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Comparison of serum metabolite compositions between obese and lean growing pigs using an NMR-based metabonomic approach

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Abstract

Childhood obesity has become a prevalent risk to health of children and teenagers. To develop biomarkers in serum for altered lipid metabolism, genetically obese (Ningxiang strain) and lean (Duroc×Landrace×Large Yorkshire strain) growing pigs were used as models to identify potential differences in the serum metabonome between the two strains of pigs after consuming the same diet for 46 days. At the end of the study, pigs were euthanized for analysis of the serum metabonome and determination of body composition. Obese pigs had higher fat mass (42.3±8.8% vs. 21.9±4.5%) and lower muscle mass (35.4±4.5% vs. 58.9±2.5%) than lean pigs ($P<.01$). Serum concentrations of insulin and glucagon were higher ($P<.02$) in obese than in lean pigs. With the use of an NMR-based metabonomic technology, orthogonal projection to latent structure with discriminant analysis showed that serum HDL, VLDL, lipids, unsaturated lipids, glycoprotein, *myo*-inositol, pyruvate, threonine, tyrosine and creatine were higher in obese than in lean pigs ($P<.05$), while serum glucose and urea were lower in obese pigs ($P<.05$). In addition, changes in gut microbiota-related metabolites, including trimethylamine-*N*-oxide and choline, were observed in sera of obese pigs relatively to lean pigs ($P<.05$). These novel findings indicate that obese pigs have distinct metabolism, including lipogenesis, lipid oxidation, energy utilization and partition, protein and amino acid metabolism, and fermentation of gastrointestinal microbes, compared with lean pigs. The obese Ningxiang pig may be a useful model for childhood obesity research.

Keywords: Metabonomics; Obese pigs; Lean pigs; Obesity; Serum

1. Introduction

Obesity is one of the greatest public health challenges of the 21st century [1]. Obesity and overweight pose a major risk for serious diet-related chronic diseases, including type 2 diabetes, cardiovascular diseases, hypertension, stroke, certain forms of cancer, and other obesity-associated problems [2]. The prevalence of obesity increases with age, and there is a greater likelihood that obesity beginning in early childhood will persist through the life span [3]. However, experimental animal models to study childhood obesity are not fully established. Obesity and related health risks were presumably attributable to an excess of energy substrates from overeating [3]. However, the gut microbiota, lifestyle and genetic background also influence this process [4]. To enhance the understanding of relationships between these factors and obesity in humans, some animal species have been evaluated as experimental models for obesity research. Those animal models included C57BL/KsJ *db*/*db* mice [5–7], obese Zucker diabetic fatty rats [8], obese fatty (fa/fa) Zucker rats [9],

Abbreviations: BPP-LED, bipolar-pair longitudinal eddy current; COSY, correlated spectroscopy; CPMG, Carr-Purcell-Meiboom-Gill; DLL, Duroc×Landrace×Large Yorkshire; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; 1D, one-dimensional; 1DOPLS-DA, orthogonal projection to latent structure with discriminant analysis; PCA, principal component analysis; RD, recycle delay; TMAO, trimethylamine-*N*-oxide; TOCSY, total correlation spectroscopy; VLDL, very low density lipoprotein.

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rabbits [10], Ossabaw pigs [11] and primates [12]. Because of similarities in nutrition and metabolism between pigs and humans [13,14], genetically obese and lean pigs are useful in childhood obesity research to understand the mechanisms responsible for development of adiposity [6,15].

Metabonomics provides a useful systems approach to understanding global changes in metabolites in animals in response to alterations in genetics, nutrition, environments and gut microbiota [16–19]. Despite many studies on obese and lean pigs [20,21], a comprehensive analysis of metabonomes as potential indicators for the utilization of glucose and amino acids, lipid synthesis, as well as the turnover and storage of fat and protein in obese and lean pigs has not been performed, to the best of our knowledge. The serum metabonome could be used to develop biomarkers to identify early obesity and other associated health risks to facilitate prevention and treatment of obesity.

The Ningxiang pig is a regional swine strain in China and has excessive fat deposition genetically [22,23]. In contrast, the Duroc×Landrace×Large Yorkshire (DLL) hybrid pig is recognized as a genetically lean strain [23]. The present study was designed to compare serum metabonome between the genetically obese and lean pigs using a nuclear magnetic resonance (NMR)-based metabonomic method.

