Comparison of the [^{13}C]Glucose Breath Test to the Hyperinsulinemic-Euglycemic Clamp When Determining Insulin Resistance

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OBJECTIVE — With increasing emphasis on the recognition of the metabolic syndrome and early type 2 diabetes, a clinically useful measure of insulin resistance is desirable. The purpose of this study was to evaluate whether an index of glucose metabolism, as measured by $^{13}CO_2$ generation from ingested [^{13}C]glucose, would correlate with indexes from the hyperinsulinemic-euglycemic clamp.

RESEARCH DESIGN AND METHODS — A total of 26 subjects with varying degrees of insulin sensitivity underwent both the [^{13}C]glucose breath test and the hyperinsulinemic-euglycemic clamp. Results from the [^{13}C]glucose breath test were compared with measures of insulin sensitivity from the glucose clamp as well as with other commonly used indexes of insulin sensitivity.

RESULTS — There was a strong correlation between the [^{13}C]glucose breath test result and the glucose disposal rate ($r = 0.69$, $P < 0.0001$) and insulin sensitivity index ($r = 0.69$, $P < 0.0001$) from the insulin clamp. The magnitude of these correlations compared favorably with QUICKI and were superior to the homeostasis model assessment.

CONCLUSIONS — The [^{13}C]glucose breath test may provide a useful noninvasive assessment of insulin sensitivity.

T ype 2 diabetes is a common condition that is becoming increasingly prevalent (1,2). It is now recognized that clinically apparent type 2 diabetes is often preceded by a period of glucose intolerance that is due to a combination of β-cell dysfunction and insulin resistance (3–6). It is estimated that this preclinical phase of type 2 diabetes may antedate the onset of overt diabetes by 10–12 years (7). Furthermore, insulin resistance has been put forth as a major component of the metabolic syndrome (8) and is associated with significant cardiovascular morbidity and mortality (9–13). Recent and ongoing studies have targeted prediabetic individuals for early therapeutic intervention in the hopes of preventing progression to overt type 2 diabetes. These studies in individuals with impaired glucose tolerance have shown benefits from both lifestyle and pharmacological interventions (14–17). Indeed, these studies have shown the benefits of both approaches in preventing or delaying the onset of type 2 diabetes. It is thus hypothesized that timely intervention in individuals with insulin resistance, with or without impaired glucose tolerance, may prevent the development of type 2 diabetes and its attendant complications.

A difficulty arises in identifying individuals at risk for type 2 diabetes. Most disease prevention strategies rely on intervention in the prediseased state. In the case of type 2 diabetes, early intervention might therefore be most appropriate at the stage of disease progression when insulin resistance is present but before glucose intolerance occurs. However, the clinical diagnosis of insulin resistance is challenging. The gold standard diagnostic test is the hyperinsulinemic-euglycemic clamp, but this technique is clearly unsuitable for routine clinical use. To provide clinically accessible testing for insulin resistance, indexes such as the homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI) have been devised (18,19). Results from these tests correlate with results from the hyperinsulinemic clamp and allow for identification of individuals with insulin resistance. However, these and most other indexes of insulin resistance require serum insulin and glucose measurements and variably complex calculations. Thus, these indexes have not yet made major inroads into general medical practice.

To address the issue of detecting insulin resistance, a simple sensitive test of glucose metabolism was proposed. In normal individuals, in the presence of insulin, glucose is taken up by a variety of cells, where it undergoes glycolysis and then enters the tricarboxylic acid cycle or is shunted to fat synthesis. In either case, CO_2 is produced as a metabolic byproduct. This CO_2 then enters the circulation and is eliminated in the lungs. We hypothesized that ingested glucose, labeled with nonradioactive ^{13}C, would result in the expiration of $^{13}CO_2$ that could be detected in expired air. In type 2 diabetes

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and other states of insulin resistance, glucose uptake would be impaired and the generation of $^{13}CO_2$ would therefore be blunted. To test this hypothesis, we proceeded to develop and optimize a [^{13}C]glucose breath test based on this principle. This early report compares the performance of this [^{13}C]glucose breath test with results from the hyperinsulinemic-euglycemic clamp. In addition, performance of the [^{13}C]glucose breath test is compared with the HOMA and QUICKI indexes.

