Published in final edited form as:

J Diabetes Complications. 2015 March ; 29(2): 238–244. doi:10.1016/j.jdiacomp.2014.11.009.

Failure of Hyperglycemia and Hyperinsulinemia to Compensate for Impaired Metabolic Response to an Oral Glucose Load

M Hussain¹, M Janghorbani^{2,3}, S Schuette², RV Considine¹, RL Chisholm¹, and KJ Mather¹

¹Indiana University School of Medicine, Indianapolis, IN

²BioChem Analysis Inc., Chicago IL

³Center for Stable Isotope Research Inc, Chicago IL

Abstract

Objective—To evaluate whether the augmented insulin and glucose response to a glucose challenge is sufficient to compensate for defects in glucose utilization in obesity and type 2 diabetes, using a breath test measurement of integrated glucose metabolism.

Methods—Non-obese, obese normoglycemic and obese Type 2 diabetic subjects were studied on 2 consecutive days. A 75g oral glucose load spiked with ¹³C-glucose was administered, measuring exhaled breath ¹³CO₂ as an integrated measure of glucose metabolism and oxidation. A hyperinsulinemic euglycemic clamp was performed, measuring whole body glucose disposal rate. Body composition was measured by DEXA. Multivariable analyses were performed to evaluate the determinants of the breath ¹³CO₂.

Results—Breath ¹³CO₂ was reduced in obese and type 2 diabetic subjects despite hyperglycemia and hyperinsulinemia. The primary determinants of breath response were lean mass, fat mass, fasting FFA concentrations, and OGTT glucose excursion. Multiple approaches to analysis showed that hyperglycemia and hyperinsulinemia were not sufficient to compensate for the defect in glucose metabolism in obesity and diabetes.

Conclusions—Augmented insulin and glucose responses during an OGTT are not sufficient to overcome the underlying defects in glucose metabolism in obesity and diabetes.

Keywords

Glucose oxidation; breath test; insulin resistance; compensation; hyperinsulinemia; hyperglycemia

© 2014 Elsevier Inc. All rights reserved.

Address for Correspondence: Kieren J Mather MD FRCPC Professor of Medicine Indiana University School of Medicine 541 North Clinical Dr, CL365 Indianapolis IN 46202 Tel 317-278-7826 Fax 317-274-2695 kmather@iu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author contributions: Study design KJM, RLC, MJ; data collection KJM, RLC, RVC; data analysis MH, KJM; manuscript preparation MH, MJ, SS, RVC, RLC, KJM.

Conflicts of Interest: MJ and SS are employees of BioChemAnalysis Corp, which was the recipient of the NIH SBIR grant that supported these studies. Processes of data collection and data analysis were independent of this company. The remaining authors have no conflicts to declare.

Introduction

Impaired carbohydrate oxidation is a well-recognized feature of obesity and diabetes. Investigations to date have implicated defects in multiple stages of glucose handling and metabolism, including the pancreatic response to ingestion (1), glucose absorption (2), glucose and insulin delivery to peripheral tissues (3, 4), tissue insulin receptor signaling responses (5-7), and transmembrane transport and intracellular glucose trapping by phosphorylation (7, 8) following insulin stimulation (insulin-mediated glucose uptake) or driven by mass action (glucose-mediated glucose uptake) (9-11). Most recently impaired mitochondrial function (12-14) has been added to this list.

The relative importance of these phenomena in impaired net carbohydrate oxidation in obesity and type 2 diabetes remains unclear. One argument has been that net tissue glucose uptake and oxidation are normalized by compensatory hyperinsulinemia and/or hyperglycemia, an idea that has found some experimental support (15, 16). However, other studies suggested that neither postprandial hyperinsulinemia nor postprandial hyperglycemia were sufficient to overcome the net metabolic defects in obesity and type 2 DM (17-19). Our group demonstrated using limb balance methods in humans that the net glucose delivery to skeletal muscle under steady-state conditions did not differ across insulin resistance groupings, suggesting that hyperinsulinemia was sufficient to compensate for this aspect of glucose handling (20). In a recent paper, Galgani and Ravussin (21) studied integrated whole-body glucose metabolism, measuring total carbohydrate oxidation by indirect calorimetry and production of labeled water following ingestion of labeled glucose as part of a traditional 75 gram glucose load. Nondiabetic subjects were studied, divided by clamp-derived insulin sensitivity into insulin resistant and insulin sensitive groups. There was no difference in rates of net glucose oxidation following glucose ingestion between these groups. There was however a lower glucose oxidation rate in insulin resistant subjects when adjusted for their greater glucose exposure following glucose ingestion. These results suggest that the modest hyperglycemic response was compensatory in this nondiabetic group.

We have recently utilized exhaled labeled CO₂ following ingestion of labeled glucose as an index of integrated carbohydrate handling (22-24). This method has been found to distinguish between lean, obese and obese/type 2 diabetic subjects, showing stepped decrements in areas under the curve for net glucose utilization (22, 24). Analogous to the Galgani method, by measuring a post-metabolism product of ingested glucose the breath method integrates all of the components of tissue and cellular glucose handling. This method is clearly dependent on the glucose excursion in response to ingestion, and in fact can serve as a surrogate measure of this phenomenon (24); we have also recently shown that it is inversely correlated to fasting indices of insulin resistance and to clamp-derived measures of insulin resistance (23). In contrast to effects observed using the deuterium labeling method of Galgani, our method demonstrates early and continuing divergence of tracer appearance between subject groups (arguing that this is not simply an effect of delayed glucose metabolism) (22). For current purposes the breath method offers a new opportunity to

evaluate the question whether hyperinsulinemia and/or hyperglycemia following an oral challenge are sufficient to normalize tissue glucose metabolism.

