

Reference values of biochemical and hematological parameters for Guizhou minipigs

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Abstract

The pig is not only an economically important livestock animal but is also a valuable model animal for biomedical research and xenotransplantation. Reference values for clinical biochemical and hematological parameters are required for accurate data interpretation while using a pig model. In this study, whole blood samples were collected from 54 healthy Chinese Guizhou minipigs. We analyzed routine biochemical and hematological parameters and special coagulation parameters, including thrombelastography and coagulation factor activities, and have presented the baseline values of these parameters. These data provide valuable information for investigators using minipigs as animal models in biomedical studies and useful physiological data for veterinarians and livestock producers. We also compared all the results for the minipigs with the corresponding data from healthy humans. The bilirubin, uric acid and cholesterol levels of minipigs were significantly lower than those of humans (14%, 0.086% and 48% of human levels, respectively), whereas the serum enzyme levels were much higher than those in humans (e.g. the hydroxybutyrate dehydrogenase and creatine kinase levels of the minipigs were 19- and 8.4-fold higher than the human reference values). The red blood cell counts, platelet counts and white blood cell counts of the minipigs were significantly higher than those of the humans. The coagulation activities of factor VII and factor X were higher in minipigs than in humans. The significant differences observed between minipigs and humans for many of these parameters suggest substantial interspecies disparities in organs and tissues. These differences merit greater attention in biomedical research involving minipigs, particularly in the area of pig-to-human transplantation.

Keywords: Guizhou minipig, reference values, clinical biochemistry, hematology, coagulation

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Introduction

The minipig (*Sus scrofa domestica*) is an important model organism¹ and is widely used in many areas of medical research, including obesity,² cardiovascular disease, endocrinology, alcoholism, diabetes, nephropathy and organ transplantation. Comprehensive anatomical, physiological and pathological analyses of various breeds of minipigs are required to develop appropriate disease models and to accurately analyze and use experimental results involving minipigs.

The minipig has great potential as a xenotransplant donor and using it as an organ donor can help resolve the shortage of human organ donors.³ However, incompatibility between porcine and human physiological processes may adversely

affect the recipient's outcome.⁴ Therefore, understanding species-specific physiological processes and the differences between pigs and humans is essential for identifying and resolving problems that need to be addressed before xenotransplantation can be used in the clinical setting.

Clinical biochemical and hematological parameters are important physiological indicators, and baseline values for these parameters in experimental minipigs have not yet been clearly established. Researchers refer to human reference values for analyzing the experimental results from minipigs, which could complicate interpretation of data from minipigs.

Guizhou minipigs have stable characteristics, small size and clear genetic background; they originate from a closed

colony of animals in China and are widely used in various kinds of biological research. In this study, we report the baseline values of clinical biochemical and hematological parameters of healthy Guizhou minipigs. These baseline values will be useful in research conducted using minipigs as animal models or xenograft donors and will also provide valuable physiological data for veterinarians and livestock producers.

Materials and methods

Animals

All the procedures used in this study are in compliance with the Animal Welfare Act and the Guide for Care and Use of Laboratory Animals. The experimental treatment of the animals was reviewed and approved by the Animal Care and Welfare Committee of West China Hospital, Sichuan University. The study included 54 Chinese Guizhou minipigs: 27 healthy Chinese Guizhou minipigs (males, 17; females, 10) were obtained from the Agriculture Institute of Guizhou University and were used for biochemical and hematological tests; another 27 Guizhou minipigs (males, 19; females, 8) were obtained from Dashuo Biotechnology Co. Ltd (Sichuan, China) and were used for thrombelastograph (TEG) tests. The animals were 6–10 months old, with weights ranging from 10 to 15 kg. The minipigs were housed in clean cages and given *ad libitum* access to water and pelleted food. All the pigs bred in these facilities can be used for biomedical research and are free of specific pathogenic microorganisms such as the hog cholera virus, pseudorabies virus, *Ascaris suum*, pathogenic dermal fungi, *Mycobacterium tuberculosis* and *Shigella* spp.

Humans

We included clinical biochemical data for 30 healthy humans (men, 15; women, 15; age, 17–59 y) and hematological data for 53 healthy humans (men, 27; women, 25; age, 23–52 y); these data were obtained from West China Hospital, Sichuan University, China. While comparing the coagulation and TEG parameter data for humans and minipigs, the human data were presented as the range of normal values provided by the manufacturers.