RESEARCH DESIGN AND METHODS

— This study was carried out in 26 adults, aged $\geq$18 years, chosen based on the likelihood that they would represent a spectrum of insulin sensitivities. Healthy nonobese subjects ($n = 10$), obese subjects ($n = 7$), and subjects with known type 2 diabetes ($n = 9$) were included in this study. Type 2 diabetic subjects refrained from metformin for a minimum of 12 h before the study and from sulfonylureas for 24 h before the study. Exclusion criteria included the presence of any significant pulmonary, gastrointestinal, or endocrine disorders or the use of insulin or any other medications known to affect insulin sensitivity. Subjects on thiazolidinediones were excluded from participation because of the long clinical half-life of these drugs. Diabetic status for diabetic subjects was verified by chart review according to American Diabetes Association (ADA) criteria. Normal control subjects were included only if euglycemic according to ADA criteria. Obese subjects were excluded if they had a history of diabetes or if they met ADA criteria for diabetes at any point in the study. The project received approval from the Research Ethics Board of the University of Alberta Faculty of Medicine, and all subjects gave their informed consent before participating.

[^{13}C]glucose breath test

The [^{13}C]glucose breath test and hyperinsulinemic-euglycemic clamp were carried out within 2 days of each other. In most cases, the [^{13}C]glucose breath test was carried out the day before the insulin clamp. After an overnight fast, study subjects attended the Clinical Trials Center at the University of Alberta Hospital. On arrival, a baseline breath sample was taken and then subjects consumed 100 ml of the breath test solution. A second breath

sample was obtained 90 min after consumption of the breath test solution. The [^{13}C]glucose breath test consists of 25 mg [^{13}C]glucose mixed with 15 g dextrose and orange flavoring. Immediately before testing, 100 ml tap water was added to the powdered ingredients, and the solution was stirred until dissolved. The [^{13}C]glucose (Martek Biosciences Corporation, Columbia, MD) is universally labeled, meaning that ^{13}C occupies all six carbon positions in the molecule. Previous optimization studies had demonstrated that 25 mg [^{13}C]glucose was sufficient for diagnostic purposes (data on file, Isotechnika Inc.). The 15 g unlabelled dextrose was used for purposes of palatability and as an indicator to ensure the complete dissolution of [^{13}C]glucose. The expired $^{13}CO_2$ after test drink ingestion was compared with the baseline value, and results were expressed as an absolute increase in ^{13}C in δ ‰. $^{13}CO_2$ was measured in breath samples using an AP2003 isotope ratio mass spectrometer (Analytical Precision Limited, Cheshire, U.K.), although it can also be measured using nondispersive infrared spectrometry. To obtain breath samples, subjects were asked to blow the volume of a normal exhalation through a short straw into 10-ml gas sampling tubes (Labco Exetainer system; ^{13}C and gas testing vials, Labco Limited, Buckinghamshire, U.K.). The tubes were then immediately stoppered until analyzed. These tubes are known to be impermeable to gasses for up to 90 days after sealing. Gas sampling from the tubes occurs via a needle in the AP2003 machine permeating a rubber membrane present in the cap of the tube. The same apparatus and overall method is commonly used in other ^{13}C breath tests such as the ^{13}C urea breath test for *Helicobacter pylori*. Laboratory personnel were blinded to the clinical status of the subjects.

