A Novel Insulin Resistance Index to Monitor Changes in Insulin Sensitivity and Glucose Tolerance: the ACT NOW Study

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Objective: The objective was to test the clinical utility of Quantose M^Q to monitor changes in insulin sensitivity after pioglitazone therapy in prediabetic subjects. Quantose M^Q is derived from fasting measurements of insulin, α-hydroxybutyrate, linoleoyl-glycerophosphocholine, and oleate, three nonglucose metabolites shown to correlate with insulin-stimulated glucose disposal.

Research Design and Methods: Participants were 428 of the total of 602 ACT NOW impaired glucose tolerance (IGT) subjects randomized to pioglitazone (45 mg/d) or placebo and followed for 2.4 years. At baseline and study end, fasting plasma metabolites required for determination of Quantose, glycated hemoglobin, and oral glucose tolerance test with frequent plasma insulin and glucose measurements to calculate the Matsuda index of insulin sensitivity were obtained.

Results: Pioglitazone treatment lowered IGT conversion to diabetes (hazard ratio = 0.25; 95% confidence interval = 0.13–0.50; $P < .0001$). Although glycated hemoglobin did not track with insulin sensitivity, Quantose M^Q increased in pioglitazone-treated subjects (by 1.45 [3.45] $mg{\cdot}min^{-1}{\cdot}kg_{wbm}{}^{-1}$) (median [interquartile range]) ($P < .001$ vs placebo), as did the Matsuda index (by 3.05 [4.77] units; $P < .0001$). Quantose M^Q correlated with the Matsuda index at baseline and change in the Matsuda index from baseline (*rho*, 0.85 and 0.79, respectively; $P < .0001$) and was progressively higher across closeout glucose tolerance status (diabetes, IGT, normal glucose tolerance). In logistic models including only anthropometric and fasting measurements, Quantose M^Q outperformed both Matsuda and fasting insulin in predicting incident diabetes.

Conclusions: In IGT subjects, Quantose M^Q parallels changes in insulin sensitivity and glucose tolerance with pioglitazone therapy. Due to its strong correlation with improved insulin sensitivity and its ease of use, Quantose M^Q may serve as a useful clinical test to identify and monitor therapy in insulin-resistant patients. (***J Clin Endocrinol Metab*** **100: 1855–1862, 2015)**

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Abbreviations: AIR, acute insulin response; AUC, area-under-the concentration curve; AUC/AUC_G, insulin-to-glucose AUC ratio; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; FFA, free fatty acid; FPG, fasting plasma glucose; FSIVGTT, frequently-sampled iv glucose tolerance test; α-HB, α-hydroxybutyrate; HbA_{1c}, glycated hemoglobin; IGT, impaired glucose tolerance or tolerant; L-GPC, L-linoleoyl-glycerophosphocholine; NGT, normal glucose tolerance or tolerant; OGTT, oral glucose tolerance test; ROC, receiver operating characteristics; S_I, insulin sensitivity from the FSIVGTT; T2DM, type 2 diabetes mellitus.

Insulin resistance is a characteristic feature of type 2 diabetes mellitus (T2DM) (1). Individuals in the upper tertile of impaired glucose tolerance (IGT) also manifest marked insulin resistance and have lost approximately 70–80% of their β-cell function (1–3). Subjects with IGT progress to T2DM with rates varying from 5–15% per year (4). Multiple studies have shown that lifestyle intervention or pharmacotherapy with metformin, thiazolidinediones, or acarbose can prevent or delay the progression of IGT to T2DM (5–9). Of the available antidiabetic agents, thiazolidinediones appear to be the most effective (1). Thus, in the ACT NOW study, pioglitazone reduced IGT conversion to T2DM by 72% (7).

