Journal of Diabetes Science and Technology
Volume 7, Issue 1, January 2013

ORIGINAL ARTICLE

A Novel Fasting Blood Test for Insulin Resistance and Prediabetes

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Abstract

Background:

Insulin resistance (IR) can precede the dysglycemic states of prediabetes and type 2 diabetes mellitus (T2DM) by a number of years and is an early marker of risk for metabolic and cardiovascular disease. There is an unmet need for a simple method to measure IR that can be used for routine screening, prospective study, risk assessment, and therapeutic monitoring. We have reported several metabolites whose fasting plasma levels correlated with insulin sensitivity. These metabolites were used in the development of a novel test for IR and prediabetes.

Methods:

Data from the Relationship between Insulin Sensitivity and Cardiovascular Disease Study were used in an iterative process of algorithm development to define the best combination of metabolites for predicting the M value derived from the hyperinsulinemic euglycemic clamp, the gold standard measure of IR. Subjects were divided into a training set and a test set for algorithm development and validation. The resulting calculated M score, M^Q, was utilized to predict IR and the risk of progressing from normal glucose tolerance to impaired glucose tolerance (IGT) over a 3 year period.

Results:

M^Q correlated with actual M values, with an *r* value of 0.66. In addition, the test detects IR and predicts 3 year IGT progression with areas under the curve of 0.79 and 0.70, respectively, outperforming other simple measures such as fasting insulin, fasting glucose, homeostatic model assessment of IR, or body mass index.

Conclusions:

The result, Quantose™, is a simple test for IR based on a single fasting blood sample and may have value as an early indicator of risk for the development of prediabetes and T2DM.

J Diabetes Sci Technol 2013;7(1):100–110

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Abbreviations: (α-HB) α-hydroxybutyric acid, (AUC) area under curve, (BMI) body mass index, (FPG) fasting plasma glucose, (HESI) heated electrospray ionization, (HOMA-IR) homeostatic model assessment of insulin resistance, (IGT) impaired glucose tolerance, (IR) insulin resistance, (L-GPC) linoleoylglycerophosphocholine, (NGT) normal glucose tolerance, (OGIS) oral glucose insulin sensitivity, (OGTT) oral glucose tolerance test, (QUICKI) quantitative insulin sensitivity check index, (RISC) Relationship between Insulin Sensitivity and Cardiovascular Disease, (T2DM) type 2 diabetes mellitus, (UHPLC-MS-MS) ultra-high performance liquid chromatographic tandem mass spectroscopy

Keywords: biomarkers, insulin resistance, metabolomics, prediabetes, Quantose

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Introduction

Insulin resistance (IR) is an early and important factor in the development of type 2 diabetes mellitus (T2DM) and may be present for years before the emergence of any changes in glycemic control.[1–3] A practical measure of IR would be valuable for early identification of individuals at risk for T2DM and cardiovascular disease in the general population and as a tool for monitoring progress in intervention strategies to prevent or delay these diseases. The gold standard measure of insulin sensitivity is the hyperinsulinemic euglycemic clamp.[4] The clamp has been used to demonstrate the range of insulin sensitivities within a population, and IR has generally been defined as the lower end of this distribution (e.g., bottom tertile).[5] The clamp is an important research tool but is not practical for routine assessment of insulin sensitivity. A simple surrogate for insulin sensitivity in nondiabetics is the fasting insulin level, but this measure loses value in the context of β-cell decompensation as T2DM progresses and does not capture the level of insulinemia needed to dispose of a glucose load.[6,7] Obesity is closely linked to IR, with body mass index (BMI) and waist circumference being good predictors of IR.[8]

A number of models have been proposed and utilized as simplified measures of insulin sensitivity.[9] Several steady-state (fasting) models based only on insulin and glucose levels have been generated, including the homeostatic model assessment of insulin resistance (HOMA-IR),[10,11] quantitative insulin sensitivity check index (QUICKI),[12] and fasting insulin resistance index.[13] Related models use fasting insulin plus various lipids measures, such as free fatty acids (revised QUICKI),[14] triglycerides (McAuley index),[15] or high-density lipoprotein/total cholesterol and free fatty acids.[16] These models are simple, requiring only a single blood sample, but it is not clear that they offer advantages over fasting insulin alone. Other models are based on the oral glucose tolerance test (OGTT) and use various combinations of glucose and insulin values from the fasting state and during the OGTT, including the Matsuda index,[17] the Stumvoll index,[18] and oral glucose insulin sensitivity (OGIS).[19] More complex methods include the insulin tolerance test and the frequently sampled intravenous glucose tolerance test.[20] These latter methods all require multiple blood samples, are complicated, and, like the clamp, are not practical for routine screening purposes. Therefore, there remains a need for a simple IR test for routine screening, prospective studies, risk assessment, and therapeutic monitoring.[21,22]

We reported a nontargeted metabolomics study using fasting plasma samples obtained from a healthy, nondiabetic population [Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) Study], searching for novel biomarkers of insulin sensitivity.[23] A number of small molecule metabolites were identified that correlated with the M values derived from the hyperinsulinemic euglycemic clamp. This work details subsequent efforts to utilize these findings to create a model for predicting the clamp M value using only data derived from a single fasting blood sample. The development, testing, validation, and utility of the Quantose™ IR diagnostic are reported herein.