Hyperinsulinemic-euglycemic clamp

The hyperinsulinemic-euglycemic clamp was carried out as described in a similar comparative study (20). After an overnight fast, subjects were admitted to the Clinical Investigation Unit of the University of Alberta Hospital, where intravenous catheters were placed in both arms for insulin and glucose infusion and for blood sampling. The insulin infusion was carried out with a 10-min priming dose of insulin (80 mU $\cdot$ m^{-2} body surface area $\cdot$ min^{-1} for 5 min followed by 40 mU $\cdot$ m^{-2}

body surface area $\cdot$ min^{-1} for 5 min) and then maintained at a rate of 20 mU $\cdot$ m^{-2} body surface area $\cdot$ min^{-1} for 240 min. Blood glucose was clamped at a level of 5.0 mmol based on results from blood samples taken every 5 min and analyzed using a YSI 2300 Statplus blood glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Serum glucose and insulin levels were measured at baseline and hourly thereafter. The M value (in μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}) was based on the amount of glucose infused during the last 30 min of the study. The insulin sensitivity index (ISI) was defined as the ratio of M to the measured insulin concentration at the end of the study. Both HOMA and QUICKI indexes were calculated based on the basal serum glucose and insulin levels measured at the beginning of the insulin clamp according to methods originally described (13,14). The coefficient of variation of glucose in the last hour of the clamp was <3%.

Statistical analysis

All values are expressed as means $\pm$ SD. The principal hypothesis of this study was that results of the [^{13}C]glucose breath test would correlate with indexes from the hyperinsulinemic clamp. Thus, the principal analysis was by weighted linear univariate regression. In addition, multiple and logistic regression was carried out to explore the influence of other variables on insulin sensitivity. A Pearson's correlation matrix was likewise constructed to compare the relative predictive value of the surrogate measurements of insulin sensitivity with the variables obtained from the insulin clamp. Although not part of the a priori study design, subjects were also categorized according to clinical status as normal (BMI <30 kg/m^2), obese (BMI $\geq$30 kg/m^2), or diabetic. Differences in continuous variables between groups were then compared using ANOVA and ANCOVA, or in the case of non-normally distributed data, the Kruskall-Wallis test. Post hoc testing for ANOVA and ANCOVA was carried out using the Tukey test. Categorical data were assessed by Fisher's exact test. A P value of <0.05 was considered statistically significant. Statistical analysis was carried out using Statview version 5.0.1 (SAS Institute, Cary, NC).

RESULTS — Subject characteristics according to clinical status are shown in

Table 1—*Clinical and metabolic characteristics of study subjects*

	Normal	Obese	Diabetic	P
Sex (M/F)	5/5	4/5	4/3	0.88
Age (years)	32.4 ± 15.8	47.4 ± 18.5	50.0 ± 23.2	0.12
Weight (kg)	72.4 ± 17.8	100.2 ± 17.8	94.6 ± 21.3	0.006
BMI (kg/m^2)	23.5 ± 2.5	36.7 ± 6.5	32.1 ± 3.6	<0.0001
Fasting serum glucose (mmol/l)	4.7 ± 0.5	6.5 ± 1.9	8.1 ± 2.4	0.002
Fasting serum insulin (mU/l)	5.2 ± 3.9	17.3 ± 18.8	9.1 ± 3.2	0.02

Data are means ± SD.

Table 1. A correlation matrix for the various experimental variables is contained in Table 2. Principal results of the study and those of interest based on the correlation matrix are plotted in Figs. 1-3. These results show that the [13C]glucose breath test correlates with both *M* and the ISI from the hyperinsulinemic clamp with a correlation coefficient of 0.69 in both cases (*P* < 0.0001 for both cases). Comparatively, the QUICKI correlated with *M* to a similar degree as the [13C]glucose breath test (*r* = 0.69, *P* < 0.0001) but showed a stronger correlation for ISI (*r* = 0.79, *P* < 0.0001). The correlation between the QUICKI and the [13C]glucose breath test was also significant (*r* = 0.73, *P* < 0.0001). Correlations between the HOMA index and the insulin clamp variables were not as great as for either the QUICKI or the [13C]glucose breath test. Three diabetic subjects were on oral hypoglycemic medications. Removing these three subjects from the analysis had only trivial effects on the correlation coefficients for all variables studied.