By measuring a large number of metabolites from a single fasting plasma sample (10), metabolomics has the potential to identify biomarkers that can provide insights into the pathophysiology of complex metabolic diseases and to monitor and predict responses to therapeutic interventions. In patients with T2DM, a number of novel biomarkers have been shown to be elevated and to correlate with insulin resistance (11–17). These include branched-chain amino acids, which are elevated in animal models of obesity and T2DM and in nondiabetic obese and T2DM humans (18). Raised plasma branched-chain amino acid levels also predict incident T2DM and improvement in insulin resistance with weight loss (18, 19).

Using fasting plasma samples from the healthy, nondiabetic population of the Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study, we identified novel biomarkers that correlated strongly with the rate of whole body insulin-mediated glucose disposal (M value, insulin stimulated glucose metabolism) derived from the euglycemic insulin clamp technique (13). Individually, α-hydroxybutyrate (α-HB), oleate, and insulin were negatively correlated with insulin-stimulated glucose metabolism (M), whereas L-linoleoyl-glycerophosphocholine (L-GPC) was positively correlated with M. Collectively, these four variables (called Quantose M) (20) predicted the 3-year progression from normal glucose tolerance (NGT) to IGT in RISC and to overt diabetes in the Botnia cohort (13).

The aims of the present study were to examine, for the first time: 1) the relationship between Quantose M^Q and insulin resistance in a North American population; and 2) the effect of a pharmacological intervention with the insulin sensitizer pioglitazone in a prediabetic population (ACT NOW Study) (21) on these novel insulin sensitivity biomarkers.

Subjects and Methods

Subjects

In ACT NOW (21), 602 high-risk individuals with IGT were recruited over 2 years and followed for a mean of 2.4 years. The inclusion/exclusion criteria and subject characteristics have been published (7, 21). The study population consisted of 57% Caucasians, 24% Mexican Americans, 16% African Americans, and 3% Asians. Eight centers participated in the study, which was approved by the Institutional Review Board at each site.

A total of 441 IGT patients completed the study, and baseline metabolite measurements were available for 428 subjects (210 treated with pioglitazone and 218 with placebo); follow-up metabolite measurements were available for 404 patients (199 pioglitazone and 205 placebo).

Methods

At baseline, all subjects received a 2-hour oral glucose tolerance test (OGTT) after an overnight fast, and plasma samples were obtained at −30, −15, 0, and every 15 minutes for 2 hours for determination of plasma glucose and insulin concentrations. On a separate day, after an overnight fast, a subgroup of 260 subjects also received a frequently-sampled iv glucose tolerance test (FSIVGTT) (22). Samples for plasma insulin and glucose concentrations were obtained every 2 minutes for the first 10 minutes and every 10 minutes for the subsequent 80 minutes. Participants were randomized to pioglitazone (30 mg/d) or placebo; 1 month after randomization, pioglitazone was increased to 45 mg/d. Fasting plasma glucose (FPG) was measured at each 3-month follow-up visit, glycated hemoglobin (HbA_{1c}) was measured every 6 months, and OGTT was repeated annually and at study end or at the time of conversion to diabetes. FSIVGTT was repeated at study end or at the time of conversion to diabetes.

Measurements

Plasma glucose was measured by the glucose oxidase reaction, plasma insulin by RIA (Diagnostic Products) (interassay and intra-assay coefficients of variation [CVs] = 7.1 and 5.1%, respectively), plasma C-peptide by RIA (Diagnostic Systems) (interassay and intra-assay CVs = 4.3 and 2.4%, respectively), and HbA_{1c} with DCA 2000 Analyzer (Bayer).

