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Pharmacometabonomic Phenotyping Reveals Different Responses to Xenobiotic Intervention in Rats

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In conventional pharmacological studies, intersubject differences within an animal strain are normally neglected, leading to variations in pharmacological outcomes in response to the same stimulus. Using two classical experimental models, the Streptozotocin (STZ)-induced diabetic model of Wistar rats and the high-energy, diet-induced obesity model of Sprague−Dawley rats, we demonstrate that the different outcomes of STZ or diet intervention are closely associated with variation in predose (baseline) urinary metabolic profiles of the rats. The pharmacometabonomic analysis of predose metabolic profiles indicates that the intersubject difference is, to a great extent, associated with gut-microbiota, which predisposes different pathophysiological outcomes upon diet alteration or chemical stimulus. We hypothesize that there may exist an important association between observations from these two models and the obese/diabetic human population in that subtle variations in metabolic phenotype may predetermine different systems' responses to xenobiotic perturbation, ultimately leading to varied pathophysiological processes. Results from two independent models also suggest that the pharmacometabonomics approach is of great importance in the study of pharmacology and clinical drug evaluations, where endogenous metabolite signatures of predose individuals should be taken into consideration to minimize intersubject difference and the resulting variation in the postdose pharmacological outcomes.

Keywords: pharmacometabonomics • metabolic phenotyping • GC/MS • intersubject difference • predose metabolic profile • xenobiotic intervention

Introduction

In drug preclinical studies, the ability to detect treatment-related changes depends on a stable, uniform animal population in which the endogenous metabolic profile of biofluids is regarded as constant.[1] However, the metabolic profile of normal animals such as C57BL10J, ApfCD mice, and Sprague−Dawley rats is well-documented to show a wide variety of strain or gender-related differences, which may lead to significant variation in the pharmacological outcomes.[1,2] Thus, it is of vital importance to investigate 'intersubject/metabolic variation' and to understand its biological significance. A 'pharmacometabonomics' approach to personalize drug treatment was recently proposed by Clayton et al.,[3] which is defined as "the prediction of the drug metabolism and toxicity in an individual using a mathematical model of preintervention metabolite signatures." Pharmacometabonomics takes into account both genomic and environmental factors and, therefore, provides an advantageous alternative or complementary procedure to pharmacogenomics for personalized human healthcare in the future.[4]

In pharmacological or nutritional studies, 'intersubject variation' is often observed among laboratory animals, in which the induced effect (such as diabetes or obesity) is so variable that the animals could be classified as either 'responders' or 'nonresponders'. Additionally, regardless of the number of experimental rats used, only about a quarter to one-half of Wistar and Sprague−Dawley rats show an obese phenotype under the same conditions of nutrition and environment.[5−7] Similarly, differential responses to Streptozotocin (STZ) in blood glucose level have also been noticed in mice.[8] So, it has been hypothesized that the disparity of intersubject response to diet or chemical stimulus is the result of variation in predose metabolic phenotype of the animals.[3]

In this study, we have employed a combined gas chromatography and mass spectrometry (GC/MS) method coupled with multivariate statistical techniques,[9,10] to study the predose metabolic profile and postdose pharmacological outcomes in two well-established experimental models, the Streptozotocin (STZ)-induced diabetic model of Wistar rats and the high-energy, diet-induced obesity model of Sprague−Dawley rats. In addition, the significant metabolites contributing to the

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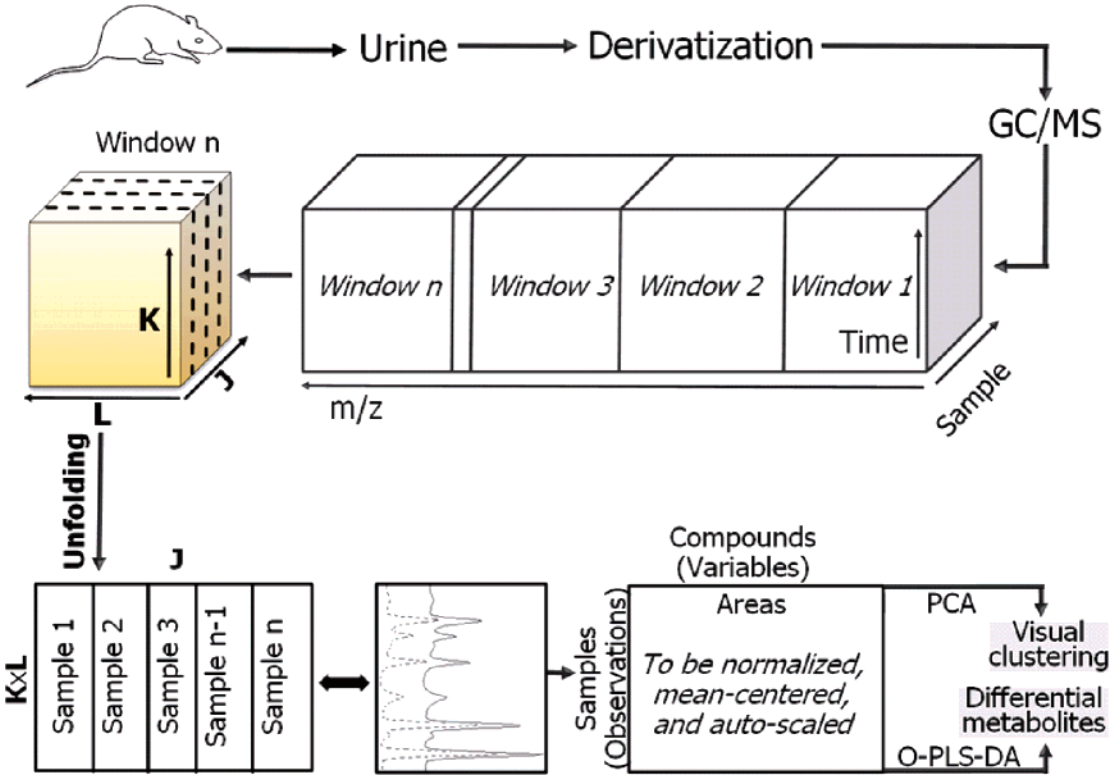

