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ORIGINAL ARTICLE

Accuracy of the ^{13}C-glucose breath test to identify insulin resistance in non-diabetic adults

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Abstract

Aims To assess the validity of the ^{13}C-glucose breath test (^{13}C-GBT) to identify insulin resistance (IR) in non-diabetic individuals, using hyperinsulinemic–euglycemic clamps as gold standard. This validity was compared with that of other IR surrogates.

Methodology Non-diabetic adults were studied in a cross-sectional design. In a first appointment, oral glucose tolerance tests were conducted simultaneously with ^{13}C-GBTs. Oral 75 g glucose dissolved in 150 ml water, followed by 1.5 mg/Kg body weight U-^{13}C-glucose dissolved in 50 ml water, was administered. Breath and blood samples were collected at baseline and at 30-min intervals. The percentages of glucose-oxidized dose at given periods were calculated. Clamps were conducted a week later. A clamp-derived M value ≤ 6.0 mg/kg*min was used as cut-off. ROC curves were constructed for ^{13}C-GBT, fasting insulin, HOMA, and ISI-composite.

Results Thirty-eight subjects completed the study protocol. The correlation coefficient between the ^{13}C-GBT derived glucose-oxidized dose at 180 min and M values was 0.524 ($p = 0.001$). The optimal value to identify IR with the ^{13}C-GBT was 4.23% (AUC 0.81; $_{95}$CI 0.66, 0.96; accuracy 0.82, $_{95}$CI 0.66, 0.92). The ^{13}C-GBT sensitivity (0.88) was higher than HOMA and fasting insulin sensitivities (0.83 and 0.75 respectively), while their specificities were comparable (0.71, 0.71, and 0.79, respectively). The sensitivity of ISI-C was higher (0.92) than that of the ^{13}C-GBT, but its specificity was poor (0.36). The accuracy of the ^{13}C-GBT was superior to that of the other studied surrogates.

Conclusions The ^{13}C-GBT is a valid and accurate method to detect IR in non-diabetic adults. Therefore, it is potentially useful in clinical and community settings.

Keywords Insulin resistance · ^{13}C-glucose breath test · Hyperinsulinemic–euglycemic clamp · ISI-composite · Accuracy · ROC curve

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic diseases worldwide with high rates of mortality [1]. Insulin resistance (IR) is an early and reversible condition in the pathogenesis of T2DM [2, 3]; thus, it is expected that the early identification of individuals with IR would help to stop or delay the onset of diabetes. Useful methods to identify IR in clinical and community settings are still needed. Therefore, the development of practical and accurate methods to recognize IR is crucial to prevent T2DM [4, 5].

The gold standard to measure IR is the hyperinsulinemic–euglycemic clamp, because it provides a direct measurement of tissue insulin sensitivity [6]. However, it is an invasive, time-consuming, complex, expensive procedure,

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and, consequently, inadequate in the field and in clinical practice [7]. These circumstances have prompted the development of technically simple methods to identify IR, although with poorer accuracy than clamp [8]. Surrogates of IR such as fasting insulin and the homeostasis model assessment (HOMA) derived from fasting insulin and glucose concentrations [9], as well as other dynamic test like ISI-composite calculated in a post-glucose load scenario [10], are used to search for IR in routine practice, but their reproducibility is poor [11–13] and requires blood sampling which, in the case of dynamic tests, the necessary sampling is multiple [14].

The ^{13}C-glucose breath test (^{13}C-GBT) has been recognized as an adequate, simple and non-invasive method to assess several points of glucose metabolism [15]. The test is based on the assumption that the ingestion of isotopically labeled glucose (U-^{13}C-glucose) produces the expiration of labeled CO_2 ($^{13}CO_2$), which can be measured in breath samples. Since $^{13}CO_2$ abundance in exhaled air represents an indirect measurement of glucose oxidation via Krebs cycle, it is expected to be low in states with impaired glucose metabolism, such as T2DM and IR [15, 16]. Preliminary studies reported that the test is altered in patients with T2DM, suggesting its potential to diagnose the disease [16–18]. The intra-subject variability of the ^{13}C-GBT was assessed in healthy individuals, either lean or with obesity, as well as in subjects with diabetes, showing that this test exhibited a lower variability than that of an OGTT [19]. Our research team also studied the variability of the ^{13}C-GBT by repeating it in two occasions during a 1-week period in subjects without diabetes, finding that it was reproducible. In that study, we also confirmed that the variability was lower than that of other IR surrogates [20].