Subjects, Materials and Methods

We performed secondary analyses of data from a recently published study (23). Full details of the methodology and participant characteristics are previously published (23); for convenience we provide a brief summary here. Participants were men and women, 18–65 years of age. Participants were categorized as non-obese (BMI <30 kg/m²), or obese (BMI ≥30 kg/m²). Diabetes mellitus was determined by prior physician diagnosis or defined at screening using a 2-hour 75-g oral glucose tolerance test (OGTT) under fasting conditions, applying the American Diabetes Association criteria of fasting glucose level >126 mg/dL or 2-h glucose level >200 mg/dL). Participants with diabetes could be treated with any combination of diet, exercise, insulin, or antidiabetes medications except thiazolidinediones or metformin. Volunteers were excluded if they had used thiazolidinediones within 6 months or metformin within 4 weeks. Stable antihypertensive or antihyperlipidemic medications were allowed. Volunteers were also excluded if not weight-stable for at least 6 months, if they had type 1 diabetes or rare variant forms of diabetes, were pregnant, had a concurrent acute or chronic medical illness likely to affect systemic fuel metabolism, used psychotropic medication including antidepressants, or had pulmonary disease or a history of current or past smoking.

Volunteers who qualified after this screening were scheduled for clinical research center admission on 2 consecutive days to undergo study measurements. Volunteers provided written informed consent for screening and main study participation. This project was overseen and approved by the Indiana University Institutional Review Board.

Measurements

The methodology has been previously published (23); a brief summary is provided. Body composition was measured by dual energy x-ray absorptiometry at the time of screening evaluation. OGTT and clamp studies were done in the morning following an overnight fast; participants consumed their usual diet except where provided by the study while admitted to the clinical research center. Participants with diabetes continued to take their diabetes medications, except morning treatments were withheld on each of the 2 measurement days until after the completion of the studies.

Anthropomorphic and blood pressure measures were performed the morning of the first testing procedure. Beginning at 7:30 a.m., baseline blood samples were drawn through an indwelling intravenous catheter, followed by a standard 75-g OGTT containing an added 150mg of [¹³C₆] glucose (catalog number CLM-1396; Cambridge Isotope Laboratories, Andover, MA). Breath samples were obtained at half-hourly intervals during the following 3 h. Blood samples were obtained concurrent with these breath samples. Upon completion of the OGTT, subjects were fed lunch and dinner ad libitum. Fasting from 8 p.m. was again implemented in anticipation of the second measurement day.

A 4-hour hyperinsulinemic euglycemic clamp procedure was performed on the second day using established procedures in our laboratory (20), based on the original method of

DeFronzo (25). An insulin infusion rate of 120 mU/m²/min was used to ensure full suppression of endogenous glucose production in all subject populations (26, 27).

Analytical procedures

Breath [¹³C] glucose enrichment was measured using isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH). Screening laboratory measurements were performed by the Indiana University Health clinical laboratory using standard methodologies. Glucose measurements were performed at the bedside using a glucose oxidase method (YSI 2500 STAT glucose analyzer; Yellow Springs Instruments, Yellow Springs OH). Serum free fatty acids were measured using a colorimetric assay (Roche Diagnostics, Indianapolis, IN). Serum insulin levels were measured by radioimmunoassay (Millipore-Linco, St. Charles MO).

End points

Fasting and 2-hour glucose values and the area under the curve for glucose excursion above baseline to 180 min (glucose AUC₁₈₀, calculated using the trapezoidal rule) were derived from OGTT glucose data. Similarly, breath measures of enrichment of exhaled breath for ¹³CO₂ were used to calculate breath AUC. In prior work we determined that the breath AUC₁₈₀ value is the preferred single endpoint measure for this breath testing method (22-24). This value was used as the dependent variable for the current set of analyses.

The clamp glucose infusion rate-based calculation of glucose disposal rate (GDR) was adjusted for shifts in and out of the glucose space over each 20-min interval, with the average of values from the fourth hour of the clamp procedure taken as the GDR for each individual (23). This measure was then expressed per Kg fat-free mass (GDR_{ffm}) for the current set of analyses. The oral disposition index (oDI) was calculated using the OGTT-derived insulinogenic index (insulin₃₀-insulin₀/glucose₃₀-glucose₀) multiplied by the clamp-derived GDR_{ffm}. Free Fatty Acids (FFA) were measured in blood samples from the OGTT and the glucose clamp procedure. From these measures we calculated the Adipose Insulin Resistance Index (Adipose-IR_i = FFA₀ min OGTT × Fasting plasma insulin) (28), and estimated adipose insulin sensitivity as the ability of insulin to suppress lipolysis (FFA₁₂₀) = Free Fatty Acid_{0min} – Free Fatty acid_{120min} using clamp data.