Quantose metabolite analysis

For absolute quantitation, metabolites were analyzed by an analytically and clinically validated isotope dilution ultra-HPLC tandem mass spectrometry (UHPLC-MS-MS) assay developed and carried out in a Clinical Laboratory Improvement Amendments/College of American Pathologists-accredited laboratory, as reported previously (12, 13). In brief, 50 μL of EDTA plasma samples were spiked with internal standards and subsequently subjected to protein precipitation by mixing with 250 μL of methanol. After centrifugation, aliquots of clear supernatant were injected onto an UHPLC-MS-MS system, consisting of a Thermo TSQ Quantum Ultra Mass Spectrometer (Thermo Fisher Scientific Inc, Waltham, MA) and a Waters Acquity UHPLC system (Waters Corporation, Milford, MA) equipped with a column manager module in 2.5-minute assay. α-HB, L-GPC, and oleic acid were eluted with a gradient on a Waters Acquity single RP C-18 column (2.1 mm × 50 mm, 1.7-mm particle size) at a mobile phase flow rate of 0.4 mL/min at 40°C. Ionization was achieved by heated electrospray ionization source. Quantitation was performed based on the 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 before each run. Stable isotope-labeled compounds (α-HB-D_3, L-GPC-D_9,

and oleic acid-$^{13}C_{18}$) were used as internal standards. The inter-run CVs for α-HB, L-GPC, and oleic acid were 4.0, 6.3, and 4.6%, respectively (based on 146 replicates over 9 mo).

Calculations

Area-under-the-concentration curves (AUCs) were calculated using the trapezoidal rule. Insulin sensitivity was estimated as the Matsuda index from the OGTT (23), and the S_I parameter from the FSIVGTT (22). β-Cell function was indexed as the insulin-to-glucose AUC ratio (AUC_I/AUC_G) during the OGTT (24) and the acute insulin response (AIR) during the FSIVGTT (22). The Quantose M index (M^Q) is derived from a multiple linear regression based on fasting measurements (logarithmically transformed) of plasma α-HB, L-GPC, oleic acid, and insulin, as previously described (20). We chose the metabolites that had the highest correlation with insulin sensitivity obtained from hyperinsulinemic euglycemic clamp studies (α-HB, −0.36; L-GPC, 0.33; and oleate, −0.22) (20). Quantose M^Q is designed to estimate the clamp-derived M value.

Statistical analysis

Two-group differences were analyzed by Mann-Whitney test, multiple-group differences by Kruskal-Wallis test, and proportions by Fisher's exact test. Differences between values before and after treatment were analyzed using an analysis of covariance model, with the difference as the dependent variable and with baseline value and group as the independent variables. Simple associations were tested by Spearman's correlation coefficient (*rho*). The independent influence of treatment and closeout glucose tolerance status was tested by two-way ANOVA. Prediction of incident diabetes was analyzed by logistic regression; *c* statistic was indexed as the area under the receiver operating characteristics (ROC). A P value $\leq .05$ was considered statistically significant; all analyses were carried out using JMP version 7.0 (SAS Institute Inc).

Results

Baseline

Pioglitazone and placebo groups were well matched with regard to age, gender, and body mass index (BMI) (Table 1). Fasting and 2-hour plasma glucose levels, estimates of insulin sensitivity (Matsuda index and S_I), β-cell function (AUC_I/AUC_G and AIR), and the Quantose index (Quantose M^Q) and its components were very similar between the two groups. In the group as a whole, the Matsuda index and S_I were correlated with one another (*rho* = 0.52; n = 260; $P < .0001$), and Quantose M^Q was positively correlated with both S_I (*rho* = 0.42; n = 260; $P < .0001$) and the Matsuda index (*rho* = 0.85; n = 428; $P < .0001$). Likewise, AUC_I/AUC_G and AIR were correlated with one another (*rho* = 0.49; n = 260; $P < .0001$). Across quartiles of baseline 2-hour plasma glucose concentrations (mean ± SEM, 146 ± 4, 161 ± 5, 176 ± 4, and 193 ± 5 mg/dL), baseline Quantose M^Q declined gradually from 5.25 ± 2.58 to 5.08 ± 2.63 to 4.71 ± 2.49 to 4.49 ± 1.98 mg/dL ($P < .03$).

