

Whole-Body Glycolysis Measured by the Deuterated-Glucose Disposal Test
Correlates Highly with Insulin Resistance *in vivo*

Received for publication 29 August 2006 and accepted in revised form 17 January 2007.

Running title:

Glycolysis as a measure of insulin resistance

Carine Beysen, DPhil¹
Elizabeth J. Murphy, MD, DPhil^{1,2}
Tracey McLaughlin, MD³
Timothy Riiff¹
Cindy Lamendola³
Holly C. Turner¹
Mohamad Awada¹
Scott M. Turner, PhD¹
Gerald Reaven, MD³
Marc K. Hellerstein, MD, PhD^{2,4,5}

¹ KineMed, Inc., Emeryville, CA

² SF General Hospital

University of California at San Francisco,
San Francisco, CA 94110

³ Stanford University
Palo Alto, CA 94305

⁴ Nutritional Sciences and Toxicology
University of California at Berkeley, CA 94720

⁵ Corresponding author:

Marc Hellerstein, MD, PhD
Nutritional Sciences and Toxicology
309 Morgan Hall
University of California at Berkeley
CA 94720
Email: march@nature.Berkeley.edu

Abstract

OBJECTIVE To compare an *in vivo* test of whole-body glycolysis, the deuterated-glucose disposal test (^2H -GDT), with insulin sensitivity measured by the euglycemic-hyperinsulinemic glucose clamp and the steady-state plasma glucose (SSPG) test.

RESEARCH DESIGN AND METHODS The ^2H -GDT consists of an oral glucose challenge containing deuterated-glucose, followed by measurement of heavy water ($^2\text{H}_2\text{O}$) production, which represents whole-body glycolytic disposal of the glucose load. $^2\text{H}_2\text{O}$ production is corrected for ambient insulin concentration as an index of tissue insulin sensitivity. The ^2H -GDT was compared to euglycemic-hyperinsulinemic glucose clamps in healthy lean subjects (n=8) and subjects diagnosed with the metabolic syndrome (n=9), and to the SSPG in overweight (n=12) and obese (n=6) subjects.

RESULTS A strong correlation with the clamp was observed for the 75g and 30g ^2H -GDT ($r=0.95$, $P<0.0001$ and $r=0.88$, $P<0.0001$ respectively). The ^2H -GDT and clamp studies revealed marked insulin resistance in metabolic syndrome subjects compared to lean controls. The correlation with the clamp was maintained in each group (lean, $r=0.86$, $P<0.01$; metabolic syndrome, $r=0.81$, $P<0.01$) for the 75g test. The ^2H -GDT also correlated strongly with the SSPG ($r=0.87$, $P<0.0001$) in overweight and obese subjects.

CONCLUSIONS The ^2H -GDT, which measures whole-body glycolysis in humans in a quantitative manner, correlates highly with the euglycemic-hyperinsulinemic glucose clamp and the SSPG. Impaired insulin-mediated whole-body glycolysis is a feature of insulin resistance, providing a means of assessing insulin sensitivity *in vivo*.

Insulin resistance (IR) is a highly prevalent condition (1) and an important risk factor for the development of type 2 diabetes mellitus (2; 3) and cardiovascular disease (4; 5). IR has thus become a public health issue of central importance (6). Several approaches have been used for measurement of IR *in vivo*.

The gold-standard technique, the euglycemic-hyperinsulinemic glucose clamp (7) measures insulin-mediated glucose disposal by peripheral tissues. The clamp is labor-intensive and burdensome on subjects, precluding routine use. Other direct measures of IR such as the steady-state plasma glucose (SSPG) test (8; 9) and the frequently sampled intravenous glucose tolerance test (FS IVGTT) (10), also primarily measure whole-body glucose disposal and are similarly labor intensive. Indirect markers of IR, such as serum insulin concentrations, body mass index (BMI), waist circumference, and serum triacylglycerol concentrations have limited utility. BMI, for example, is not a good surrogate for IR, as 16% of IR individuals are lean, while 30% of insulin sensitive persons are obese or overweight (11). Other surrogate measures model the relationship between glucose and insulin. The homeostatic model assessment (HOMA) (12; 13), quantitative insulin sensitivity check index (QUICKI) (14), and models based on the oral glucose tolerance test (OGTT) (e.g., (15)) have correlated well with the clamp in some studies but, often, the correlation has been poor ($r^2 \sim 0.50$) (16-19), especially in normal weight individuals (19). Attempts to define a clinical entity (i.e. the metabolic syndrome (20)) or combine parameters (e.g., BMI and HOMA-IR) (21) to establish IR have proven to be insensitive for detecting IR (22; 23) and do not provide a continuous measure for monitoring treatment response (21).