Figure 1. Summary of the general procedure for the metabonomic study used in this experiment.

variation in the corresponding metabolic phenotype were analyzed. The general procedure of the metabonomic studies used in these experiments was summarized in Figure 1.

Experimental Section

Experiment I: Streptozotocin (STZ)-Induced Diabetic Model. A total of 26 9-week-old male Wistar rats (210 ± 10 g) was commercially obtained from Shanghai Laboratory Animal Co., Ltd. (SLAC, Shanghai, China). All animals were kept in a barrier system with regulated temperature (23−24 °C) and humidity (60 ± 10%) and on a 12/12-h light−dark cycle with lights on at 08:00 a.m. The rats were fed certified standard rat chow and tap water *ad libitum*. After 1 week of acclimation, 24-h predose urine samples were collected for predose metabolic profile analysis. After 16-h fasting , 17 rats (one rat was excluded because of poor health) were randomly selected and injected ip with STZ (Sigma, NY) freshly prepared in acetate buffer (0.10 mol/L, pH = 4.5) at a single dosage of 55 mg/kg of body weight, whereas the remaining animals were taken as a control group and injected with the same volume of vehicle. In accordance with the published protocol, rats at 96-h postdose presenting a blood glucose level higher than 16.70 mmol/L are defined as diabetic rats (responders),[11] and those with a blood glucose level lower than 16.70 mmol/L are defined as as nonresponders. The tail-blood glucose value at 96-h postdose was immediately determined with OneTouch Ultra Meter (Lifescan Inc., CA). Predose urine samples were centrifuged at 10 000 rpm for 10 min to remove suspended debris and stored at −80 °C pending GC/MS analysis.

Experiment II: High Energy (HE) Diet-Induced Obesity Model. Twenty 8-week-old male Sprague−Dawley rats (185 ± 5 g) were purchased from SLAC and raised under protocols in accordance with Experiment I. Following 1 week of acclimation, rats were individually placed in metabolic cages to collect 24-h urine samples for the predose (baseline) metabolic profile analysis. Fourteen animals were randomly chosen and switched to HE diet (which was composed of 10% lard, 5% corn oil, 10% sucrose, 10% casein, and 65% standard rat chow), while the rest of the rats were kept on standard rat chow over the experimental period. Body weight of each animal was measured weekly. Animals with body weight in the upper quartile were labeled as obesity-prone (OP) rats, while animals in the lower quartile were labeled as obesity-resistant (OR) rats.[12] The handling of urine samples was in line with Experiment I. Animal experiments were carried out under the Guidelines for Animal Experiment of Shanghai University of Traditional Chinese Medicine (Shanghai, China), and the protocol was approved by the local Animal Ethics Committee.

GC/MS Sample Preparation, Derivatization, and Spectral Acquisition. The urine samples were selected for GC/MS sample preparation, and relevant spectral acquisition was used as the method[13] with some modifications. An amount of 300 μL of each urine sample was used with addition of 100 μL of L-2-chlorophenylalanine (0.10 mg/mL, internal standard for batch quality control), and derivatized with 50 μL of ethyl chloroformate (ECF) at 20.0 ± 0.1 °C, with sonication at 40 kHz for 60 s. The derivatives were extracted with 300 μL of chloroform, and the pH was adjusted with 100 μL of NaOH (7 mol/L). The derivatization process was repeated by adding an additional 50 μL of ECF. The resultant mixtures were centrifuged at 3000 rpm for 3 min. The aqueous layer was aspirated, while the chloroform layer containing derivatives was dehydrated with anhydrous sodium sulfate for subsequent analysis. Each 1-μL aliquot of analytes was injected into a DB-5MS capillary column coated with 5% diphenyl cross-linked 95% dimethylpolysiloxane (30 m × 250 μm id, 0.25-μm film thick-