Blood sample preparation

Venous blood samples were obtained from the precaval veins of the minipigs. Subsequently, the blood samples were pooled into plastic tubes containing 0.11 mol/L trisodium citrate for coagulation tests, plastic tubes with 2.0 mg/mL dipotassium ethylenediaminetetraacetic acid (EDTA-K₂) for complete blood count testing, and plastic tubes without anticoagulants for biochemical tests. Sera were separated by centrifugation at 2500g for 15 min for use in biochemical and coagulation tests; whole blood samples were used for routine hematological tests. The samples, except for those used in TEG, were stored for 24 h at 4°C before testing.

Biochemical analysis

Biochemical analysis was performed on serum samples with an Olympus AU5400 autoanalyzer (Tokyo, Japan). Analytes included serum concentrations of total bilirubin (Tbil, $\mu\text{mol/L}$), direct bilirubin (Dbil, $\mu\text{mol/L}$), indirect bilirubin (Ibil, $\mu\text{mol/L}$), total serum bile acid (TBA, $\mu\text{mol/L}$), total protein (TP, g/L), albumin (Alb, g/L), globulin (Glb, g/L), glucose (Glu, mmol/L), blood urea nitrogen (Bun, mmol/L), creatinine (Crea, $\mu\text{mol/L}$), uric acid (Uric, $\mu\text{mol/L}$), triglyceride (TG, mmol/L), cholesterol (Chol, mmol/L), high-density lipoprotein cholesterol (HDL-C, mmol/L), low-density lipoprotein cholesterol (LDL-C, mmol/L), sodium (Na, mmol/L), potassium (K, mmol/L), chloride (Cl, mmol/L), calcium (Ca, mmol/L), magnesium (Mg, mmol/L), ferrum (Fe, $\mu\text{mol/L}$), inorganic phosphorus (PO₄, mg/L) and total carbon dioxide (CO₂, mmol/L); serum activities (IU/L) of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH), and hydroxybutyrate dehydrogenase (HBDH), as well as the ratio of Alb and Glb (A/G) and ratio of AST and ALT activities (AST/ALT); and anion gap (AG, mmol/L).

Complete blood count analysis

Complete blood count was analyzed in Hematology XE-2100 (Sysmex Corporation, Kobe, Japan), with EDTA-K₂ as the anticoagulant. This analysis included the following variables: hemoglobin (HB, g/dL), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, %), red cell distribution width coefficient of variation (RDW-CV, %), and counts of red blood cell (RBC, $\times 10^{12}/\text{L}$), platelet (PLT, $\times 10^9/\text{L}$), white blood cell (WBC, $\times 10^9/\text{L}$), neutrophil (NEUT, both $\times 10^9/\text{L}$ and %), lymphocyte (LYM, both $\times 10^9/\text{L}$ and %), monocyte (MON, both $\times 10^9/\text{L}$ and %), eosinophil (EOS, both $\times 10^9/\text{L}$ and %) and basophil (BAS, both $\times 10^9/\text{L}$ and %).

Liver coagulation function analysis

Coagulation function analysis was performed using plasma samples separated from blood, with trisodium citrate as an anticoagulant. The prothrombin time (PT) and activated partial thromboplastin time (APTT) values were determined using an STA-Stago autoanalyzer (Stago, Paris, France). The clotting activities of factors II (FII), VII (FVII) and X (FX) were determined using FII-, FVII- and FX-deficient human plasma samples, and the coagulation factor activities were calculated as the PT or APTT of test plasma samples versus the PT or APTT, respectively, of standard human plasma samples. Antithrombin III (AT-III) assays were performed using the AT-III assay kit (Diagnostic Stago, Paris, France) with an automated system, i.e. the STA-Stago autoanalyzer, at 405 nm.

Thrombelastography

TEG, a measure of global hemostasis, is routinely used during cardiac and hepatic surgeries for optimizing blood product selection and usage.⁵ In this study, TEG of the whole blood samples were obtained to evaluate the hemostasis status in minipigs. We used the Thrombelastograph Coagulation Analyzer (Haemoscope, Niles, IL, USA) and TEG analytical software for the tests, which were performed according to the manufacturer's standard protocol.