2. Materials and methods

2.1. Pigs, diets, housing and experimental design

Ten castrated male Ningxiang growing pigs (obese type pig) at 4 months of age with an average body weight of 55±6 kg and eight castrated male DLL growing pigs (lean type pig) at 4 months of age with an average body weight of 47±4 kg were obtained from two local commercial swine herds, respectively. They were fed the same corn- and soybean meal-based diet (Table 1) (Tianke Company, Guangzhou, China), which met or exceeded the nutrient recommendations of National Research Council [24]. Pigs were housed individually in an environmentally controlled facility (23–28°C; 40–60% relative humidity; 12-h dark and 12-h light cycle) with hard-plastic slotted flooring and had free access to feed and drinking water. The experiment was carried out in accordance with the Chinese guidelines for animal welfare and experimental protocol [25] and approved by the Animal Care and Use Committee of the Chinese Academy of Sciences.

2.2. Serum collection and storage

At the end of a 46-day experimental period (approximately body weight of 80 kg), blood samples (5 ml) were collected by venipuncture of the jugular vein between 0800 and 1000 following a 12-h food deprivation period. Sera were separated from whole blood by centrifugation at 1000×g and 4°C for 10 min and stored in 1-ml aliquots at −80°C until NMR analysis and other biochemical analyses.

Table 1
Composition and nutrient concentrations of the basal diet (g/kg)

Items	Content
Ingredients	
Corn	633.2
Soybean meal	253
Wheat bran	50
Soybean oil	34
Salt	3
Calcium hydrogen phosphate	7
Calcium carbonate	9.8
Vitamin–mineral premix[a]	10
Nutrients[b]	
Crude protein	172.3
Total phosphorus	5.2
Total calcium	7.5
L-Lysine	9.7
Digestible energy, kJ/g	14.28

[a] Supplying the following (mg/kg diet): Cu (as $CuSO_4$), 15; Zn (as $ZnSO_4$), 104; Fe (as $FeSO_4$), 100; Mn (as $MnSO_4$), 19; vitamin A, 10,000 IU; vitamin D, 1000 IU; vitamin E, 40 IU; vitamin K, 2.5; choline, 570; pantothenic acid, 16; riboflavin, 5; folic acid, 2; niacin, 25; thiamine, 1.6; vitamin B_6, 1.8; biotin, 0.2; vitamin B_{12}, 0.25; choline chloride (50%), 1000; preservative, 1000; antioxidant, 10; and carrier, 6590.
[b] Calculated values according to National Research Council [24].

2.3. ^{1}H NMR Spectroscopic measurement of serum

One hundred microlitres of 0.9% saline in D_2O was mixed with 500 μl serum (D_2O was added for locking signal) in 5-mm NMR tubes. Proton NMR spectra of serum were recorded at 298 K on a Bruker Avance AVIII 600 spectrometer equipped with an inverse cryogenic triple-resonance high-resolution probe (Bruker Biospin, Rheinstetten, Germany) operating at a ^{1}H frequency of 600.11 MHz. A total of 10 min was allowed for the temperature to reach equilibration for each sample before spectra were acquired. The 90°C pulse length (~10.0 μs) was adjusted individually for each sample. A total of 32 transients were collected into 32 K data points for each spectrum with a spectral width of 20 ppm and a recycle delay (RD) of 2.0 s [26].

Three ^{1}H NMR spectra were acquired for each sample. A standard one-dimensional (1D) NMR spectrum, which is a general representation of the total metabolite composition, was acquired using the first increment of the standard 1D pulse sequence to achieve water presaturation [90°–t_1–90°–t_m–90°–acq] [27]. The inter-pulse delay t_1 was 3 μs, the mixing time t_m was 100 ms and irradiation of the water resonance was used during t_m and RD. A T_2-edited spectrum was recorded to attenuate signals from macromolecules with short spin–spin relaxation times using the CPMG pulse sequence [90°–(τ–180°–τ)$_n$–acq] [28]. A total spin–spin relaxation delay ($2n\tau$) of 200 ms and water peak irradiation were applied during RD. A diffusion-edited NMR spectrum, which selectively detects large macromolecules, was acquired using the bipolar-pair longitudinal eddy current (BPP-LED) pulse sequence [RD-90°-G_1-τ-180°-G_2-τ-90°-Δ-90°-G_3-τ-180°-G_4-τ-90°-T_e-90°- acq] [29]. A pulsed field gradient of 2.5 ms was used for diffusion-edited spectra, followed by a delay (τ) of 400 μs to allow for the decay of eddy currents. A diffusion time (Δ) of 100 ms and a delay T_e of 5 ms were used together with water peak irradiation during RD. For resonance assignment purposes, two-dimensional ^{1}H–^{1}H correlation spectroscopy (COSY) [30] and total correlation spectroscopy (TOCSY) [31] were also performed for selected serum samples.

