

Metabonomic Variations in the Drug-Treated Type 2 Diabetes Mellitus Patients and Healthy Volunteers

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The pathological development and the drug intervention of type 2 diabetes mellitus (T2DM) involve altered expression of downstream low molecular weight metabolites including lipids and amino acids, and carbohydrates such as glucose. Currently, a small number of markers used for clinical assessment of T2DM treatment may be insufficient to reflect global variations in pathophysiology. In this study, a metabonomic study was performed to determine metabolic variations associated with T2DM and the drug treatments on 74 patients who were newly diagnosed with T2DM and received a 48 week treatment of a single drug, repaglinide, metformin or rosiglitazone. Fasting overnight and 2 h postprandial blood serum of patients were collected at 24 and 48 weeks to monitor the biochemical indices (FPG, 2hPG, HbA_{1c}, etc.). Gas chromatography/mass spectrometer coupled with multivariate statistical analysis was used to identify the alteration of global serum metabolites associated with T2DM as compared to healthy controls and responses to drug treatment. Significantly altered serum metabolites in diabetic subjects include increased valine, maltose, glutamate, urate, butanoate and long-chain fatty acid (C16:0, C18:1, C18:0, octadecanoate and arachidonate), and decreased glucuronolactone, lysine and lactate. All of the three treatments were able to down-regulate the high level of glutamate to a lower level in serum of T2DM patients, but rosiglitazone treatment was able to reverse more abnormal levels of metabolites, such as valine, lysine, glucuronolactone, C16:0, C18:1, urate, and octadecanoate, suggesting that it is more efficient to alter the metabolism of T2DM patients than the other two drugs.

Keywords: metabonomics • Type 2 diabetes mellitus • repaglinide • metformin • rosiglitazone • gas chromatography/mass spectrometry

Introduction

Chronic perturbations of metabolic regulatory system are the basis for many metabolic disorders such as obesity and type 2 diabetes mellitus (T2DM). Successful treatment of these conditions with drugs requires restoring the perturbed metabolism without generating defects in other metabolic pathways. In most cases, however, such medication is not able to normalize the dysregulated metabolic system, but is likely to maintain its functions at certain levels for the basic physiological needs.¹ Therefore, accurate assessments of therapeutic effectiveness and its impact on physiology must involve a comprehensive measurement of the global metabolism, a goal that is not to be accomplished by the analysis of a single biomarker. Quantitative and comprehensive analyses of the metabolome can

assess metabolic response to a therapy with much more information and power than biomarker approaches, and readily reveal distinct differences in metabolism between diseased individuals and healthy ones.^{2,3} Furthermore, identification of the differential metabolites accountable for the intergroup variation will help us obtain valuable insights into molecular mechanisms of certain pathophysiological variations under treatment. In this study, a GC/MS-based metabonomic analysis was applied to identify significant changes in global metabolite profiles in serum associated with T2DM and to evaluate the impact of the long-term medication on metabolism and physiology of T2DM patients.

Currently, a number of therapeutic drugs are used for the treatment of T2DM patients.⁴ As a first-line treatment, metformin, whose mechanisms are still not entirely clear, exerts its hypoglycemic effects by lowering hepatic glucose output with decreased gluconeogenesis and, to a lesser extent, increased glucose uptake by skeletal muscles.⁵ Repaglinide, another commonly used medicine, stimulates insulin secretion primarily via closure of ATP-dependent potassium channels

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(KATP channels) of the outer membrane of β -cells.⁶ Rosiglitazone, a representative of the emerging thiazolidinediones family, functions as a ligand for the peroxisome proliferator-activated receptor gamma (PPAR γ) most highly expressed in adipocytes. These nuclear receptors, which are ligand-activated transcription factors, play an integral part in the regulation of the expression of a variety of genes involved in carbohydrate and lipid metabolism, resulting in improved insulin sensitivity, particularly in the peripheral tissues.⁷ Side effects of the medication with thiazolidinediones were observed concomitantly, for instance, weight gain, edema, anemia, pulmonary edema and congestive heart failure and an unacceptable risk of fulminant hepatic failure.^{7,8} Long-term pharmacological effects of the above-mentioned three antidiabetic drugs appear to be diverse, owing to their different therapeutic mechanisms. These drugs have been used clinically for several years to decades with reasonably good knowledge of therapeutic effectiveness. However, the pharmacological impacts on the global metabolism and physiology upon the long-term administration are yet to be understood.

The common method used to assess the clinical effects of drug treatments is based on the measurement of a single or several biochemical marker(s), which do not sufficiently reflect the overall physiological status of the patients. On the basis of our recent studies,^{7,9–12} the combined global metabolic analysis and multivariate statistical technique has become a robust metabonomics strategy, providing comprehensive metabolic information for classification of different physiological states and understanding of important molecular mechanisms associated with pathological variations. To date, metabonomics has been applied in disease diagnosis, therapy monitoring and R&D of new drugs.^{13,14} In this study, we performed a gas chromatography/mass spectrometry (GC/MS) metabolic profiling coupled with the analysis of clinical biochemical indices for a comprehensive evaluation of the three antidiabetic drugs, repaglinide, metformin and rosiglitazone, on newly diagnosed T2DM patients over 48 weeks.