Methods

Clinical Study Design

The RISC Study subjects were utilized to generate and validate the diagnostic test. The methodology and objectives of this study and the 3 year follow-up data have been published.[24,25] In brief, RISC is a prospective, observational, cohort study in clinically healthy people between the ages of 30 and 60 years recruited from 13 European countries. An OGTT was performed at the initial examination followed by a hyperinsulinemic euglycemic clamp within 1 week. A second OGTT was performed at a 3 year follow-up examination. Fasted blood samples were obtained at each examination.

Local ethics committee approval was obtained at each recruiting center. Subjects were given a written and an oral explanation of the study, and all provided informed consent.

Hyperinsulinemic Euglycemic Clamp

Insulin was infused at a rate of 240 pmol/min^{-1}/m^{-2} along with a simultaneous 20% dextrose infusion, whose rate was adjusted every 5–10 min to maintain plasma glucose within ±15% of the targeted range of 81–99 mg/dl. Insulin-mediated whole body glucose disposal was reported as the value M_{wbm} (mg/kg^{-1}/min^{-1}, whole body mass)

derived from the mean of the glucose infusion rate over the final 40 min of the 2 h clamp. The values for M_{wbm} ranged from 0.89 to 23.86 $mg/kg^{-1}/min^{-1}$. The RISC cohort was divided into tertiles based on M_{wbm}, and the members of the lowest tertile, T1, were defined as being insulin resistant (**Table 1**).

Table 1.
Relationship between Insulin Sensitivity and Cardiovascular Disease Study: Baseline Anthropometric and Metabolic Parameters by M_{wbm} Tertile[a]

	T1	T2	T3
n	426	426	425
M_{wbm} cutoffs	<5.703		>8.063
M_{wbm}	4.16 ± 1.08	6.90 ± 0.63	10.46 ± 2.32
Women (%)	49	58[b]	59[b]
Age (years)	44 ± 8	44 ± 8	43 ± 8[c]
Family history of diabetes (%)	36.5	25.3[c]	19.98[d]
BMI (kg/m^2)	28.1 ± 4.3	25.1 ± 3.3[d]	23.3 ± 2.8[d]
Fasting glucose (mg/dl)	92 ± 10	91 ± 10	90 ± 9[b]
Fasting insulin (pmol/liter)	39 (28)	26 (15)[d]	20 (12)[d]
α-HB (μg/ml)	5.01 (2.37)	4.27 (2.00)[d]	3.68 (1.97)[d]
L-GPC (μg/ml)	12.95 (5.42)	15.10 (6.42)[d]	16.92 (6.54)[d]
Oleate (μg/ml)	91.8 (42.0)	82.4 (37.8)[c]	72.3 (42.6)[d]

[a] Entries are mean ± standard deviation or median (interquartile range). All *p* values are for two-sided *t* tests, and the usual χ^2 test for proportions for sex and family history were employed (without the continuity correction). All subjects had diastolic/systolic blood pressure <140/90 mmHg, FPG <126 mg/dl, 2 h plasma glucose <200 mg/dl, total cholesterol <300 mg/dl, triglycerides <400 mg/dl, and no electrocardiogram abnormalities as per RISC Study exclusion criteria.
[b] $p < .05$ versus T1.
[c] $p < .001$ versus T1.
[d] $p < .00001$ versus T1.

Analytical Procedures

Blood samples were divided into both plasma and serum fractions, aliquoted, and stored at -80 °C. Plasma glucose was measured using the glucose oxidase method. Serum insulin was measured using a dissociation enhanced lanthanide fluoroimmunoassay (AutoDELFIA insulin kit, Wallac, Turku, Finland).