2.4. Confirmation of selected metabolites by CX-4 auto-blood biochemical analyzer

Serum biochemical metabolites, including glucose, low-density lipoprotein (LDL), very low density lipoprotein (VLDL), high-density lipoprotein (HDL), triglycerides, total cholesterol, total protein, albumin and urea, were analyzed using a CX-4 Auto-Blood Biochemical Analyzer (Beckman, Inc., Fullerton, CA, USA), according to the manufacturer's instructions (Beijing Leadman Biochemistry Technology Co. Ltd., Beijing, China).

2.5. Carcass composition

All pigs were euthanized and eviscerated. The head and skin were removed. Then, the carcass was split longitudinally. Each right carcass side was weighed and then physically dissected into muscle, fat and bone. Muscle, fat and bone were weighed and recorded.

2.6. Analysis of serum hormones

Insulin, glucagon, growth hormone, insulin-like growth factor I, triiodothyronine and thyroxine were determined by radioimmunoassays using kits from Tianjin Nine Tripods Biomedical Engineering, Inc. (Tianjin, China).

2.7. Analysis of NMR data

Free induction decays were multiplied by an exponential window function of 1.0 Hz prior to Fourier transformation and were corrected for phase and baseline distortions using TopSpin 2.0 (Bruker). Chemical shift was referenced to the peak of the methyl proton of L-lactate at δ 1.33.

NMR spectra (δ 0.5–8.5) were binned with each region of 0.002 ppm wide and automatically integrated with the AMIX package (v. 3.8.3, Bruker Biospin). The region δ 4.54–5.20 was removed to avoid the effects of imperfect water suppression. The regions δ 1.16–1.20 and δ 3.63–3.69 were also removed due to the presence of peaks from ethanol which was used in the process of blood collection. Consequently, the spectra over the ranges δ 0.5–1.16, 1.20–3.63, 3.69–4.54 and 5.20–8.5 were selected and reduced to 3623 regions, each 0.002 ppm wide. Each integral region was normalized to the sum of all integral regions for each spectrum prior to pattern recognition analyzes.

An overview of the data distribution and intersample similarities (e.g., clusterings and outliers) for each serum was firstly investigated by principal component analysis (PCA), which was performed with the software Simca-P 11.0 (Umetrics, Sweden) [26,32]. Further analysis on NMR spectral data was processed using the orthogonal projection to latent structure with discriminant analysis (OPLS-DA) method with unit variance scaling [33], and the loadings in the coefficient plots were calculated from the coefficients combining the weight of the variables contributing to the sample clustering in the model [34]. The coefficient plots were generated using an in-house developed MATLAB script and were color coded with absolute value of coefficients (|r|).

The coefficient plot showed the variables (resonances) that contributed to clustering and the significance of such contribution. In the present study, a correlation coefficient of $|r|>0.67$ was used as the cutoff value for the statistical significance based on the discrimination significance at the level of $P<.05$, which was determined according to the test for the significance of the Pearson's product-moment correlation coefficient. The quality of the sevenfold cross-validated OPLS-DA models was described by the parameters R^2X, R^2Y and Q^2 (Fig. 2). The models were further assessed rigorously with permutation tests [34,35] (200 permutations) as valid (Figs. S1–S2). Since OPLS-DA results of the BPP-LED spectra from serum are similar to those of standard 1D spectra, analysis for the BPP-LED spectra will not be discussed in the Results section.

2.8. Statistical analysis

Concentrations of serum biochemical metabolites, serum hormones, carcass composition and the relative integrals from selected metabolites contributing to the classification of genetically obese and lean pigs are expressed as mean±S.D. and were subjected to Student's *t* test (SAS, Institute, Cary, NC, USA). $P<.05$ was taken to indicate significance.

3. Results

3.1. ^{1}H NMR Spectra of serum samples

Examples of a ^{1}H NMR CPMG (Fig. 1A), standard 1D (Fig. 1B) and BPP-LED (Fig. 1C) spectrum from an obese pig are illustrated in Fig. 1. From these spectra, 34 metabolites were unambiguously assigned, and their chemical shifts and peak multiplicity are given in Table 2 along with the corresponding ^{1}H NMR chemical shifts and signal multiplicities. Assignment of metabolites was made by comparison with the published literature [27,36–42] and confirmed by two-dimensional ^{1}H–^{1}H COSY and TOCSY methods (data not shown).