Materials and Methods

Human Sample Collection. A total of 82 patients with newly diagnosed type 2 diabetes mellitus and no treatment with hypoglycaemic agents or lipid drugs received the drug treatment: 35 received repaglinide, 22 received metformin and 25 received rosiglitazone. There were no significant differences in clinical parameters among the three groups upon recruitment as indicated in Table 1 and Supplemental Data Table S1 (Supporting Information). All these subjects experienced a 2-week observation (wash-out) period during which they were treated with diet and exercise alone before the first sample was taken. The glycated hemoglobin value (HbA_{1c}) of patients selected for the metabolic profiling was no less than 6.5% at the end of this 2-week observation period. Serum samples for metabolic profiling were taken from these patients, and also from 36 healthy volunteers (as determined by medical history, physical examination and routine laboratory tests) as controls of GC/MS metabolic profiles.

At the end of the observation period (baseline, 0 week), patients were randomly assigned to one of three treatment groups for a 48-week study comprising 8 visits: repaglinide (1.5–6 mg/d), metformin (0.75–1.5 g/d) or rosiglitazone (4–8 mg/d) groups. Fasting overnight and 2 h postprandial blood serum samples of patients were collected to monitor the biochemical indices (FPG, 2hPG, HbA_{1c}, etc.) at patients' visit

Table 1. Comparison of Clinical and Biochemical Parameters and T-Predicted Score Values of Prediction Model at Baseline, 24 and 48 Weeks after Treatment among Repaglinide, Metformin and Rosiglitazone^a

markers	repaglinide			metformin			rosiglitazone		
	baseline	24 week	48 week	baseline	24 week	48 week	baseline	24 week	48 week
Weight (kg)	68.94 ± 8.47	69.79 ± 8.99	70.08 ± 9.74	74.2 ± 11	71.57 ± 11.56	71.07 ± 12.13	71.25 ± 9.03	70.87 ± 8.79	73.4 ± 8.14
Waist (cm)	89.29 ± 7.16	88.73 ± 7.02	88.94 ± 7.54	94.23 ± 6.2	89.19 ± 5.51	89.64 ± 6.25	86.17 ± 6.2	86.73 ± 6.37	88.62 ± 6.33
BMI (kg/m ²)	24.64 ± 1.97	24.95 ± 2.13	24.96 ± 2.37	26.96 ± 2.07	25.95 ± 2.39	24.51 ± 3.68	25.09 ± 2.33	24.94 ± 2.45	25.79 ± 2.29
HbA _{1c} (%)	8.61 ± 1.29	6.26 ± 0.53**	6.46 ± 0.67**	8.21 ± 1.11	6.19 ± 0.38**	6.29 ± 0.55**	8.8 ± 1.34	6.31 ± 0.52**	6.33 ± 0.39**
FPG (mmol/L)	9.74 ± 1.46	6.45 ± 0.77**	7.25 ± 1.15**	8.82 ± 1.51	6.09 ± 0.71**	6.37 ± 0.76**	9.16 ± 1.43	6.35 ± 0.81**	6.46 ± 0.99**
2hFG (mmol/L)	14.09 ± 2.28	8.59 ± 1.75**	9.51 ± 2.11**	12.81 ± 3.19	8.37 ± 1.82**	7.51 ± 1.44**	14.14 ± 2.41	9.16 ± 1.81**	8.78 ± 1.56**
Systolic blood pressure (mmHg)	123.17 ± 9.75	120.23 ± 7.53	121.15 ± 11.57	125.64 ± 11.75	125.91 ± 10.45	120.47 ± 10.08	124.4 ± 8.93	122.43 ± 9.31	119.24 ± 10.65
Diastolic blood pressure (mmHg)	78.57 ± 5.27	77.54 ± 6.75	75.67 ± 5.49	81.86 ± 6.93	81.41 ± 7.41	79.89 ± 6.45	80.68 ± 5.4	79.83 ± 6.33	76.62 ± 6.02
Heart rate (times/min)	75 ± 7.09	74.26 ± 5.05	75.42 ± 4.62	75.27 ± 6.82	74.27 ± 6.69	74.26 ± 5.15	75.56 ± 7.37	74.91 ± 5.9	74.05 ± 5.01
Total cholesterol (mmol/L)	5.23 ± 0.63	5.03 ± 0.74	5.04 ± 0.66	5.42 ± 0.75	5.1 ± 0.59	5.02 ± 0.78	5.21 ± 0.84	5.2 ± 0.83	5.39 ± 1.05
TG (mmol/L)	2.27 ± 0.93	2.77 ± 1.58	2.71 ± 1.51	2.44 ± 1.06	2.08 ± 0.76	2.07 ± 0.74	1.84 ± 0.68	1.95 ± 0.74	2.10 ± 1.13
HDL cholesterol (mmol/L)	1.19 ± 0.17	1.17 ± 0.18	1.16 ± 0.21	1.29 ± 0.26	1.29 ± 0.24	1.32 ± 0.35	1.19 ± 0.18	1.22 ± 0.22	1.22 ± 0.23
LDL cholesterol (mmol/L)	3.25 ± 0.52	2.82 ± 0.75	2.83 ± 0.59	3.31 ± 0.77	2.93 ± 0.45	2.82 ± 0.52	3.31 ± 0.75	3.09 ± 0.61	3.07 ± 0.68
T-predicted score	—	0.42 ± 1.15	0.59 ± 1.23	—	0.9 ± 1.15	0.52 ± 0.89	—	−0.37 ± 0.81 ^{ΔΔ}	−0.16 ± 0.92 ^Δ