Baseline HbA_{1c} was weakly related to the Matsuda index and Quantose M^Q in the whole dataset, as well as in each group separately (with *rho* values ranging between 0.14 and 0.25). However, it should be noted that mean HbA_{1c} varied only slightly (from 5.40 to 5.61%; $P = .0131$) across quartiles of 2-hour plasma glucose concentrations. Furthermore, the change in HbA_{1c} at closeout was unrelated to the changes in the Matsuda index in

Table 1. Clinical, Anthropometric, and Laboratory Data at Baseline

	Pioglitazone	Placebo	*P* Value
n	210	218	
Gender, F/M, %	56/44	59/42	.66
Age, y	54 ± 10	53 ± 12	.29
BMI, kg/m^2	33.5 ± 5.4	34.3 ± 6.4	.52
Waist, cm			
Male	109 ± 12	112 ± 14	.29
Female	102 ± 12	103 ± 14	.60
HbA_{1c}, %	5.52 ± 0.42	5.47 ± 0.39	.16
FPG, mg/dL	105 ± 7	105 ± 8	.45
2-hour PG, mg/dL	170 ± 17	169 ± 18	.53
FPI, mU/L	8.3 [8.1]	8.4 [9.2]	.77
Matsuda index	3.13 [3.29]	3.23 [3.31]	.94
AUC_I/AUC_G, mU/g	38 [26]	40 [28]	.64
S_I, $min^{-1}\cdot\mu U\cdot mL^{-1}$)[a]	2.29 [1.81]	2.35 [1.73]	.51
AIR, mU/L[a]	307 [330]	291 [310]	.33
α-HB, μg/L	4.17 [1.95]	4.42 [1.94]	.43
L-GPC, μg/L	10.81 [4.87]	10.44 [5.16]	.16
Oleic acid, μg/L	79 [40]	77 [38]	.67
M^Q ($mg\cdot min^{-1}\cdot kg_{wbm}{}^{-1}$)	4.92 [1.21]	4.77 [2.50]	.50

Abbreviations: F, female; M, male; PG, plasma glucose; FPI, fasting plasma insulin; M^Q, Quantose index of insulin sensitivity; wbm, whole body mass. Data are expressed as mean ± SD or median [interquartile range].

[a] 123 subjects in the pioglitazone group and 137 in the placebo group.

either the pioglitazone (*rho* = −0.14; *P* = .06) or placebo group (*rho* = −0.14; *P* = .06).

Indices of insulin sensitivity were inversely associated with indices of β-cell function; in particular, baseline Quantose M^Q was reciprocally related to both AIR (*rho* = −0.15; n = 260; *P* = .015) and AUC_I/AUC_G (*rho* = −0.60; n = 428; *P* < .0001).

Closeout

During a median follow-up of 2.4 years, 42 individuals in the placebo group and 12 in the pioglitazone group developed diabetes (hazard ratio = 0.25; 95% confidence interval [CI] = 0.13–0.50; *P* < .0001). Of the other 374 subjects, 181 regressed to NGT (110 with pioglitazone vs 71 with placebo; *P* < .02).

Subjects randomized to pioglitazone had significantly greater declines in fasting and 2-hour plasma glucose concentrations, HbA_{1c}, and fasting plasma insulin concentration compared to subjects in the placebo group (Table 2). Insulin sensitivity (both the Matsuda index and S_I) increased significantly more in the pioglitazone vs placebo group, whereas β-cell function declined more in the placebo group. Quantose M^Q increased significantly more with pioglitazone than placebo (Table 2). Each individual component of Quantose M^Q (ie, fasting insulin, α-HB, and oleic acid decreased, and L-GPC increased) changed significantly more with pioglitazone compared to placebo (Table 2). Moreover, the change in Quantose M^Q at study end was significantly correlated with the change in AUC_I/AUC_G (*rho* = −0.39; *P* < .0001).