We measured four main TEG parameters: reaction time (*R*), distance from the start of the sample run to the point of the first significant clot formation with an amplitude of 2 mm; *K* value, distance (mm) taken to achieve clot strength with an amplitude of 20 mm; alpha angle (α), which reflects the kinetics of clot formation; and maximum amplitude (MA), a measure of maximum clot strength. We also calculated the coagulation index (CI) that reflects the overall coagulation status of the animal by using the *R*, *K*, MA and α values of native or celite-activated whole blood.

Statistical analysis

For statistical analysis, we have calculated the mean (standard deviation) values for all parameters for minipigs and humans; the data for male and female individuals have been segregated in the tables. Subsequently, independent-samples *t*-tests were performed to compare interspecies data and to identify gender-based differences in the minipigs and humans. One-sample *t*-tests were performed to compare the mean values for the two species in cases where only the normal reference range values were available for humans. Statistical significance was defined as $P \leq 0.05$. Data were analyzed using the SPSS version 11.5 statistical software.

Results

The data are provided in Tables 1–4. The data were analyzed for statistically significant differences between minipigs and humans and between males and females; these differences are indicated in the tables.

Clinical biochemistry

The Bun and TG levels in male minipigs were significantly higher than those in the female minipigs. In contrast, the serum Ca levels in male minipigs were significantly lower than those in female minipigs. The ALT, Alb, Glu, Crea, Uric, ALP, CK, K and Ca values for men were significantly higher than those for women, whereas the AST/ALT, HDL-C and Cl values for men were significantly lower than those for women.

Comparison of the data for healthy humans and minipigs of both sexes revealed statistically significant differences in all biochemical parameters, except in the Glu and Crea values (Table 1). The human values for Uric, Tbil, Dbil, Ibil, LDL-C and Chol levels were substantially higher (i.e. 1156-, 7.0-, 4.0-, 3.3-, 2.3- and 2.1-fold, respectively) than those for minipigs. However, for most other parameters, the values for minipigs were higher than those for

humans. The minipigs had 19.2-, 8.4-, 5.2-, 4.5-, 4.3-, 3.6-, 2.8- and 2.4-fold higher HBDH, CK, GGT, AST, LDH, TBA, ALT and PO₄ values, respectively, than did humans (Table 1); these differences were significant.

Complete blood count

Only the HB and HCT values for female minipigs were statistically higher than those for male minipigs. This result contrasts to humans, where the RBC, HB, MCH and MCHC values for men were statistically higher than those for women.

Comparisons between healthy humans and minipigs of both sexes indicated statistically significant differences in most of the blood parameters. The MON and NEUT percentage values for humans were 3.4- and 1.8-fold higher, respectively, than those for minipigs, and these differences were statistically significant. In contrast, the values for most other parameters were higher in minipigs than in humans. The EOS, LYM, WBC, RBC, LYM percentage and PLT values in minipigs were 6.1-, 4.8-, 2.7-, 1.8-, 1.8- and 1.7-fold higher, respectively, than those in humans (Table 2).

Liver coagulation function

The differences between the values for PT, APTT and coagulation factor activities for male and female minipigs were not statistically significant; however, significant differences were observed between the human and minipig values. The PT and APTT in minipigs were slightly longer than those in humans. The median activity of porcine FII was considerably lower than the median activity of human FII and was even outside the lower limit of the normal range for humans (humans, 70–120; minipigs, 59 ± 9.53). In contrast, the mean values for porcine FVII and FX activities in minipigs were significantly higher than the corresponding mean values in humans and were even outside the upper limit of the normal range for humans. The anticoagulation activity of porcine AT-III was within the normal range for human AT-III (Table 3).

Thrombelastography

The difference between the TEG test results for the male and female minipigs were not statistically significant. Significant differences were noted between the median values of the parameters for minipigs and humans. The *R* and *K* values for minipigs were slightly lower than those for humans, and the *K* value for pigs was outside the normal range of *K* values for humans. The mean MA, α and CI values for minipigs were considerably higher than the corresponding median values for humans and were even outside the normal ranges of the corresponding parameters for humans (Table 4).