Targeted Metabolite Analysis

Metabolites were analyzed by isotope dilution ultra-high performance liquid chromatographic tandem mass spectroscopy (UHPLC-MS-MS) for absolute quantitation. Fifty microliters of ethylenediaminetetraacetic acid plasma samples were spiked with internal standard solution and subsequently subjected to protein precipitation by mixing with 250 μl of methanol. Following centrifugation, an aliquot of the clear supernatant was injected onto an UHPLC-MS-MS system consisting of a Thermo TSQ Quantum Ultra Mass Spectrometer and a Waters Acquity UHPLC system equipped with a column manager module and two different columns. α-Hydroxybutyric acid (α-HB) was eluted with a 0.01% formic acid in water/acetonitrile-methanol (1:1) gradient on a Waters Acquity BEH C18 column (100 × 2.1 mm^2, 1.7 μm) at a mobile phase flow rate of 0.4 ml/min at 40 °C. Ionization was achieved by negative heated electrospray ionization (HESI) mode. 1-linoleoylglycerophosphocholine (L-GPC) was eluted with a 0.01% formic acid in water/acetonitrile-water-ammonium formate (700:300:2.7) gradient on a Thermo BioBasic SCX column (50 × 2.1 mm^2, 5 μm) at a mobile phase flow rate of 0.5 ml/min at 40 °C. Ionization was achieved by positive HESI mode. Oleic acid was eluted isocratically with 15% 5 mM ammonium bicarbonate in water and 85% acetonitrile-methanol (1:1) on a Waters Acquity BEH C18 column (100 × 2.1 mm^2, 1.7 μm) at a mobile phase flow rate of 0.4 ml/min at 40 °C. Ionization was achieved by negative HESI mode. Quantitation was performed based on area ratios of analyte and internal standard

peaks using a weighted linear least squares regression analysis generated from fortified calibration standards in an artificial matrix prepared immediately prior to each run. The following corresponding stable labeled compounds were used as internal standards: α-HB-D_3, oleic acid-$^{13}C_{18}$, and L-GPC-D_9.

Algorithm Development Background

In a previous study, fasting plasma samples from a representative subset of the RISC cohort (n = 399) underwent nontargeted metabolomic[26,27] profiling using mass spectrometry.[23] A number of metabolites were identified whose plasma levels correlated with IR as measured by M_{ffm} (M normalized by fat-free mass; M_{wbm} gave similar correlations) obtained from the hyperinsulinemic euglycemic clamp. The highest correlating metabolite was α-HB. Other highly correlated metabolites included L-GPC, glycine, and creatine. A total of 26 metabolites from this study were collated and investigated for potential inclusion into an IR (M-predicting) algorithm along with BMI and insulin. These 26 metabolites were chosen based on statistical and biological relevance and analytical characteristics.

A total of 1277 subjects from the RISC Study had complete baseline values for M_{wbm}, insulin, glucose, BMI, age, sex, and the 26 candidate metabolite biomarkers. This group was divided into a training set (n = 894) and a test set (n = 383) for algorithm generation and validation, respectively (**Table 2**). These two sets were evenly matched for sex, age, BMI, M_{wbm}, fasting glucose, and fasting insulin.

Table 2.
Pearson Correlation of the Metabolites to M_{wbm}[a]

Univariate correlations, entire data set				
Variable	Correlation	95% CI_L	95% CI_U	*p* value
Insulin	-0.531	-0.569	-0.490	7.95E-94
α-HB	-0.368	-0.414	-0.319	3.29E-42
L-GPC	0.325	0.276	0.374	6.89E-33
Oleate	-0.217	-0.269	-0.164	4.12E-15
BMI	-0.517	-0.556	-0.476	2.66E-88
FPG	-0.121	-0.174	-0.066	1.55E-05
Univariate correlations, training set				
Variable	Correlation	95% CI_L	95% CI_U	*p* value
Insulin	-0.545	-0.589	-0.497	3.32E-70
α-HB	-0.404	-0.457	-0.347	2.32E-36
L-GPC	0.342	0.283	0.399	5.79E-26
Oleate	-0.234	-0.295	-0.171	1.36E-12
BMI	-0.515	-0.562	-0.465	9.00E-62
FPG	-0.111	-0.175	-0.046	0.000908
Univariate correlations, test set				
Variable	Correlation	95% CI_L	95% CI_U	*p* value
Insulin	-0.500	-0.571	-0.420	1.45E-25
α-HB	-0.282	-0.372	-0.187	1.99E-08
L-GPC	0.284	0.190	0.374	1.48E-08
Oleate	-0.178	-0.273	-0.079	0.000473
BMI	-0.522	-0.592	-0.445	3.42E-28
FPG	-0.143	-0.240	-0.044	0.004941

CI, confidence interval.
[a] All correlations are log–log.

Results

Algorithm Development Using the Training Set

A small set of variables were examined to see what transformations were best suited for use in the algorithm. Leverage plots suggested a natural log transformation of the input metabolite variables and a Box–Cox transformation suggested a log transformation of the response variable, M_{wbm}. Forward-selection models for predicting M_{wbm} were generated in R with the lars package.[28] Many models were generated and cross validated in an iterative manner, and the optimal number of variables was determined to be four. The final model was developed and optimized using forward selection limited to four variables. The following variables were selected in this order: fasting insulin, α-HB, L-GPC, and stearate. Stearate was later found to have the undesirable analytical property of high background levels, and interestingly, the related fatty acid oleate was determined to be a suitable replacement. Individually, insulin, α-HB, and oleate are negatively correlated with M_{wbm} and L-GPC is positively correlated with it. The Quantose algorithm consists of a multiple linear regression (natural log transformed) on insulin, α-HB, L-GPC, and oleate used to calculate ln(M). At this point, the test's calculated M (M^Q) correlated with M_{wbm} with an *r* value of 0.68.