Any measurement test must be based on the known physiology of IR. Most

attention has been given to the effects of IR on glycogen storage in tissues such as skeletal muscle, but glycolytic disposal of glucose shares the steps of glucose transport and phosphorylation, which are likely to play key roles in insulin sensitivity (24), and glycolytic enzymes are sensitive to insulin. Moreover, at serum insulin concentrations in the “dynamic range” between basal and maximal (i.e., between approximately 100-1000 pM), glycolytic disposal of glucose closely parallels glycogen storage in clamp studies and is impaired to a similar extent by IR (25; 26). Measurement of glycolysis has a major advantage over glycogen synthesis, however, since tissue glycogen is not readily accessible to sampling, whereas an immediate product of glycolysis (hydrogen atoms released to tissue water), can be tagged, traced and sampled in body fluids.

Here, we describe a stable isotope-mass spectrometric test of whole-body glycolysis, the deuterated-glucose disposal test (^2H -GDT), and compare this index of *in vivo* glucose utilization to established measures of IR. The ^2H -GDT measures the rate of uptake, phosphorylation and glycolytic metabolism of glucose in response to a physiologic glucose load. Our goal here was to determine whether insulin-mediated whole-body glycolysis is a quantitative measure of IR in humans, based on correlation with the clamp and SSPG in subjects across a range of insulin sensitivities.

RESEARCH DESIGN AND METHODS

Model

The ^2H -GDT consists of a 75g or 30g glucose load containing 15g of [6,6- $^2\text{H}_2$] glucose. For C-6 labeled glucose, >90% of C-H hydrogen atoms are lost to tissue water after glycolytic metabolism to pyruvate and oxaloacetate, but C-H hydrogen atoms are otherwise non-labile and retained in the glucose molecule (27). Complete glycolytic

disposal of 15g of [6,6-²H₂] glucose results in the release of 0.0824 moles of ²H₂O. Dispersion in the body water pool (about 2200 moles/70 kg human) results in body water enrichment of about 0.0037% (250 delta units) in humans - well above the detection limit (ca. 1 delta) for ²H₂O by isotope-ratio mass spectrometry (IR-MS).

The ²H-GDT was designed to adhere to the following principles: *a*) ambient glucose and insulin concentrations should reflect metabolic conditions present physiologically in daily life, *b*) the test should primarily quantify insulin-mediated glucose utilization by tissues, rather than other aspects of insulin action, such as suppression of hepatic glucose production *c*) the metabolic conditions should be comparable to those present in tests proven to be predictive for cardiovascular outcomes and type 2 diabetes risk (i.e., euglycemic-hyperinsulinemic clamp and SSPG).

Some features of glucose homeostasis are worth noting. First, suppression of endogenous glucose production (EGP) is essentially complete during a clamp or SSPG (28). Second, insulin concentrations influence the relative contributions from glycolytic and glycogenic routes of glucose disposal. At supraphysiologic concentrations (e.g., >1000 pM), glucose disposal is dominated by the pathway in the body with the greatest glucose utilization capacity (muscle glycogen storage), whereas glycolysis reaches a maximum and becomes less informative. At low insulin concentrations (e.g., <120-180 pM), hepatic glucose production is not fully suppressed (29) and insulin-independent glucose utilization (e.g., by the brain) makes a proportionately greater contribution to glucose utilization. Accordingly, our criteria for a glycolysis-based test of IR are best fulfilled at serum insulin concentrations in the dynamic range, as achieved by a 75g oral glucose load (15).

Operationally, any measure of tissue insulin sensitivity must either control insulin

concentrations (e.g., the clamp or SSPG) or correct for them (e.g., HOMA or FS IVGTT). Glucose utilization rates cannot be compared directly as a metric of tissue insulin sensitivity if insulin levels are different. Accordingly, the observed glycolytic rate was corrected here for ambient insulin concentrations, to calculate insulin sensitivity of tissues. If glucose concentrations are variable or out of the normal range, correction for glucose concentrations can also be performed to account for glucose effectiveness (30).