Discussion

In this paper, we report the baseline values of clinical biochemical, hematological and coagulation parameters for

Table 1 Chemistry values in healthy humans and pigs

Parameter (unit)	Healthy humans			Pigs		
	Males and females (n = 30)	Males (n = 15)	Females (n = 15)	Males and females (n = 27)	Males (n = 17)	Females (n = 10)
Tbil ($\mu\text{mol/L}$)	13.7 $\pm$ 4.6*	14.4 $\pm$ 5.4	13.2 $\pm$ 3.8	1.9 $\pm$ 0.7	1.9 $\pm$ 0.6	2.1 $\pm$ 0.8
Dbil ($\mu\text{mol/L}$)	3.6 $\pm$ 1.1*	3.8 $\pm$ 1.3	3.3 $\pm$ 0.9	0.9 $\pm$ 0.7	0.8 $\pm$ 0.6	1.1 $\pm$ 0.8
Ibil ($\mu\text{mol/L}$)	3.5 $\pm$ 0.7*	10.6 $\pm$ 4.1	9.9 $\pm$ 3.0	1.1 $\pm$ 0.6	1.1 $\pm$ 0.6	1.1 $\pm$ 0.6
TBA ($\mu\text{mol/L}$)	5.8 $\pm$ 8.5*	3.5 $\pm$ 1.6	4.2 $\pm$ 1.7	20.5 $\pm$ 17.8	19.6 $\pm$ 15.6	22.1 $\pm$ 21.9
ALT (IU/L)	24.8 $\pm$ 11.6*	30.0 $\pm$ 11.0 [†]	19.5 $\pm$ 9.9	68.30 $\pm$ 37.5	77.5 $\pm$ 43.1	52.6 $\pm$ 18.2
AST (IU/L)	21.6 $\pm$ 4.9*	22.7 $\pm$ 4.8	20.6 $\pm$ 4.9	100.0 $\pm$ 55.6	112.6 $\pm$ 66.4	78.7 $\pm$ 17.4
AST/ALT	1.1 $\pm$ 0.6*	0.8 $\pm$ 0.3 [†]	1.3 $\pm$ 0.8	1.6 $\pm$ 0.6	1.5 $\pm$ 0.6	1.6 $\pm$ 0.4
TP (g/L)	72.3 $\pm$ 3.9*	73.0 $\pm$ 3.0	71.6 $\pm$ 4.7	76.1 $\pm$ 5.5	76.1 $\pm$ 4.8	76.0 $\pm$ 6.8
Alb (g/L)	45.5 $\pm$ 2.8*	46.6 $\pm$ 2.2 [†]	44.5 $\pm$ 2.9	38.2 $\pm$ 6.3	37.2 $\pm$ 6.8	39.9 $\pm$ 5.3
Glb (g/L)	26.8 $\pm$ 2.8*	26.5 $\pm$ 2.5	27.1 $\pm$ 3.1	37.9 $\pm$ 8.9	38.9 $\pm$ 8.1	36.1 $\pm$ 10.4
A/G	1.7 $\pm$ 0.2*	1.8 $\pm$ 0.2	1.7 $\pm$ 0.2	1.1 $\pm$ 0.4	1.0 $\pm$ 0.4	1.2 $\pm$ 0.5
Glu (mmol/L)	4.9 $\pm$ 0.4	5.1 $\pm$ 0.4 [†]	4.6 $\pm$ 0.4	5.5 $\pm$ 1.7	5.6 $\pm$ 1.2	5.3 $\pm$ 2.5
Bun (mmol/L)	5.0 $\pm$ 1.3*	4.9 $\pm$ 1.2	5.2 $\pm$ 1.4	4.4 $\pm$ 1.1	4.8 $\pm$ 1.2 [†]	3.8 $\pm$ 0.8
Crea ($\mu\text{mol/L}$)	65.0 $\pm$ 13.5	73.9 $\pm$ 10.6 [†]	56.1 $\pm$ 9.7	69.0 $\pm$ 12.6	69.3 $\pm$ 14.9	68.5 $\pm$ 7.7
Uric ($\mu\text{mol/L}$)	300.6 $\pm$ 62*	336.6 $\pm$ 55 [†]	264.6 $\pm$ 47	0.3 $\pm$ 2.8	0.2 $\pm$ 1.9	0.4 $\pm$ 4.0