Multiple regression analysis using the various insulin clamp results as dependent variables and the [13C]glucose breath test, fasting serum insulin, fasting plasma glucose, age, sex, weight or BMI, and clinical status as independent variables consistently showed that the [13C]glucose breath test was the strongest predictor of insulin sensitivity. The only other variable that was noted to be of statistical significance was sex. However, when BMI was taken into account, the effect of sex was removed.

Table 3 shows ANOVA results for categorical analysis of the various variables. In all cases, both the obese and diabetic groups differed significantly from the nonobese. The one exception was for the HOMA, where only the normal subjects differed from the obese subjects. In no case did the obese and diabetic subjects differ significantly. The introduction of covariables such as age generally did not influence the primary relationships. A notable exception was that correction for body mass eliminated any differences between groups for both HOMA and QUICKI, whereas the [13C]glucose breath test results remained significantly different between groups (*P* = 0.027) with no significant breath test–weight interaction.

Multiple and logistic regression models using either the clamp glucose disposal rate or the ISI generally gave total correlations in the range of 0.85 (*R*^{2} = 0.72) when the [13C]glucose breath test, fasting plasma glucose, fasting serum insulin, body weight, sex, and age were entered as independent variables. In these cases, the partial correlation coefficient for the [13C]glucose breath test was in the order of 0.69, with sex and fasting blood glucose generally having partial correlation coefficients of ~0.25. Other variables, or the substitution of either BMI or body surface area for weight, did not contribute significantly to the overall models.

CONCLUSIONS — Results of this study show that [13C]glucose breath test results correlate with variables from the hyperinsulinemic-euglycemic clamp. The degree of correlation is similar to that demonstrated by the QUICKI but superior to that of the HOMA index. Overall,

results from this study for the latter two variables compare favorably to those reported in the literature. For example, in the original description of QUICKI by Katz et al. (19), the correlation coefficients between QUICKI and HOMA versus the clamp ISI were 0.78 and −0.60, respectively, which are virtually identical to the respective values of 0.79 and −0.60 obtained in this study. Katsuki et al. (21) reported a QUICKI–clamp *M* correlation of −0.598 in 60 Japanese diabetic patients, which increased to −0.649 when 5 subjects treated with sulfonylureas were excluded from the analysis. Bonora et al. (20) found a HOMA–clamp *M* correlation of −0.627 in 115 unselected individuals, although the correlation increased to −0.820 when both variables were log-transformed. Carrying out the same transformation on data from the current study similarly increased the correlation to −0.700. Thus, the consistency of results between the current clamp data and conventional measures of insulin resistance would imply that the [13C]glucose breath test–clamp relationships are valid as well.

Despite correlation coefficients between the clamp and the [13C]glucose breath test in the range of 0.69, such correlations give an *R*^{2} value of 0.48, indicating that only about half the value of insulin resistance as measured by the clamp variables could be accounted for by the [13C]glucose breath test. The remaining variability could be due to chance or other factors. Indeed, in the various regression models tested, sex and differences in fasting plasma glucose accounted for an additional proportion of the variability, increasing *R*^{2} values up to the range of 0.7. The remaining variability could otherwise be due to unmeasured factors such as serum triglycerides, as has been reported (22), or could indeed

Table 2—*Correlation coefficients between study variables*

	Breath	FPG	Insulin	HOMA	QUICKI	Weight	BMI	*M*	ISI
Breath	1	—	—	—	—	—	—	—	—
FPG	−0.607	1	—	—	—	—	—	—	—
Insulin	−0.401	0.128	1	—	—	—	—	—	—
HOMA	−0.510	0.402	0.947	1	—	—	—	—	—
QUICKI	−0.731	−0.593	−0.647	−0.744	1	—	—	—	—
Weight	−0.834	0.310	0.445	0.478	−0.708	1	—	—	—
BMI	−0.716	0.189	0.493	0.468	−0.585	0.791	1	—	—
M	0.690	−0.672	−0.398	−0.550	0.691	−0.548	−0.559	1	—
ISI	0.694	−0.588	−0.488	−0.600	0.792	−0.619	−0.639	0.955	1