^a **P* < 0.05, compared with baseline, ***P* < 0.01, compared with the other two groups, ^Δ*P* < 0.05, compared with the other two groups, ^{ΔΔ}*P* < 0.01, compared with the other two groups. T-predicted score was also illustrated in Supplemental Data Figure 1 in Supporting Information.

of 2 (visit 1), 4 (visit 2), 6 (visit 3), ..., 24 (visit 6), 36 (visit 7), 48 (visit 8) weeks of treatment. Metabolic analysis was performed using GC/MS at 24 weeks and 48 weeks. Antihypertensive drugs or other concurrent treatments, including dietary regimens, remained unchanged throughout the study. The number of patients taking antihypertensive medications were 16 (42%) for the repaglinide group, 10 (45%) for the metformin group, and 11(44%) for the rosiglitazone group, respectively. None of the participants was on lipid-lowering therapy. Of all the patients recruited, 80 patients completed the 24 week treatment and 74 patients completed the 48 weeks treatment, whereas the rest participants failed to follow up the protocol (two subjects from rosiglitazone group at 24 week failed to return for follow up, and two subjects in each group were lost to follow-up at 48 week). The protocol for this study was in accordance with the Helsinki Declaration and was approved by the ethical committee of the hospital, and all subjects recruited provided written informed consent.

Biochemical Measurement. Plasma glucose levels [fasting plasma glucose (FPG) and 2-h postprandial plasma glucose (2hPG)], Serum lipid profiles, including total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein-cholesterol (HDL-cholesterol), and low-density lipoprotein-cholesterol (LDL-cholesterol), HbA_{1c}, heart rate, blood pressure, weight and body mass index (BMI) were determined as previously described.⁷

Sample Preparation and GC/MS Analysis. Serum samples collected from fasting subjects were stored frozen at -80 °C until use, at which point the samples were thawed on ice. Each 200- μ L aliquot of serum sample was added into a 1.5 mL of tube followed by the addition of 400 μ L of acetone for protein precipitation. The mixture was stirred by vortex for 30 s and centrifuged at 10 000 rpm for 10 min. A 400- μ L supernatant was transferred to a 500 μ L of glass tube and dried under vacuum. The dried analytes were dissolved in 80 μ L of methoxylamine hydrochloride (15 mg/mL, dissolved in pyridine) for 90 min at 30 °C and then silylated with 80 μ L *N,O*-bis-trimethylsilyl- trifluoroacetamide (BSTFA) and Trimethylchlorosilane (at a ratio of 99:1) (Supelco) for 2 h at 70 °C. Each 70- μ L aliquot of hexane was added to the derivatization bottles. After the sample was stirred for 1 min and kept at room temperature for an hour, 1- μ L aliquot of the solution was injected into a PerkinElmer gas chromatography coupled with a TurboMass-Autosystem XL mass spectrometer (PerkinElmer, Inc.) in the splitless mode. A DB-5MS capillary column coated with 5% Diphenyl cross-linked 95% dimethylpolysiloxane (30 m \times 250 μ m i.d., 0.25- μ m film thickness; Agilent J&W Scientific, Folsom, CA) was used for separation. Both the injection temperature and the interface temperature were set to 260 °C, and the ion source temperature was adjusted to 200 °C. Initial GC oven temperature was set at 80 °C for 2 min after injection, and was raised up to 285 °C with 5 °C/min and maintained at 285 °C for 7 min. Helium at a flow rate of 1 mL/min was used as the carrier gas. The measurements were made with electron impact ionization (70 eV) in the full scan mode (*m/z* 30–550).

Data Analysis. GC/MS data files were converted into the NetCDF format via DataBridge (Perkin-Elmer, Inc.) and a pretreatment was conducted as previously described.⁹ The mean-centered and autoscaled data were then introduced into the SIMCA-P 11.5 Software (Umetrics, Umeå, Sweden) for multivariate statistical analysis. Principal component analysis (PCA) was used to obtain an overview of variations among the different groups. Orthogonal projections to latent structures discriminant analysis (OPLS-DA), a supervised pattern recogni-

tion approach, was utilized to construct a predictive model to evaluate the effect of individual drug and identify the differential metabolites accountable for the disease or the pharmacological effects. To avoid the overfitting of the models, the OPLS-DA model was carefully validated by the following three steps: first, an iterative 7-round cross-validation¹⁵ with one-seventh of the samples being excluded from the model in each round; second, 1000 random permutations test;¹⁶ finally, blind prediction test in which the data set was randomly divided into training set (70%) and test set (30%), and the model built on the training set was applied to build the classification model to predict the class membership of the test set.¹⁷ T-Score value which is the predictive result of the treatment by the established model was used as an index of metabonomic assessment to calculate and compare with other indices among the three treatment groups.