Statistical analysis

Due to technical or scheduling problems we were not able to obtain DEXA measures on all participants. For the purposes of the current analyses involving body composition measures, we were therefore limited to 45 of the original 69 participants. The characteristics of this subset are described in Table 1. Where specific analyses could be done with the full dataset, these were performed in parallel; there were no systematic differences in the results obtained from the full dataset versus the subset with body composition measures available (not shown).

Descriptive statistics were compared across groups using one-way analysis of variance, with subsequent pairwise testing using the Bonferroni method for variables with homogenous variance across group, or the Games-Howell method for variables with nonhomogenous

variance. Age differed across the phenotypic groupings (Table 1, $p=0.011$), and age and sex were significant univariate determinants of the breath response ($r=0.34$, $p=0.005$ and $r=-0.34$, $p=0.003$ respectively). Therefore the analyses were adjusted for these demographic parameters. Age- and sex-adjusted univariate correlations were evaluated between the breath index of glucose oxidation and the metabolic variables of interest listed above.

Multivariable linear modeling analyses were performed to evaluate the primary determinants of the breath response, first in a hierarchical stepwise approach that allowed forced inclusion of variables of interest and then in standard forward stepwise fashion to extract the primary independent determinants. In the hierarchical modeling, age and sex were included as potential confounders. Insulin and glucose areas under the curve were evaluated initially to address the question of a compensatory effect; these parameters were also forced into the hierarchical models. To construct parsimonious models and minimize collinearity among parameters, 120 minute glucose and FFA concentrations were not included in the hierarchical models. The forward stepwise model did not include any forced parameters, and included age, sex, and variables that exhibited univariate associations with $p<0.10$. Adjusted breath responses were calculated from this model, for the further evaluation of relationships of adjusted breath response with insulin resistance, and to compare adjusted values across phenotypic groups. To allow implicit comparisons of the effects of incremental excursions versus total excursions of glucose and insulin, the modeling approaches were replicated using only incremental AUC parameters.

Results

The characteristics of the study participants evaluated are presented in **Table 1**. The expected group differences were seen in measures of obesity, glucose and insulin excursions after glucose ingestion, and fatty acid concentrations. Total fat mass differed between non-obese versus obese, and non-obese versus diabetic ($P<0.0001$ for ANOVA comparing all groups; $p<0.05$ for pairwise comparisons). There was a more modest but statistically significant difference in lean mass across groups ($p=0.04$), greater in the obese and diabetic subjects but not achieving significance in pairwise comparisons. Breath parameters also differed across groups, with graded reductions in tracer enrichment as obesity/dysglycemia increased ($P<0.0001$ across groups, all pairwise comparisons $p<0.05$), indicating progressively impaired net glucose uptake and oxidation despite the concurrent magnified glucose and/or insulin excursions.

Age- and sex-adjusted correlations of the breath data and various anthropomorphic and metabolic variables of interest are presented in **Table 2**. BMI (inversely) and insulin sensitivity (directly) were significantly related to breath $^{13}\text{CO}_2$ production. Total lean mass and total fat mass were also strongly inversely related to breath $^{13}\text{CO}_2$ production. Fasting and OGTT glucose values were inversely related to breath production of $^{13}\text{CO}_2$ following ingestion of labeled glucose. OGTT insulin area under the curve was not significantly related to breath $^{13}\text{CO}_2$ production, but the oral disposition index (a measure of early insulin production with the OGTT) was directly associated (i.e. greater early insulin production was related to greater breath $^{13}\text{CO}_2$). Free fatty acid concentrations were not significantly

associated, but the fasting adipose insulin sensitivity index was related to breath $^{13}\text{CO}_2$ production.

The results of multivariable modeling to identify the main determinants of breath $^{13}\text{CO}_2$ production are presented in **Table 3** (Models 1-4, incorporating total glucose and insulin areas under the curve) and **Table 4** (Models 5-8, incorporating incremental glucose and insulin areas under the curve). A hierarchical approach was taken to isolate effects of glucose absorption from those related to insulin sensitivity and body composition.

Age and sex, while related to parameters of interest in univariate analyses, were not important determinants of $^{13}\text{CO}_2$ production in the hierarchical models. Model 1 evaluated the contributions of total glucose and insulin exposures; this model explained approximately 15% of the variance in the $^{13}\text{CO}_2$ production, due to a contribution of glucose excursion but not insulin. Model 2 added metabolic parameters related to obesity and insulin resistance (which were individually strongly related to the breath parameter), early insulin response to the glucose load, and fasting glucose and fatty acid parameters. 120 minute glucose and free fatty acid values were not included due to concerns of collinearity with other included variables. Insulin sensitivity expressed per kg fat free mass was not significantly related to breath $^{13}\text{CO}_2$ production in this or the subsequent multiply adjusted model. Model 3 added DEXA-derived measures of body composition. Doing so removed the contribution of body mass index revealing a significant effect of lean mass but not fat mass as a determinant of $^{13}\text{CO}_2$ production.

With traditional forward stepwise modeling (Model 4), lean mass was again the most potent determinant of the breath response, with an inverse relationship (lower breath response with greater lean mass). Total fat mass was also modestly associated with the breath response in this model. Fasting free fatty acids, 120 minute OGTT glucose, and oDI (early insulin response to glucose) were also significant contributors to the breath response. A direct relationship with glucose excursion emerged from the selection process, along with an inverse relationship with 120 minute glucose. As with the hierarchical modeling, in this approach to modeling insulin resistance and insulin excursion were not independent determinants of the breath response.