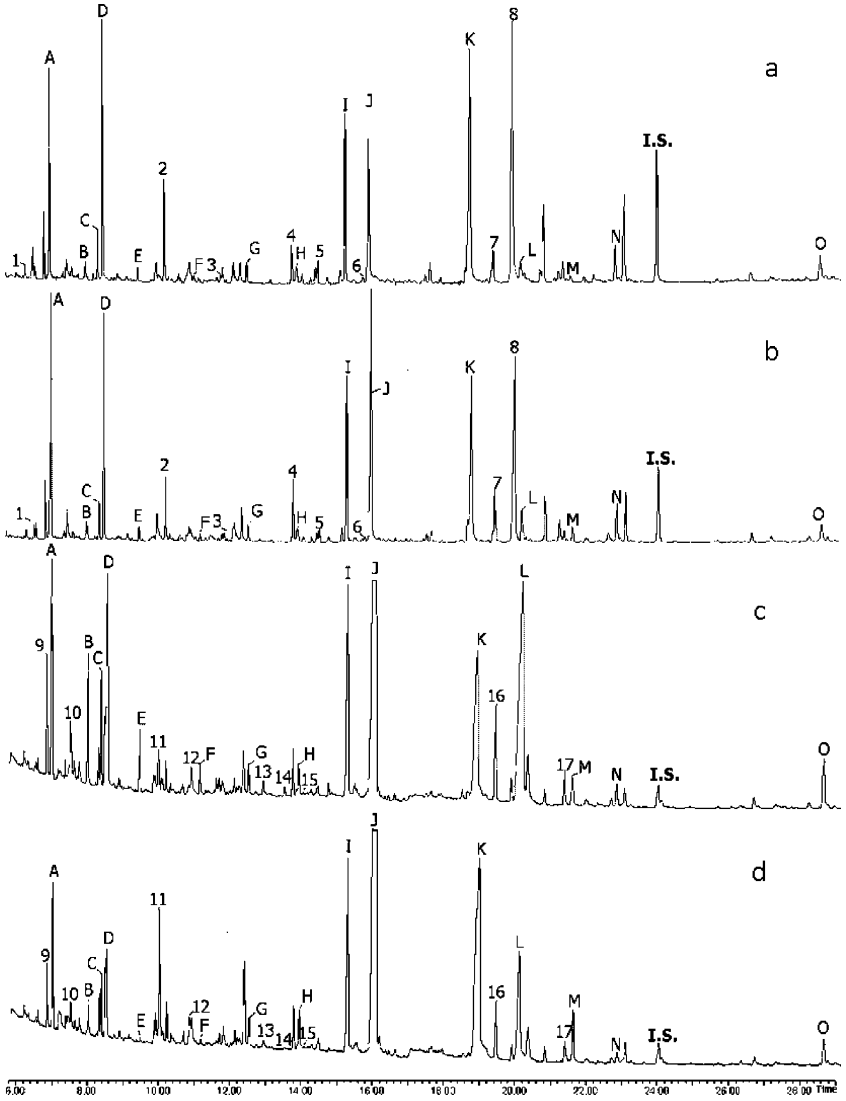

Figure 2. Total ion current chromatograms of GC/MS of typical urine samples from (a) OP SD rat, (b) OR SD rat, (c) DP Wistar rat, and (d) DR Wistar rat. Key: representative metabolites labeled as A-O are commonly identified in the four phenotypes of two strains in this study; 1−8 are only in OP and OR rats; 9−17 are only in DP and DR rats. A = succinate; B = phenylacetate; C = alanine; D = glycine; E = *p*-cresol; F = 4-ethyl-phenol; G = proline; H = malate; I = aconitate; J = citrate; K = hippurate; L = putrescine; M = acetyl citrate; N = 4-oxo-pimelate; O = lysine; 1 = hexanoate; 2 = adipate; 3 = glutamine; 4 = glutamate; 5 = dodecanoate; 6 = aspartate; 7 = 4-hydroxy-phenylacetate; 8 = *N*-phenylacetyl-glycine; 9 = lactate; 10−12 = unidentified; 13 = asparagine; 14−16 = unidentified; 17 = indole acetate; I.S. = L-2-chlorophenylalanine (internal standard).

ness; Agilent J&W Scientific, Folsom, CA) and conducted by a combined gas chromatography (Perkin-Elmer, Inc.) and mass spectrometer (TurboMass−Autosystem XL, Perkin-Elmer, Inc.). The injection and the interface temperature were set at 260 °C; and the ion source was adjusted to 200 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The analysis was performed under the following temperature program: 2 min isothermal heating at 80 °C, followed by 10 °C/min oven temperature ramps to 140 °C, 4 °C/min to 240 °C, and 10 °C/min to 280 °C, and a final 3-min heating at 280 °C. Electron energy was 70 eV, and detection was conducted in full scan mode (m/z 30−550).

Spectral Processing and Multivariate Modeling. All the GC/MS raw files were converted to CDF format via DataBridge (Perkin-Elmer, Inc.) and subsequently processed by the XCMS toolbox (http://metlin.scripps.edu/download/) using XCMS's default settings with the following exceptions: xcmsSet (fwhm = 4, snthresh = 8, max = 20), group (bw = 5).[14,15] The resulting table (TSV file) was exported into Matlab software 7.0 (The MathWorks, Inc.), and each detected peak was normalized to the total sum of spectrum. The three-dimensional matrix consisting of peak index (RT−m/z pair), sample names (observations), and normalized peak area was introduced into SIMCA-P 11.0 Software package (Umetrics, Umeå, Sweden) in which multivariate statistical analyses such as Principal Component Analysis (PCA) and Orthogonal-Projection to Latent Structures-Discriminant Analysis (O-PLS-DA)[16−18] were performed. Additionally, a majority of the metabolites detected were identified using the reference compounds available and the commercial compound libraries: NIST and Wiley in Turbomass 4.1.1 software (Perkin-Elmer, Inc.).