Breath, [13C]glucose breath result; FPG, fasting plasma glucose; insulin, fasting serum insulin.
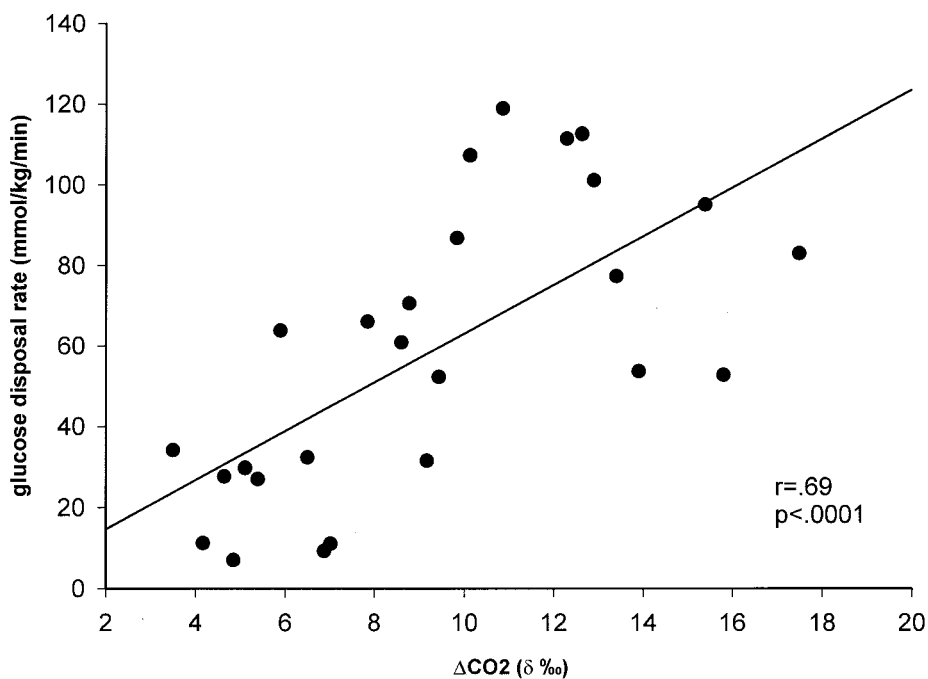
Figure 1—*Correlation between a [^{13}C]glucose breath result and the glucose disposal rate (M) from a hyperinsulinemic-euglycemic clamp.*

be due to chance. Nevertheless, the same drawback in predicting insulin resistance applies to QUICKI and even more so to HOMA.

In examining Table 2, it is clear that a strong relationship exists between body weight and the [^{13}C]glucose breath test results. However, a similarly strong cor-