Thus, it seems that the ^{13}C-GBT is a reproducible technique that is altered in subjects with diabetes, but its validity to identify IR before it progresses to diabetes is not as clear, because a valid cut-off point has not been properly established. For instance, Ibarra-Pastrana et al., proposed a cut-off point of 9.99 glucose-oxidized dose (*A*%OD) at 90 min to detect IR, but used HOMA as the gold standard [21]. Gosh et al. suggest a cut-off limit of 24.5 dose of ^{13}C at 120 min over basal using ISI-composite for comparison [22], and our research group identified a cut-off point of 16.3 *A*%OD at 180 min, using fasting insulin as gold standard, because it was conducted in children [23]. From our point of view, all these comparisons appear inappropriate given the low reliability of the IR surrogates used as references and the differences in the times of comparisons, which, besides to likely explain the high variability of the suggested limits, it makes evident the need to identify a trustable cut-off point.

Thus, the aim of the current study was to assess the validity of the ^{13}C-GBT to identify IR using the hyperinsulinemic–euglycemic clamp as the gold standard in a sample of healthy adults within a wide range of insulin sensitivity. The validity of the ^{13}C-GBT was compared with that of other IR surrogates such as fasting insulin, HOMA, and ISI-composite.

Methods

The study was conducted in the Unit of Research in Medical Nutrition of the Instituto Mexicano del Seguro Social in Mexico City. The protocol was approved by the Ethics Committee of the Institute (Approval Number: R-2007-3603-18). Informed consent was obtained from all individual participants included in the study before the study protocol initiated.

In a cross-sectional design, voluntary adults within a broad range of body mass index (BMI) to assure a wide spectrum of insulin sensitivity, but healthy otherwise, were invited to participate. Subjects whose capillary blood glucose was ≥ 126 mg/dl (OneTouch Ultra® glucometer; LifeScan, Inc., Milpitas, CA) were excluded. Those who met the selection criteria and accepted the protocol procedures signed an informed consent form and were asked to attend the Unit of Research in two different occasions separated by 1 week. In the first visit, an oral glucose tolerance test (OGTT) and the ^{13}C-GBT were simultaneously performed. On the second visit, a hyperinsulinemic–euglycemic clamp was conducted.

Study procedures

Day 1, OGGT and ^{13}C-GBT

Subjects were asked to arrive at 8:00 AM after 12 h fast to conduct a standard OGTT and the ^{13}C-GBT. An oral load of 75 g glucose (ACS reagent; Sigma-Aldrich, St Louis, MO) dissolved in 150 ml water was administered, followed by a dose of 1.5 mg/Kg body weight U-^{13}C-glucose (Cambridge Isotope Laboratories, Inc., Andover, MA) dissolved in 50 ml water. Breath and blood samples were collected at baseline and at 30, 60, 90, 120, 150, and 180 min following glucose loads. Breath samples were collected in Exetainer ® test tubes (Labco Ltd., High Wycombe UK) with a common straw. Blood samples were collected via an antecubital venous catheter. Plasma was stored at − 70 °C, and breath samples at room temperature, until analyses.