PCA was conducted on auto-scaled, normalized predose spectral data. The scores plot of PCA exhibited the general clustering, trends, or outliers in the observations (or samples). Each coordinate of the plot represents one subject. Essentially, auto-scaling somewhat magnifies the influence of the system-

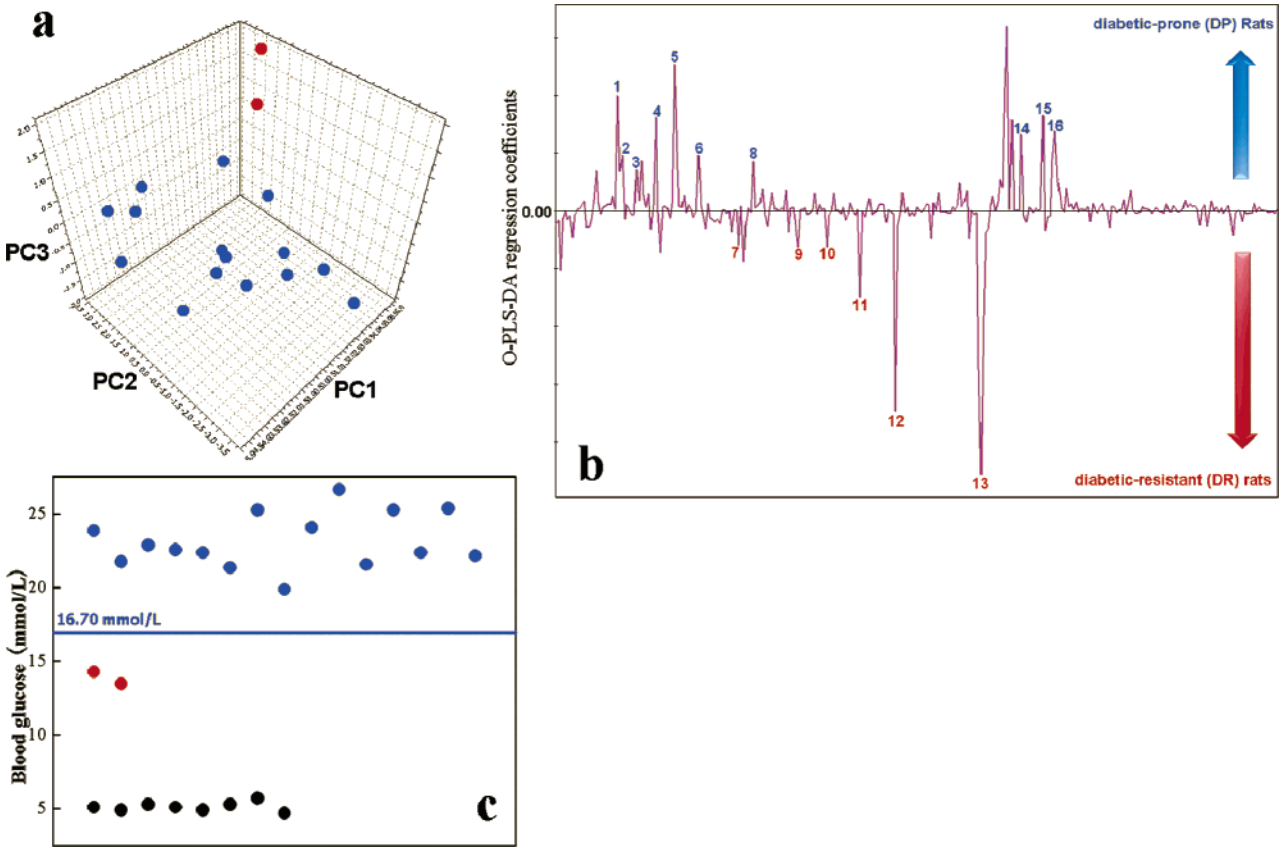

Figure 3. (a) PCA scores plot from GC/MS spectra of urine samples obtained from male Wistar rats before streptozotocin (ip, 55 mg/kg) injection. Each colored point represents an individual rat according to its postdose behavior (the red dots denote diabetic-resistant (DR) rats, while the blue dots are diabetic-prone (DP) rats). (b) An O-PLS-DA regression coefficient plot of the GC/MS data set presented significant urinary metabolites (peaks) contributing to the differentiation of metabolic profiles between predose DP and DR rats. These metabolites are labeled in blue, indicating they are in relatively higher concentrations in the urine sample of DP rats, and in red color, indicating relatively higher concentrations in DR rats. The labeled metabolites are the following: 1, lactate; 2, succinate; 3, unidentified; 4, phenylacetate; 5, glycine; 6, *p*-cresol; 7, unidentified; 8, 4-ethyl-phenol; 9, proline; 10, malate; 11, aconitate; 12, citrate; 13, hippurate; 14, putrescine; 15, indole acetate; 16, acetyl citrate; 17, hexanoate; 18, alanine; 19, adipate; 20, dodecanoate; 21, 4-hydroxy-phenylacetate; 22, *N*-phenylacetyl-glycine; 23, lysine. (c) Blood glucose levels of all of Wistar rats at 96-h postdose. Fifteen of the total 17 rats injected with STZ showed a dramatically increased blood glucose level (23.19 mmol/L, blue dots), while two rats, with different predose urinary metabolic profiles displayed in panel a, showed a relatively lower blood glucose level (13.90 mmol/L, red dots). The mean blood glucose level in control animals treated with vehicle was 5.12 mmol/L (black dots).