Univariate statistical analysis, one-way analysis of variance (ANOVA), was used to find the differentially expressed metabolites after treatment with repaglinide and metformin whose OPLSDA models of 24 and 48 weeks treatment were hard to be constructed. One-way ANOVA was also employed to compare the biochemical indices and T-predicted score between the pretreatment (baseline) and post-treatment (24 and 48 weeks), and among the treatment groups. Differences are considered significant at *P* < 0.05. SPSS version 15.0 (SPSS, Chicago, IL) was used for univariate statistical analyses.

Results

A total of 212 individual metabolites were consistently detected in at least 90% of the serum samples using our optimized GC/MS analysis protocol. Compound identification was carried out either by comparing mass spectra and retention time with those obtained with commercially available reference compounds or based on commercial libraries of NIST, NBS and Wiley. We were able to identify 67 (31.6%) of the 212 metabolites, most of which were organic acids/alcohol, amines, amino acids, free fatty acids and sugars. Because the statistical contribution of glucose to the division of diabetic and healthy individuals is too strong, four peaks corresponding to the glucose were removed from the original GC/MS data matrix prior to PCA so that the contributions from other differential metabolites, with good statistical significance, can be identified between the diabetic group and healthy control/treatment group. Orthogonal projections to latent structures discriminant analysis (OPLSDA), a newly developed supervised pattern recognition method, was used to capture the subtle intergroup variations (Figure 1A) and establish a prediction model to assess the physiological impact by drug treatment. The cumulative Q²Y of the model was 0.50 (see also Supplemental Data Table S2 in Supporting Information), the permutation test result was shown in Supplemental Data Figure (Supporting Information), and the sensitivity and the specificity of the model are 95.85% and 81.36%, respectively.

The OPLSDA model calculated from the GC/MS data of diabetic versus healthy subjects was employed to evaluate the effectiveness of each drug treatment by predicting the class membership in the data set of treatment groups. To test the predose metabolic status for the three treatment groups, the OPLSDA models were used to compare one treatment group with another one at predose stage. Such OPLSDA models to compare the intergroup variations were not successfully constructed, suggesting that the initial metabolic status of the three groups prior to the drug treatment was of no statistical