Visual inspection of the ^{1}H NMR spectra showed visible differences in serum metabolites between obese and lean pigs. For example, concentrations of lipoproteins, lipids, unsaturated lipids and glycoprotein were higher, but concentrations of glucose and urea were lower in the serum of obese pigs compared with lean pigs. To obtain more detailed analysis of metabolic differences between these two swine strains, multivariate data analyses including PCA and OPLS-DA were further performed.

3.2. Multivariate data analysis

The PCA of serum CPMG and standard 1D spectral data between obese and lean pig groups showed clear clustering (data not shown).

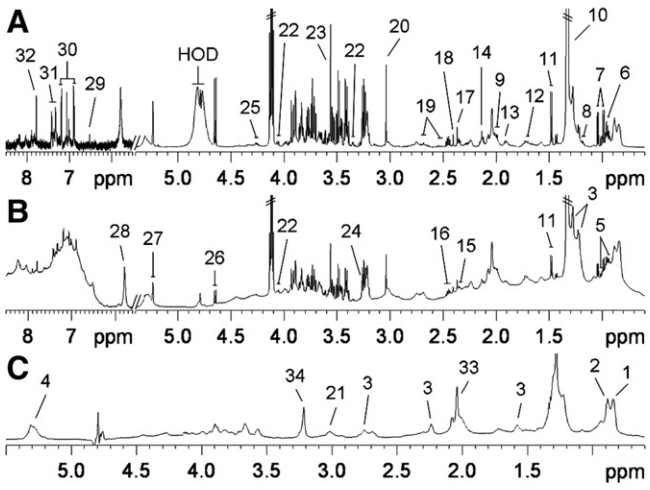

Fig. 1. Typical 600 MHz ^{1}H NMR spectra of serum taken from an obese pig from CPMG (A), standard 1D (B) and BPP-LED (C) experiments. The spectra in the aromatic region (δ 5.6–8.5) were magnified 16 times (A) or four times (B) compared to the aliphatic region (δ 0.6–5.4). Keys for metabolites are given in Table 2.

Table 2
Assignments of serum metabolites in pigs (chemical shift was referenced to the methyl group of lactate at δ 1.33)

Key	Metabolites	Moieties	δ ^{1}H (ppm) and multiplicity[a]
1	HDL	$CH_3^*(CH_2)_n$	0.84(t)
2	VLDL	$CH_3^*CH_2CH_2C=$	0.88(t)
3	Lipids (triglycerides and fatty acids)	$CH_3(CH_2)_n$,* $CH_2^*CH_2CO$, $CH_2-C=C$, $CH_2-C=O$, $=C-CH_2-C=$	1.29(m), 1.58(m), 2.04(m), 2.24(m), 2.75(m)
4	Unsaturated lipids	$-CH=CH-$	5.31(m)
5	Isoleucine	αCH, βCH, γCH_2, γCH_3, δCH_3	3.65(d), 1.95(m), 1.25(m)–1.45(m), 1.01(d), 0.94(t)
6	Leucine	αCH, βCH_2, γCH, δCH_3, δCH_3	3.72(t), 1.96(m), 1.63(m), 1.69(m), 0.91(d), 0.96(d)
7	Valine	αCH, βCH, γCH_3	3.61(d), 2.26(m), 0.99(d), 1.04(d)
8	Ethanol	CH_2, CH_3	3.65(q), 1.18(t)
9	Proline	αCH, βCH_2, γCH_2, δCH_2	4.11(t), 2.02(m)–2.33(m), 2.00(m), 3.35(t)
10	Lactate	αCH, βCH_3	4.11(q), 1.33(d)
11	Alanine	αCH, βCH_3	3.77(q), 1.48(d)
12	Arginine	αCH, βCH_2, γCH_2, δCH_2	3.76(t), 1.89(m), 1.73(m), 3.25(t)
13	Acetate	$CH_3-C=O$	1.92(s)
14	Methionine	αCH, βCH_2, γCH_2, δCH_3	3.78(m), 2.16(m), 2.64(dd), 2.13(s)
15	Glutamate	αCH, βCH_2, γCH_2	3.75(m), 2.08(m), 2.37(m)
16	Glutamine	αCH, βCH_2, γCH_2	3.68(t), 2.15(m), 2.45(m)
17	Pyruvate	CH_3	2.37(s)
18	Succinate	α, βCH_2	2.41(s)
19	Citrate	Half CH_2, half CH_2	2.52(d), 2.68(d)
20	Creatine	$N-CH_3$, CH_2	3.04(s), 3.93(s)
21	Albumin	Lysyl-CH_2	3.02(m)
22	*myo*-Inositol	5-CH, 4,6-CH, 1,3-CH, 2-CH	3.35(t), 3.63(t), 3.53(dd), 4.06(t)
23	Glycine	CH_2	3.56(s)
24	TMAO	CH_3	3.26(s)
25	Threonine	αCH, βCH, γCH_3	3.58(d), 4.28(m), 1.32(d)
26	β-Glucose	1-CH	4.64(d)
27	α-Glucose	1-CH	5.23(d)
28	Urea	NH_2	5.78(s)
29	Fumarate	CH	6.52(s)
30	Tyrosine	CH, CH	7.18(m), 6.88(m)
31	Phenylalanine	2,6-CH, 3,5-CH, 4-CH	7.33(m), 7.38(m), 7.42(m)
32	1-Methylhistidine	4-CH, 2-CH	7.05(s), 7.76(s)
33	Glycoprotein	$CH_3-C=O$	2.05(s), 2.08(s), 2.15(s)
34	Choline	$N-(CH_3)_3$, αCH_2, βCH_2	3.2(s), 4.05(t), 3.51(t)