Human subjects

All subjects gave written, informed consent. Protocols were approved by the appropriate institutional review boards. Two studies were performed:

1) Euglycemic-hyperinsulinemic glucose clamp. Ten healthy non-obese subjects and ten subjects with the metabolic syndrome were recruited and studied at the Diabetes and Glandular Disease Center (San Antonio, TX, a fee for service clinical trial site). Metabolic syndrome was defined by the presence of three out of five modified ATP III criteria (20): blood pressure $\geq 130/85$ mmHg or taking antihypertensive medication; fasting triacylglycerol concentrations ≥ 1.7 mmol/l (150 mg/dl) or treated with gemfibrozil or fenofibrate; fasting glucose concentrations ≥ 5.5 mmol/l (100 mg/dl); fasting HDL cholesterol ≤ 1.0 mmol/l (40 mg/dl) for men or ≤ 1.3 mmol/l (50 mg/dl) for women; waist ≥ 102 cm for men or ≥ 88 cm for women. Subjects with known type 2 diabetes, fasting glucose ≥ 7.0 mmol/l (126 mg/dl), or treated with medications known to alter insulin sensitivity were excluded.

Subjects underwent a 75g ²H-GDT followed within 2 weeks by a clamp study. Subjects then returned 6-8 months later for a 30g ²H-GDT.

2) SSPG studies. Overweight (n=12) and obese subjects (n=6) screened for a weight loss study at Stanford University underwent both an SSPG test and a 75g ²H-GDT.

Subjects with BMI >35 kg/m², fasting plasma glucose ≥7.0 mmol/l, variable weight, use of drugs known to alter insulin sensitivity, or history of major organ disease were excluded.

²H-GDT protocol

After a 10-12h overnight fast, subjects drank 75g or 30g glucose, of which 15g was [6,6-²H₂] glucose, dissolved in 300 ml of water. Plasma samples for glucose, insulin and ²H₂O content were obtained at baseline and hourly for up to 4 hours.

Euglycemic-hyperinsulinemic glucose clamp protocol

The clamps were performed as described by DeFronzo et al (7). Briefly, after a 10h overnight fast subjects received a primed, continuous insulin infusion (40 mU/m²/min) for 110 min. Blood glucose concentrations were clamped at 5 mmol/l with an exogenous glucose infusion (20% wt/vol).

SSPG protocol

Insulin-mediated glucose disposal was quantified as previously described (11). Briefly, subjects were admitted to the Stanford GCRC after a 12 hour overnight fast for 180 minute infusions of octreotide (0.27 µg/m²/min), insulin (25 mU/m²/min), and glucose (240 mg/m² min).

Total body water (TBW) determination

TBW in the clamp study was measured by bioelectrical impedance analysis (Tanita model TBF 300A). As a comparison, TBW was also calculated from weight and height using the Hume formula (31). TBW for the SSPG study was calculated using the Hume formula.

Analytic determinations

For ²H₂O content, 100 µL aliquots of plasma in the cap of an inverted vial were placed in a 70°C glass bead filled heating block overnight. Water distillate inside the

vial was then collected. Samples were run in triplicate.

Plasma glucose was measured by the glucose-oxidase method. Plasma insulin concentrations were measured by radioimmunoassay (Linco, St. Charles, MO).

IR-MS analyses

The deuterium content of plasma samples was determined using a Thermo Finnigan High Temperature Conversion/Elemental Analyzer coupled with a Thermo Finnigan MAT 253 IR-MS via a Conflo-III Interface. The deuterium isotope abundance is first calculated in δ ²H values relative to the international VSMOW standard, and then transformed to APE by using a calibration curve of standards.

Calculations

²H₂O enrichment from IR-MS was converted to mmoles by multiplying enrichment by the TBW pool size and dividing by 20 (MW ²H₂O). Plasma insulin (INS AUC) and glucose (GLU AUC) areas under the curves were calculated using the trapezoidal method.

The ²H-GDT parameter of insulin sensitivity was calculated as ²H₂O production (mmol) per unit of insulin exposure (INS AUC). M-value (mg/kg/min), SSPG and steady-state plasma insulin (SSPI) concentrations were determined during the last 30 min of the clamp. HOMA (12), QUICKI (14) and ISI (Matsuda) (15) were calculated as referenced.

Plasma glucose was determined during the last 30 minutes of SSPG; the higher the SSPG concentration, the more insulin-resistant the individual.

Statistical analysis

Differences between independent groups were determined by the Mann-Whitney-*U* test. Correlations were calculated

with the Pearson's correlation test. $P \leq 0.05$ was considered statistically significant.

