

Reference Values of Hematological and Biochemical Parameters for the World Smallest Microminipigs

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ABSTRACT. In this study, we demonstrated growth curves and reference values for hematological and serum biochemical parameters of Microminipigs, the world smallest experimental minipigs. In both male and female animals, the body weights (BW) at 3 and 6 months of age were <5 kg and <10 kg, respectively, and growth curve revealed almost plateau (approximately 20 kg BW) after 18 months of age. Major hematological and serum biochemical parameters showed no gender differences and the values were very similar to those in Göttingen and Yucatan minipigs. The values obtained in this study can serve as fundamental reference, and thereby facilitate the use of Microminipig in life science research.

KEY WORDS: biochemistry, gender difference, growth curve, hematology, swine.

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Swine have been used extensively in biomedical research with a significant increase in recent decades, more than 60,000 pigs having been used in a year in the EU [1, 11]. Because of their physiological and anatomical similarities to humans [5], swine are becoming increasingly attractive animal models in toxicological and pharmacological research. Recently, minipigs defined that they are smaller than domestic swine and their body weight (BW) is <100 kg, have been developed including the strains of Göttingen, Yucatan, Sinclair, Clawd, and others [2, 9, 14]. However, most minipigs are difficult to manage due to their large size, e.g. the Göttingen minipigs of adult are 30–40 kg in BW under conditions of restricted diet [11, 12]. Microminipig (brand name; registered with the Japanese Ministry of Agriculture, Forestry and Fisheries as a novel variety of swine; Fuji Micra Inc., Shizuoka, Japan) has been emerged as a possible experimental animal model for non-clinical pharmacological/toxicological use [4, 8, 10]. The BW of young mature Microminipig is <10 kg, enabling easy handling [5, 7, 13]. The “Eve” of Microminipig (a female minipig named “Cath-

erin”) was born by the mating of Pot-bellied pig and another type of minipig [4]. The feature of Microminipig (about a half of body weight of general minipigs) is inherited at the sixth generation. The breeder produces hybrids between six strains of Microminipigs, which correspond to from third to sixth generation. Animals which analyzed in this study were randomly selected from population of hybrids by age. The aim of this study was to establish reference values for growth curves, hematological (23 parameters) and serum biochemical (19 parameters) in the healthy Microminipigs.

All animals were maintained in the same animal unit at 24 ± 3°C and relative humidity at 50 ± 20%, with a 12 hr light/dark cycle in the breeder. The breeding space was 0.5–1.2 m²/an animal. Amount of porcine diet (Marubeni Nisshin Feed Co., Tokyo, Japan) was set to 4–8%, 2–4%, and 1–3% of BW according to one’s age, correspond to 1–3, 4–6, and after 7 months, respectively. The diet was composed of >13.0% crude protein, >2.0% crude fat, <8.0% crude fiber, <10.0% crude ash, >1.1% calcium, and >0.9% phosphorus. Tap water was available *ad libitum*. Animals used in this study were found to be in good health and free of clinical signs of illness. They were not given any treatment and medication other than vaccination thorough the study. All the data were presented as mean ± SD and statistical analysis of the differences was assessed by F-test and Student’s *t*-test or Welch’s *t*-test, and considered significant at *P*<0.05.

BWs of animals were measured once monthly (0–12

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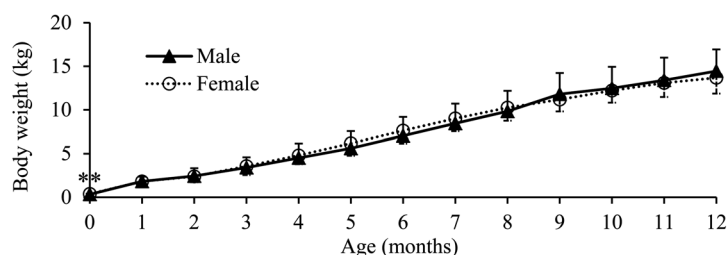


Fig. 1. Body weight of the Microminipig. ** $P < 0.01$; significantly different for males vs. females.