Test Performance: Estimating M_{wbm} with the Test Set

Without refitting the coefficients from the training set, the algorithm's performance for estimating M_{wbm} was evaluated using the test set. Regression analysis on the results of these calculations showed an *r* value of 0.62. In contrast, fasting insulin, BMI, fasting glucose, HOMA-IR, and the OGTT-derived OGIS[19,25] index were all significantly less correlated with M_{wbm} when compared with M^Q (**Table 3**). These data validate the algorithm and the similar *r* values suggest that it is well fitted.

Table 3.
Pearson Correlations to the Actual ln(M_{wbm}) Values in the Test Set[a]

Variable	Correlation	95% CI, lower limit	95% CI, upper limit	*p* value versus M^Q
ln(M^Q)	0.615	0.548	0.673	—
-1*ln(HOMA-IR)	0.489	0.409	0.562	1.002E-6
-1*ln(fasting insulin)	0.500	0.420	0.571	4.449E-6
-1*ln(fasting glucose)	0.143	0.044	0.240	1.076E-19
-1*ln(BMI)	0.522	0.445	0.592	0.0072
ln(OGIS)	0.510	0.428	0.583	0.0049

CI, confidence interval.
[a] The correlation of test M^Q predictions was evaluated with the correlation of the other four variables. The Hotelling–Williams test was performed for each of these using the "r.test" function from the "psych" package in R.[28] One-sided tests were performed in order to evaluate if the correlation of calculated M is higher than the other correlation. The HOMA-IR was evaluated as fasting glucose × fasting insulin × constant. Oral glucose insulin sensitivity was calculated from data taken in the initial OGTT (*n* = 352).

Predicting Insulin Resistance in the Test Set

The areas under curve (AUCs) for detecting the bottom tertile of M_{wbm} (<5.703 $mg/kg^{-1}/min^{-1}$) were computed (**Table 4**) as an assessment of the test's ability to predict IR. The algorithm's derived AUC was 0.79. This is significantly better than the AUCs calculated for HOMA-IR, fasting insulin, fasting glucose, and the OGIS index, but not for BMI.

The Final Algorithm

At this point, the algorithm's coefficients were refitted to the entire data set (*n* = 1277). M^Q calculated from the final optimized algorithm correlated with M_{wbm}, with an *r* value of 0.66. The correlation plots for M^Q (and HOMA-IR) versus M_{wbm} are shown in **Figure 1**. The M^Q score is a simple IR test and was not adjusted for age, sex, family history of diabetes, BMI, or level of physical activity.

Table 4.
Areas under the Curve for Detecting the Bottom Tertile of M_{wbm} in the Test Set (Predicting Insulin Resistance)[a]

Variable	AUC	95% CI, lower limit	95% CI, upper limit	*p* value versus M^Q
M^Q	0.794	0.746	0.843	—
HOMA-IR	0.725	0.669	0.782	9.87E-05
Fasting insulin	0.736	0.680	0.791	0.0007
Fasting glucose	0.548	0.486	0.611	1.77E-14
BMI	0.754	0.702	0.806	0.0671
OGIS	0.731	0.672	0.790	0.012

CI, confidence interval.
[a] Areas under the curve for predicting IR, as defined by the bottom tertile of M_{wbm} values (<5.703) using the algorithm-derived M^Q from the training set and the other four variables. The AUCs and confidence intervals were computed in R[28] from the package "pROC."[29] The DeLong method[30] was used to construct the 95% confidence intervals. The significance test for comparing the predicted M to the other AUCs was implemented with "roc.test" from the pROC package using the DeLong method with the "paired" option given that these are ROC curves from the same subjects. One-sided tests were performed to test the hypothesis that the AUC is greater for test predicted M. Oral glucose insulin sensitivity was calculated from data taken in the initial OGTT (n = 352).