RESULTS

Euglycemic-hyperinsulinemic glucose clamp study

Healthy lean subjects and subjects with the metabolic syndrome were recruited, to compare the 75g $^2\text{H-GDT}$ with the clamp across a range of insulin sensitivities. Technical problems occurred with the clamp in 3 subjects preventing completion of the study, leaving 17 subjects. Fourteen subjects also had a follow-up 30g $^2\text{H-GDT}$ to determine if a lower glucose load allowed a shorter $^2\text{H-GDT}$. Clinical characteristics of subjects are shown (Table 1).

During the clamp, SSPG concentrations were 5.1 ± 0.3 mmol/l for controls vs 4.9 ± 0.3 mmol/l for metabolic syndrome subjects and SSPI concentrations were 382 ± 54 pmol/l vs 502 ± 116 pmol/l, respectively ($P < 0.05$). Peak plasma insulin concentrations after the 75g oral glucose challenge were 309 ± 123 pmol/l for controls and 837 ± 352 pmol/l for metabolic syndrome subjects. After the 30g oral glucose challenge, peak insulin concentrations were 155 ± 73 pmol/l for controls and 371 ± 191 pmol/l for metabolic syndrome subjects. No significant difference between groups was seen in GLU AUC for the 75g $^2\text{H-GDT}$ although a significant difference between groups was seen after a 30g glucose load (2h, $P < 0.01$).

The M-value from the clamp was significantly lower in subjects with metabolic syndrome (Fig. 1A). The 75g (Fig. 1B) and the 30g (data not shown) $^2\text{H-GDT}$ results revealed an almost identical proportional decrease in insulin sensitivity as the clamp in metabolic syndrome subjects. The 75g $^2\text{H-GDT}$ correlated extremely closely with the M-value across all subjects ($r = 0.93$ at 3h and $r = 0.95$ at 4h) (Fig. 1C and D). Use of the Hume formula instead of BIA to determine TBW did not change correlations. A

significant correlation with the M-value was seen for the 30g $^2\text{H-GDT}$ as early as 1-2h after the glucose load ($r = 0.88$) (Fig. 1E and Table 2), better than the 2h correlation seen with the 75g test ($r = 0.75$). Adjusting the 75g $^2\text{H-GDT}$ values for differences in glucose concentrations (GLU AUC) did not improve correlations, although a slight improvement was seen for 30g $^2\text{H-GDT}$ values ($r = 0.92$).

Correlations between the clamp, $^2\text{H-GDT}$ and other indices of IR are shown (Table 2). When including data from all subjects, the best correlation was with the 75g $^2\text{H-GDT}$ whereas only modest correlations were seen for fasting indices of IR and Matsuda ISI. Considering subjects in the metabolic syndrome group alone, only the 75g $^2\text{H-GDT}$ correlated significantly with the M-value. Correlation coefficients improved slightly when GLU AUC was included. For subjects in the control group alone, both the 75g and the 30g $^2\text{H-GDT}$ correlated significantly with the clamp. INS AUC had a weaker but significant correlation at 4h after the 75g glucose load. When differences in GLUC AUC were taken into account in the control group, the correlation coefficients improved for the 30g but not for the 75g $^2\text{H-GDT}$.

SSPG study

Clinical characteristics of subjects are shown (Table 1). SSPG values in these obese and overweight subjects ranged from 2.7-14.9 mmol/l, indicating a broad range of insulin sensitivities. Previous studies have shown that SSPG values > 10 mmol/l represent the top tertile denoting IR (32). Correlation between the SSPG value and the $^2\text{H-GDT}$ (Fig. 1F) was excellent ($r = -0.874$).

DISCUSSION

We demonstrate here that, in humans, insulin-mediated whole-body glycolysis correlates closely with insulin sensitivity in states of normal and reduced insulin sensitivity and may be useful as a potential metric of IR.

Insulin mediated whole-body glycolytic disposal of a glucose load was measured by the ^2H -GDT in IR and insulin sensitive individuals and correlated extremely well with M-values during the clamp. Correlations between the clamp and ^2H -GDT were stronger than correlations with indirect markers of IR and remained strong even in lean controls alone, where correlations with other markers have historically been poor (19). The SSPG also correlated well with the ^2H -GDT, while other markers of IR have traditionally correlated poorly (33).

Metabolic influences on glycolysis are worth considering. Factors that reduce glucose transport and/or phosphorylation should impair glycolysis and glycogen synthesis in parallel. In contrast, high-fat diet feeding to rats was reported (34) to reduce glycolytic disposal prior to reducing glycogen synthesis. It is possible that fatty acid oxidation products may inhibit glycolytic enzymes, such as phosphofruktokinase, more than glucose phosphorylation or transport. We did not see dissociation between insulin-mediated glycolysis and insulin-mediated total glucose disposal in humans with IR, however, based on comparisons of the ^2H -GDT to clamps and SSPG.