TG (mmol/L)	1.1 $\pm$ 0.3*	1.2 $\pm$ 0.4	1.0 $\pm$ 0.3	0.6 $\pm$ 0.2	0.6 $\pm$ 0.3 [†]	0.4 $\pm$ 0.1
Chol (mmol/L)	4.1 $\pm$ 0.6*	4.2 $\pm$ 0.7	4.1 $\pm$ 0.6	2.0 $\pm$ 0.4	2.1 $\pm$ 0.3	1.8 $\pm$ 0.4
HDL-C (mmol/L)	1.7 $\pm$ 0.3*	1.6 $\pm$ 0.3 [†]	1.9 $\pm$ 0.3	1.0 $\pm$ 0.2	1.1 $\pm$ 0.2	0.9 $\pm$ 0.2
LDL-C (mmol/L)	2.5 $\pm$ 0.7*	2.7 $\pm$ 0.7	2.4 $\pm$ 0.6	1.1 $\pm$ 0.3	1.1 $\pm$ 0.3	1.0 $\pm$ 0.3
ALP (IU/L)	68.4 $\pm$ 19*	75.7 $\pm$ 19 [†]	61.1 $\pm$ 17	128.7 $\pm$ 61	132.6 $\pm$ 65	122.0 $\pm$ 55
GGT (IU/L)	20.4 $\pm$ 12*	19.7 $\pm$ 5.4	16.0 $\pm$ 7.2	105.8 $\pm$ 64	101.9 $\pm$ 61	112.3 $\pm$ 72
CK (IU/L)	78.5 $\pm$ 37*	95.2 $\pm$ 43 [†]	61.9 $\pm$ 21	662.3 $\pm$ 276	613.4 $\pm$ 301	745.5 $\pm$ 215
LDH (IU/L)	157.3 $\pm$ 43*	171.3 $\pm$ 38	143.3 $\pm$ 45	678.2 $\pm$ 120	676.2 $\pm$ 83	681.6 $\pm$ 172
HBDH (IU/L)	25.5 $\pm$ 4.7*	135.9 $\pm$ 22	129.8 $\pm$ 22	489.7 $\pm$ 107	483.7 $\pm$ 96	500.1 $\pm$ 129
Na (mmol/L)	143.1 $\pm$ 2.2*	142.9 $\pm$ 1.9	143.2 $\pm$ 2.6	149.5 $\pm$ 9.8	148.6 $\pm$ 7.8	151.0 $\pm$ 13
K (mmol/L)	4.1 $\pm$ 0.3*	4.2 $\pm$ 0.3 [†]	4.0 $\pm$ 0.3	5.2 $\pm$ 0.6	5.1 $\pm$ 0.7	5.3 $\pm$ 0.5
Cl (mmol/L)	105.5 $\pm$ 2.0*	104.6 $\pm$ 1.8 [†]	106.4 $\pm$ 1.9	100.4 $\pm$ 4.9	101.1 $\pm$ 4.9	99.4 $\pm$ 4.8
Ca (mmol/L)	2.3 $\pm$ 0.1*	2.3 $\pm$ 0.1 [†]	2.2 $\pm$ 0.1	2.2 $\pm$ 0.1	2.1 $\pm$ 0.1 [†]	2.2 $\pm$ 0.1
Mg (mmol/L)	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.2
Fe ($\mu\text{mol/L}$)	19.6 $\pm$ 5.5*	20.7 $\pm$ 7.2	18.3 $\pm$ 2.6	30.9 $\pm$ 12.5	28.1 $\pm$ 6.8	35.7 $\pm$ 18.1
PO ₄ (mg/L)	1.1 $\pm$ 0.2	1.1 $\pm$ 0.2	1.1 $\pm$ 0.1	2.7 $\pm$ 0.3	2.6 $\pm$ 0.3	2.8 $\pm$ 0.4
CO ₂ (mmol/L)	22.8 $\pm$ 2.3	23.3 $\pm$ 1.5	22.2 $\pm$ 2.8	15.0 $\pm$ 3.8	15.4 $\pm$ 3.7	14.2 $\pm$ 4.0
AG (mmol/L)	18.9 $\pm$ 2.4	19.2 $\pm$ 2.7	18.6 $\pm$ 2.0	39.3 $\pm$ 15.8	37.2 $\pm$ 13.2	42.8 $\pm$ 19.7