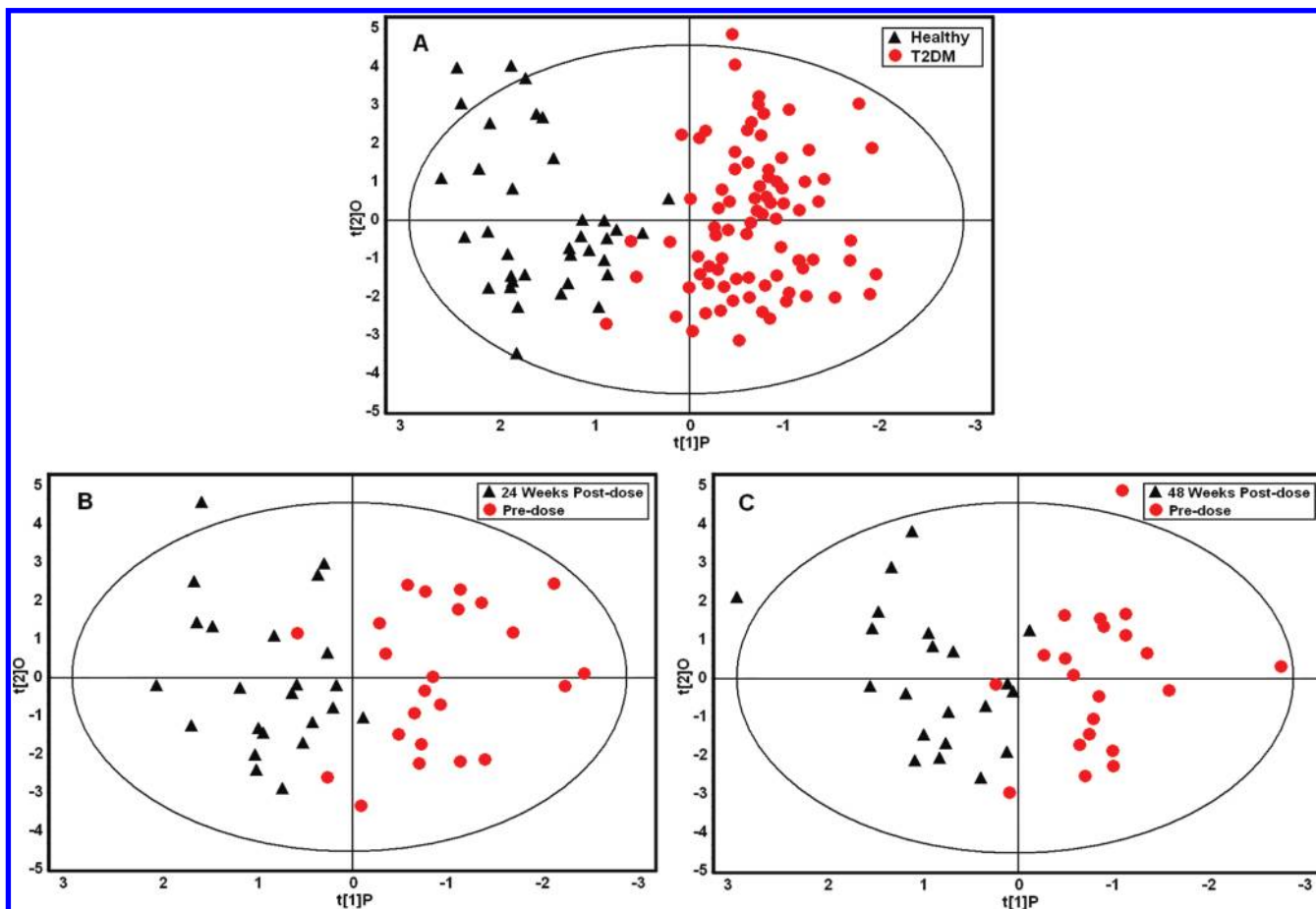


Figure 1. Scores plot of the OPLS-DA model of metabolic profile of 82 newly diagnosed T2DM group vs 36 healthy volunteers (A), rosiglitazone treatment 24 week postdose vs predose group (B) and 48 week postdose vs predose group (C).

difference. The T-predicted scatter plot (see Figure 2) from the OPLS-DA model assigned the samples to either the healthy group or the diabetic group using an a priori cutoff of 0, providing a visible view of the effect of each treatment on the metabolic profile of diabetic patients. Such scatter plot suggests that rosiglitazone treatment yields more effective results at 24 and 48 weeks than the repaglinide and metformin groups, as more serum metabolites were normalized and more individuals in the rosiglitazone group were localized below the baseline. The differentially expressed metabolites in T2DM individuals and the three treatment groups deduced from the retention time and m/z of variant were summarized in Table 2, which mainly involve free fatty acids (FFAs), amino acids and organic acids in serum.

The OPLS-DA was also applied to analyze the serum samples of patients before and after treatment in the study. From the scores plot, only the rosiglitazone group at 24-week and 48-week was able to separate from their predose profile (Figure 1B,C; cumulative $Q^2Y = 0.39$ and 0.30 , respectively; see Supplemental Data Table S2 in Supporting Information), indicating that the rosiglitazone treatment was able to significantly alter the metabolism of T2DM patients whereas the other two drugs were not. There were more differentially expressed metabolites identified in diabetic patients normalized toward healthy levels in the rosiglitazone group than the other two (Tables 2 and 3), such as valine, lysine, glucuronolactone, C16:0, C18:1, urate, and octadecanoate. The differential metabolites in repaglinide and metformin groups in the table were only

identified by one-way ANOVA since the multivariate model can not be constructed successfully, indicating that these two treatments were not able to significantly improve the perturbed metabolic profiles.

Clinical and biochemical parameters of the three treatment groups at 24 weeks and 48 weeks were shown in Table 1. Common clinical indices for diabetes, such as HbA_{1c} , FPG, and 2hFG, were found to be normalized by the drug treatment. The three treatment groups showed no significant differences in the above parameters at 24 and 48 weeks, except for 2hFG, which is relatively lower in metformin treatment group.

Therapeutic effects and physiological improvement were also assessed using four important parameters, HbA_{1c} , FPG, 2hPG and T-predicted score (Figure 3). At 24 and 48 weeks, the percentile of patients in each treatment group meeting with the following standard, $HbA_{1c} < 6.5\%$, $FPG < 7.0$ mmol/L, $2hPG < 11.1$ mmol/L and T-predicted Score < 0 , was calculated, respectively. While the improvement in three common biochemical indices appears to be similar in three treatment groups, T-predicted score, reflecting the comprehensive metabolic alterations, indicates different impacts on global metabolic network by three treatments, respectively. Treatment with rosiglitazone appears to yield more significant improvement at 24 and 48 weeks than the repaglinide and metformin groups.

Discussion

Since T2DM is a metabolic disorder that unbalances the metabolism of carbohydrates, lipids and amino acids, the