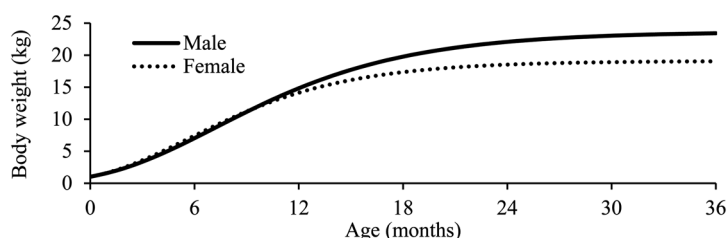


Fig. 2. Growth curve of the Microminipig predicted by Gompertz function.

months of age) by the breeder from 2009 to 2010. Animals aged >13 months could not weigh because of shipment. In total, the original dataset contained 324 and 492 BW measurements of 27 males and 41 females, respectively. Growth curves were fitted by Gompertz function, using KaleidaGraph (4.1J demo version) [6]. BWs in males and females at birth were 336 ± 83 g and 385 ± 65 g, respectively, and showed significant gender differences ($P < 0.01$). However, BWs in males and females from 1 to 12 months of age were almost equal and showed no gender differences. The BW in both males and females at 3 and 6 months of age were <5 kg and <10 kg, respectively (Fig. 1). Growth curve revealed almost plateau (approximately 20 kg) over 18 months of age (Fig. 2).

The fasting blood samples were collected from the cranial vena cava of 125 individual conscious animals (58 males and 67 females) aged 0–34 months. The numbers of males in 0–2, 3–5, 6–8, 9–12, 13–24, and 25–34 months of age were 9, 6, 9, 14, 14, and 6, respectively. The numbers of females in 0–2, 3–5, 6–8, 9–12, 13–24, and 25–34 months of age were 11, 8, 17, 13, 10, and 8, respectively. For measurement of 23 hematological parameters except prothrombin time (PT) and activated partial thromboplastin time (APTT) (Table 1), 1 ml of blood was collected with an anticoagulant, EDTA-2K and then applied to an automatic analyzer (ADVIA 120, Simens Healthcare Diagnostics Manufacturing Ltd., Dublin, Ireland). For measurement of PT and APTT, 1.5 ml of blood was collected with 150 μ l of 3.8 w/v% sodium citrate solution as an anticoagulant. The plasma was obtained by centrifugation (4°C , $1,710 \times g$, 3,000 rpm, 15 min) and analyzed in an automatic analyzer (CA-7000, Sysmex Corporation, Kobe, Japan). For measurement of 19 serum biochemical parameters (Table 2), the serum was ob-

tained by centrifugation (room temperature, $1,710 \times g$, 3,000 rpm, 15 min) and applied to an automatic analyzer (JCA-BM8, JEOL Co., Ltd., Tokyo, Japan). As shown in Table 1, major hematological parameters including erythrocyte and leukocyte count showed no gender differences except for the percentage of basophils, lymphocytes and neutrophils. The former 2 were higher in females and the latter 1 was higher in males. As shown in Table 2, major serum biochemical parameters showed no gender differences. Some biochemical values however, showed significant gender differences. The serum levels of alanine aminotransferase, globulin, and total cholesterol were higher in females, and those of urea nitrogen and sodium were higher in males. The major hematological and biochemical parameters in Microminipigs were similar to those in Göttingen and Yucatan minipig, although alkaline phosphatase and albumin showed higher tendency, and APTT and total bilirubin showed lower tendency [2, 3, 9]. These differences on the hematology and biochemistry between Microminipigs and other minipigs may be related to environmental factors such as diets or genetic factors.

Recent development of minipigs and their physiological and anatomical similarities to humans make minipigs a suitable species for toxicological/pharmacological studies; however, despite continuous efforts from breeders, minipigs are not yet widely used in life science research and one possible reason is the lack of reference values [14]. In this study, we provided useful reference values of Microminipigs, and thereby it would facilitate the use Microminipigs in various life science researches.

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Table 1. Reference values of hematological parameters in Microminipig