The ^2H -GDT was designed to measure glycolysis, not complete oxidation of a glucose load. Another stable isotope method has recently been described for assessing IR, based on measurement of whole-body oxidation of [$\text{U-}^{13}\text{C}_6$] glucose to $^{13}\text{CO}_2$. This approach gave a lower correlation with clamps ($r=0.69$) (35) than the ^2H -GDT. Several factors may account for the lower correlation. A $^{13}\text{CO}_2$ collection represents oxidation at a single time point, while measurement of $^2\text{H}_2\text{O}$ production reveals integrated glycolytic flux over the preceding 3-4h period. In addition, extensive exchange of ^{13}C -label from $^{13}\text{CO}_2$ into cellular metabolites reduces recovery of $^{13}\text{CO}_2$ in breath in an unpredictable manner (36),

whereas $^2\text{H}_2\text{O}$ distributes predictably into body water. Dietary and endogenous fatty acids may also affect pyruvate dehydrogenase activity, independent of insulin sensitivity (37), so that complete oxidation of glucose may be dissociated from glycolysis or glycogen synthesis. Finally, the low total glucose load given (15g) for the breath $^{13}\text{CO}_2$ test (35) interrogates a different physiologic state and insulinemic level than is present in clamps (e.g., EGP or insulin-independent glucose utilization may confound results).

Hyperglycemia per se can stimulate uptake and glycolytic disposal of glucose ("glucose effectiveness" (30)), potentially increasing $^2\text{H}_2\text{O}$ production. Accordingly, we corrected for glucose AUC, although this had at most a minor impact on calculated insulin sensitivity in the euglycemic subjects studied here. Conversely, hyperglycemia can also reduce $^2\text{H}_2\text{O}$ production. A glucose load mixes into whole-body pool of free glucose of 15-20g, so that the throughput of glucose after a 75g glucose challenge is much higher than the pool size present before the load. If fasting blood glucose concentration is 11 mmol/l, the pool size increases to 30-40g; above 17 mmol/l, the fasting pool size of glucose becomes quantitatively significant in comparison to the glucose load. Thus, the presence of fasting hyperglycemia may dilute exogenous labeled glucose and reduce recovery of $^2\text{H}_2\text{O}$ from an exogenous load, but this effect is modest unless hyperglycemia is severe.

The effects of EGP on the ^2H -GDT are also worth considering. One of the advantages of the ^2H -GDT (compared to glucose concentrations, for example) is that it primarily reflects glucose utilization, not hepatic IR. EGP might dilute exogenous ^2H -glucose, however, and reduce recovery of $^2\text{H}_2\text{O}$. An oral glucose load normally suppresses fasting EGP by about 60%, from ~ 2 mg/kg/min to ~ 0.8 mg/kg/min during the next 3-4 hours (38). Endogenously produced

glucose (roughly 14g over 4 h) mixes with exogenous glucose (75g), for a total flux rate of about 90g glucose through the bloodstream. This, in turn, mixes with the ca. 20g of free glucose in the body prior to the glucose load. Total “exposure” to glucose over the 3-4h after a 75g glucose load is therefore normally about 110g, with EGP providing about 11-14g, so that dilution of labeled glucose by EGP normally affects $^2\text{H}_2\text{O}$ recovery only modestly. If a euglycemic, insulin resistant subject starts with a higher EGP (2.5–3.0 mg/kg/min, for example) and only suppresses EGP by 40% (to 1.5-1.8 mg/kg/min), EGP will be about twice-normal (~25-30 g over 4h). Total flux of glucose will increase to ~100-105g/4h and the total glucose exposure to 120-125g. Thus, if EGP after a glucose load is twice-normal, the net effect is to dilute exogenous label by about 15-20% and potentially reduce $^2\text{H}_2\text{O}$ production proportionately. This is less than the differences observed between lean controls and subjects with metabolic syndrome (Fig.1B). In diabetic patients, EGP may be even higher and less suppressible. Although we did not measure EGP here, this could be measured after a ^2H -GDT based on the die-away curve of plasma ^2H -glucose. Given these factors, the ^2H -GDT is most easily interpreted in the presence of normal or near-normal glucose concentrations, which is the setting where metrics of IR are most lacking. Independent validation of the ^2H -GDT in diabetes will be required.