[a] Key: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd: doublet of doublets.

Further analysis using OPLS-DA indicated that concentrations of serum HDL, VLDL, lipids, unsaturated lipids, glycoprotein, *myo*-inositol, choline, pyruvate, threonine, tyrosine and creatine were higher in obese than in lean pigs ($P<.05$), while concentrations of serum glucose, urea and trimethylamine-*N*-oxide (TMAO) were lower in obese pigs ($P<.05$) (Fig. 2 and Table 3).

3.3. Conventional biochemical assay for some metabolites in serum

Serum biochemical metabolites of obese and lean pigs were measured by conventional assays (Table 4). Serum glucose and urea concentrations in obese pigs were lower than those in lean pigs ($P<.01$), which confirmed the relative signal integrals of glucose and urea in the metabonomic analysis noted above. Serum concentrations of VLDL, LDL, cholesterol and protein in obese pigs were higher than those in lean pigs ($P<.05$). Trends of the relative signal integrals of glucose, urea, VLDL and lipid (Table 3) in obese and lean pigs were consistent with the conventional measurements of serum biochemical metabolites (Table 4), which displayed the robustness of the NMR-based metabonomic technique.

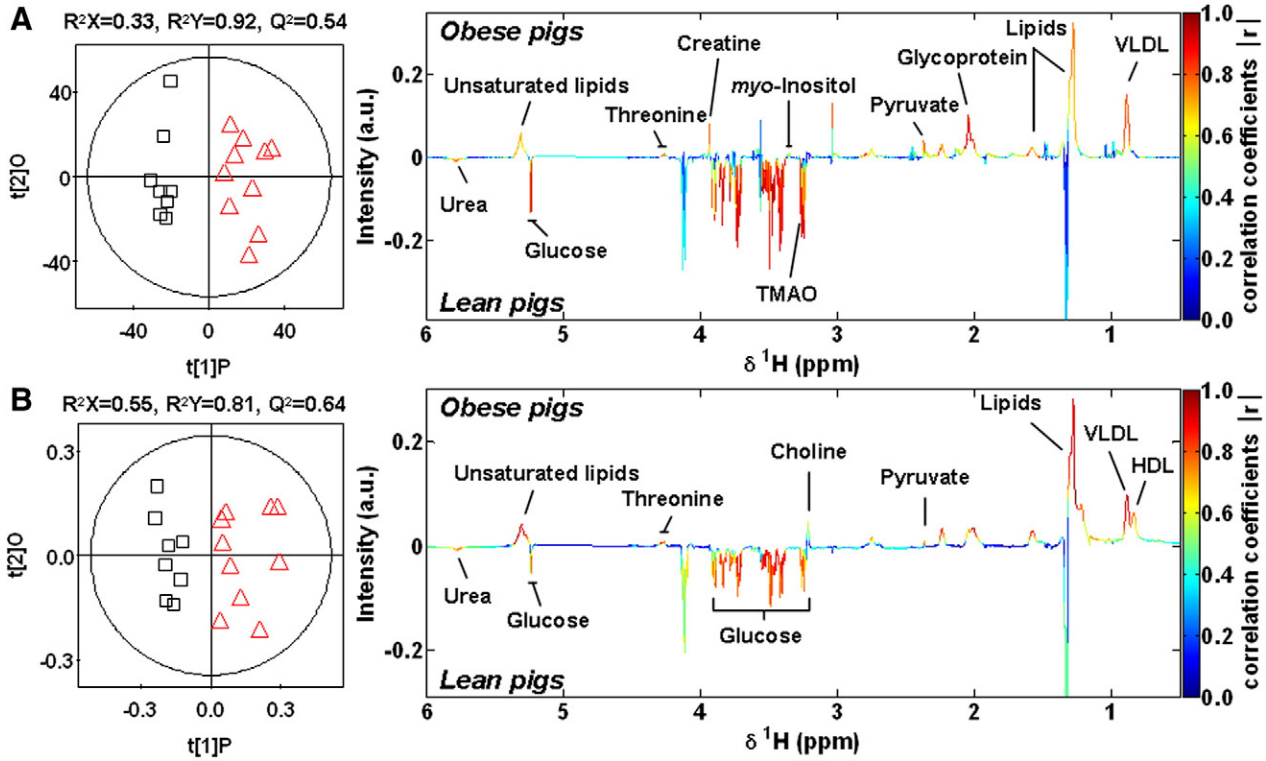

Fig. 2. OPLS-DA scores (left) and coefficient plots (right) for CPMG (A) and standard 1D (B) spectra of obese (Δ) and lean (□) pig groups. The colored scale in coefficient plots shows the significance of metabolite variations discriminating between the obese (positive) and lean pigs (negative).

3.4. Body composition

Carcass measurements showed that obese pigs had a higher percentage of fat tissue (42.3±8.8% vs. 21.9±4.5%; $P<.01$) and a low percentage of muscle tissue (35.4±4.5% vs. 58.9±2.5%; $P<.001$), compared with lean pigs.

3.5. Concentrations of serum hormones

Genetically obese pigs had higher concentrations of insulin and glucagon in serum ($P<.05$), compared with genetically lean pigs. Serum concentrations of growth hormone, insulin-like growth factor I, triiodothyronine or thyroxine did not differ between obese and lean pigs (Table 5).