Parameter	Unit	Male (n=58)		Female (n=66)		Whole (n=124)	
		Mean $\pm$ SD	(Range)	Mean $\pm$ SD	(Range)	Mean $\pm$ SD	(Range)
Erythrocyte count	10 ⁶ /mm ³	7.85 $\pm$ 0.75	(5.08–9.46)	7.66 $\pm$ 0.89	(4.28–9.39)	7.75 $\pm$ 0.83	(4.28–9.46)
Leukocyte count	10 ³ /mm ³	12.41 $\pm$ 5.11	(5.95–35.44)	12.64 $\pm$ 3.92	(6.66–28.07)	12.53 $\pm$ 4.50	(5.95–35.44)
Hematocrit	%	45.60 $\pm$ 5.48	(23.20–57.40)	44.72 $\pm$ 5.60	(21.60–55.90)	45.13 $\pm$ 5.54	(21.60–57.40)
Hemoglobin	g/dl	14.84 $\pm$ 1.85	(8.10–18.80)	14.69 $\pm$ 1.98	(7.70–18.50)	14.76 $\pm$ 1.91	(7.70–18.80)
Platelet	10 ³ /mm ³	426.1 $\pm$ 126.3	(87.0–700.0)	439.5 $\pm$ 132.9	(129.0–786.0)	433.2 $\pm$ 129.5	(87.0–786.0)
Mean corpuscular volume	fL	58.06 $\pm$ 3.85	(45.60–64.60)	58.50 $\pm$ 4.44	(47.50–68.80)	58.29 $\pm$ 4.17	(45.60–68.80)
Mean corpuscular hemoglobin	pg	18.93 $\pm$ 1.60	(14.20–21.80)	19.22 $\pm$ 1.75	(14.10–22.30)	19.08 $\pm$ 1.68	(14.10–22.30)
Mean corpuscular hemoglobin concentration	g/dl	32.58 $\pm$ 1.39	(28.30–34.90)	32.84 $\pm$ 1.27	(28.90–36.70)	32.72 $\pm$ 1.33	(28.30–36.70)
Reticulocytes	%	1.85 $\pm$ 3.91	(0.20–25.20)	1.44 $\pm$ 3.00	(0.20–22.60)	1.63 $\pm$ 3.45	(0.20–25.20)
Eosinophils	%	2.53 $\pm$ 1.46	(0.40–6.40)	2.42 $\pm$ 1.48	(0.30–6.60)	2.47 $\pm$ 1.47	(0.30–6.60)
Basophils	%	0.91 $\pm$ 0.32	(0.40–2.10)	1.02 $\pm$ 0.30*	(0.40–1.90)	0.97 $\pm$ 0.31	(0.40–2.10)
Monocytes	%	5.20 $\pm$ 1.36	(1.90–7.80)	5.18 $\pm$ 1.50	(2.70–10.50)	5.19 $\pm$ 1.43	(1.90–10.50)
Lymphocytes	%	56.57 $\pm$ 13.01	(23.80–82.50)	60.88 $\pm$ 9.91*	(28.80–82.20)	58.86 $\pm$ 11.62	(23.80–82.50)
Neutrophils	%	33.29 $\pm$ 12.10	(10.80–63.80)	28.82 $\pm$ 9.81*	(9.40–61.90)	30.91 $\pm$ 11.12	(9.40–63.80)
Large unstained cells	%	1.51 $\pm$ 0.68	(0.30–3.40)	1.69 $\pm$ 0.93	(0.30–4.10)	1.60 $\pm$ 0.83	(0.30–4.10)
Eosinophils	10 ³ /mm ³	0.30 $\pm$ 0.21	(0.03–1.38)	0.30 $\pm$ 0.21	(0.03–1.05)	0.30 $\pm$ 0.21	(0.03–1.38)
Basophils	10 ³ /mm ³	0.12 $\pm$ 0.08	(0.03–0.42)	0.13 $\pm$ 0.05	(0.05–0.29)	0.12 $\pm$ 0.07	(0.03–0.42)
Monocytes	10 ³ /mm ³	0.63 $\pm$ 0.26	(0.12–1.79)	0.66 $\pm$ 0.33	(0.24–2.46)	0.65 $\pm$ 0.30	(0.12–2.46)
Lymphocytes	10 ³ /mm ³	7.04 $\pm$ 3.56	(3.46–19.77)	7.62 $\pm$ 2.53	(3.99–18.47)	7.35 $\pm$ 3.06	(3.46–19.77)
Neutrophils	10 ³ /mm ³	4.15 $\pm$ 2.50	(0.94–15.41)	3.72 $\pm$ 1.98	(1.32–10.65)	3.92 $\pm$ 2.24	(0.94–15.41)
Large unstained cells	10 ³ /mm ³	0.18 $\pm$ 0.11	(0.02–0.59)	0.21 $\pm$ 0.13	(0.04–0.63)	0.20 $\pm$ 0.12	(0.02–0.63)
Prothrombin time [#]	s	13.05 $\pm$ 0.93	(11.20–16.00)	12.87 $\pm$ 0.88	(10.60–14.70)	12.95 $\pm$ 0.90	(10.60–16.00)
Activated partial thromboplastin time [#]	s	11.79 $\pm$ 1.63	(7.80–17.00)	11.46 $\pm$ 1.19	(8.90–13.70)	11.61 $\pm$ 1.42	(7.80–17.00)

* $P < 0.05$; significantly different from male. [#]Male (n=55), Female (n=64), Whole (n=119).