relation is seen between QUICKI and body weight as well. Indeed, this latter phenomenon has been suggested in other studies (14,22). It might therefore be sug-
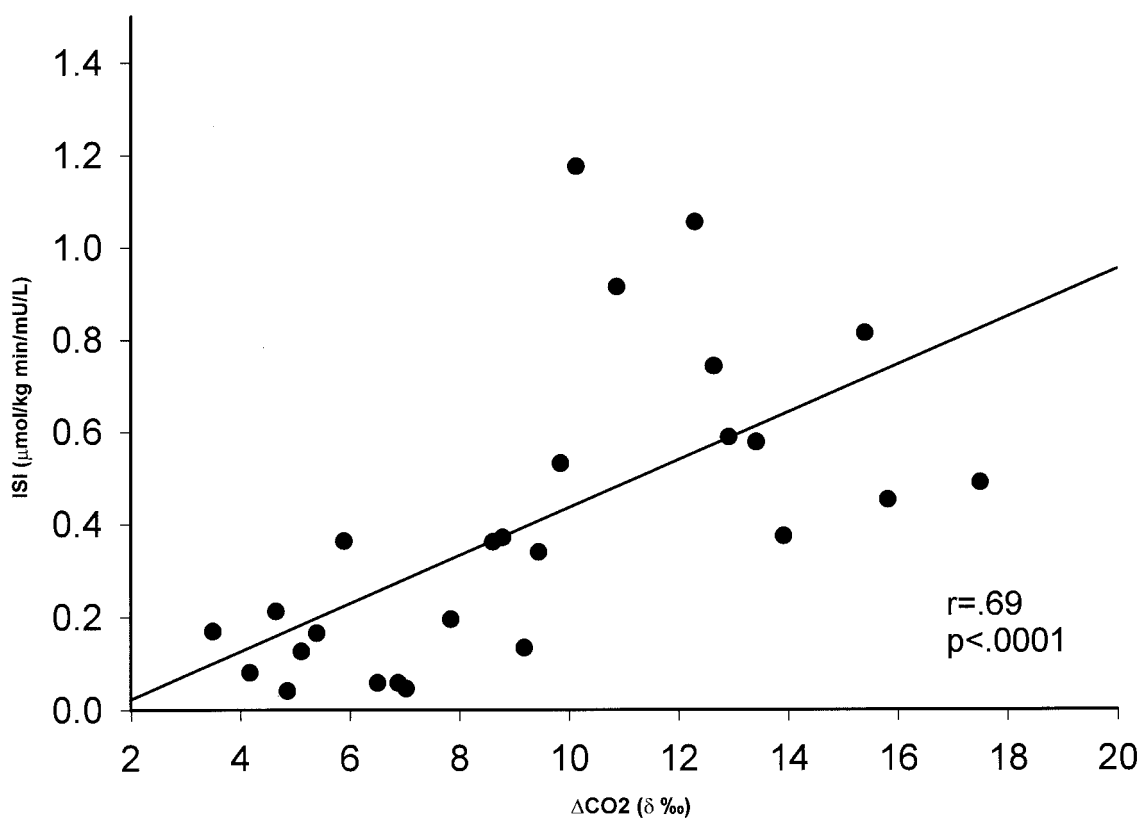
Figure 2—*Correlation between a [^{13}C]glucose breath result and an ISI from a hyperinsulinemic-euglycemic clamp.*
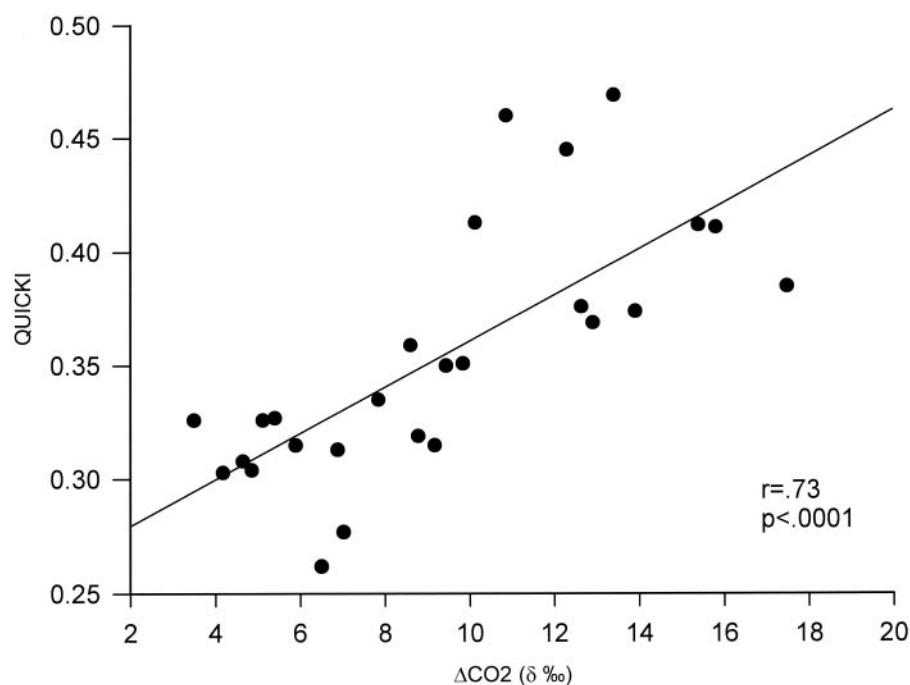
Figure 3—*Correlation between a [13C]glucose breath result and QUICKI.*