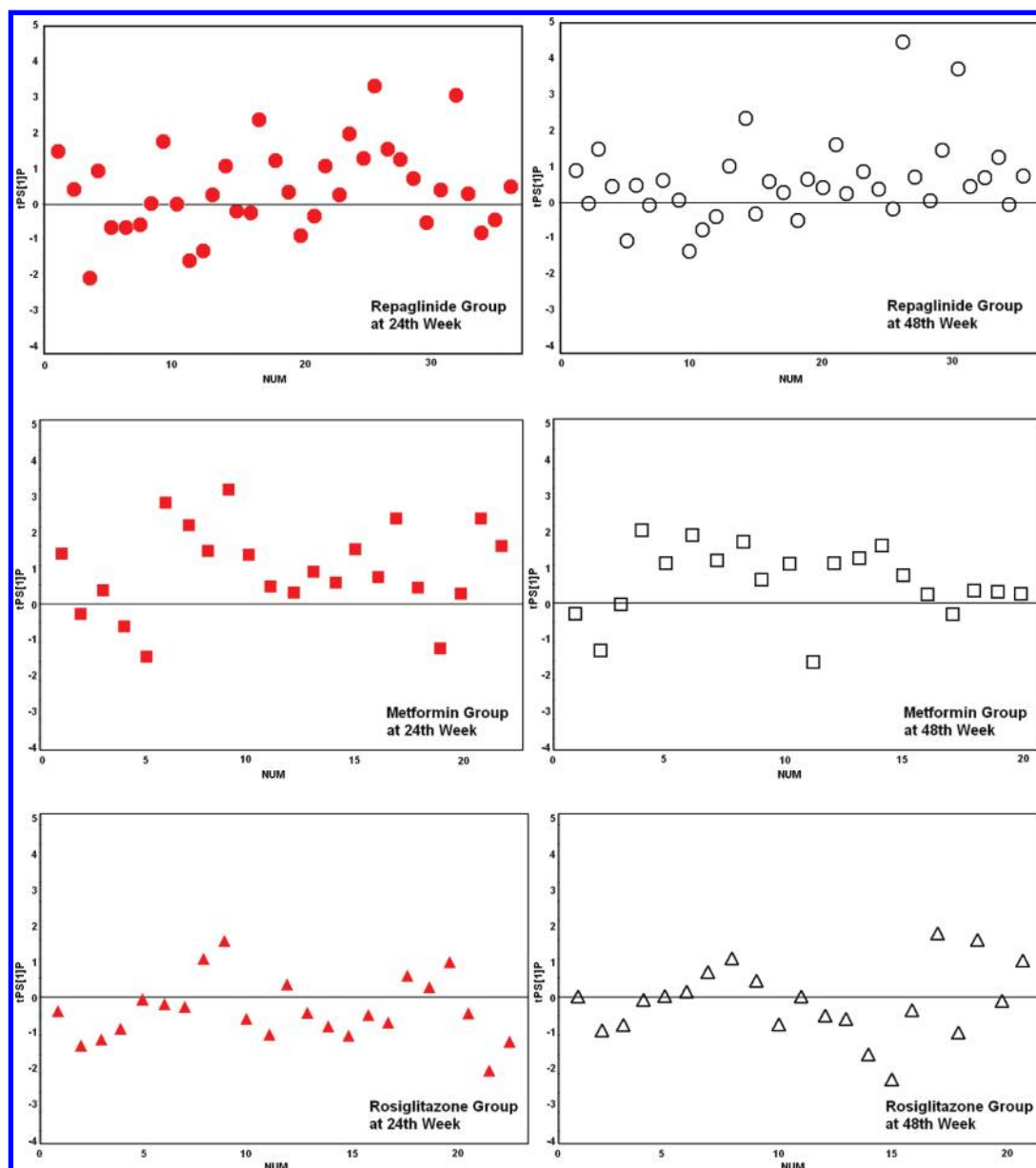


Figure 2. T-predicted scatter plot of the three treatment groups at the 24th week and 48th week.

pathological process and the drug intervention must involve altered expressions of downstream low molecular weight metabolites including glucose. The present study is designed to visualize the alteration of global serum metabolites associated with the pathophysiology of T2DM and three drug treatments in comparison with the conventional biochemical indices. As compared to healthy controls, the altered serum metabolites in diabetic subjects (in Table 3), include the significantly increased valine, maltose, glutamate, urate, butanoate and long-chain fatty acid (C16:0, C18:1, C18:0, octadecanoate and arachidonate), and decreased glucuronolactone, lysine and lactate. These findings suggest a hypercatabolic state in T2DM patients which is consistent with previously reported results.¹⁸ However, these differentially expressed metabolites observed in our study were not in good agreement with recently reported metabolite profiles of T2DM patients.¹⁹

The mean value and the HbA_{1c}, FPG and 2hPG of the three treatment groups were improved to a similar level, as seen in Figure 3 and Table 1. However, the variations of the global

metabolic profiles were significantly different among the groups. Multivariate statistics indicated the rosiglitazone-mediated normalization of 8–10 key metabolites at 24 and 48 weeks, which were differentially expressed in diabetic subjects, involving glutamate, maltose, butanoate, glucuronolactone, C16:0, C18:1. In repaglinide and metformin groups, only glutamate and lysine (in repaglinide group at 48 week only) were brought to the normal level. All of the three treatments were able to down-regulate the high level of glutamate to a lower level in serum, suggesting a possible effect on prevention and amelioration of diabetic complications in T2DM patients, since it has been reported that a high level of serum glutamate is involved in immunodeficiency, amyotrophic lateral sclerosis, and neurological complications.²⁰ The results also indicate that concentrations of several carbohydrates including fructose, α -galactose, mannose and maltose were significantly altered, in addition to glucose levels, by rosiglitazone and repaglinide treatments.