Day 2, hyperinsulinemic–euglycemic clamp

A week after, a hyperinsulinemic–euglycemic clamp was conducted as previously described by DeFronzo et al. [6]. Briefly, subjects were instructed to arrive at 8:00 after 10 h fast, and an antecubital venous catheter was placed to administer glucose and insulin infusions. A second retrograde catheter was placed in the opposite hand for sample collection, while the hand was kept in a heating device. Blood samples were drawn at 5-min intervals for glucose determinations (0.5 ml) and at 30-min intervals for insulin determinations (3 ml). Insulin infusion was started with a priming dose of 80 mU/m^2 body surface area (BSA) during 10 min, followed by a constant infusion of 40 mU/m^2 BSA. A solution of 20% glucose was administered at a variable rate to maintain plasma glucose at 90 mg/dl. The mean glucose infusion rate during the steady state (*M*, mg/kg*min) was assessed during the last 30 min when the steady state was reached (glucose concentration at 90 ± 3 mg/dl). An *M* value ≤ 6.0 mg/kg*min was considered as the reference limit to detect IR [24].

Biochemical determinations

Plasma glucose was measured with an enzymatic method (YSI 2300 Stat Plus Glucose Analyzer, YSI Inc., Yellow Springs OH, USA), and plasma insulin was determined by radioimmunoassay using commercial kits (Millipore, Billerica MA, USA). Coefficients of variation were 3.9% and 7.5%, respectively.

Breath-derived measurements

$^{13}CO_2$ abundance in breath samples was measured with an isotope ratio mass spectrometer (Finnigan Breath Mat Plus, Bremen, Germany). Intra- and inter-assay coefficient of variation (CV) was < 1% in an internal breath sample control. As reported previously in several manuscripts published by our group, breath test results are expressed as percentage oxidized dose at a given time period. Raw $^{13}C/^{12}C$ data were expressed as delta per mille versus the Pee Dee Belemnite standard (δ13 PDB) and transformed to percentage of oxidized dose using the equations described by Braden et al. [25]. All oxidation data were adjusted by the amount of anhydrous glucose received per kg body weight (refer to Eq. 3 below).

Equations The following equations were used:

1. HOMA = (fasting glucose [mmol/L] × fasting insulin [μU/mL])/22.5 [5]
2. ISI-composite = 10,000/√{(fasting glucose [mg/dl] × fasting insulin [μU/ml]) × (mean OGTT glucose [mg/dl] × mean OGTT insulin [μU/ml])} [9]
3. Adjusted oxidized dose (%) = (oxidized dose at 180 min [%]) × (75 g/kg body weight [kg]) [24]

Statistical analysis

Statistical analysis was performed with SPSS software (SPSS In., version 19, Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD) and minimum and maximum for description. Qualitative variables were presented as simple frequency and percentages, and statistical differences were analyzed by Chi-square test. The correlations among the IR surrogates were determined by *r*-Pearson coefficient. Sensitivity (Se), specificity (Sp), predictive values (positive and negative), likelihood ratios (positive and negative), and accuracy with their 95% confidence intervals were calculated for each studied IR surrogate using clamps as the gold standard. Receiver-Operator Characteristics' (ROC) curves were constructed for the ^{13}C-GBT using the *A%OD* at 180 min and an *M* ≤ 6.0 mg/kg*min. ROC curves were also constructed for ISI-composite, HOMA, and fasting insulin. We propose the best cut-off point for the ^{13}C-GBT in the maximum sensitivity–specificity value in the ROC curve. Areas under curves with their 95% interval confidence were calculated. Statistical significance was determined to be a probability value less than 0.05.

Results

Thirty-eight individuals out of forty who were invited to participate met the selection criteria and completed the study protocol. Studied individuals were within a wide range of age, BMI, and insulin sensitivity (Table 1). According to BMI, 32% were lean (< 25 Kg/m^2), 50% overweight (≥ 25, < 30 Kg/m^2), and 18% presented obesity (≥ 30 Kg/m^2). According with waist circumference, 49% presented

Table 1 Clinical and metabolic characteristics of study subjects (*n* = 38)