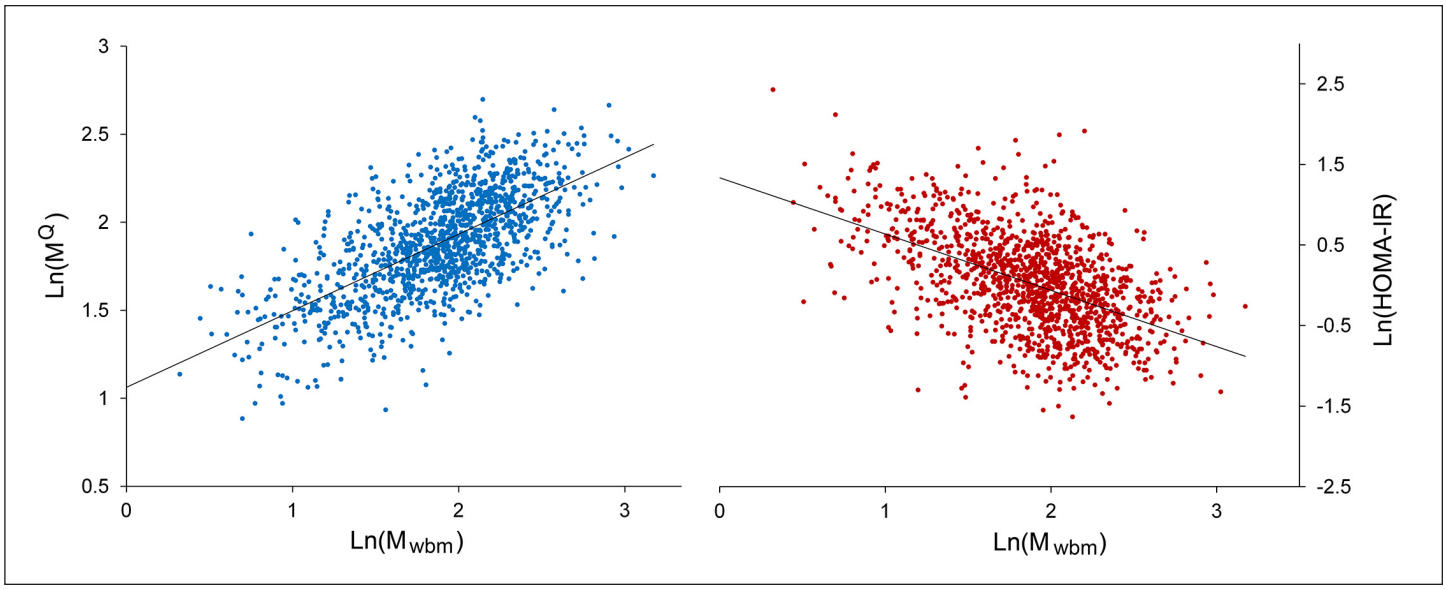

Figure 1. Correlation Plots: Ln(M^Q) left or Ln(HOMA-IR) right versus Ln(M_{wbm}). Ln(M^Q): r = 0.66 versus Ln(Mwbm). Ln(HOMA-IR): r = 0.51 versus Ln(M_{wbm}). N = 1277, and HOMA-IR = (fasting plasma insulin in mU/l × FPG in mM)/22.5.

Predicting Normal Glucose Tolerance to Impaired Glucose Tolerance Progression

In order to determine the ability of the algorithm to identify individuals at risk for developing impaired glucose tolerance (IGT), normal glucose tolerance (NGT) subjects at baseline were evaluated at the three-year follow-up. This analysis included only study participants who had complete values for insulin, glucose, BMI, age, sex, and the three metabolite biomarkers at the 3 year follow-up (**Table 5**). Based on the follow-up 2 h glucose values, the 899 subjects were reclassified as "stable" normal glucose tolerant (n = 815) or "progressors" to IGT (n = 84). M^Q values were used to predict progression from NGT to IGT. The AUCs for progression to IGT were computed using baseline variable values calculated from the baseline OGTT time point. The algorithm-derived M^Q had an AUC of 0.697, which is significantly better than the AUCs derived from baseline fasting insulin, fasting glucose, HOMA-IR, or BMI, but not significantly different from the OGIS index (**Table 6**). M^Q values calculated at the clamp time point and baseline M_{wbm} values gave similar and not significantly different AUCs of 0.704 and 0.707, respectively. M^Q scores of <5.703, denoting IR, were found in 47% of the IGT progressors versus 18% having a BMI ≥30 or 25% having elevated fasting plasma glucose

Table 5.
Relationship between Insulin Sensitivity and Cardiovascular Disease Study: Baseline Anthropometric and Metabolic Parameters by Outcome[a]

Variable	Stable NGT	NGT to IGT	*p* value
n	815	84	
Women (%)	53	51	0.69
Age (years)	44 ± 8	46 ± 8	0.13
FHD (%)	25.5	28.9	0.50
BMI (kg/m^2)	25.1 ± 3.7	26.3 ± 4.0	0.0062
2 h glucose (mg/dl)	96 ± 20	114 ± 16	1.38E-17
OGIS (μmol min^{-1} [kg fat-free mass]$^{-1}$)	11.9 ± 2.6	10.1 ± 2.0	2.07E-9
Fasting glucose (mg/dl)	91 ± 10	95 ± 9	0.00077
Fasting insulin (pmol/liter)	29 (20)	37.5 (25.5)	1.66E-05
α-HB (μg/ml)	3.49 (1.72)	4.16 (1.82)	0.00043
L-GPC (μg/ml)	15.65 (7.60)	12.56 (4.76)	5.21E-07
Oleate (μg/ml)	73.8 (40.7)	83.7 (43.3)	0.0147
M^Q	7.23 ± 2.06	5.91 ± 1.52	1.44E-09