Table 1. Summary of the GC/MS Urine Data Sets Used in PCA and O-PLS-DA Modeling[a]

	PCA model		O-PLS-DA model		
	No. of components	$\mathbf{R^2X}$ (cum)	No. of components	$\mathbf{R^2Y}$	$\mathbf{Q^2Y}$
OP vs OR group	5	0.850	2	0.965	0.787
DP vs DR group	5	0.834	3	0.868	0.548

[a] $\mathbf{R^2X}$ (cum) represents the cumulative Sum of Squares (SS) of the **X** explained by all extracted components, $\mathbf{R^2Y}$ provides an estimate of how well the model fits the **Y** data, and $\mathbf{Q^2Y}$ is an estimate of how well the model predicts the **Y**. Cumulative values of $\mathbf{R^2X}$, $\mathbf{R^2Y}$, and $\mathbf{Q^2Y}$ close to 1 indicate an excellent model.

atic noise and strongly distorts the relative values of loadings, thus, Pareto scaling (the scaling factor is the square root of standard deviation) is favored for the subsequent analysis. These Pareto-scaled, normalized data were further analyzed using an O-PLS-DA model, a supervised method capable of removing information from an input data set **X** unrelated to the response set **Y** so as to improve the interpretation of metabolites responsible for the separation. Here, an O-PLS-DA regression coefficient plot was used to depict the up- or down-regulated metabolite corresponding to one class. As with regression coefficients, the weight value (e.g., the variable importance in the project (VIP)) of each variable in the model is able to reveal the correlation of the **X** variables with **Y** and is commonly employed to select the metabolites of interest. The sum of the square of all VIP values is equal to the number of terms in the model; hence, the average VIP would be equal to 1. That is, terms with a VIP value larger than 1 are more relevant for explaining **Y**. In general, metabolites contributing to the variation in the current study were identified by the rank of VIP values, and the signal orientation in the regression coefficient plot denoted the concentration variation (i.e., a positive peak represents a higher concentration of the metabolite in the corresponding group). To validate the model against overfitting, a typical 7-round cross-validation was carried out with 1/7 of the samples being excluded from the model in each round. This procedure was repeated in an iterative manner until each sample had been excluded once and Q^2 was calculated from the results in SIMCA-P package. $\mathbf{R^2Y}$ provides an estimate of how well the model fits the **Y** data, whereas $\mathbf{Q^2Y}$

Table 2. Rat Body Weights during Experimental Period (g, Mean ± SD)[a]

	week						
group	0	1	2	3	4	5	6
OP	218 ± 8.5	312 ± 11.2[b]	364 ± 17.3[c]	392 ± 13.9[c]	430 ± 11.4[c]	477 ± 13.8[c]	493 ± 12.6[d,e]
Control	213 ± 6.7	305 ± 11.3	349 ± 18.9	373 ± 11.1	393 ± 23.3	426 ± 27.9	432 ± 25.3
OR	218 ± 13.4	286 ± 13.9	326 ± 10.6	340 ± 9.0	365 ± 11.6	402 ± 8.9	413 ± 9.3

[a] The body weight of the two phenotypes (obesity-prone, OP, $n = 4$, and obesity-resistant, OR, $n = 4$) on high energy (HE) diet and the control group ($n = 6$) on standard rat chow. There was no difference among the rats at the beginning of the experiment ($P > 0.05$). However, a significant difference was observed between the OP and OR phenotypes after 1 week of the HE diet ($P < 0.05$), and this trend increased with time during the experimental period. Data points are means ± SD. [b] $P < 0.05$. [c] $P < 0.01$. [d] $P < 0.001$ compared with OR group. [e] $P < 0.05$ compared with control group.