Characteristic	Mean ± SD	Minimum	Maximum
Gender (M|F)	13|25		
Age (years)	37.45 ± 10.65	19	62
BMI (Kg/m^2)	26.87 ± 3.59	21.05	37.95
HOMA	4.42 ± 2.63	1.00	15.73
Fasting glucose (mg/dL)	94.89 ± 12.51	74	119
2 h OGTT glucose (mg/dL)	122.87 ± 27.46	71	192
Fasting insulin (μU/ml)	18.26 ± 9.33	5	59
2 h OGTT insulin (μU/ml)	95.32 ± 60.23	22	288
ISI-composite	2.97 ± 1.97	0.63	9.89
M (mg/kg*min)	5.94 ± 2.47	2.54	16.64
M/I (mg/kg*min/μU)	5.85 ± 3.41	1.85	20.84
A%OD (%)	3.97 ± 1.67	0.88	6.31

HOMA homeostasis model assessment, *OGTT* oral glucose tolerance test, *ISI* insulin sensitivity index, *A%OD* glucose-oxidized dose at 180 min central obesity (> 88 for female and > 94 for male). Based on clamp measurements, 63% participants were identified as with IR, which was detected in all the subjects with obesity, 74% overweight, and 25% lean ($\chi^2 = 12.49$, $p = 0.001$). The breath-test-derived oxidation rate was higher for lean than for overweight and obese individuals, just as it was the *M* value. In contrast, for the other IR surrogates, the results for overweight patients were not different than those of lean or obese (Table 2).

We first investigated the correlations of *M* values with anthropometric parameters and with the different measurements of IR. As expected, *M* was strongly associated with BMI ($r = -0.785$) and waist circumference ($r = -0.643$). Likewise, *M* correlated significantly with HOMA ($r = -0.543$), fasting insulin ($r = -0.596$), ISI-composite ($r = 0.698$), and with *A*%OD at 180 min ($r = 0.524$). Regression analysis demonstrated a robust positive association between the glucose oxidation rates obtained with clamps and with the ^{13}C-GBT (Fig. 1). We further explored the association of *A*%OD with the studied IR surrogates. Significant correlations between breath values and HOMA ($r = -0.479$), fasting insulin ($r = -0.528$), and ISI-composite ($r = 0.476$) were observed.

A ROC curve was created to assess the accuracy of the ^{13}C-GBT to predict M ≤ 6.5 mg/kg*min. The area under the curve (AUC) was 0.81 ($_{95}$CI = 0.66–0.96) with an optimal *A*%OD at 180 min cut-off point of 4.23%. ROC curves were also constructed for ISI-composite, HOMA, and fasting insulin (Fig. 2). ^{13}C-GBT demonstrated the best accuracy than the other studied IR surrogates. Although ISI-composite exhibited the best Se, its Sp, positive likelihood ratio, and predictive values were lower than those of the ^{13}C-GBT (Table 2).

Discussion

Our results demonstrate that the ^{13}C-GBT is an accurate test to detect IR in non-diabetic individuals. Therefore, it is a potentially useful tool to be used in community and clinical

Table 2 Comparisons of insulin resistance surrogates among nutritional status groups

	Lean Mean (95% CI)	Overweight	Obese	*p* value
HOMA	2.87 (1.45, 4.29)[a]	4.85 (3.71, 5.98)[ab]	5.90 (4.04, 7.76)[b]	0.026
FPI (μU/ml)	12.83 (7.80, 17.87)[a]	19.47 (15.47, 23.48)[ab]	24.29 (17.69, 30.88)[b]	0.021
ISI-composite	4.04 (2.99, 5.11)[a]	2.81 (1.96, 3.66)[ab]	1.60 (0.19, 3.00)[b]	0.025
M (mg/kg*min)	8.04 (6.84, 9.23)[a]	5.31 (4.36, 6.26)[b]	3.93 (2.37, 5.50)[b]	< 0.001
A%OD (%)	5.04 (4.50, 5.58)[a]	3.55 (3.12, 3.98)[b]	3.28 (2.57, 3.99)[b]	< 0.001