A parallel set of analyses were performed using incremental AUC for glucose and insulin from the OGTT rather than total AUC (**Table 4**). The overall pattern that emerged from the hierarchical modeling was similar to those of Models 1-3, including a contribution from the incremental OGTT glucose excursion and BMI that were no longer significant after adding body composition to the model, and a final model with only lean body mass contributing significantly. The results of the forward stepwise modeling (Model 8) differed from analyses using total glucose excursion only in that the incremental glucose excursion parameter did not survive the selection process. .

Next we turned to the question of whether post challenge hyperinsulinemia and/or hyperglycemia are sufficient to overcome the effects of insulin resistance on net tissue glucose metabolism. Simple inspection of the raw data (**Figure 1**, left panels) suggests that the groupwise differences in insulin and glucose excursions were insufficient to compensate,

as the raw breath data differed. Adjusting the breath response for the glucose or insulin exposures across the post-OGTT interval individually (**Figure 1** right middle and lower panels) did not remove the group differences in breath response.

It is evident from Table 1 and the modeling approaches that the groups differed in other parameters that could be contributing to the group differences in breath response. We therefore used the regression modeling results to adjust for the main determinants of the breath response (i.e. Models 4 and 8), and evaluate whether these adjusted breath values account for group differences. The results from this exercise using Model 4 are presented; essentially identical results were seen when adjustments using Model 8 were used (not shown). **Figure 2** upper panel presents the adjusted breath response plotted against clamp-derived insulin sensitivity. The adjusted breath response remains significantly related to GDR_{ffm}, suggesting that this set of adjustments is not sufficient to explain the relationship of breath $^{13}\text{CO}_2$ production with insulin resistance. Further, the adjusted breath response remained significantly different across the subject groups (**Figure 2** lower panel), suggesting that the primary factors underlying the breath response, including lean body mass, early insulin response, 120 minute OGTT glucose levels, and total glucose excursion are insufficient to account for the net metabolic defect in postprandial glucose utilization in obesity and type 2 diabetes. The lack of effect from adjusting for glucose and insulin excursion, combined with the observation that these excursions were not individually strong contributors to the breath response, argues that the hyperglycemia and hyperinsulinemia of obesity and type 2 diabetes are not sufficient to overcome the metabolic defect that is evident with an oral glucose tolerance test.

Discussion

Exhaled $^{13}\text{CO}_2$ following ingestion of ^{13}C -labeled glucose provides a measure of integrated glucose metabolism, from absorption through cellular oxidation and waste CO_2 disposal. We observed significant stepped reductions in this response in obese and obese/Type 2 diabetic individuals, but these groups also differ in a number of relevant phenomena including the insulin and glucose excursions following glucose ingestion, and tissue responsiveness to insulin. The current analyses were aimed at adjusting the breath response for these differences in order to rigorously evaluate the question of whether the augmented insulin and glucose responses were sufficient to overcome the intrinsic metabolic defects that underlie obesity and type 2 diabetes. The primary determinants of the breath response in this dataset included total lean mass, total fat mass, fasting fatty acid concentrations and measures of glucose and insulin response. In analyses adjusting for these effects, breath responses remained different across groups, arguing that overall the exaggerated insulin and glucose responses are not fully compensatory for the underlying metabolic defects.

We and others have previously found differences by obesity or insulin resistance status in the breath response following ^{13}C -labeled glucose (22, 24, 29, 30). These observations, along with those currently presented, are in contrast to a recent study using an analogous method, ingesting ^2H -labeled glucose and measuring appearance of labeled $^2\text{H}_2\text{O}$ in total body water (21). In that study of 28 healthy men and women, divided into insulin resistant versus insulin sensitive subgroups by the median clamp-derived measure of insulin-

stimulated glucose disposal, there was no significant difference in the rate of appearance of tracer in body water by insulin resistance status. However, there was a lower rate of tracer appearance in the insulin resistant subgroup after adjustment for the overall post-challenge glucose concentrations, suggesting that increased glucose excursions were compensating for an underlying metabolic defect in the more insulin resistant subset of their subjects. The differences between results from our studies and these data likely reflect important differences in the populations studied, with our studies evaluating a broader range of metabolic dysfunction. This interpretation is bolstered by prior observations using a water labeling method that showed significantly lower net glucose oxidation in type 2 diabetes (31), and a continuous relationship between the rate of appearance of tracer in body water and clamp-measured insulin resistance (32). Methodologic differences may also contribute to the contrasting observations; for example, ^{13}C -labeled intermediates can be redirected to metabolic fates other than immediate mitochondrial uptake and oxidation. Also it is possible that there are differences in timing of the detectable appearance of the label in the detection medium.