[a] Entries are mean ± standard deviation or median (interquartile range). A 2 h, 75 g OGTT was performed after a 10–12 h overnight fast. Blood samples were obtained before and 30, 60, 90, and 120 min into the test. Two-sided *t*-tests were performed on the continuous variables and a χ^2 test (without continuity correction) was performed on the categorical variables. Stable NGT = subjects whose glucose tolerance was normal at baseline and follow-up (2 h glucose <140 mg/dl); NGT to IGT = subjects whose glucose tolerance was normal at baseline and impaired (2 h glucose ≥140 mg/dl) at follow-up; FHD = family history of diabetes; M^Q = M (insulin-mediated glucose disposal rate) calculated using the test algorithm using data acquired at the time of the original OGTT; OGIS data = (*n* = 805 for stable NGT and *n* = 82 for NGT to IGT).

(FPG; ≥100 mg/dl) at baseline. Subjects with test scores <5.703 had a relative risk of 5.12 for progression to IGT when compared with subjects having a score >8.06, reflecting the most insulin-sensitive tertile of M_{wbm} (**Table 7**). At the 3 year follow-up, M^Q, α-HB, FPG, and 2 h glucose had all significantly worsened in the IGT progressors (**Figure 2**).

Discussion

Predicting Insulin Resistance

The Quantose algorithm was developed within the context of an essentially healthy Caucasian population displaying a wide range of insulin sensitivities indexed as M_{wbm}. The traditional predictors of IR were evident in this population, with BMI, fasting insulin, and family history of diabetes all being significantly different in the lowest tertile of insulin sensitivity (T1) when compared with the other two tertiles (**Table 1**). The three metabolite biomarkers in the test displayed a similar pattern of significant differences for insulin-resistant T1 subjects versus the others. α-Hydroxybutyric acid and oleate levels are increased in the insulin-resistant subjects, and L-GPC is decreased in a manner consistent with their correlations with M_{wbm} (**Table 2**). Although the individual metabolites do not correlate as well as insulin alone with M_{wbm}, their inclusion into the algorithm significantly improves its correlation with M_{wbm} in comparison with insulin alone (**Table 3**). In addition, the test M^Q score is significantly better for detecting IR, as defined by the T1 criterion, than insulin alone (AUC of 0.79 versus 0.74; **Table 4**). For these two measures, HOMA-IR has no advantage over insulin alone due to the nominal value of fasting glucose in these cases. Additionally, M^Q correlates significantly better with M_{wbm} than BMI and performs equally well for identifying IR.

Predicting Progression to Impaired Glucose Tolerance

The three metabolite biomarkers and insulin were all significantly different at baseline for NGT to IGT progressors when compared with the stable NGT subjects at the 3 year follow-up. The data suggest that the test score may be an

early indicator of risk for progression to IGT, as there were few clear signals of risk in the group at baseline. For example, the mean age and family history of diabetes for the IGT progressors were not significantly different from the NGT group, mean BMI was at the low end of overweight, and mean fasting glucose was in the normal range. In fact, the test identified subjects at risk who were lean and had normal fasting glucose values. M^Q score decreased by 8% in the IGT progressors over the course of the 3 year period, which is indicative of worsening IR. In contrast, it decreased by only 2% in the stable NGT group. The test score may be useful for monitoring patient progression toward prediabetes or T2DM and, potentially, improvements in their glycemic status as well.

Table 6.
Areas Under the Curve for Predicting Progression from Normal Glucose Tolerance to Impaired Glucose Tolerance[a]

Variable	AUC	95% CI, lower limit	95% CI, upper limit	*p* value versus M^Q
M^Q	0.697	0.641	0.753	—
Fasting insulin	0.640	0.580	0.701	0.0022
Fasting glucose	0.605	0.543	0.667	0.0031
BMI	0.590	0.526	0.654	0.0009
HOMA-IR	0.648	0.587	0.709	0.007
OGIS	0.703	0.646	0.761	0.36

CI, confidence interval.
[a] M^Q was calculated using data obtained at the initial OGTT time point. Areas under the curve were calculated as in **Table 3** to compare stable NGT (n = 815) with NGT subjects at baseline that were IGT at the three-year follow-up (n = 84). One-sided t-tests were performed to test the hypothesis that the AUC is greater for M^Q. The baseline 2 h glucose has an AUC of 0.76. N = 887 for OGIS.