Tbil, total bilirubin; Dbil, direct bilirubin; Ibil, indirect bilirubin; TBA, total serum bile acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; Alb, albumin; Glb, globulin; A/G, ratio of Alb and Glb; Glu, glucose; Bun, blood urea nitrogen; Crea, creatinine; Uric, uric acid; TG, triglyceride; Chol, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; CK, creatine kinase; LDH, lactate dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; Na, sodium; K, potassium; Cl, chloride; Ca, calcium; Mg, magnesium; Fe, ferrum; PO₄, inorganic phosphorus; CO₂, total carbon dioxide; AG, anion gap

Data were presented as arithmetic mean values $\pm$ SD. The statistical significance is indicated with '*' in the column 'healthy human' when comparing data of healthy humans with pigs of both sexes (* means $P < 0.05$). The statistical significance is indicated with '†' in the column 'males' when comparing data of male with female of humans and pigs, separately († means $P < 0.05$)

Chinese Guizhou minipigs. These data will be valuable references while evaluating systemic metabolic and immunological responses as well as the functions of the liver, kidney, pancreas and heart in experimental minipigs.

In addition, we compared the minipig baseline values with the corresponding reference values for healthy humans. Routine hepatic function tests showed that the levels of Tbil, Dbil and Ibil in the minipigs were much lower than those in humans (14–29% of human values), whereas the ALT, AST, ALP and GGT levels in minipigs were higher than those in humans (2.76–5.18-fold higher than the values for humans); this difference in values is consistent with that reported in a study on Chinese Banna minipigs by Zhang *et al.*⁶ Increases in liver enzyme levels are always due to hypohepatia, which may be caused by jaundice, hepatitis or liver cancer. However, the high levels of these enzymes in healthy minipigs may be attributable to the fact that pigs have a higher body temperature (39°C) than do humans (37°C); this difference may lead to faster

metabolism in pigs than in humans and a greater disparity between humans and pigs with respect to clearance of enzymes from plasma.⁶ Moreover, the porcine TP values are similar to those of humans and monkeys, but the porcine Alb levels are significantly lower, and consequently, the Alb/Glb ratio of pigs is also lower than that of primates. Thus, in pig-to-primate liver transplantation, hypoalbuminemia may develop because of the low serum concentrations of Alb.⁷

Increases in the CK, LDH, AST and HBDH values are sensitive and specific to myocardial infarction; of these parameters, increases in CK are most specific for infarcts, and the CK value may increase by several folds after an angina episode. The physiologically high level of these enzymes in pigs can interfere with the accurate diagnosis of angina in recipients after cardiac xenotransplantation.

The porcine RBC, WBC and PLT counts were 1.76-, 2.68- and 1.73-fold higher, respectively, than the corresponding

