also, 2h-glucose post-OGTT and the AUC-OGTT were higher in HFD rats (*p* < 0.001) at the 19th week, while fasting plasma

glucose did not differ between groups (Table 1). Mean HOMA-IR for three determinations was higher in the HFD group at the 19th week (*p* < 0.001). The mean coefficient of variance of HOMA-IR was 34.8%. HOMA-IR was strongly correlated with AUC-ITT (*p* < 0.0001 and *p* = 0.0248). The best correlation was found when the mean HOMA-IR for three determinations was considered and the outlier value (if present) was ruled out: (*r* = 0.637; *p* < 0.0001) (Figure 1A). Agreement between AUC-ITT and HOMA-IR ROC curves was highly significant (Kappa = 0.469; *p* = 0.009) (Figure 2). The best agreement for the curves was at the highest HOMA-IR values above the median. A 90% sensitivity was observed considering the cut-off value for AUC-ITT > 6200 and HOMA-IR > 3.9.

Table 1. Weight and metabolic parameters at baseline and after 19 weeks

n	Control diet		High-fat diet		p1	p2
	Baseline	19 th week	Baseline	19 th week		
	15	15	15	15		
Body weight (g)	392.9 ± 31.7	493.6 ± 34.1	385.9 ± 32.6	507.5 ± 47.3	0.525	0.332
AUC ITT	8437.5 [7965.5-9718.3]	6015 [5938.3-6818.7]	7687.5 [7296.1-8419.9]	8145 [7317.7-8525.3]	0.072	< 0.001
FPG (mg/dL)	82 ± 9	94 ± 8	81 ± 5	95 ± 6	0.616	0.866
2hPG (mg/dL)	-	113 ± 12	-	131 ± 14	-	< 0.001
AUC OGTT	-	12782 ± 1056.6	-	14172.2 ± 1303.7	-	0.003
FI (pmol/L)	-	455.8 [437.2-716.8]	-	818.9 [668.2-183.1]	-	0.010
HOMA-IR	-	2.32 ± 0.75	-	4.58 ± 1.85	-	< 0.001

AUC: area under curve; ITT: insulin tolerance test; AUC-OGTT: area under the curve for the oral glucose tolerance test; HOMA-IR: homeostasis assessment model of insulin resistance; FPG: fasting plasma glucose; 2hPG: glucose 2h after OGTT; FI: fasting insulin; P1: baseline comparisons; P2: 19th week comparisons. Non-parametric Mann-Whitney Test for independent samples was performed in AUC-ITT and fasting insulin variables, and data are presented as medians and confidence intervals. Student T-test was performed in weight, FPG, 2hPG, AUC-OGTT, and HOMA-IR variables, and data are expressed as means and standard deviations.

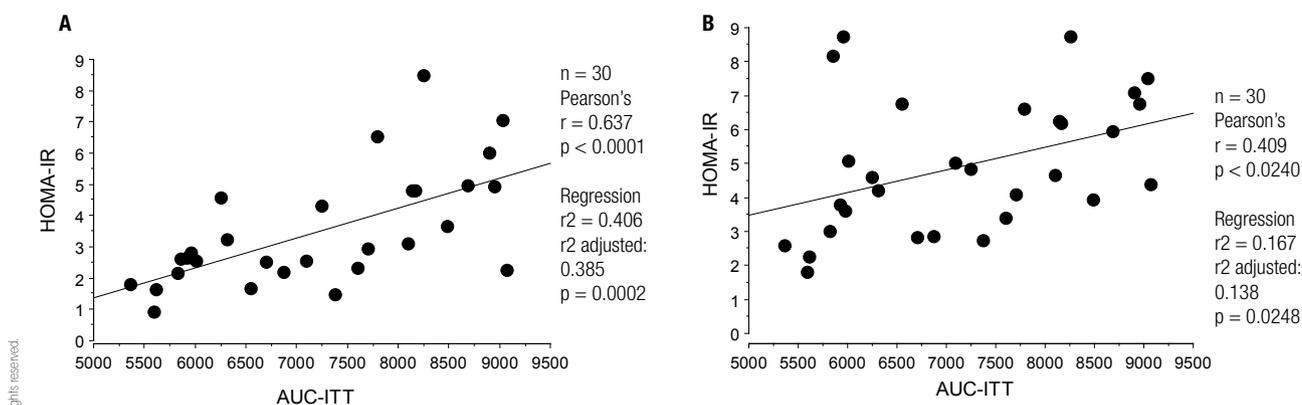
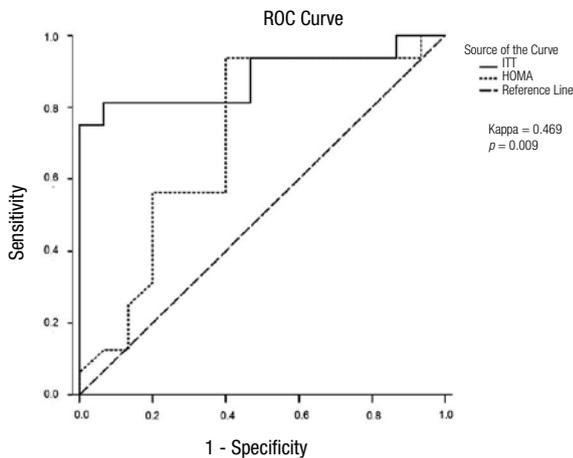


Figure 1. Describes the linear regression between AUC-ITT and HOMA-IR defined as the smallest value in three determinations (A), or as the mean of three determinations (B). Pearson's correlation is described aside.