4. Discussion

Obesity has become a serious and growing public health problem. Childhood obesity is attributable to a variety of nutritional, psychological, familial and physiological factors. However, heredity has been reported to influence fatness, regional fat distribution and response to overfeeding [43]. The objectives of the present study were to

Table 3
OPLS-DA coefficients of metabolites from obese pigs relatively to lean pigs

Metabolites	Correlation coefficient (r)[a]		Relative integrals (%)[b]			
	CPMG	Standard 1D	Lean	Obese	Lean	Obese
Glucose (δ 5.23)	−0.83	−0.80	1.30±0.11	1.00±0.16[c]	0.35±0.04	0.28±0.04[c]
HDL (δ 0.84)		0.69	1.01±0.36	1.11±0.33	1.91±0.12	2.19±0.23[c]
VLDL (δ 0.88)	0.74	0.89	1.77±0.35	2.56±0.68[c]	1.75±0.09	2.07±0.18[c]
Lipids (δ 1.29)	0.67	0.87	4.14±0.94	5.89±1.75[c]	2.94±0.27	3.99±0.49[c]
Glycoprotein (δ 2.05)	0.84	0.88	1.52±0.23	1.99±0.20[c]	1.14±0.11	1.24±0.08[c]
Pyruvate (δ 2.37)	0.72	0.74	0.252±0.077	0.322±0.045[c]	0.265±0.010	0.276±0.009[c]
Choline (δ 3.20)		0.67	0.23±0.09	0.25±0.07	0.45±0.08	0.55±0.06[c]
TMAO (δ 3.26)	−0.79	−0.72	0.450±0.097	0.337±0.104[c]	0.174±0.031	0.140±0.027[c]
myo-Inositol (δ 3.35)	0.68		0.164±0.023	0.193±0.022[c]	0.152±0.013	0.159±0.014
Unsaturated lipids (δ 5.31)	0.68	0.9	1.15±0.34	1.63±0.50[c]	0.66±0.08	0.94±0.11[c]
Urea (δ 5.78)	−0.74		0.31±0.06	0.21±0.07[c]	0.30±0.05	0.24±0.08[c]
Threonine (δ 4.28)	0.78	0.85	0.23±0.03	0.28±0.03[c]	0.466±0.014	0.489±0.013[c]
Creatine (δ 3.93)	0.67		0.34±0.08	0.42±0.07[c]	0.233±0.017	0.241±0.022
Tyrosine (δ 6.88)	0.76		0.063±0.006	0.072±0.008[c]	0.189±0.012	0.180±0.016

[a] The coefficients from OPLS-DA results; positive and negative signs respectively indicate positive and negative correlation in the concentrations of serum metabolites in obese relatively to lean pigs. The coefficient of 0.67 was used as the cutoff value for the significant difference evaluation ($P<.05$).
[b] Data are means±S.D. Normalized integral of metabolites in spectrum (normalized to 100, chemical shift region over the ranges of δ 0.5–1.16, 1.20–3.63, 3.69–4.54 and 5.20–8.50).
[c] Significantly different from lean pigs, $P<.05$.