HOMA homeostasis model assessment, *FPI* fasting plasma insulin, *ISI* insulin sensitivity index, *M* clamp-derived glucose oxidation, *A%OD*, glucose-oxidized dose at 180 min from breath test

[ab]ANOVA, different letters mean $p < 0.050$

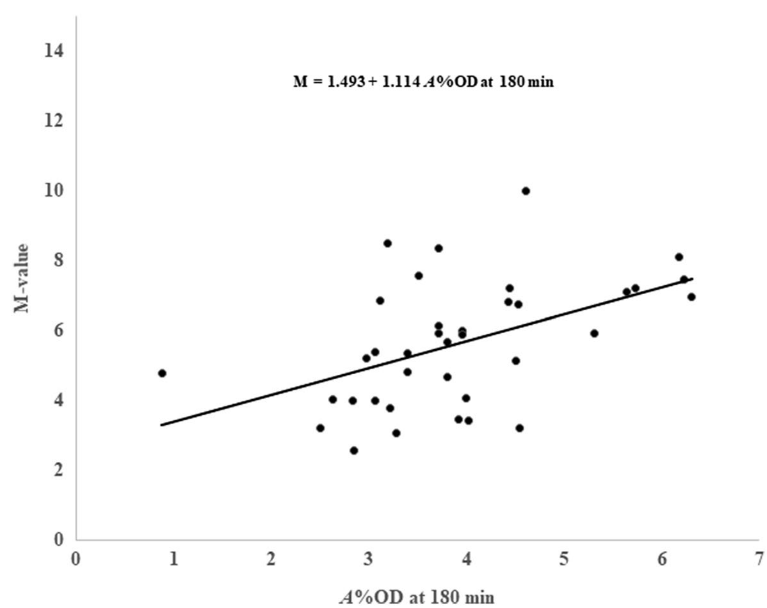

Fig. 1 The glucose oxidation rate (*A*%OD) at 180 min, obtained with the 13C-GBT, was directly associated with the glucose oxidation rate measured with the clamp technique (*M* values). Regression analysis: $R^2 = 0.27$, $p = 0.001$

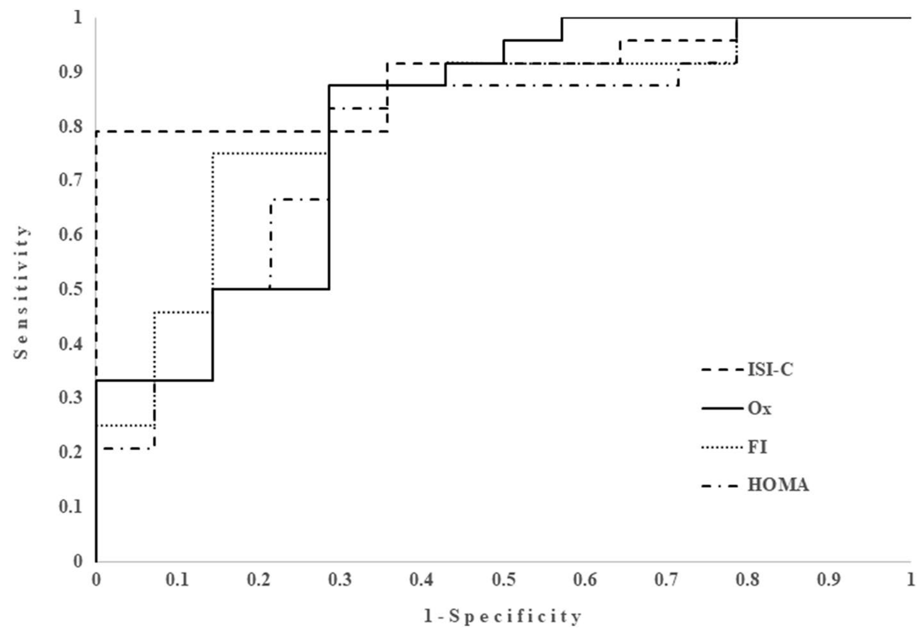

Fig. 2 ROC curves for 13C-GBT, ISI-composite, HOMA, and fasting plasma insulin. The cut-off for calculation was: *M* value ≤ 6.5 mg/kg*min

settings. With this purpose, we propose a cut-off point of 4.23% oxidized dose at 180 min. We and others, previously, demonstrated that the ^{13}C-GBT is a reliable method, because it exhibited low variability after two or three repetitions of the tests were conducted within 1-week period [19, 20]. However, its validity, i.e., how close it actually measures IR, had not been defined, mainly because inappropriate references were used for validation, but also because studies analyze together individuals with IR and with diabetes.