ITT: insulin tolerance test; HOMA-IR: homeostasis assessment model of insulin resistance; ROC curve was performed in AUC ITT and HOMA-IR. Significance level, $p < 0.05$.

Figure 2. ROC curve comparing sensitivity and specificity between AUC-ITT and HOMA-IR.

DISCUSSION

The present study shows that HOMA-IR has a strong, direct correlation with the insulin tolerance test (ITT) in Wistar rats, and can be used as a surrogate marker of insulin resistance in rats. The current experiment provides evidence that HOMA-IR is as accurate as ITT to detect relative insulin-resistance in rats with 90% sensitivity, which is evidenced by a significant agreement of both ROC curves.

We found few similar studies evaluating the association between HOMA-IR and the hyperinsulinemic euglycemic glucose clamp (HEGC), and none with the ITT. In a study using pregnant female Wistar and Sprague-Dawley rats (14) in which HOMA-IR was validated against the HEGC, there was a strong association with HOMA-IR, which is in similar to our findings. In mice, however, the correlations are only modest due to increased variability and technical difficulties for performing clamp studies (15). In another study comparing insulin-based indexes in cats, HOMA-IR was considered the most useful predictor of insulin resistance (16).

The present study has some limitations. It is important to mention that we did not use the HEGC as the gold standard, but the insulin tolerance test (ITT). The ITT determines the sensitivity of insulin receptors in tissues by measuring the rate of decrease in blood glucose levels before and after intra-venous insulin administration (8). This fall yields a curve along time

creating an area under the curve (AUC) which is used as the indicator of insulin sensitivity. The greater the AUC, the lower is the sensitivity to insulin. ITT strongly correlates with the HEGC (8,10,11) and is highly reproducible in humans (12,13). More recently, it was also validated in mice with few adaptations, including injection of insulin by intra-peritoneal route (17). ITT may have some pitfalls such as the interference of the level of the fasting plasma glucose and the possibility of late glucose counter-regulation in response to hypoglycemia due to prolonged fasting (17). However, ITT is considered a valid and well-established reproducible method for the assessment of insulin sensitivity in animals, so that it is reasonable to use it as a reference method to validate other surrogate markers of insulin sensitivity or resistance.

A potentially important topic is that when using HOMA-IR in rodents there may be a considerable variability due to changes in insulin and glucose levels, according to the duration of fasting (17). In mice, the counter-regulatory system response is activated when blood glucose is just below 80 mg/dL (18), so that a prolonged fast could induce activation, leading to false interpretation. In the present study, despite the fact we used a 12h overnight fast for logistical reasons, we substantially minimized this potentially source of variability by using a mean of triplicate HOMA-IR values in three different days, ruling out occasional outlier values, if adequate, which may have had a possible impact in the variability of HOMA-IR. A shorter period of fasting, such as 5 to 6h, is however, recommended by some authors (17,19).

Another important point is related to the interpretation of HOMA-IR in rodents. In humans, as HOMA-IR is determined in the fasting, it and provides little information about the insulin sensitivity in the post-prandial state. This is confirmed by studies showing poor correlation between HOMA-IR and post-prandial excursions of blood glucose in treated diabetic patients (20). The HOMA-IR model is based on the premise that fasting circulating glucose/insulin levels are determined by a crosstalk between the liver and the pancreas. This reflects changes in hepatic insulin-sensitivity, but is limited for reflecting changes in peripheral insulin sensitivity. Even though it can be considered a good predictor of total insulin-sensitivity (14), considering the metabolic differences between humans and rodents, it remains to be established if all the inferences in humans can be extrapolated to rodents.

The advantages of using HOMA-IR for studying insulin sensitivity in animal research are notorious. It is a simple method with no need for special expertise, causing minimal stress to animals, and it is virtually free from the risk of hypoglycemia, which is a common problem when using HEGC or ITT. HOMA-IR may then be used in experimental research in rodents, as a surrogate marker of insulin resistance. Future studies are still necessary, however, to standardize methodology and interpretation of data for a broader use of HOMA-IR in rodents and in other species.

Contributor statements: Luciana C. Antunes designed the study, wrote the protocol, reviewed studies, drafted the article, conducted the statistical analysis, and interpreted the data. Manoela N. Jornada, Jessica L. Elkfury and Kelly C. Foletto collected data. Marcello C. Bertoluci suggested the study and participated in the study design, interpreted the data, drafted the article, and was responsible for the final approval of this version.

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