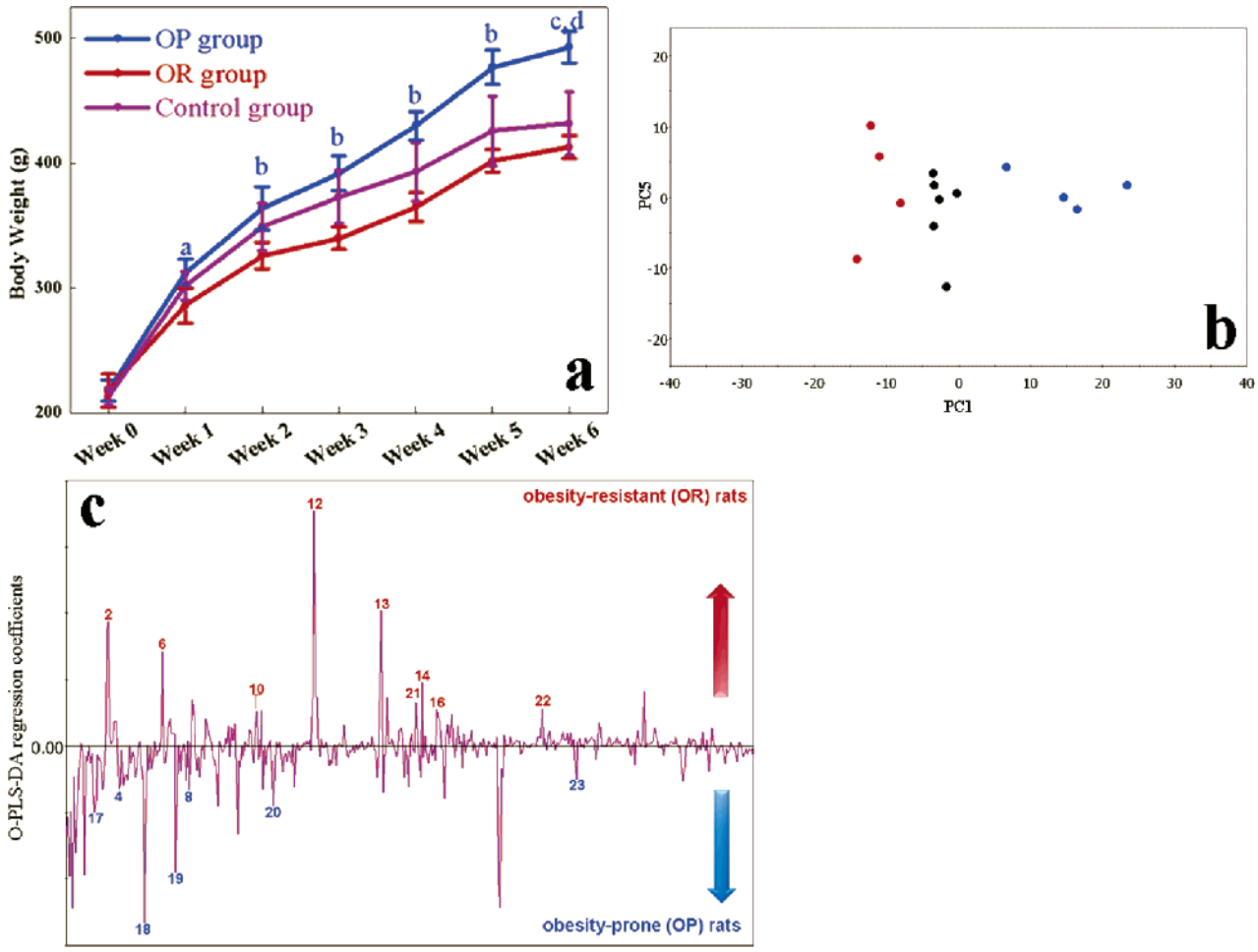

Figure 4. (a) Body weight in rats over the 6 weeks. Significant body weight variation was observed after 1 week of high energy (HE) diet between a group of rats with greater weight gain (blue, $n = 4$, defined as obesity-prone rats, OP group) and a group of rats with minimal weight gain (red, $n = 4$, named obesity-resistant rats, OR group) ($P < 0.05$). This trend increased with time during the experimental period. Data points are expressed as means ± SD. a, $p < 0.05$; b, $p < 0.01$; and c, $p < 0.001$, denoting the statistical difference between OP group and OR group; d, $p < 0.05$, significantly different between OP group and control group. Statistical analysis was performed by ANOVA. (b) PCA scores plot from GC/MS spectra of urine samples obtained from male Sprague−Dawley rats before exposure to HE diet. Each colored point represents an individual rat according to postdose body weight gain (red are OR rats, while blue are OP rats). (c) An O-PLS-DA regression coefficient plot of the data set showed significant metabolites contributing to the differentiation of OP and OR predose metabolic phenotypes. The differential urinary metabolites labeled on the plot are provided in Figure 3b.

is an estimate of how well the model predicts the **Y**. The cumulative values of $\mathbf{R^2Y}$ and $\mathbf{Q^2Y}$ close to 1 indicate an excellent model.

Univariate Statistical Analysis. The one-way analysis of variance (One-Way ANOVA) was utilized to compare the mean body weight between the different phenotypes. The blood glucose values were presented with a simple scatter dot plot. All the figures were obtained by OriginPro 7.5 software (OriginLab, Co., MA).

Results and Discussion

Predose Metabolic Profiling and Blood Glucose Values at 96-h Postdose in STZ-Induced Diabetic Rats. Figure 2c,d shows the typical GC/MS total ion current chromatograms

(TIC) from the analysis of urine samples of Wistar rats before they were dosed with Streptozotocin, revealing differences in metabolites between two types of rats as responders and nonresponders according to the extent of postdose blood glucose levels. A scores plot from PCA of the GC/MS spectra of predose urine samples showed that there were two rats with somewhat different metabolic profiles (Figure 3a) from the rest of animals. These two rats exhibited a significantly lower blood glucose level (13.90 mmol/L, named as diabetic-resistant or DR in this paper) than that of others (23.19 mmol/L, named as diabetic-prone or DP) at 96-h postdose, while the control was 5.12 mmol/L. The significant metabolites responsible for the metabolic profile variation between predose DP and DR rats were illustrated in the O-PLS-DA regression coefficient plot (Figure 3b). The PCA and the O-PLS-DA modeling were summarized in Table 1.

Predose Metabolic Profiling of Urine and Body Weight Follow-Up in HE Diet-Induced Obesity Rats. Before the HE diet intervention, all animals presented the same body weight. Significant difference in body weight gain was observed among experimental rats after a 1 week exposure to HE diet ($P < 0.05$). Four rats with body weight in the upper quartile were labeled as obesity-prone (OP) rats, while four rats with body weight in the lower quartile were labeled as obesity-resistant (OR) rats as previously described.[12] The remainder of the animals presented intermediate body weight between the OR and OP groups. The division of body weight gain between OP and OR rats increased with time during the experimental period (Table 2, Figure 4a). PCA of GC/MS spectra of urine samples acquired from the 14 rats before HE diet intervention showed distinct differences between OP (blue dot) and OR (red dot) rats (Figure 4b), which was consistent with TIC of GC/MS spectra of typical responders and nonresponders (Figure 2a,b). A further O-PLS-DA regression coefficient plot of the whole data demonstrated the differential metabolites between OP and OR rats (Figure 4c).