For instance, the study of Ibarra-Pastrana [21] used as reference the HOMA index to recommend a cut-off point, which seems inadequate, because, while the ^{13}C-GBT is a dynamic test that measures the response after a glucose load, HOMA is an index that uses glucose and insulin in the fasting state. Likewise, our research team proposed a cut-off point for IR, but the study was conducted in healthy children in whom conducting the invasive clamp was unjustified; therefore, fasting insulin, which is also a fasting scenario, was used as reference [23]. The problem of comparisons between dynamic tests and tests obtained at fasting states lie in that the mechanisms that regulate plasma glucose concentration in the post-absorptive state (fasting) are different from those that regulate plasma glucose concentration after a meal. While plasma glucose is primarily determined by the rate of hepatic glucose production in the fasting state, glucose after a meal is determined by the rate of glucose-stimulated insulin secretion and skeletal muscle insulin sensitivity. Thus, comparing a fasting test with a dynamic test does not seem appropriate. In addition, the cut-off point proposed by Ibarra-Pastrana [21] was measured 90 min after the basal dose. Similarly, in another study conducted in India by Gosh and coworkers [22], even though a post-challenge test was used as reference (ISI-Composite), breath samples were measured at 120 min over basal. On this regard, we previously demonstrated that the ^{13}C-GBT was reliable only at 180 min, because the intra-subject variability was high at earlier times [20]. Besides, the Gosh study proposes a cut-off limit based on deltas of ^{13}C, which represent a raw (unadjusted) measurement. This is important, because the unadjusted delta values may vary with body weight. In our study, the oxidized dose was corrected by body weight to avoid a dilution effect of the labeled glucose, because subjects were within a wide range of BMIs.

As in our study, the studies of Lewanczuk [16], Dillion [18], and Singal [19] reported that breath test correlated with clamp and other IR surrogates, but no reference limits were proposed. These three studies included together individuals with IR or diabetes, which may be interpreted as if the test is valid to identify individuals with IR, but it is not clear from their results if the test discriminates IR from diabetes. To our knowledge, only the Gosh study [22] assessed the validity of the ^{13}C-GBT to detect IR before diabetes was developed. In his study, Gosh claims that the exhaled breath test is a feasible and accurate test to identify not only type 2 diabetes, but also IR. While our results are consistent with such statement, Gosh et al. used unadjusted values to assess the cut-off limit and, therefore, the cut-off points are not comparable.

Finally, we found that, opposite to the IR surrogates, the breath test and M values were different for lean than for overweight and obese individuals, suggesting that the breath test is able to detect IR at the early stages. Nevertheless, the small samples for each nutritional status group do not allow to make such inference.