Table 2. The Statistical Analysis Result of the Differentially Expressed Endogenous Metabolites Correlated with T2DM (DM Patients vs Healthy Controls), Rosiglitazone Treatment at 24 Weeks (24 Week Postdose vs Predose) and Rosiglitazone Treatment at 48 Weeks (48 Week Postdose vs Predose)^a

rt/min	metabolites	DM patients vs healthy controls		24th week postdose vs predose		48th week postdose vs predose	
		correlation coefficient	fold change	correlation coefficient	fold change	correlation coefficient	fold change
6.00	Lactate	−0.16	−1.2	—	—	—	—
8.17	Butanoate	0.18	2.6	−0.37	−2.6	−0.33	−2.2
9.38	valine	0.32	1.7	—	—	—	—
9.76	unidentified	0.25	1.8	−0.19	−1.4	−0.34	−1.6
11.4	Isoleucine	—	—	—	—	−0.25	−1.5
13.05	serine	—	—	0.29	1.4	0.22	1.3
13.67	theronine	—	—	0.35	1.5	0.24	1.3
14.08	3–Amine-alanine	—	—	−0.26	−1.4	—	—
14.52	lysine	−0.17	−1.7	0.22	1.8	—	—
14.72	GABA	—	—	−0.25	−1.3	−0.19	−1.2
15.76	aspartate	—	—	—	—	0.19	1.5
16.24	Malate	—	—	−0.3	−1.4	−0.26	−1.3
17.00	proline	—	—	0.3	1.4	0.31	1.4
17.36	glutamate	0.19	1.3	−0.35	−1.4	−0.45	−1.5
20.61	fructose	—	—	0.22	1.3	0.39	1.9
22.49	α-Glycerophosphate	—	—	—	—	0.22	1.3
22.52	Glucuronolactone	−0.29	−1.6	0.20	1.2	—	—
27.84	α-galactose	—	—	−0.26	−1.8	−0.21	−1.5
28.23	C16:0	0.38	1.6	−0.30	−1.2	−0.25	−1.2
29.21	Urate	0.14	1.7	—	—	—	—
30.80	unidentified	−0.14	−2.7	−0.34	−2.0	−0.21	−1.5
31.32	C18:1	0.32	1.7	−0.19	−1.2	−0.18	−1.1
31.78	C18:0	0.26	1.3	−0.29	−1.2	—	—
33.48	unidentified	—	—	−0.32	−1.9	−0.33	−2.1
33.82	Arachidonate	0.19	1.3	—	—	—	—
36.82	C22:6	—	—	−0.33	−2.0	—	—
39.37	Maltose	0.21	1.8	−0.32	−1.6	−0.39	−1.8
39.79	unidentified	0.19	1.4	—	—	—	—
40.19	Octadecanoate	−0.14	−1.7	—	—	—	—

^a The correlation coefficients shown are based on O-PLS-DA analysis of the two-group model (group 1 vs group 2), positive value indicate an increase of the metabolite in group 1. The “—” represents statistically nonsignificant values (VIP < 1).

Table 3. The One-Way ANOVA Result of the Differentially Expressed Endogenous Metabolites Correlated with Repaglinide and Metformin Treatment at 24 Weeks (24 Week Postdose vs Predose) and 48 Weeks (48 Week Post-dose vs Predose)

rt/min	metabolites	24 week postdose vs predose		48 week postdose vs predose	
		<i>P</i>	fold change	<i>P</i>	fold change
Repaglinide Treatment					
14.52	lysine	—	—	0.038	2.0
14.72	mannose	0.013	−1.9	—	—
15.28	unidentified	—	—	0.035	1.4
16.5	valine	—	—	0.035	2.0
17.36	glutamate	0.013	−1.6	—	—
17.68	unidentified	—	—	0.008	1.5
19.36	GABA	0.033	−1.5	0.042	−1.5
20.61	fructose	—	—	0.018	1.9
23.66	unidentified	0.049	−1.3	—	—
27.84	α-galactose	0.008	−1.3	—	—
Metformin Treatment					
17.36	glutamate	0.008	−1.4	0.037	−1.3

The results of quantitative assessment of the serum metabolome demonstrated that a number of metabolites in glutamate pathways as seen in Figure 4 were down-regulated following 24 and 48-week treatment of rosiglitazone, whereas those in aspartate pathways were up-regulated. As an important hydro-transfer shuttle reaction crossing mitochondrial matrix within cells, malate and aspartate were mutually biosynthesized by

glutamate and 2-oxo-glutarate. Interestingly, α-glycerophosphate, an important participant of α-glycerophosphoric acid shuttle system, was increased by rosiglitazone, too. Among these altered metabolites, aspartate and α-glycerophosphate were only discovered in the 48-week treatment group, suggesting that such alteration may be the outcome of long-term rosiglitazone administration.