Table 2. Reference values of serum biochemical parameters in Microminipig

Parameter	Unit	Male (n=58)		Female (n=67)		Whole (n=125)	
		Mean $\pm$ SD	(Range)	Mean $\pm$ SD	(Range)	Mean $\pm$ SD	(Range)
Aspartate aminotransferase	IU/l	39.72 $\pm$ 15.64	(20.0–124.0)	41.19 $\pm$ 27.54	(19.0–225.0)	40.51 $\pm$ 22.73	(19.0–225.0)
Alanine aminotransferase	IU/l	42.52 $\pm$ 12.46	(21.0–74.0)	47.96 $\pm$ 17.67*	(16.0–97.0)	45.43 $\pm$ 15.65	(16.0–97.0)
Alkaline phosphatase	IU/l	725.0 $\pm$ 708.2	(176.0–4246.0)	560.9 $\pm$ 373.2	(175.0–2087.0)	637.0 $\pm$ 558.0	(175.0–4246.0)
Creatinine kinase	IU/l	724.6 $\pm$ 929.1	(134.0–5102.0)	761.3 $\pm$ 1220.8	(158.0–8446.0)	744.3 $\pm$ 1085.1	(134.0–8446.0)
Total bilirubin	mg/dl	0.031 $\pm$ 0.043	(0.000–0.320)	0.032 $\pm$ 0.040	(0.000–0.270)	0.032 $\pm$ 0.041	(0.000–0.320)
Total protein	g/dl	7.69 $\pm$ 0.97	(5.50–10.40)	7.67 $\pm$ 0.97	(4.90–10.30)	7.68 $\pm$ 0.97	(4.90–10.40)
Albumin	g/dl	4.48 $\pm$ 0.47	(3.40–5.50)	4.30 $\pm$ 0.52	(2.40–5.20)	4.38 $\pm$ 0.50	(2.40–5.50)
Globulin	g/dl	3.21 $\pm$ 0.92	(1.30–5.30)	3.37 $\pm$ 0.96*	(1.40–6.80)	3.30 $\pm$ 0.94	(1.30–6.80)
Albumin-globulin ratio	ratio	1.53 $\pm$ 0.53	(0.71–3.46)	1.40 $\pm$ 0.47	(0.51–3.00)	1.46 $\pm$ 0.50	(0.51–3.46)
Total cholesterol [#]	mg/dl	74.36 $\pm$ 16.56	(43.0–121.0)	96.70 $\pm$ 61.20**	(57.0–533.0)	86.30 $\pm$ 47.20	(43.0–533.0)
Triglycerides	mg/dl	41.71 $\pm$ 30.30	(17.0–236.0)	47.36 $\pm$ 21.84	(19.0–117.0)	44.74 $\pm$ 26.15	(17.0–236.0)
Glucose	mg/dl	91.02 $\pm$ 25.65	(69.0–224.0)	90.03 $\pm$ 15.66	(66.0–146.0)	90.49 $\pm$ 20.81	(66.0–224.0)
Urea nitrogen	mg/dl	14.55 $\pm$ 3.65	(6.80–25.40)	13.06 $\pm$ 2.94*	(7.80–24.20)	13.75 $\pm$ 3.36	(6.80–25.40)
Creatinine	mg/dl	0.97 $\pm$ 0.34	(0.49–1.94)	0.87 $\pm$ 0.25	(0.31–1.78)	0.91 $\pm$ 0.30	(0.31–1.94)
Phosphorus	mg/dl	6.69 $\pm$ 1.57	(4.77–13.21)	6.92 $\pm$ 1.40	(3.85–11.74)	6.82 $\pm$ 1.48	(3.85–13.21)
Calcium	mg/dl	10.71 $\pm$ 0.59	(9.80–12.30)	10.63 $\pm$ 0.52	(9.30–12.30)	10.67 $\pm$ 0.56	(9.30–12.30)
Sodium	mEq/l	145.5 $\pm$ 3.0	(136.0–153.0)	144.1 $\pm$ 4.0*	(119.0–151.0)	144.8 $\pm$ 3.6	(119.0–153.0)
Potassium	mEq/l	5.75 $\pm$ 0.65	(4.00–7.50)	5.73 $\pm$ 0.78	(4.30–7.40)	5.74 $\pm$ 0.72	(4.00–7.50)
Chloride	mEq/l	103.4 $\pm$ 3.2	(93.0–111.0)	103.3 $\pm$ 4.2	(76.0–108.0)	103.4 $\pm$ 3.8	(76.0–111.0)

* $P < 0.05$, ** $P < 0.01$; significantly different from male. [#]Female (n=66), Whole (n=124).

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