r=.73
p<.0001

gested that recognition of the syndrome of insulin resistance could just as easily be accomplished by determining an individual's weight or BMI. This would not seem to be the case because both the QUICKI and breath test correlate more strongly with the insulin clamp results than does body weight. Moreover, in multiple and logistic regression models, the [13C]glucose breath test result consistently showed a greater partial correlation with both the clamp glucose disposal rate or ISI compared with body weight or BMI. Finally, after adjusting for body weight in ANCOVA, there were still differences between the various groups of subjects and their [13C]glucose breath test results. Interestingly, a similar adjustment for body weight eliminated any differences between groups for HOMA and QUICKI. Thus, the [13C]glucose breath test would seem to detect differences among the control, obese, and diabetic subjects beyond what could be accounted for by just body weight.

Although we simplistically hypothesize that the [13C]glucose breath test results are explicable because of insulin-mediated glucose uptake and subsequent oxidation via the tricarboxylic acid cycle, the actual physiological processes that are being measured are not really known. We are confident that insulin-mediated glucose uptake is being measured, because we have carried out the [13C]glucose breath test in two nonobese type 1 diabetic individuals in the absence of insulin and could not detect any change in expired $^{13}CO_2$ (values were <1 δ ‰). Thus, the contribution of non–insulin-mediated glucose uptake to the expired $^{13}CO_2$ pool would appear to be inconsequential. A number of intracellular glucose disposal pathways may be abnormal in type 2 diabetes and insulin resistance, but whether the [13C]glucose breath re-

sults are influenced by such abnormalities is unknown. For example, glycogen synthesis has been reported to be reduced in type 2 diabetes (23). However, the glycogen synthetic pathway does not result in the generation of CO_2. Thus, any abnormalities in this pathway would lead to enhanced glucose flux down CO_2-generating pathways, which would result in increased, rather than decreased, amounts of $^{13}CO_2$ being produced, as is observed in the current study. It would therefore seem unlikely that abnormalities in this pathway are of significance in determining the [13C]glucose breath results. Other pathways, such as the pentose monophosphate shunt, may be altered in type 2 diabetes (24). However, preferential metabolism via this pathway also results in CO_2 generation and would likely not influence the [13C]glucose breath results either.

We presume that as for HOMA and

Table 3—*ANOVA results for study variables when analyzed by subject category*

	Nonobese	Obese	Diabetic	F	P
[13C]glucose breath (δ ‰)	12.7 ± 2.9	7.9 ± 3.4*	6.3 ± 2.0*	11.7	0.0003
HOMA	1.12 ± 0.90	5.03 ± 5.00‡	3.17 ± 1.30	3.8	0.036
QUICKI	0.395 ± 0.042	0.330 ± 0.061†	0.327 ± 0.021†	6.4	0.0063
M (μmol · kg^{-1} · min^{-1})	76.8 ± 28.5	36.0 ± 27.9*	26.3 ± 17.4*	9.6	0.0009
ISI (μmol · kg^{-1} · min^{-1} · mU^{-1} · l^{-1})	2.7 ± 1.1	1.0 ± 1.1*	0.8 ± 0.5*	9.8	0.0008

Data are means ± SD. *P < 0.005 vs. nonobese, †P < 0.01 vs. nonobese, ‡P < 0.05 vs. nonobese.