Determinants of Integrated Glucose Handling

The particular set of determinants of the breath response that emerged from multivariable modeling is perhaps surprising. Most immediately, although 2hr OGTT glucose was inversely related to the breath response, the total glucose and insulin excursions, and the measures of insulin resistance, did not emerge as important determinants of the breath response when other factors were included. Further, while it is predictable on first principles that the total muscle mass is an important determinant of total tissue metabolism, the final relationship of this term was unexpectedly inverse in our study – the greater the muscle mass, the lower the breath response. This is presumably due to the fact that the obese and diabetic individuals, who demonstrate the lowest breath responses, have the greatest lean body mass as well as the greatest fat mass. One parameter that exhibited an expected relationship was fasting fatty acid concentration (reduced breath response with higher FFA), which emerged as a strong determinant in the multivariable modeling. This effect may reflect the balance of fuel availability and competition for mitochondrial oxidation; however, we did not design any aspect of this study to ask detailed questions about this interplay of fuel sources. Specifically designed studies will be needed to explore these possibilities.

Is the hyperinsulinemic/hyperglycemic response compensatory?

The principal result of these analyses is that the breath response remains reduced in obese normoglycemic and obese/Type 2 diabetic subjects compared to lean subjects after adjustment for relevant covariates, specifically including the glucose and insulin excursions. This indicates that the hyperglycemic and hyperinsulinemic responses following an oral glucose load are not sufficient to overcome the underlying metabolic defect in obese and type 2 diabetic subjects. This result is concordant with prior reports using dynamic insulin clamps (17, 18, 33) to mimic the OGTT response, with the obvious advantage that our results reflect native in vivo responses. Our analyses suggest that neither insulin resistance nor impairments in glucose-mediated glucose disposal are overcome.

Impaired tissue responses to insulin-mediated glucose disposal are well described features of obesity and type 2 diabetes. Our findings highlight the parallel impairment in glucose disposal, including glucose-mediated glucose disposal, which has been previously described as an important contributor to dysglycemia in Type 2 diabetes (9-11). It is also possible that other aspects of non-insulin mediated glucose handling contribute to the residual defect. The latter would include differences in intermediary metabolism, and mitochondrial defects in glucose oxidation (12, 19). Another potentially relevant factor is the meal-related change in metabolic rate (and, more generally, dynamic changes in the metabolic response following meal ingestion) (34-36) which is known to differ by insulin resistance status and could also contribute to residual group differences independent of insulin resistance. To determine whether such factors explain the quantitative differences in glucose handling we observe will require specifically designed studies that assess these phenomena.

Limitations

The principal limitations in these analyses are the reduced dataset due to unavailable body composition measures. This dataset was originally collected for the purpose of evaluating the relationship of the breath response with insulin resistance (23) and included a precise measure of insulin sensitivity, a precise measure of the breath response, and the current collection of potentially relevant covariates to allow for detailed analyses such as those presented. Given the high precision of the measures of insulin resistance the relationship with breath response in the adjusted models was unequivocally negative with the current sample size. The current analyses highlight measures that will be needed in order to further explore the questions raised here, such as calorimetry, measures of fatty acid dynamics, or measures of mitochondrial number or function.

Conclusions

The breath measurement of total glucose metabolism and oxidation is reduced in obesity and type 2 diabetes, reflecting impaired whole-body glucose metabolism following an oral glucose load. Multiple approaches to analyzing the contributions of exaggerated glucose and insulin exposure demonstrated that augmented insulin and glucose responses during an OGTT are not sufficient to overcome the underlying defects in glucose metabolism in obesity and diabetes.

Acknowledgements

This project was supported by NIDDK 2R44DK072637, and by the Indiana Clinical and Translational Sciences Institute funded in part by Grant Number TR000006 (Clinical Research Center support) from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award. We gratefully acknowledge the contributions of study staff and Clinical Research Center support staff, and the willing participation of our study volunteers.

References

1. Del Prato S, Marchetti P. Beta- and alpha-cell dysfunction in type 2 diabetes. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2004; 36(11-12):775-81. [PubMed: 15655708]