Table 3 ROC curve derived data for insulin resistance surrogates

	^{13}C-GBT (*A*%OD)	ISI-composite	HOMA	FPI (µU/ml)
Cut-off point	4.23	4.00	4.10	16.50
AUC	0.81 (0.66–0.96)	0.90 (0.80–1.00)	0.78 (0.62–0.93)	0.83 (0.69–0.96)
Se	0.88 (0.68, 0.97)	0.92 (0.73, 0.99)	0.67 (0.45, 0.84)	0.75 (0.53, 0.90)
Sp	0.71 (0.42, 0.92)	0.64 (0.35, 0.87)	0.71 (0.42, 0.92)	0.79 (0.49, 0.95)
+LR	3.06 (1.32, 7.11)	1.43 (0.95, 2.15)	2.33 (0.97, 5.60)	3.50 (1.25, 9.80)
−LR	0.17 (0.06, 0.53)	0.23 (0.05, 1.05)	0.47 (0.24, 0.90)	0.32 (0.15, 0.67)
+PV	0.84 (0.69, 0.92)	0.71 (0.62, 0.79)	0.80 (0.63, 0.91)	0.86 (0.68, 0.94)
−PV	0.77 (0.52, 0.91)	0.71 (0.36, 0.92)	0.56 (0.39, 0.71)	0.65 (0.47, 0.79)
Accuracy	0.82 (0.66, 0.92)	0.71 (0.54, 0.85)	0.68 (0.51, 0.83)	0.76 (0.60, 0.89)

VALUES include ($_{95}$CI)

CI confidence interval, *^{13}C-GBT* ^{13}C-glucose breath test, *FPI* fasting plasma insulin, *AUC* area under the curve, *Se* sensitivity, *Sp* specificity, *+LR* positive likelihood ratio, *−LR* negative likelihood ratio, *+PV* positive predictive value, *−PV* negative predictive value

Thus, we recognize that the small sample which we studied is the main limitation. However, our sample size is comparable to that of most of the other studies here mentioned [16–18]. In addition, the consistency of our results with those of others in the literature, for instance the strong correlations among anthropometry and the different IR surrogates, demonstrates a good quality of our measurements, suggesting that our results are trustworthy. Another limitation, though, is that although we studied non-diabetic subjects, yet 37% presented fasting plasma glucose (FPG) between 102 and 119 mg/dL. The mean *A*%OD at 180 min obtained for the altered FPG group was not different than that of those with normal FPG (3.71 ± 1.02 vs. 4.12 ± 1.24, respectively, $p = 0.283$). Likewise, the number of subjects with *A*%OD at 180 min above the identified cut-off point was comparable between the altered and normal FPG groups ($\chi^2 = 0.313$, $p = 0.573$. Fisher's exact test $p = 0.728$), suggesting that altered FPG does not alter the result of the test.

Moreover, our study has several important strengths. For instance, we evaluated the breath test against the hyperinsulinemic–euglycemic clamp, which is the gold standard for IR; second, we included a sample of individuals within a wide range of insulin sensitivity, but without diabetes; third, the detected cut-off points for the other known IR surrogates are in the range of those reported in the literature (5, 8–13). Thus, it seems that the ^{13}C-GBT is a reliable and accurate method to detect IR that performs better than the other studied IR surrogates. In addition, it exhibits several other advantages, since it constitutes a non-invasive alternative that does not require blood sampling. Therefore, it may be used in epidemiological studies or in community settings were blood sampling and processing is difficult to perform. Furthermore, breath samples can be stored at room temperature and do not require any special handling prior to their analysis.

We recognize that other tests may perform better in different circumstances. For instance, the high LR+ and PV+ for fasting plasma insulin suggest that this surrogate is likely useful for screening in places where blood sampling and handling are not a problem. In fact, we are providing enough information for researchers to select the appropriate test according to their requirements (Table 3).

Thus, we propose the ^{13}C-GBT as a valid and accurate method to screen for IR in non-diabetic adults. Nevertheless, we recognize that the cut-off which we are proposing is appropriate only if the ^{13}C-GBT is carried out in similar conditions than those we used in our study; that is, if the administered dose is adjusted by body weight and the result is evaluated at 180 min over basal. This finding could be relevant to confront the epidemic of diabetes worldwide, because it offers a reliable, inexpensive, non-invasive test to detect insulin resistance.

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Compliance with ethical standards

Conflict of interest Authors declare that no competing financial interests exist.

Ethical approval The protocol was approved by the Ethics Committee of the Instituto Mexicano del Seguro Social and with the 1964 Helsinki declaration (Approval Number: R-2007-3603-18).

Informed consent Informed consent was obtained from all individual participants included in the study before the study protocol initiated.

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