Based on these findings, the ^2H -GDT is of interest as an index of IR. The ^2H -GDT correlates closely with the euglycemic-hyperinsulinemic glucose clamp and the SSPG. In addition, this measurement approach has a fundamental advantage over measures of IR that are based on serum insulin concentrations, including derived parameters such as HOMA or QUICKI. The

loss of beta-cell function that develops in the progression to type 2 diabetes (39; 40) represents a fundamental problem for use of insulin concentrations alone as markers of IR. An individual who exhibits progressively lower insulin levels over time might either be improving insulin sensitivity or progressing toward beta-cell failure. Indeed, a low insulin sensitivity index is better at predicting cardiovascular disease (41) than high post-challenge insulin concentrations.

In contrast, the ^2H -GDT overcomes the problem of pancreatic response, because the ratio of $^2\text{H}_2\text{O}$ production/Insulin AUC remains low even if insulin secretion is failing.

The ^2H -GDT does have some limitations. IR-MS is not a routine technique in clinical laboratories, but requires special expertise. Future analytic advances (e.g. laser-based spectroscopic instruments) may overcome this limitation. Other limitations include the 2-3 hours required to complete the test, with blood draws during that time. While the ^2H -GDT is considerably easier than the clamp, it does not have the ease of a single blood draw.

In summary, the ^2H -GDT, a measure of whole-body glycolysis in response to a physiologic glucose load, has an excellent correlation with the euglycemic-hyperinsulinemic glucose clamp and SSPG in humans across a wide range of insulin sensitivities. Accordingly, impaired insulin-mediated whole-body glycolytic disposal of a glucose load is a feature of IR in humans and provides a quantitative metric of insulin sensitivity.

ACKNOWLEDGEMENTS

We would like to thank Mark Kipnes and the Diabetes and Glandular Disease Center in San Antonio for performing the clamps and Alex Fong for his assistance.

REFERENCES

1. Reaven GM: Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 37:1595-1607, 1988
2. Warram JH, Martin BC, Krolewski AS, Soeldner JS, Kahn CR: Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med* 113:909-915, 1990
3. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C: Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 329:1988-1992, 1993
4. Yip J, Facchini FS, Reaven GM: Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. *J Clin Endocrinol Metab* 83:2773-2776, 1998
5. Howard G, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R: Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* 93:1809-1817, 1996
6. Ford ES: Prevalence of the metabolic syndrome in US populations. *Endocrinol Metab Clin North Am* 33:333-350, 2004
7. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214-223, 1979
8. Greenfield MS, Doberne L, Kraemer F, Tobey T, Reaven G: Assessment of insulin resistance with the insulin suppression test and the euglycemic clamp. *Diabetes* 30:387-392, 1981
9. Shen SW, Reaven GM, Farquhar JW: Comparison of impedance to insulin-mediated glucose uptake in normal subjects and in subjects with latent diabetes. *J Clin Invest* 49:2151-2160, 1970
10. Bergman RN: Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 38:1512-1527, 1989
11. McLaughlin T, Allison G, Abbasi F, Lamendola C, Reaven G: Prevalence of insulin resistance and associated cardiovascular disease risk factors among normal weight, overweight, and obese individuals. *Metabolism* 53:495-499, 2004
12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419, 1985
13. Wallace TM, Levy JC, Matthews DR: Use and abuse of HOMA modeling. *Diabetes Care* 27:1487-1495, 2004
14. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ: Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85:2402-2410, 2000
15. Matsuda M, DeFronzo RA: Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462-1470, 1999
16. Tripathy D, Almgren P, Tuomi T, Groop L: Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. *Diabetes Care* 27:2204-2210, 2004
17. Ferrara CM, Goldberg AP: Limited value of the homeostasis model assessment to predict insulin resistance in older men with impaired glucose tolerance. *Diabetes Care* 24:245-249, 2001
18. Skrha J, Haas T, Sindelka G, Prazny M, Widimsky J, Cibula D, Svacina S: Comparison of the insulin action parameters from hyperinsulinemic clamps with homeostasis model assessment

and QUICKI indexes in subjects with different endocrine disorders. *J Clin Endocrinol Metab* 89:135-141, 2004

19. Kim SH, Abbasi F, Reaven GM: Impact of degree of obesity on surrogate estimates of insulin resistance. *Diabetes Care* 27:1998-2002, 2004

20. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *Jama* 285:2486-2497, 2001

21. Stern SE, Williams K, Ferrannini E, DeFronzo RA, Bogardus C, Stern MP: Identification of individuals with insulin resistance using routine clinical measurements. *Diabetes* 54:333-339, 2005