2. Nielsen MF, Nyholm B, Caumo A, Chandramouli V, Schumann WC, Cobelli C, et al. Prandial glucose effectiveness and fasting gluconeogenesis in insulin-resistant first-degree relatives of patients with type 2 diabetes. *Diabetes*. 2000; 49(12):2135–41. [PubMed: 11118017]
3. Baron AD, Clark MG. Role of blood flow in the regulation of muscle glucose uptake. *Annual review of nutrition*. 1997; 17:487–99.
4. Hallsten K, Yki-Jarvinen H, Peltoniemi P, Oikonen V, Takala T, Kempainen J, et al. Insulin- and exercise-stimulated skeletal muscle blood flow and glucose uptake in obese men. *Obes Res*. 2003; 11(2):257–65. [PubMed: 12582222]
5. Caro JF, Sinha MK, Raju SM, Ittoop O, Pories WJ, Flickinger EG, et al. Insulin receptor kinase in human skeletal muscle from obese subjects with and without noninsulin dependent diabetes. *The Journal of clinical investigation*. 1987; 79(5):1330–7. [PubMed: 3033021]
6. Draznin B. Molecular mechanisms of insulin resistance: serine phosphorylation of insulin receptor substrate-1 and increased expression of p85alpha: the two sides of a coin. *Diabetes*. 2006; 55(8):2392–7. [PubMed: 16873706]
7. Petersen KF, Shulman GI. Etiology of insulin resistance. *The American journal of medicine*. 2006; 119(5 Suppl 1):S10–6. [PubMed: 16563942]
8. Williams KV, Bertoldo A, Mattioni B, Price JC, Cobelli C, Kelley DE. Glucose transport and phosphorylation in skeletal muscle in obesity: insight from a muscle-specific positron emission tomography model. *The Journal of clinical endocrinology and metabolism*. 2003; 88(3):1271–9. [PubMed: 12629118]
9. Williams KV, Price JC, Kelley DE. Interactions of impaired glucose transport and phosphorylation in skeletal muscle insulin resistance: a dose-response assessment using positron emission tomography. *Diabetes*. 2001; 50(9):2069–79. [PubMed: 11522673]
10. Ahren B, Pacini G. Insufficient islet compensation to insulin resistance vs. reduced glucose effectiveness in glucose-intolerant mice. *American journal of physiology Endocrinology and metabolism*. 2002; 283(4):E738–44. [PubMed: 12217891]
11. Viviani GL, Pacini G. Reduced glucose effectiveness as a feature of glucose intolerance: evidence in elderly type-2 diabetic subjects. *Aging*. 1999; 11(3):169–75. [PubMed: 10476312]
12. Coletta DK, Mandarino LJ. Mitochondrial dysfunction and insulin resistance from the outside in: extracellular matrix, the cytoskeleton, and mitochondria. *American journal of physiology Endocrinology and metabolism*. 2011; 301(5):E749–55. [PubMed: 21862724]
13. Goodpaster BH. Mitochondrial deficiency is associated with insulin resistance. *Diabetes*. 2013; 62(4):1032–5. [PubMed: 23520282]
14. Parish R, Petersen KF. Mitochondrial dysfunction and type 2 diabetes. *Current diabetes reports*. 2005; 5(3):177–83. [PubMed: 15929863]
15. Egan BM, Stepniakowski K. Compensatory hyperinsulinemia and the forearm vasodilator response during an oral glucose-tolerance test in obese hypertensives. *J Hypertens*. 1994; 12(9):1061–7. [PubMed: 7852750]
16. Natalucci S, Boemi M, Fumelli D, Fumelli P, Burattini R. Interaction between glucose metabolism and endogenous insulin release in hypertension. *Metabolism: clinical and experimental*. 2002; 51(3):297–303. [PubMed: 11887163]
17. Klauser R, Prager R, Schernthaner G, Olefsky JM. Contribution of postprandial insulin and glucose to glucose disposal in normal and insulin-resistant obese subjects. *The Journal of clinical endocrinology and metabolism*. 1991; 73(4):758–64. [PubMed: 1890150]
18. Prager R, Wallace P, Olefsky JM. Hyperinsulinemia does not compensate for peripheral insulin resistance in obesity. *Diabetes*. 1987; 36(3):327–34. [PubMed: 3542654]
19. Mandarino LJ, Consoli A, Jain A, Kelley DE. Interaction of carbohydrate and fat fuels in human skeletal muscle: impact of obesity and NIDDM. *Am J Physiol*. 1996; 270(3 Pt 1):E463–70. [PubMed: 8638694]
20. Mather K, Laakso M, Edelman S, Hook G, Baron A. Evidence for physiological coupling of insulin-mediated glucose metabolism and limb blood flow. *American journal of physiology Endocrinology and metabolism*. 2000; 279(6):E1264–70. [PubMed: 11093913]
21. Galgani JE, Ravussin E. Postprandial whole-body glycolysis is similar in insulin-resistant and insulin-sensitive non-diabetic humans. *Diabetologia*. 2012; 55(3):737–42. [PubMed: 22173627]