Accordingly, with regard to the effect of rosiglitazone on glycol-lipid metabolisms,^{6,21} we speculate that this impact on the two shuttle reactions may be due to the augmented glucose utility and glycolysis. On the other hand, as a product of glycometabolism and a substrate of triglyceride synthesis, α-glycerophosphate was increased, indicating an up-regulated stearo-generation that is also a potential adverse reaction of adiposis hepatica and another reason for lipid metabolism dysfunction.^{22,23} Additionally, it was reported that rosiglitazone decreased the rate of loss of β-cell function and improved insulin sensitivity to a greater extent than metformin, maintaining a longer glycemic control.²⁴

The rosiglitazone induced depletion of GABA, an important neurotransmitter in vasodilatation and neurotransmission,^{25,26} suggesting that rosiglitazone treatment may involve the interferences of lipometabolism and GABA-mediated vasodilatation.

Although biochemical markers such as HbA_{1c}, FPG and 2hPG were normalized in the treatment groups of repaglinide and metformin, the metabolic profiles did not alter significantly between pre- and post-treatment states, especially in met-

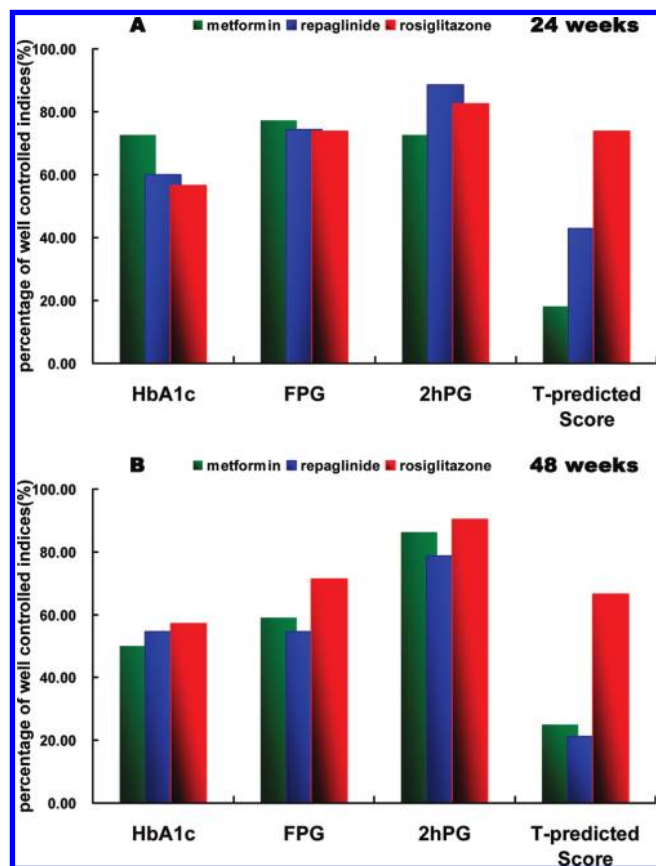


Figure 3. The percentages of the well-controlled biochemical indices (HbA_{1c}, FPG, 2hPG) and T-predicted scores of the repaglinide (green), metformin (blue) and rosiglitazone (red) treatment groups at 24 weeks (A) and 48 weeks (B), respectively.

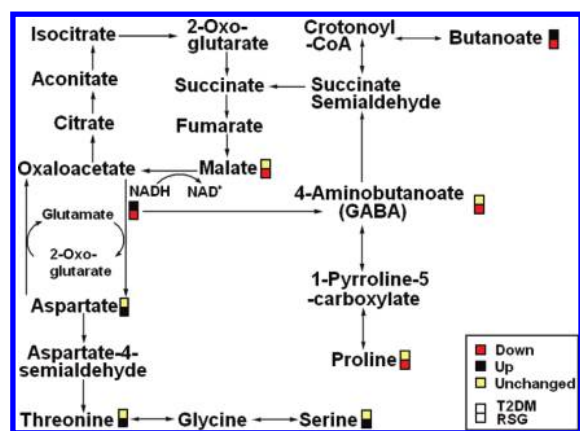


Figure 4. Simplified pathway illustrating the metabolic effect of rosiglitazone.

formin group. Such results suggest that the long-term administration of the two antidiabetic drugs is to moderate the metabolic activity of patients to sustain their physiological functions at certain levels, rather than completely reverse the dysregulation of the metabolic network.

In conclusion, the current study demonstrates that the metabonomic analysis can reveal different treatment effects on physiology and can yield a novel, nonglucose based evaluation strategy for the systemic treatment effect in T2DM patients, which distinguished from conventional clinical indices. Significantly altered serum metabolites in diabetic subjects were detected including increased valine, maltose, glutamate, urate,

butanoate and long-chain fatty acid (C16:0, C18:1, C18:0, octadecanoate and arachidonate), and decreased glucuronolactone, lysine and lactate. Rosiglitazone treatment was able to reverse more abnormally expressed metabolites, such as valine, lysine, glucuronolactone, C16:0, C18:1, urate, and octadecanoate, than the other two drugs.

Abbreviations: T2DM, Type 2 diabetes mellitus; HbA_{1c}, glycated hemoglobin; BMI, body mass index; TC, total cholesterol; TG, triacylglycerol; HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; BMI, body mass index; OPLSDA, orthogonal projections to latent structures discriminant analysis.

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Supporting Information Available: Baseline demographics of study populations; parameters of OPLSDA model; validation model of 1000 random permutations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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