22. Cheal KL, Abbasi F, Lamendola C, McLaughlin T, Reaven GM, Ford ES: Relationship to insulin resistance of the adult treatment panel III diagnostic criteria for identification of the metabolic syndrome. *Diabetes* 53:1195-1200, 2004

23. Liao Y, Kwon S, Shaughnessy S, Wallace P, Hutto A, Jenkins AJ, Klein RL, Garvey WT: Critical evaluation of adult treatment panel III criteria in identifying insulin resistance with dyslipidemia. *Diabetes Care* 27:978-983, 2004

24. Shulman GI: Cellular mechanisms of insulin resistance. *J Clin Invest* 106:171-176, 2000

25. Golay A, Felber JP, Jequier E, DeFronzo RA, Ferrannini E: Metabolic basis of obesity and noninsulin-dependent diabetes mellitus. *Diabetes Metab Rev* 4:727-747, 1988

26. Golay A, DeFronzo RA, Ferrannini E, Simonson DC, Thorin D, Acheson K, Thiebaut D, Curchod B, Jequier E, Felber JP: Oxidative and non-oxidative glucose metabolism in non-obese type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 31:585-591, 1988

27. Katz J, Rognstad R: Futile cycles in the metabolism of glucose. *Curr Top Cell Regul* 10:237-289, 1976

28. Matsuda M, DeFronzo RA: In vivo measurement of insulin sensitivity in humans. In *Diabetes and Obesity* Draznin B, Rizza R, Eds. Totowa, New Jersey, Humana Press, 1997, p. 23-65

29. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J: Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* 32:35-45, 1983

30. Best JD, Kahn SE, Ader M, Watanabe RM, Ni TC, Bergman RN: Role of glucose effectiveness in the determination of glucose tolerance. *Diabetes Care* 19:1018-1030, 1996

31. Hume R, Weyers E: Relationship between total body water and surface area in normal and obese subjects. *J Clin Pathol* 24:234-238, 1971

32. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G: Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med* 139:802-809, 2003

33. Yeni-Komshian H, Carantoni M, Abbasi F, Reaven GM: Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. *Diabetes Care* 23:171-175, 2000

34. Kim JK, Wi JK, Youn JH: Metabolic impairment precedes insulin resistance in skeletal muscle during high-fat feeding in rats. *Diabetes* 45:651-658, 1996

35. Lewanczuk RZ, Paty BW, Toth EL: Comparison of the [13C]glucose breath test to the hyperinsulinemic-euglycemic clamp when determining insulin resistance. *Diabetes Care* 27:441-447, 2004

36. Tounian P, Schneiter P, Henry S, Tappy L: Effects of infused glucose on glycogen metabolism in healthy humans. *Clin Physiol* 16:403-416, 1996

37. Randle PJ: Fuel selection in animals. *Biochem Soc Trans* 14:799-806, 1986
38. Mitrakou A, Kelley D, Veneman T, Jenssen T, Pangburn T, Reilly J, Gerich J: Contribution of abnormal muscle and liver glucose metabolism to postprandial hyperglycemia in NIDDM. *Diabetes* 39:1381-1390, 1990
39. Kahn SE: The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia* 46:3-19, 2003
40. Bergman RN, Finegood DT, Kahn SE: The evolution of beta-cell dysfunction and insulin resistance in type 2 diabetes. *Eur J Clin Invest* 32 Suppl 3:35-45, 2002
41. Rewers M, Zaccaro D, D'Agostino R, Haffner S, Saad MF, Selby JV, Bergman R, Savage P: Insulin sensitivity, insulinemia, and coronary artery disease: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 27:781-787, 2004

Table 1 Clinical characteristics of subjects who participated in the euglycemic-hyperinsulinemic glucose clamp study and the SSPG study.

	Clamp study		SSPG study
	<i>Controls</i>	<i>Metabolic syndrome</i>	<i>Overweight and Obese</i>
Sex (M/F)	1/7	4/5	3/15
Age (years)	28 ± 6	44 ± 14*	54 ± 7
Weight (kg)	57 ± 9	100 ± 19*	82 ± 11
BMI (kg/m ²)	22 ± 2	35 ± 4*	29 ± 2
Waist circumference (cm)	77 ± 8	111 ± 12*	98 ± 10
Fasting Glucose (mmol/l)	4.8 ± 0.3	5.9 ± 0.5*	5.2 ± 0.5
Fasting Insulin (pmol/l)	24 ± 12	159 ± 72*	71 ± 24
Fasting triacylglycerols (mmol/l)	0.86 ± 0.35	2.01 ± 1.13*	1.35 ± 0.74
Fasting total cholesterol (mmol/l)	4.8 ± 0.9	4.5 ± 0.9	5.6 ± 1.7
Fasting LDL cholesterol (mmol/l)	3.2 ± 0.9	2.6 ± 0.8	3.3 ± 1.3
Fasting HDL cholesterol (mmol/l)	1.7 ± 0.3	1.2 ± 0.2*	1.2 ± 0.3
Systolic BP (mmHg)	107 ± 7	126 ± 12*	123 ± 15
Diastolic BP (mmHg)	67 ± 11	78 ± 8*	71 ± 9