Table 4
Serum biochemical metabolites of obese and lean pigs[a]

Parameters	Lean pig (n=8)	Obese pig (n=10)	P value
Glucose, mmol/L	8.07±1.41	5.78±0.92	.001
LDL, mmol/L	1.69±0.27	2.45±0.27	.001
VLDL, mmol/L	0.212±0.023	0.292±0.034	.001
HDL, mmol/L	1.27±0.09	1.26±0.15	.94
Triglycerides, mmol/L	0.65±0.13	0.76±0.12	.10
Total cholesterol, mmol/L	2.94±0.43	3.79±0.42	.001
Total protein, g/L	82.1±5.9	90.1±7.3	.03
Albumin, g/L	33.8±4.3	32.5±3.0	.41
BUN, mmol/L	5.46±0.62	4.63±0.39	.001

BUN, Blood urea nitrogen.
[a] Values are means±S.D.

investigate metabonomic differences in the serum of genetically obese and lean growing pigs and to explore the feasibility of using the obese Ningxiang pig as an animal model for childhood obesity research. Although both obese Ningxiang pigs and lean pigs were fed the same diet and raised under the same environmental conditions to eliminate any nutritional or environmental differences except appetite and food intake, the present study clearly demonstrated marked differences in serum metabolites, hormones and body composition between obese and lean pigs. A substantial increase in body fat and a decrease in whole-body protein were observed in obese pigs, which indicate that obese pigs converted more dietary energy to fat deposition in adipose tissue, while lean pigs utilized more dietary energy to synthesize protein in skeletal muscle.

Elevated lipid concentration and reduced glucose concentration in the serum of obese pigs suggest a high rate of glucose utilization for fat synthesis in obese pigs. Although the key enzymes in lipogenesis were not determined in the present study, there is a high likelihood that lipogenesis in white adipose tissue was stimulated in the obese strain, due to high concentrations of insulin, VLDL, triglycerides and fatty acids in serum. These observations were in agreement with obese Zucker diabetic fatty rats and rabbits [10,39,44]. Decreased serum glucose was observed in obese pigs in the present study, which has been reported for other obese animal models such as nondiabetic Zucker rats [39,44]. In the present study, lean pigs exhibited a relatively high concentration of glucose in serum (8.07 mmol/L), which is within the range of values reported by other investigators [20,45]. This might be related to the genetic background, metabolism and circadian rhythms of these pigs.

Another interesting observation from the current study is that obese pigs had elevated concentrations of VLDL, lipids, unsaturated lipids, glycoprotein and *myo*-inositol (P<.05) in serum, suggesting an increase in fat synthesis. Enhanced fat accretion contributed to the development of obesity in the Ningxiang pig. Results of the published work show that genetically obese pigs have higher concentrations of serum triglyceride, but lower concentrations of glucose than genetically lean pigs [20,21]. The elevated levels of lipids, VLDL and insulin in the serum of obese pigs were consistent with increased concentrations of lipids, VLDL and insulin in the circulation of obese children [46,47]. Similarly, increased concentrations of lipids were also observed in the plasma of obese Zucker diabetic fatty rats as well as obese rabbits and primates [9,10,12,48,49]. As a popular genetically obese model for studying the metabolic syndrome and obesity, the Ossabaw pig also exhibited a higher percentage of carcass fat mass and high plasma concentrations of insulin, cholesterol, triglycerides and VLDL relative to lean animals [11]. In the current study, obese Ningxiang pigs have increased body fat and elevated serum concentrations of insulin, VLDL, lipids, and unsaturated lipids, glycoprotein and *myo*-inositol, which provided a basis for the use of the Ningxiang pig as an animal model in obesity research.

Table 5
Serum hormones in obese and lean pigs[a]

Parameters	Lean pig (n=8)	Obese pig (n=10)	P value
Insulin (μU/ml)	14.9±1.9	25.9±2.6	.001
Glucagon (pg/ml)	29.4±4.9	36.7±6.6	.02
Growth hormone (ng/ml)	2.04±0.27	1.81±0.20	.06
Insulin-like growth factor I (g/ml)	562±50	525±53	.16
Triiodothyronine (nmol/ml)	0.38±0.04	0.35±0.05	.20
Thyroxine (nmol/ml)	12.5±2.8	10.9±2.6	.25

[a] Values are means±S.D.