QUICKI, the current [¹³C]glucose breath result evaluates basal unstimulated insulin sensitivity as opposed to indexes derived from a glucose challenge. The amount of glucose ingested during the test is small, and measurement of plasma glucose results at the 90-min point showed a net change of -0.01 mmol ($P = 0.66$). Unfortunately, corresponding serum insulin levels are not available, and, thus, it cannot be determined whether an insulinemic response does not occur. It would therefore be of interest to compare $^{13}CO_2$ output when even less glucose is provided versus a more substantial glucose load. However, although mechanistically intriguing and perhaps providing insight into peripheral versus hepatic insulin sensitivity, for practical purposes, there is generally a good correlation between steady-state versus dynamic measures of insulin resistance (25). Nevertheless, further work will be necessary to fully determine the physiological processes that the [¹³C]glucose breath test measures.

It is important to emphasize that the [¹³C]glucose breath test has been carried out under conditions similar to those of the oral glucose tolerance test (namely, after an overnight fast and with no more than light activity). Additionally, the [¹³C]glucose breath has not been used to assess the effects of interventions designed to alter insulin sensitivity. Thus, the performance of the [¹³C]glucose breath test under conditions of altered metabolic activity or in response to metabolic changes is unknown. This may be of significance because one study indicated a lack of correlation between QUICKI and the effects of chronic exercise on insulin sensitivity (26). On the other hand, changes in QUICKI reflective of changes in insulin sensitivity were seen by another study examining the effects of diet and exercise in diabetic subjects (21). Similarly, we did recruit diabetic individuals on oral hypoglycemic agents. It is possible that the use of these medications may have influenced the correlations. However, as mentioned, exclusion of these individuals from the analysis affected the various correlation coefficients by no more than 0.05. Additionally, because the [¹³C]glucose breath test and the insulin clamp were carried out at the same time of day after similar periods of withholding medication, it can be anticipated that the relative insulin sensitivities during those times were also similar. Nevertheless, further study regarding the performance characteristics of the [¹³C]glucose breath test in response to metabolic changes or medications will be necessary.

Although QUICKI may be a somewhat better predictor of insulin resistance based on the ISI, the [¹³C]glucose breath test offers some advantages. Whereas both QUICKI and HOMA require blood sampling, the [¹³C]glucose breath test requires only breath samples. Thus, there is no need for specially trained personnel, for blood precautions, or for specialized handling and storage of the sample. With written instructions, patients themselves could potentially carry out the [¹³C]glucose breath test. Indeed, this is analogous to the commonly used commercial [¹³C]urea breath tests for *H. pylori*. Similarly, once a breath sample is taken, it is stable for 90 days and requires no specialized handling. For both HOMA and QUICKI, serum must be processed within a few hours. The [¹³C]glucose breath test may therefore be more useful in remote locations than either of the preceding tests. Similarly, in large cohort studies, it may be more advantageous to use the [¹³C]glucose breath test whereby a number of samples can be collected and batched over time before shipment to a central analytical facility. Clearly, though, the breath test also has the disadvantage of requiring 90 min to perform versus a single sampling for both HOMA and QUICKI. On the other hand, this compares favorably with the 120 min required for an oral glucose tolerance test, the latter also requiring prompt processing of samples. The cost of the [¹³C]glucose breath test may be considered to be of concern. Whereas an economic evaluation is beyond the scope of the current article, the manufacturer indicates that the cost is somewhat less that the commonly used [¹³C]urea breath test for *H. pylori* and is comparable, given caveats regarding variations in laboratory pricing, with the cost of carrying out a HOMA measurement (D. Kinniburgh, personal communication). Additionally, although we carried out $^{13}CO_2$ measurement using an isotope ratio mass spectrometer, the same measurement can be carried out just as accurately using a relatively cheaper nondispersive infrared spectrometer, available as a point-of-care instrument. Thus, the cost of the [¹³C]glucose breath test will likely not be prohibitive.

In conclusion, results from the [¹³C]glucose breath test correlate with insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp in a manner comparable to other surrogate measures of insulin sensitivity. Under certain circumstances, the [¹³C]glucose breath test may therefore prove useful as a measure of insulin resistance. However, further studies are necessary to validate this test in a variety of settings and in a variety of circumstances.

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