When examining insulin sensitivity according to glucose tolerance status at study end, baseline Matsuda values only tended to be higher in subjects with NGT at follow-up than in those who remained IGT or progressed to T2DM. By contrast, Quantose M^Q was significantly higher in subjects who were NGT at follow-up than in those who remained IGT or progressed to T2DM for both pioglitazone- and placebo-treated subjects. On the other hand, the changes at closeout in both the Matsuda index and Quantose M^Q were significantly larger in NGT than IGT or T2DM subjects and significantly more positive with pioglitazone than placebo (Figure 1). Underlying the changes in Quantose M^Q, levels of fasting insulin, α-HB, and oleic acid increased, and levels of L-GPC decreased across closeout NGT, IGT, and T2DM status (data not shown; *P* < .01 for each metabolite). In the whole dataset, changes in the Matsuda index and Quantose M^Q were tightly correlated with one another in both treatment groups (Figure 2).

The ability of baseline parameters to predict incident diabetes was generally low, most likely reflecting the fact that the cohort was quite homogeneous. Thus, neither gender, nor age, nor fasting insulin, nor the Matsuda index at baseline was a significant predictor of incident diabetes in univariate analysis or when including baseline BMI and waist circumference as covariates. Both models achieved statistical significance only when also including the baseline fasting glucose concentration (Table 3). In contrast, baseline Quantose M^Q was a significant predictor, even in univariate analysis, and model predictivity increased stepwise when including BMI, waist circumference, and fasting glucose. In the latter model, the ROC AUC was 0.024 U better than the same model using the Matsuda index, and it was 0.017 U better than the same model using fasting insulin (both *P* < .05). Treatment assignment raised ROC AUC in each multivariate model, with the one using Quantose M^Q remaining superior to those using the Matsuda index or fasting insulin (Table 3).

Table 2. Changes in Laboratory Data at Study Closeout

	Pioglitazone	Placebo	*P* Value
FPG, mg/dL	−12 ± 11	−8 ± 11	<.001
HbA_{1c}, %	0.06 ± 0.41	0.27 ± 0.39	<.0001
2-hour PG, mg/dL	−31 ± 35	−15 ± 33	<.0001
FPI, mU/L	−2.8 [6.1]	−0.7 [6.6]	<.0001
Matsuda index	3.05 [4.77]	0.44 [2.68]	<.0001
AUC_I/AUC_G, mU/g	−8 [20]	−3 [20]	<.0001
S_I ($min^{-1}\cdot\mu U\cdot mL^{-1}$)	1.15 [2.81]	0.54 [2.48]	.0202
AIR, mU/L	−19 [179]	−29 [163]	ns
α-HB, μg/mL	−0.47 [2.12]	−0.02 [1.97]	.0034
L-GPC, μg/mL	1.60 [4.89]	0.30 [3.73]	<.0001
Oleic acid, μg/mL	−5 [46]	5 [39]	.0009
M^Q ($mg\cdot min^{-1}\cdot kg_{wbm}^{-1}$)	1.45 [3.45]	0.08 [1.84]	<.0001

Abbreviations: PG, plasma glucose; FPI, fasting plasma insulin; wbm, whole body mass. Data are expressed as mean ± SD or median [interquartile range]; *P* values are for the difference between pioglitazone and placebo by two-way ANOVA, with change in the index variable as the dependent variable and baseline values and treatment group as the independent variables.

Discussion

Mass spectrometry-based biochemical profiling is an emerging technological approach to identifying biomarkers that may serve as metabolic signatures for complex metabolic diseases and as the basis of novel diagnostic tests (11, 12, 15, 16). For example, recent studies have used this technique to identify biomarkers predictive of the future development of T2DM (13, 14, 18) and the response to lifestyle intervention (19, 25).