Table 2 Routine blood examination of healthy humans and pigs

Parameter (unit)	Healthy humans			Pigs		
	Males and females (n = 52)	Males (n = 27)	Females (n = 25)	Males and females (n = 26)	Males (n = 18)	Females (n = 8)
RBC (10 ¹² /L)	4.8 ± 0.4	5.1 ± 0.2 [†]	4.6 ± 0.3	8.5 ± 0.9	8.3 ± 0.9	8.9 ± 0.7
HB (g/dL)	144.4 ± 10	152.6 ± 3.9	134.6 ± 6.4	150.9 ± 20	145.3 ± 20 [†]	163.4 ± 13
HCT (%)	0.44 ± 0.03	0.47 ± 0.01 [†]	0.42 ± 0.02	0.46 ± 0.05	0.45 ± 0.05 [†]	0.50 ± 0.03
MCV (fL)	92.0 ± 3.2*	92.2 ± 3.3	91.8 ± 3.2	54.6 ± 3.8	53.7 ± 4.0	56.5 ± 2.5
MCH (pg)	30.0 ± 1.1	30.3 ± 1.1	29.6 ± 0.9	17.8 ± 1.4	17.5 ± 1.5	18.4 ± 1.1
MCHC (g/L)	326.2 ± 7.7	328.8 ± 8.5	323.1 ± 5.4	325.0 ± 9.4	324.8 ± 7.7	325.3 ± 13
RDW-CV (%)	13.3 ± 0.6	13.2 ± 0.6	13.5 ± 0.5	16.0 ± 1.5	16.0 ± 1.6	16.0 ± 1.3
PLT (10 ⁹ /L)	180.7 ± 48*	175.4 ± 50	187.0 ± 48	313.1 ± 149	317.4 ± 160	303.3 ± 130
WBC (10 ⁹ /L)	5.9 ± 1.3*	5.7 ± 1.4	6.2 ± 1.2	16.0 ± 3.9	16.5 ± 4.0	14.8 ± 3.7
NEUT (10 ⁹ /L)	3.6 ± 0.9	3.4 ± 0.9	3.8 ± 0.9	5.2 ± 1.7	5.4 ± 1.9	4.9 ± 1.1
NEUT (%)	59.9 ± 4.8	58.9 ± 4.3	61.1 ± 5.1	33.0 ± 5.1	32.9 ± 5.8	33.3 ± 3.5
LYM (10 ⁹ /L)	2.0 ± 0.50*	1.9 ± 0.55	2.0 ± 0.45	9.3 ± 2.5	9.5 ± 2.5	8.8 ± 2.3
LYM (%)	32.8 ± 4.5*	33.5 ± 4.3	31.9 ± 4.8	58.2 ± 5.3	57.7 ± 6.2	59.1 ± 2.2
MON (10 ⁹ /L)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
MON (%)	4.9 ± 1.0*	5.0 ± 1.0	4.8 ± 1.0	1.5 ± 1.2	1.4 ± 1.2	1.5 ± 1.4
EOS (10 ⁹ /L)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.7 ± 0.5	0.8 ± 0.5	0.5 ± 0.5
EOS (%)	2.0 ± 1.1	2.2 ± 1.1	1.8 ± 1.0	4.4 ± 2.6	4.8 ± 2.6	3.5 ± 2.5

RBC, red blood cell; HB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width coefficient of variation; PLT, platelet; WBC, white blood cell; NEUT, neutrophile; LYM, lymphocyte; MON, monocyte; EOS, eosinophil

For explanation, see legend to Table 1

Table 3 Coagulation functions of healthy humans and pigs

Parameter (unit)	Healthy humans (n = 44)	Pigs		
		Males and females (n = 23)	Males (n = 14)	Females (n = 9)
PT (second)	13.0 ± 0.6*	14.4 ± 0.7	14.3 ± 0.5	14.4 ± 0.9
APTT (second)	37.5 ± 3.2*	48.9 ± 8.8	48.3 ± 9.6	49.90 ± 7.7
CFII	(70–120) 95*	59.0 ± 9.5	57.1 ± 10.1	62.0 ± 8.2
CFVII	(55–170) 112.5*	191.2 ± 50	189.5 ± 41	193.8 ± 64
CFX	(70–120) 95*	142.0 ± 26	140.0 ± 21	145.1 ± 34
AT-III	(80–120) 100*	117.4 ± 12	115.2 ± 13	120.8 ± 9.0

PT, prothrombin; APTT, activated partial thromboplastin times; CFII, clotting activities of factors II; CFVII, clotting activities of factors VII; CFX, clotting activities of factors X; AT-III, antithrombin-III

The values of coagulation factors activities of healthy humans were presented as the reference ranges in brackets, and the media value was indicated outside the bracket. For other explanation, see legend to Table 1

human reference values, and the greatest difference was seen in the LYM count. Thus, the physiological level of lymphocytes in healthy minipigs can be misinterpreted as a strong immunological response to infection if we use the diagnosis criteria used for humans. It is surprising that we found that the female Guizhou minipigs have higher RBC, HB and HCT than their male counterparts, and it is the opposite of the situation in human subjects. However, some previous studies, focusing on other colonies of pigs from various areas of China such as Guangxi, Yunnan and Fujian provinces, had different results.^{8,9} In their reports, no statistic differences are shown on HB and HCT between males and females, and the average values of HB and HCT of the males are higher than the females just as the situation in human subjects. Otherwise, some studies also report statistically higher RBC in female pigs than the males. The reason for the inconsistency between our study and the others is unclear. One of the possible explanations is that the relative small sample size in our study may mislead the analysis, because only eight female pigs are involved.