Obese pigs had higher serum concentrations of insulin (74%) and glucagon (25%), compared with lean pigs (Table 5). Significantly higher levels of insulin and glucagon were associated with elevated lipid concentration and reduced glucose concentration in the serum of obese pigs. Clearly, the obese pig exhibits insulin resistance, which likely results from dyslipidemia and altered energy metabolism in the whole body. Insulin resistance and dyslipidemia often occur in obese children and other obese pig models [50–52]. Insulin, glucagon and growth hormone are known to regulate fat metabolism via cAMP-dependent mechanisms in animals [53]. Glucagon can stimulate protein kinase A by activating adenylyl cyclase to generate cAMP. Protein kinase A phosphorylates hormone-sensitive lipase, which hydrolyzes triacylglycerides to free fatty acids plus glycerol. Fatty acids are then oxidized in multiple tissues via the β-oxidation pathway [53]. Glucagon can increase glucose concentration in serum and stimulate lipolysis in adipose tissue. In contrast, insulin reduces the circulating level of glucose and enhances the synthesis of fat in white adipose tissue.

This study further showed that obese pigs had higher serum concentrations of other metabolites related to energy metabolism. For example, elevated concentrations of pyruvate and creatine (P<.05) may suggest extensive glycogenolysis and glycolysis in order to accommodate the increased demands for energy [54]. Elevated serum concentrations of threonine and tyrosine in obese pigs might reflect decreased synthesis of proteins to favour lipogenesis, which indicates a shift in energy metabolism toward fat formation. These changes in amino acid metabolism have also been reported for obese people [9,55]. Decreased concentrations of urea in the serum of obese pigs might indicate that these animals have a lower rate of protein turnover in comparison with lean pigs. In contrast to obese pigs, the energy metabolism shifted to protein deposition in lean pigs, which is a highly energetic process [56]. Elevated metabolites (including serum urea and blood urea nitrogen) involved in the metabolism of protein and amino acids reflected increased turnover of protein and nitrogen. Thus, genetically obese Ningxiang pigs have altered metabolism of protein and amino acids, as previously reported for obese rats, rabbits and humans [8–10].

Finally, an unexpected exciting observation from this study is the reduced concentration of TMAO and increased concentration of choline (P<.05) in the serum of obese pigs, compared to lean pigs. These metabolites are known to be related to functions of the gut microbiota [57–59], which has been reported to be related to obesity development [60]. It is possible that the gut microbiota modulates the nutrient metabolism of the host, therefore contributing to the development of obesity [4]. These regulatory mechanisms are intriguing and ought to be investigated in future studies involving microarray, proteomic, genomic and bioinformatic technologies [61–64].

It has to be noted that different genetic backgrounds are present between Ningxiang pigs (a Chinese strain of swine) and the DLL hybrid pigs (a European strain of swine). The metabolic differences of them resulted from both such genetic differences and obesity, although it is non-trivial to dissect the metabolic contributions from these two factors. Nevertheless, the genetic background is a contributing factor for obesity in the case of DLL pigs. In this study,

both Ningxiang and DLL pigs were raised under the same nutritional and environmental conditions. Therefore, it is still valid to consider that the differences in serum metabolite and body composition for these two pig strains are associated with the development of obesity. Such notion is supported by previous observations that a high-fat diet can induce obesity for DLL pigs [23], whereas only Ningxiang pigs can spontaneously develop obesity when feeding with a conventional diet containing a normal content of fat (Table 1). Based on the novel finding in the present study, Ningxiang pigs may offer a useful model for obesity studies in terms of key genes responsible for altered energy metabolism especially in enhancement of adipogenesis.

In conclusion, the serum metabonome of obese Ningxiang pigs is distinct from that of lean pigs raised under the same nutritional and environmental conditions. The altered serum metabonome is mainly attributable to increased lipogenesis, adipose accumulation, reduced conversion of amino acid nitrogen into urea and reduced protein synthesis. Most changes observed in the genetically obese Ningxiang pigs are similar to those reported for obese rats, mice, rabbits, Ossabaw pigs, children and teenagers. These findings justify the use of the Ningxiang pig as an animal model for childhood obesity research.

Supplementary materials related to this article can be found online at doi:10.1016/j.jnutbio.2010.11.007.

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