To our knowledge, the present study is the first to employ robust physiological measurements of insulin sensitivity and insulin secretion, combined with a double-blind placebo-controlled pharmacological intervention with pi-

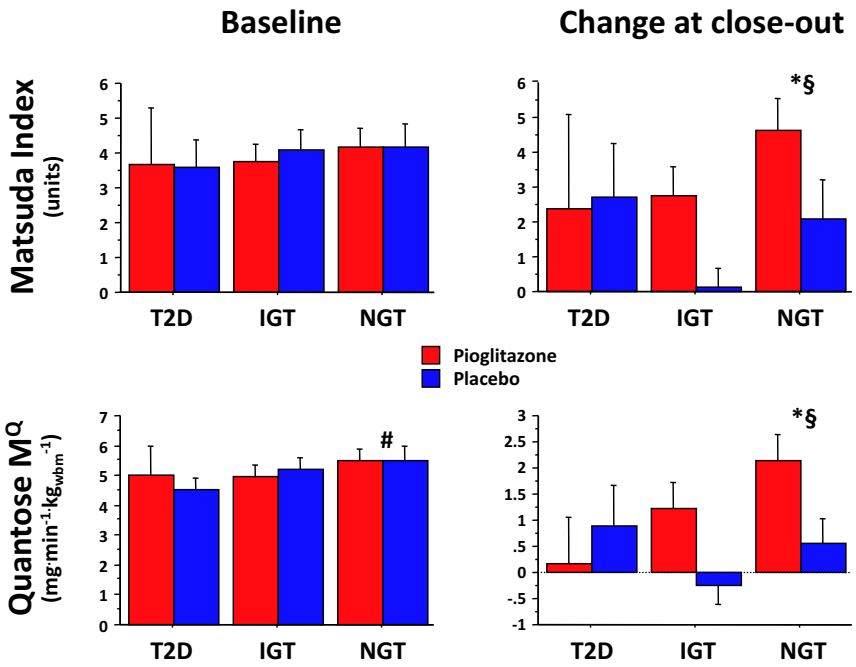

Figure 1. Baseline (left panels) and change at closeout (right panels) values for the Matsuda index (top panels) and Quantose M^Q (bottom panels) according to glucose tolerance status at closeout in subjects randomized to pioglitazone or placebo. Plots are mean + 95% CIs. #, $P = .008$ for the difference between NGT and IGT/T2D; *, $P < .01$ for the difference between NGT and IGT/T2D; and §, $P < .01$ for the difference between pioglitazone and placebo by two-way ANOVA.

oglitazone, to validate metabolites that correlate with key pathophysiological abnormalities including insulin resistance and glucose tolerance. A strength of this study is that placebo and pioglitazone groups were very well matched at baseline with respect to anthropometric measurements, measures of insulin secretion and insulin sensitivity, and plasma Quantose insulin sensitivity biomarker concentrations.

We previously developed a novel insulin sensitivity index, Quantose M^Q, based upon a single fasting measurement of plasma insulin, α-HB, L-GPC, and oleate concentrations (20). Quantose M^Q correlated well with insulin sensitivity measured from the euglycemic insulin clamp in nondiabetic healthy Europeans ($r = 0.66$; $P < .0001$) (20). In the present study, we examined application of this novel insulin sensitivity index in a prediabetic, IGT population and how this index changed after pioglitazone vs placebo treatment in relation to changes in insulin sensitivity and glucose tolerance.

Quantose M^Q correlated strongly with the Matsuda index of insulin sensitivity at baseline (*rho* = 0.85), as well as study end (*rho* = 0.89), and with the change in the Matsuda index from baseline to study end (Figure 2). In the subgroup of subjects in whom the FSIVGTT was performed, Quantose M^Q correlated with S_I at baseline (*rho* = 0.42) and follow-up (*rho* = 0.47), confirming the consistency of this index in marking for insulin sensitivity regardless of how the latter is measured. Importantly, Quantose M^Q also differentiated between glucose tolerance status, ie, NGT vs IGT vs T2DM, in pioglitazone- and placebo-treated subjects at study end (Figure 1). Finally, Quantose M^Q did significantly better than either fasting insulin alone or the Matsuda index in predictive models of incident diabetes (Table 3).