The results of the present study suggest that using reference values of humans to identify a specific pathological status may lead to incorrect conclusions in experimental studies performed using pigs as animal models. In addition, the considerable differences between the values of the biochemical and hematological parameters for pigs and

Table 4 Thrombelastography parameters of healthy humans and pigs

Parameter (unit)	Healthy humans	Pigs		
		Males and females (n = 27)	Males (n = 19)	Females (n = 8)
R (min)	(2–8) 5*	3.0 ± 1.1	2.8 ± 0.9	3.3 ± 1.5
K (min)	(1–3) 2*	0.9 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
Angel (degree)	(55–78) 66.5*	78.0 ± 4.0	77.9 ± 3.9	78.4 ± 4.4
MA (mm)	(51–69) 60*	73.4 ± 5.7	73.3 ± 5.3	73.6 ± 6.9
CI	(–3 + 3) 0*	4.8 ± 1.3	4.9 ± 1.0	4.7 ± 1.8

MA, maximum amplitude; CI, coagulation index

For explanation, see legends to Table 1 and Table 3

humans suggest interspecies differences in the physiological functions of organs and tissues, which could cause systemic functional incompatibility after pig-to-human transplantation.¹⁰ Further studies on xenotransplantation are recommended for obtaining more evidence regarding these issues.

While analyzing our results for the minipigs, it should be noted that the blood samples were collected without the use of anesthesia. This may have caused the minipigs some stress, which in turn may interfere with the final results for the parameters that are sensitive to stress. Moreover, a larger sample size would have provided better results with regard to observing individual differences and precisely defining the range of reference values.

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REFERENCES

- 1 Wang S, Liu Y, Fang D, Shi S. The miniature pig: a useful large animal model for dental and orofacial research. *Oral Dis* 2007;**13**:530–7
- 2 Bellinger DA, Merricks EP, Nichols TC. Swine models of type 2 diabetes mellitus: insulin resistance, glucose tolerance, and cardiovascular complications. *ILAR J* 2006;**47**:243–58
- 3 d'Apice AJ, Cowan PJ. Gene-modified pigs. *Xenotransplantation* 2008;**15**:87–90
- 4 Schrem H, Kleine M, Borlak J, Klempnauer J. Physiological incompatibilities of porcine hepatocytes for clinical liver support. *Liver Transplant* 2006;**12**:1832–40
- 5 Di Benedetto P, Baciarello M, Cabetti L, Martucci M, Chiaschi A, Bertini L. Thrombelastography. Present and future perspectives in clinical practice. *Minerva Anestesiol* 2003;**69**:501–9, 509–15
- 6 Zhang L, Sun X, Cheng J, Wang L, Wei Q, Li S, Zeng Y, Li Y. Study of hepatic function matching between Banna minipig inbred and humans. *Transplant Proc* 2004;**36**:2492–4
- 7 Ramirez P, Chavez R, Majado M, Munitiz V, Munoz A, Hernandez Q, Palenciano CG, Pino-Chavez G, Loba M, Minguella A, Yelamos J, Gago MR, Vizcaino AS, Asensi H, Cayuela MG, Segura B, Marin F, Rubio A, Fuente T, Robles R, Bueno FS, Sansano T, Acosta F, Rodriguez JM, Navarro F, Cabezuolo J, Cozzi E, White DJ, Calne RY, Parrilla P. Life-supporting human complement regulator decay accelerating factor transgenic pig liver xenograft maintains the metabolic function and coagulation in the nonhuman primate for up to 8 days. *Transplantation* 2000;**70**:989–98
- 8 Yang SL, Ren HY, Wang H, Feng ST, Gan SX, Wang AD, Li K. The physiological parameters of hematology in experimental minipigs in China. *J Chin Anim Sci Vet Med* 2007;**34**:38–41
- 9 Qiu JH. Detection of haemal physiological and biochemical parameters in Putian black pigs. *J Mountain Agrice Biol* 2009;**28**:317–23
- 10 Ibrahim Z, Busch J, Awwad M, Wagner R, Wells K, Cooper DK. Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. *Xenotransplantation* 2006;**13**:488–99

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