22. Dillon EL, Janghorbani M, Angel JA, Casperson SL, Grady JJ, Urban RJ, et al. Novel noninvasive breath test method for screening individuals at risk for diabetes. *Diabetes care*. 2009; 32(3):430–5. [PubMed: 19074994]
23. Hussain M, Jangorbhani M, Schuette S, Considine RV, Chisholm RL, Mather KJ. [13C]glucose breath testing provides a noninvasive measure of insulin resistance: calibration analyses against clamp studies. *Diabetes technology & therapeutics*. 2014; 16(2):102–12. [PubMed: 24116833]
24. Singal P, Janghorbani M, Schuette SA, Chisholm R, Mather KJ. Intra-individual variability of CO(2) breath isotope enrichment compared to blood glucose in the oral glucose tolerance test. *Diabetes technology & therapeutics*. 2010; 12(12):947–53. [PubMed: 21128841]
25. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*. 1979; 237(3):E214–23. [PubMed: 382871]
26. O'Shaughnessy IM, Myers TJ, Stepniakowski K, Nazzaro P, Kelly TM, Hoffmann RG, et al. Glucose metabolism in abdominally obese hypertensive and normotensive subjects. *Hypertension*. 1995; 26(1):186–92. [PubMed: 7607722]
27. Ferrannini E, Mari A. How to measure insulin sensitivity. *J Hypertens*. 1998; 16(7):895–906. [PubMed: 9794728]
28. Lomonaco R, Ortiz-Lopez C, Orsak B, Webb A, Hardies J, Darland C, et al. Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. *Hepatology*. 2012; 55(5):1389–97. [PubMed: 22183689]
29. Ghosh C, Maity A, Banik GD, Som S, Chakraborty A, Selvan C, et al. Non invasive 13C-glucose breath test using residual gas analyzer-mass spectrometry: a novel tool for screening individuals with pre-diabetes and type 2 diabetes. *Journal of breath research*. 2014; 8(3):036001. [PubMed: 24946152]
30. Jetha MM, Nzekwu U, Lewanczuk RZ, Ball GD. A novel, non-invasive 13C-glucose breath test to estimate insulin resistance in obese prepubertal children. *Journal of pediatric endocrinology & metabolism : JPEM*. 2009; 22(11):1051–9. [PubMed: 20101891]
31. Bluck LJ, Clapperton AT, Kidney CV, Coward WA. Glycogenesis and glucose oxidation during an intravenous glucose tolerance test in man. *Clinical science*. 2004; 106(6):645–52. [PubMed: 14759220]
32. Beysen C, Murphy EJ, McLaughlin T, Riiff T, Lamendola C, Turner HC, et al. Whole-body glycolysis measured by the deuterated-glucose disposal test correlates highly with insulin resistance in vivo. *Diabetes care*. 2007; 30(5):1143–9. [PubMed: 17259480]
33. Katz H, Homan M, Jensen M, Caumo A, Cobelli C, Rizza R. Assessment of insulin action in NIDDM in the presence of dynamic changes in insulin and glucose concentration. *Diabetes*. 1994; 43(2):289–96. [PubMed: 8288053]
34. Brodovicz KG, Girman CJ, Simonis-Bik AM, Rijkkelijkhuizen JM, Zelis M, Bunck MC, et al. Postprandial metabolic responses to mixed versus liquid meal tests in healthy men and men with type 2 diabetes. *Diabetes research and clinical practice*. 2011; 94(3):449–55. [PubMed: 21955958]
35. Nair KS, Webster J, Garrow JS. Effect of impaired glucose tolerance and type II diabetes on resting metabolic rate and thermic response to a glucose meal in obese women. *Metabolism: clinical and experimental*. 1986; 35(7):640–4. [PubMed: 3523118]
36. Watts NB, DiGirolamo M. Carbohydrate tolerance improves with fasting in obese subjects with noninsulin-dependent (type II) diabetes. *The American journal of the medical sciences*. 1990; 299(4):250–6. [PubMed: 2181873]

Figure 1. Oral glucose tolerance results in the evaluated population. The panels on the right present $^{13}\text{CO}_2$ AUC₁₈₀ across groups, unadjusted in the right upper panel, then following simple adjustments for glucose excursion (right middle panel) or insulin excursion (right bottom panel).

Figure 2.

Analyses of adjusted breath responses. Upper panel, adjusted breath response in relation to clamp-derived insulin resistance. Lower panel, breath responses further adjusted also for insulin resistance show persisting differences across subject groups. *Non-obese statistically different from the other groups.

Table 1

Population Descriptions. P values are presented for the one-way analysis of variance (ANOVA) comparing all three groups: ^alean versus obese, ^blean versus obese/type 2 diabetes, and ^cobese versus obese/type 2 diabetes. Adipose – IRi, adipose insulin resistance index; GDRffm, glucose disposal rate per kg fat free mass; FFA, Free Fatty Acid; OGTT, oral glucose tolerance test; FFA120=FFA at time 0 of OGTT-FFA at 120 min of OGTT; DEXA, Dual- Energy X-ray Absorptiometry measurements of, whole –body tissue mass, whole-body fat mass, and whole-body lean mass

	Non-obese (33)		Obese (22)		Type 2 DM (13)		
	Mean	SD	Mean	SD	Mean	SD	P(ANOVA)
Age (years)	39.1	12.3	40.2	10.0	53.2	4.0	0.011 (b, c)
Weight (kg)	74.2	10.1	105.0	18.9	95.3	18.2	<0.001
BMI (kg/m ²)	25.1	2.5	35.6	5.0	30.3	4.9	<0.0001 (a, b)
Percent Fat (%)	30.8	11.0	43.9	8.9	30.3	11.3	0.001 (a,b)
Total fat mass (kg)	21.1	10.5	44.7	13.4	31.1	12.0	<0.0001 (a, b)
Total lean mass (kg)	47.0	15.2	56.4	11.0	59.8	11.6	0.04
Fasting Insulin (mU/mL)	11.4	5.2	24.0	35.8	15.6	5.6	0.23
Fasting glucose (mg/dL)	95.2	13.1	102.0	12.4	145.6	70.2	0.001
120 minute glucose (mg/dL)	107.7	35.0	116.3	26.4	275.5	102.9	<0.0001 (b, c)
OGTT Glucose AUC180 (mg/dL*min)	21391	3562	22227	4307	42896	16255	<0.0001 (b, c)
OGTT Insulin AUC180 (pmol/L*min)	9263	4547	14791	9916	10296	7876	0.08

Table 2

Partial correlations of breath $^{13}\text{CO}_2\text{AUC180}$ with metabolic parameters. Pearson correlations adjusting for age and sex, presented as r value (P value). Adipose-IRi, Adipose-Insulin Resistance Index; GDRffm, glucose disposal rate per kg fat free mass; incAUC180 is the incremental AUC above baseline, whereas AUC180 is the total AUC over the 180 minute OGTT.