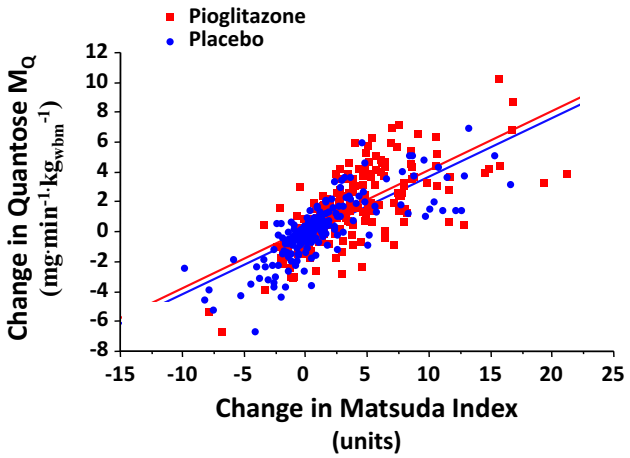

Figure 2. Relationship between closeout changes in Quantose M^Q and the Matsuda index in subjects randomized to pioglitazone or placebo. The best fit is linear in both groups ($r = 0.69$, $P < .0001$, for pioglitazone; and $r = 0.77$, $P < .0001$, for placebo); the fitted line for the pioglitazone group is significantly ($P = .01$) different from that of the placebo group.

In contrast to M^Q, HbA_{1c} did not identify IGT subjects as insulin resistant or prediabetic. Although the change in HbA_{1c} correlated with the change in insulin sensitivity (*rho* = −0.23; $P < .0001$) in the whole group, the relationship was markedly weaker than that between change in Quantose M^Q and change in the Matsuda index (Figure 2). In the pioglitazone-treated group, the change in HbA_{1c} did not correlate with a change in the Matsuda index or Quantose M^Q. This is not surprising because multiple factors, ie, β-cell function, etc (1), contribute to the mean daylong plasma glucose level as determined by HbA_{1c}. The current observations are consistent with other studies showing that the majority (approximately two-thirds) of prediabetic individuals are not diagnosed by established HbA_{1c} cutoffs (26). Therefore, Quantose M^Q may serve as an adjunct to HbA_{1c} in identifying at-risk, insulin-resistant patients (both NGT and IGT) and in monitoring their

Table 3. Prediction of Incident Diabetes

	Odds Ratio (95% CI)	ROC	*P* Value
Insulin	1.20 (0.92–1.52)	*0.582*	*.1769*
+BMI	1.18 (0.88–1.56)	*0.587*	*.2186*
+waist	0.66 (0.43–1.00)	*0.619*	*.1052*
+glucose	1.93 (1.45–2.58)	**0.693**	<.0001
+Tx	0.22 (0.16–0.49)	**0.759**	<.0001
Matsuda index	0.83 (0.58–1.12)	*0.568*	*.2326*
+BMI	1.20 (0.89–1.57)	*0.580*	*.2293*
+waist	0.69 (0.45–1.05)	*0.609*	*.1369*
+glucose	1.94 (1.45–2.63)	**0.686**	<.0001
+Tx	0.23 (0.16–0.49)	**0.754**	<.0001
M^Q	0.66 (0.46–0.91)	**0.592**	.0107
+BMI	1.10 (0.82–1.46)	**0.607**	.0301
+waist	0.62 (0.40–0.94)	**0.646**	.0105
+glucose	1.85 (1.39–2.49)	**0.710**	<.0001
+Tx	0.22 (0.16–0.49)	**0.766**	<.0001

Abbreviation: Tx, treatment (pioglitazone vs placebo). Data are expressed as odds ratio (95% CI)—calculated for 1 SD difference—and area under the ROC and its statistical significance (*P*). Predictor variables are the values measured at baseline. Insulin and glucose are fasting. Bold indicates statistically significant variables.

improvement with lifestyle and/or pharmacological interventions aimed at preventing progression to T2DM.