Gut Microbiota-Motivated Mechanisms. The most notable urinary metabolites related to predose metabolic differences in OP and OR rats were hippurate, phenylacetate, 4-hydroxyphenylacetate, *N*-phenylacetyl-glycine, 4-ethyl-phenol, and *p*-cresol. Hippurate, *p*-cresol, and a number of aromatic species present in the urine have been reported to originate from gut microbial metabolism.[19−23] This suggests that such a variation in metabolites can be attributed to the initial differences in gut microbiota under the same diet.[3] Gut flora-related differential metabolites detected in DP and DR rats were phenylacetate, *p*-cresol, 4-ethyl-phenol, indole acetate (all low in DR rats), and hippurate (high in DR rats). Mammalian metabolism is known to be significantly influenced by interaction with its complex gut microbial community.[24,25,33−35] Recent studies have indicated that gut microbiota can regulate fat storage in germ-free and conventionalized C57BL/6 mice[26] and influence the fatty liver phenotype in insulin-resistant mice.[27] We therefore deduce that there may exist different gut microbiota structures and activities between OP and OR samples, and between DP and DR samples, which may impact many metabolic pathways. In other words, the different metabolic phenotypes influenced by gut microbiota are heavily involved in, and greatly responsible for, the different systems' responses to the diet and STZ intervention.

Energy Metabolism-Related Mechanisms. It appeared that there were also marked differences between OP and OR rats, and between DP and DR rats, in energy metabolism-related metabolites before exposure to HE diet and STZ. It has been previously demonstrated that the increase of urinary organic acids reflects an impaired oxidation of fatty acids in obese Zucker rats.[28] The lower free fatty acid (FFA) in plasma in OR rats suggests a less positive fat balance by burning more and storing less fat than that seen in OP rats, a metabolic factor that may play a key role in their resistance to dietary obesity.[12] The fact that urinary organic acids such as adipate and dodecanoate in the current experiment were higher in OP rats than in OR rats was consistent with previous findings.[12] Additionally, tricarboxylic acid (TCA) cycle intermediates were significantly different between OP and OR rats and between DP and DR rats, respectively. An elevated level of citrate in urine may be an indication of more energy consumption in OR and DR rats. Lactate is the metabolite of pyruvate under anaerobic conditions, and its counterpart in aerobic conditions is acetyl-CoA, which is then metabolized through the TCA cycle. Therefore, the lower lactate level coupled with the elevated concentration of citrate in DR rats may be the result of the activation of citrate biosynthesis which indicates a more robust immunity system to xenobiotic intervention.[29]

It was generally believed that many adverse drug reactions or poor responses arise because of genetic differences in drug metabolism, transporters, receptors, ion channels, and other drug targets.[30] In this context, intersubject variations were more likely ascribed to genetic influences, which were estimated to account for only 15−30% of variations in drug metabolism and response.[31,32] However, the concept of 'pharmacometabonomics' has dramatically weakened such an incomplete biological deduction by demonstrating that the end-point metabolic phenotypes result from physiological and environmental changes including gut microbiota, aging, diet, diurnal variation, and so forth. The two independent models presented in this work demonstrated that there might exist an important linkage between the diet or chemically induced response and the variation in predose metabolite signatures partially resulting from the different gut-microbiota structures.

Conclusion

On the basis of these observations, we believe that the same animal strain may exhibit preexisting variation in metabolic phenotype, which is influenced not only by genotype, but also by the gut microbiota and environmental factors. The pharmacometabonomic analysis of predose urinary samples revealed that there was a strong involvement of gut microbial metabolism contributing to preexisting variation in the predose metabolic phenotype. The subtle variation in predose metabolic phenotype may alter an individual's metabolic response to xenobiotic intervention and, consequently, predispose these patients to different pathological outcomes such as obesity and diabetes. Furthermore, results from two independent models also suggest that the pharmacometabonomics approach is of great importance in the study of pharmacology and clinical drug evaluations, where endogenous metabolite signatures of predose individuals should be taken into consideration for minimizing intersubject difference and the resulting variation in the postdose pharmacological outcomes.