Correlations	$^{13}\text{CO}_2\text{ AUC180 min } (\% \cdot \text{min})$
BMI (kg/m^2)	-0.50 (0.001)
Percent Fat (%)	-0.16 (0.30)
Total fat mass (kg)	-0.46 (0.002)
Total lean mass (kg)	-0.59 (<0.0001)
Fasting Plasma Insulin (mU/mL)	-0.25 (0.11)
Fasting glucose (mg/dL)	-0.35 (0.02)
120 minute glucose (mg/dL)	-0.43 (0.002)
OGTT Glucose AUC180 ($\text{mg}/\text{dL} \cdot \text{min}$)	-0.40 (0.028)
OGTT Glucose incAUC180 ($\text{mg}/\text{dL} \cdot \text{min}$)	-0.38 (0.001)
OGTT Insulin AUC180 ($\text{pmol}/\text{L} \cdot \text{min}$)	-0.23 (0.14)
OGTT Insulin incAUC180 ($\text{pmol}/\text{L} \cdot \text{min}$)	+0.04 (0.72)
Oral Disposition Index	+0.30 (0.049)
GDRffm ($\text{mg}/\text{kg}/\text{min}$)	+0.39 (0.01)
Fasting FFA (mmol/L)	-0.32 (0.055)
120 min FFA (mmol/L)	-0.31 (0.065)
FFA ₁₂₀ (mmol/L)	-0.10 (0.56)
Adipose-IRi	-0.35 (0.034)

Table 3

Determinants of $^{13}\text{CO}_2$ production following ingestion of ^{13}C -labeled glucose, using total AUC for glucose and insulin. Age- and sex-adjusted models. Hierarchical linear regression modeling was performed, incorporating modules with parameters of glucose and insulin exposure (Model 1), metabolic variables (Model 2) and finally body composition measures (Model 3). Model 4 is the result of a multivariable regression incorporating using a forward stepwise approach. Values in the cells are direct beta terms (p values) for each variable as determinants of $^{13}\text{CO}_2$ AUC180 within each model.

	Model 1	Model 2	Model 3	Model 4
Total Model R^2 -adj (p value)	0.123 (0.076)	0.401 (0.004)	0.601 (9.5×10^{-5})	0.699 (2.6×10^{-8})
Change in F (p value of the change) from prior model	3.65 (0.037)	2.71 (0.033)	8.17 (0.002)	N/A
OGTT Glucose AUC180 (mg/dl*min*1000)	-1.13 (0.034)	-2.45 (0.014)	-1.35 (0.14)	+2.18 (0.044)
OGTT Insulin AUC180 (mU/dL*min*1000)	-0.81 (0.17)	+0.76 (0.42)	0.40 (0.64)	
oDI		+9.10 (0.072)	+7.36 (0.08)	+7.71 (0.018)
BMI (kg/m ²)		-2.76 (0.011)	-0.32 (0.82)	
Fasting glucose (mg/dL)		+0.47 (0.12)	+0.27 (0.30)	
120 min glucose				-0.42 (0.006)
GDRfm (mg/kg/min)		-0.51 (0.89)	+2.04 (0.60)	
Fasting FFA (mmol/L)		-7.21 (0.74)	-30.07 (0.16)	-49.12 (0.001)
Adipose-IRi		-0.50 (0.19)	-0.38 (0.26)	
DEXA total fat mass			+0.25 (0.76)	-0.37 (0.034)
DEXA total lean mass			-2.18 (0.002)	-1.63 (3.7×10^{-7})

Table 4

Determinants of $^{13}\text{CO}_2$ production following ingestion of ^{13}C -labeled glucose, using incremental AUC parameters for insulin and glucose. Models are constructed in parallel with those in Table 3. Age- and sex-adjusted models. Hierarchical linear regression modeling was performed, incorporating modules with parameters of glucose and insulin exposure (Model 5), metabolic variables (Model 6) and finally body composition measures (Model 7). Model 8 is the result of a multivariable regression incorporating using a forward stepwise approach. Values in the cells are direct beta terms (p values) for each variable as determinants of $^{13}\text{CO}_2$ AUC180 within each model.

	Model 5	Model 6	Model 7	Model 8
Total Model R^2 -adj (p value)	0.047 (0.23)	0.376 (0.006)	0.500 (1.5×10⁻⁴)	0.668 (4.3×10⁻⁶)
Change in F (p value of the change) from prior model	1.47 (0.23)	3.99 (0.005)	8.06 (0.002)	N/A
OGTT Glucose incAUC180 (mg/dL*min*1000)	-1.77 (0.069)	-2.17 (0.027)	-1.06 (0.26)	
OGTT Insulin incAUC180 (mU/dL*min*1000)	-0.33 (0.69)	+0.52 (0.52)	+0.22 (0.81)	
oDI		+8.71 (0.09)	+7.10 (0.10)	+6.54 (0.048)
BMI (kg/m ²)		-2.72(0.014)	-0.16 (0.91)	
Fasting glucose (mg/dL)		-0.009 (0.96)	-0.005 (0.98)	
120 min glucose (mg/dL)				-0.13 (0.002)
GDRfm (mg/kg/min)		-1.01 (0.79)	+1.75 (0.67)	
Fasting FFA (mmol/L)		-13.91 (0.50)	-34.93 (0.09)	-44.83 (0.002)
Adipose-IRi		-0.29 (0.36)	-0.26 (0.31)	
DEXA total fat mass			+0.21 (0.81)	-0.40 (0.029)
DEXA total lean mass			-2.22 (0.003)	-1.59 (1.0×10 ⁻⁶)