* $P < 0.05$ significantly different from control subjects

Table 2 Correlations between the euglycemic-hyperinsulinemic glucose clamp M-value, the ²H-GDT and surrogate measures of insulin sensitivity for metabolic syndrome subjects, lean control subjects and all subjects combined.

	All subjects	Metabolic syndrome subjects	Lean control subjects
	<i>r</i> -value	<i>r</i> -value	<i>r</i> -value
Fasting indices			
Fasting plasma insulin	-0.67**	0.28	0.06
HOMA	-0.66**	0.38	0.03
QUICKI	0.72**	-0.25	-0.20
ISI (Matsuda)	0.67**	0.07	0.10
75g ²H-GDT			
3 h ² H-GDT	0.93***	0.81**	0.80*
4 h ² H-GDT	0.95***	0.77*	0.86**
3 h ² H-GDT /GLU AUC	0.93***	0.86**	0.80*
4 h ² H-GDT /GLU AUC	0.95***	0.83**	0.85**
3 h INS AUC	-0.79***	-0.62	-0.52
4 h INS AUC	-0.78***	-0.63	-0.72*
30g ²H-GDT			
1 h ² H-GDT	0.88***	0.12	0.82*
2 h ² H-GDT	0.88***	0.48	0.77*
1 h ² H-GDT /GLU AUC	0.90***	0.28	0.86*
2 h ² H-GDT /GLU AUC	0.92**	0.55	0.85*
1 h INS AUC	-0.71**	-0.15	-0.66
2 h INS AUC	-0.70**	-0.35	-0.67

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ significantly correlated with clamp M-value

FIGURE 1

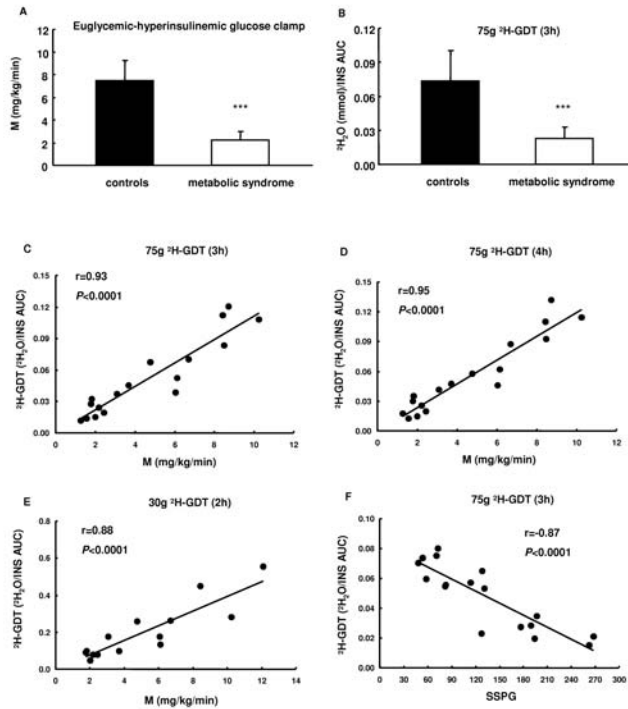


Fig. 1 M-value from the euglycemic-hyperinsulinemic glucose clamp (A) and the ²H-GDT insulin sensitivity index (²H₂O production/INS AUC) (B) calculated from the 75g ²H-GDT at 3h in lean, controls (n=8) and metabolic syndrome subjects (n=9). Correlation between the M-value and the insulin sensitivity index for the 75g ²H-GDT at 3h (C) and 4h (D) and for the 30g ²H-GDT at 2h (E). Correlation between the 75g ²H-GDT at 3h and the SSPG (F) in overweight and obese subjects (n=18). INS AUC=insulin area under the curve. Data are means ± SD. *** $P<0.001$ and ** $P<0.01$ significantly different from control subjects.