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References

(1) Robosky, L. C.; Wells, D. F.; Egnash, L. A.; Manning, M. L.; Reily, M. D.; Robertson, D. G. *Toxicol. Sci.* **2005**, *87*, 277−284.
(2) Gavaghan, McKee, C. L.; Wilson, I. D.; Nicholson, J. K. *J. Proteome Res.* **2006**, *5*, 378−384.
(3) Clayton, T. A.; Lindon, J. C.; Cloarec, O.; Antti, H.; Charuel, C.; Hanton, G.; Provost, J. P.; Le Net, J. L.; Baker, D.; Walley, R. J.; Everett, J. R.; Nicholson, J. K. *Nature* **2006**, *440*, 1073−1077.
(4) Nicholson, J. K. *J. Proteome Res.* **2006**, *5*, 2067−2069.
(5) Levin, B. E.; Keesey, R. E. *Am. J. Physiol.* **1998**, *274*, R412−419.
(6) Levin, B. E. *Am. J. Physiol.* **1999**, *276*, R382−387.
(7) Jang, I.; Hwang, D.; Lee, J.; Chae, K.; Kim, Y.; Kang, T.; Kim, C.; Shin, D.; Hwang, J.; Huh, Y.; Cho, J. *Exp. Anim.* **2003**, *52*, 99−107.
(8) Yang, Z.; Chen, M.; Fialkow, L. B.; Ellett, J. D.; Wu, R.; Nadler, J. L. *Pancreas* **2003**, *26*, e99−104.
(9) Chen, M.; Zhao, L.; Jia, W. *J. Proteome Res.* **2005**, *4*, 2391−2396.
(10) Chen, M.; Su, M.; Zhao, L.; Jiang, J.; Liu, P.; Cheng, J.; Lai, Y.; Liu, Y.; Jia, W. *J. Proteome Res.* **2006**, *5*, 995−1002.
(11) Tuitoek, P. J.; Ziari, S.; Tsin, A. T.; Rajotte, R. V.; Suh, M.; Basu, T. K. *Br. J. Nutr.* **1996**, *75*, 615−22.
(12) Chang, S.; Graham, B.; Yakubu, F.; Lin, D.; Peters, J. C.; Hill, J. O. *Am. J. Physiol.* **1990**, *259*, R1103−1110.
(13) Yunping, Qiu.; Mingming, Su.; Yumin, Liu.; Minjun, Chen.; Jingjing, Gu.; Ji, Zhang.; Wei, Jia. A*nal. Chim. Acta* **2007**, *583*, 277−283.
(14) Nordstrom, A.; O'Maille, G.; Qin, C.; Siuzdak, G. *Anal. Chem.* **2006**, *78*, 3289−3295.
(15) Smith, C. A.; Want, E. J.; O'Maille, G.; Abagyan, R.; Siuzdak, G. *Anal. Chem.* **2006**, *78*, 779−787.
(16) Wang, Y.; Holmes, E.; Nicholson, J. K.; Cloarec, O.; Chollet, J.; Tanner, M.; Singer, B. H.; Utzinger, J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12676−12681.
(17) Cloarec, O.; Dumas, M. E.; Craig, A.; Barton, R. H.; Trygg, J.; Hudson, J.; Blancher, C.; Gauguier, D.; Lindon, J. C.; Holmes, E.; Nicholson, J. *Anal. Chem.* **2005**, *77*, 1282−1289.
(18) Crockford, D. J.; Lindon, J. C.; Cloarec, O.; Plumb, R. S.; Bruce, S. J.; Zirah, S.; Rainville, P.; Stumpf, C. L.; Johnson, K.; Holmes, E.; Nicholson, J. K. *Anal. Chem.* **2006**, *78*, 4398−4408.
(19) Heavey, P. M.; Savage, S. A.; Parrett, A.; Cecchini, C.; Edwards, C. A.; Rowland, I. R. *Br. J. Nutr.* **2003**, *89*, 509−15.
(20) Phipps, A. N.; Stewart, J.; Wright, B.; Wilson, I. D. *Xenobiotica* **1998**, *28*, 527−537.
(21) Williams, R. E.; Eyton-Jones, H. W.; Farnworth, M. J.; Gallagher, R.; Provan, W. M. *Xenobiotica* **2002**, *32*, 783−794.
(22) Nicholls, A. W.; Mortishire-Smith, R. J.; Nicholson, J. K. *Chem. Res. Toxicol.* **2003**, *16*, 1395−1404.
(23) Goodwin, B. L.; Ruthven, C. R.; Sandler, M. *Biochem. Pharmacol.* **1994**, *47*, 2294−2297.
(24) Gordon, J. I.; Stappenbeck, T. S.; Hooper, L. V. *Trends Microbiol.* **2003**, *11*, 150−151.
(25) Xu, J.; Bjursell, M. K.; Himrod, J.; Deng, S.; Carmichael, L. K.; Chiang, H. C.; Hooper, L. V.; Gordon, J. I. *Science* **2003**, *299*, 2074−2076.
(26) Backhed, F.; Ding, H.; Wang, T.; Hooper, L. V.; Koh, G. Y.; Nagy, A.; Semenkovich, C. F.; Gordon, J. I. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15718−15723.
(27) Dumas, M. E.; Barton, R. H.; Toye, A.; Cloarec, O.; Blancher, C.; Rothwell, A.; Fearnside, J.; Tatoud, R.; Blanc, V.; Lindon, J. C.; Mitchell, S. C.; Holmes, E.; McCarthy, M. I.; Scott, J.; Gauguier, D.; Nicholson, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12511−12516.
(28) McDevitt, J.; Wilson, S.; Her, G. R.; Stobiecki, M.; Goldman, P. *Metabolism* **1990**, *39*, 1012−1020.
(29) Miyasaka, C. K.; Azevedo, R. B.; Curi, R.; Mancini, Filho, J.; Lajolo, F. M. *Gen. Pharmacol.* **1996**, *27*, 991−4.
(30) Eichelbaum, M.; Ingelman-Sundberg, M.; Evans, W. E. *Annu. Rev. Med.* **2006**, *57*, 119−137.
(31) Evans, W. E.; Relling, M. V. *Nature* **2004**, *429*, 464−468.
(32) Evans, W. E.; McLeod, H. L. *N. Engl. J. Med.* **2003**, *348*, 538−549.
(33) Nicholson, J. K.; Holmes, E.; Lindon, J. C.; Wilson, I. D. *Nat. Biotechnol.* **2004**, *22*, 1268−1274.
(34) Nicholson, J. K.; Wilson, I. D. *Nat. Rev. Drug Discovery* **2003**, *2*, 668−676.
(35) Nicholson, J. K.; Holmes, E.; Wilson, I. D. *Nat. Rev. Microbiol.* **2005**, *3*, 1−8.

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