Table 7.
Relative Risk for Progression to Impaired Glucose Tolerance by M^Q Value[a]

M^Q value	Progressors/category	Relative risk	95% CI	
			Low	High
<5.70	39/230	5.12	2.44	10.73
5.70–8.06	37/427	2.62	1.24	5.53
>8.06	8/242	1.00	—	—

CI, confidence interval.
[a] M^Q was calculated using data obtained at the initial OGTT time point and cutoff values come from the M_{wbm} tertiles cutoffs (**Table 1**), with scores below 5.70 being defined as insulin resistant; values above 8.06 reflect the highest tertile of insulin sensitivity. Data were calculated using JMP (version 8, SAS Institute Inc., Cary, NC).

Metabolite Biomarkers of Insulin Resistance

Metabolomic studies in metabolic diseases[31] have been used to identify novel biomarkers that associate with IR[23,32,33] and predict T2DM.[34–36] The three metabolites used in the test have a number of potential roles in metabolic pathways relevant to insulin action, insulin secretion, or β-cell function. Insulin resistance is characterized by increased lipolysis, elevated plasma free fatty acids, and increased fatty acid oxidation.[37] Oleic acid may serve as a surrogate for total fatty acids and their pathways; given that other fatty acids such as stearic acid and palmitic acid also correlate similarly with M_{wbm} lends credence to this concept. α-Hydroxybutyric acid is a shunt metabolite derived from α-ketobutyrate.[23,38] High dihydronicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide (NADH/NAD+) levels, which may occur with increased fatty acid oxidation in IR, may steer the metabolic fate of α-ketobutyrate from Krebs cycle oxidation toward conversion to α-HB. α-Ketobutyrate is a product of either threonine catabolism or of methionine metabolism via the cystathionine route, which concomitantly produces cysteine as a precursor for the antioxidant molecule glutathione. Increased flux through the latter route may be a marker of oxidative stress. α-Hydroxybutyric acid has been reported to be elevated 5.4-fold after 36 h of fasting and may serve as a general marker of a catabolic state.[39] Interestingly, α-HB correlated with baseline 2 h glucose values (r = 0.28). L-GPC is a *lyso*phosphocholine formed by the action of phospholipase A2 in the liver and by lecithin-cholesterolacyltransferase in the circulation. Phospholipase A2 activity is increased during inflammation, and related *lyso*phosphocholines have been reported to inhibit phospholipase A2 and have anti-inflammatory activity.[40] Related *lyso*phosphocholines have been reported to activate GPR119, which has a role in glucagon-like peptide-1 and insulin secretion.[41] Thus α-HB, L-GPC, and oleate may each add complementary and nonredundant information from diverse metabolic pathways related to insulin action. This may explain why each metabolite adds value to the test and improves the algorithm's estimation of M_{wbm}.

Biomarkers correlating with IR may also have value in the prediction of prediabetes (impaired fasting glucose, IGT) and T2DM. Previous reports have already noted metabolites associated with future T2DM. These include branched-