It is of interest that not only Quantose M^Q but also each of its component metabolites (α-HB, L-GPC, oleate, and fasting insulin) changed significantly after pioglitazone therapy (Table 2), and their closeout values differed significantly with respect to closeout glycemic status (Supplemental Figure 1). For example, at closeout α-HB was 4.60 ± 2.03, 4.07 ± 2.13, and 3.48 ± 1.58 μg/mL (mean ± SEM) in T2DM, IGT, and NGT subjects, respectively ($P < .0001$).

Of further interest is that Quantose M^Q was related to indices of β-cell function (AUC_I/AUC_G and AIR) and changed consensually with AUC_I/AUC_G at follow-up. This is of clinical importance because progression from IGT to T2DM is characterized by progressive β-cell failure (27–29). This in vivo observation in man is consistent with in vitro data that demonstrate that α-HB and L-GPC have dose-dependent effects on insulin secretion (13). Thus, α-HB inhibits whereas L-GPC stimulates glucose-induced insulin release in insulin β-cells. Furthermore, increased α-HB and reduced L-GPC levels are independent risk factors for insulin resistance and progression to IGT and T2DM (13). This finding is consistent with the superiority of M^Q over fasting insulin or the Matsuda index to predict incident T2DM (Table 3) even in a relatively small, homogeneous cohort of IGT subjects as the ACT NOW trial.

T2DM patients are characterized by elevated plasma free fatty acid (FFA) levels, increased FFA oxidation, and increased tissue lipid deposition. In individuals with T2DM, thiazolidinediones consistently reduce plasma FFA by approximately 30% (30, 31) and mobilize fat out of muscle and liver (32, 33). The reduction in plasma FFA concentration is associated with improved insulin sensitivity and β-cell function (34–36). Consistent with these observations, the plasma oleic acid level in the present study declined significantly more after pioglitazone therapy than placebo (Table 2). Elevated plasma FFA and increased FFA oxidation are associated with an increase in the $NADH^+$/NAD ratio, and this favors the formation of α-HB from α-ketobutyrate. Thus, the declines in plasma α-HB, as well as plasma oleate, are consistent with the action of pioglitazone to reduce the plasma FFA concentration and augment FFA oxidation. Whether the changes in α-HB, oleate, and L-GPC simply reflect, or follow, the improvement in insulin sensitivity, β-cell function, and glucose homeostasis, or whether they actually play a mechanistic role in the enhanced insulin sensitivity/β-cell function/glycemic control remains to be determined.

Association of Quantose M^Q and its metabolites with insulin resistance has been replicated in three different populations (13) and now in the current study, which is the first to examine the effect of pharmacological intervention with an insulin-sensitizing agent on Quantose M^Q insulin sensitivity index and its individual metabolites. Of note, the Matsuda index did not predict incident diabetes, whereas Quantose M^Q was a weak predictor. This is not surprising, given a relatively homogeneous population at baseline. Slightly better predictive ability of Quantose M^Q could be because of the fasting metabolites (α-HB, L-GPC, and oleate).

In summary, in ACT NOW we demonstrate that in both placebo-treated and pioglitazone-treated IGT subjects, Quantose M^Q was associated with improved insulin sensitivity and glucose tolerance. Importantly, Quantose M^Q discriminated between different stages of glucose tolerance, ie, NGT vs IGT vs T2DM, at study end.

Identification of biomarkers that predict the response to therapy or conversion of IGT to T2DM is of importance in clinical practice. Quantose M^Q and its nonglucose metabolites mark the severity of insulin resistance in IGT individuals, and their changes correlate well with changes in both insulin sensitivity and glucose tolerance status at study end. This novel fasting plasma measurement may have utility in predicting and monitoring response to therapeutic interventions.

Acknowledgments

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