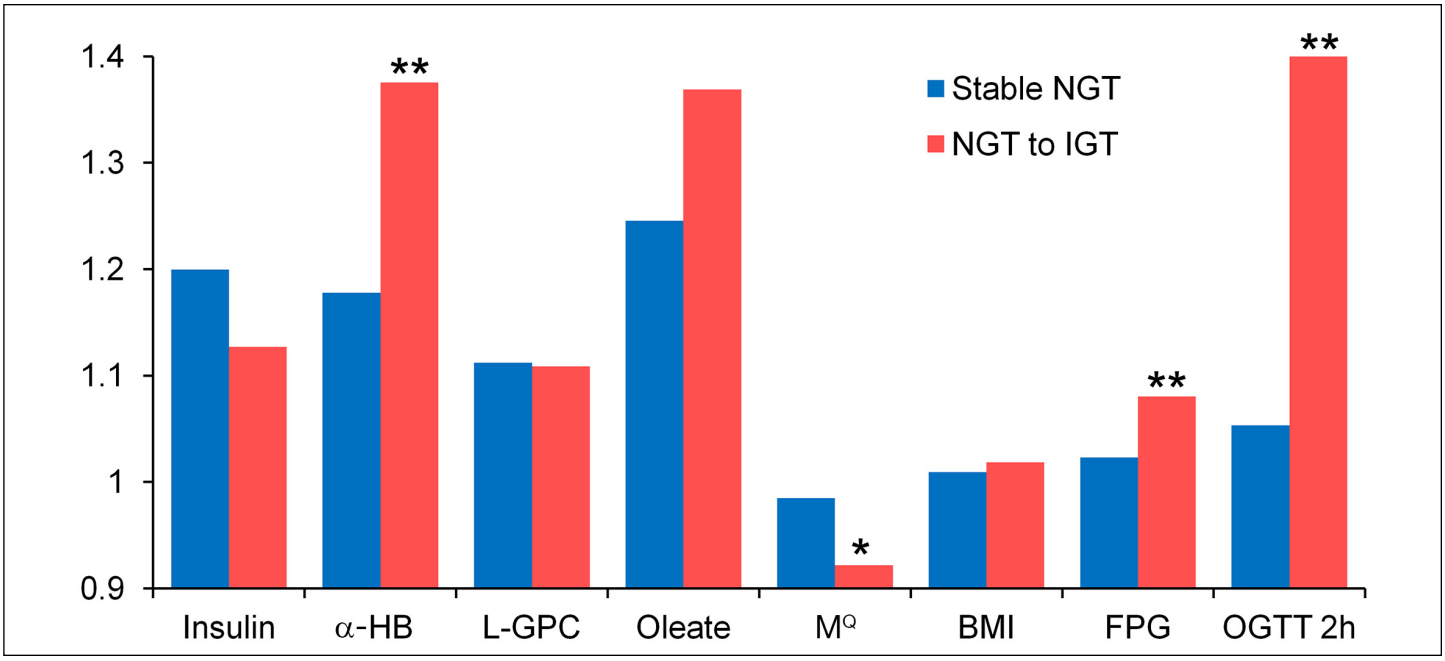

Figure 2. Three-year average individual fold change in baseline parameters: stable NGT versus NGT to IGT progressors. Stable NGT versus NGT to IGT; the single asterisk represents $p < .05$ and double asterisks represent $p < .005$ (two-sided t-tests). $N = 74$ NGT to IGT; $n = 759$ stable NGT (subjects with complete 3 year data).

chain amino acids (valine, leucine, isoleucine), aromatic amino acids (tryptophan, phenylalanine), and uric acid.[42–44] A combination of three amino acids has been used to predict T2DM.[43] In the Botnia study, α-HB was found to be a positive predictor and L-GPC a negative predictor of progression to T2DM.[45]

Body Mass Index and Insulin

In the development of the test, both insulin- and BMI-containing models were evaluated. Each gave qualitatively similar results, but the insulin algorithm was more accurate at predicting IR and identifying those at risk for IGT. Models containing both provided little additional value. The insulin-containing model was chosen with the goal of identifying at-risk, insulin-resistant individuals who may be lean or modestly overweight,[46] where a BMI-based model might not perform well.[2] Moreover, the insulin-containing model may predict changes in insulin sensitivity that are not accompanied by decreases in BMI as occurs in T2DM patients treated with thiazolidinediones. The inclusion of insulin in the test creates some challenges with respect to comparing samples that have measured insulin using different insulin assays, and this may require cross-calibration work to adjust insulin results from different studies.[47–49] Future work using the test will use a single insulin assay carried out in a consistent manner under validated conditions.

Conclusions

Quantose is a test for IR based on a single fasting blood sample developed and validated within the RISC Study population. It has shown utility in the prediction of progression from NGT to IGT (one form of prediabetes) and is superior to other simple baseline measures (fasting insulin, BMI, fasting glucose, HOMA-IR) in this regard. Prediabetes is a high-risk state for T2DM, which is currently defined in terms of glycemic variables (FPG, hemoglobin A1c, 2 h glucose) that increase relatively late in disease progression. This test may have value as an earlier predictor of prediabetes or T2DM risk when compared with these traditional glycemic markers. Such information could be used for earlier implementation of lifestyle changes or drug therapy that have been shown to be successful in the prevention of T2DM.[50] In the light of the ongoing global T2DM pandemic, such preventative actions are extremely important, and this test may have value in monitoring the progress of these interventions. Finally, clinical outcome data from diverse, longitudinal study populations are currently being analyzed to demonstrate the full clinical potential of this novel test for IR and prediabetes.

Funding:

This work was funded by Metabolon Inc., Durham, NC.

Disclosures:

Jeff Cobb, Matthew Mitchell, Klaus-Peter Adam, Pam Nakhle, Eric Button, James Hathorn, Kay Lawton, Michael Milburn, Regis Perichon, and Walter Gall are full-time employees of Metabolon Inc. Ele Ferrannini serves on advisory panels for AstraZeneca LP, Bristol-Myers Squibb Company, Boehringer Ingelheim Pharmaceuticals Inc., Sanofi-Aventis, Johnson & Johnson, GlaxoSmithKline, Novartis Pharmaceuticals Corporation, Merck Sharp & Dohme Limited, and Astellas and receives research support from Boehringer Ingelheim.Pharmaceuticals Inc., Amylin Pharmaceuticals Inc., Eli Lilly and Company, Merck Sharp, and Dohme Limited.

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