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Body surface area measurement in juvenile miniature pigs using a computed tomography scanner

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Abstract: The use of miniature pigs in non-clinical studies for medical drugs or devices has gradually been increasing in recent years. It is anticipated that the use of juvenile miniature pigs in laboratory practice will also increase. Therefore, it is important to investigate various parameters of juvenile miniature pigs. The body surface area (BSA) of an organism is one of the important parameters for evaluating physiological functions. In drug development, normalization by BSA is an appropriate method for extrapolating doses between species. The BSA of animals has generally been estimated by multiplying the *k* value by 2/3 of the power of the body weight (BW) (Meeh's formula). To our knowledge, the BSA of juvenile miniature pigs has not as yet been reported. In this study, we measured the BSA of 13 miniature pigs less than 1 month old, using a computed tomography scanner and 3-dimensional analysis software. The measurement results showed the BSAs of these 13 juvenile miniature pigs to be in the range of 386 to 1,672 cm^2 (working BW range: 278 to 3,200 g). After BSA determination, the *k* values were calculated from the BSA and the BW. The mean calculated *k* value was 8.58. We advocate using Meeh's formula, as follows, for estimating the BSA of juvenile miniature pigs less than 1 month old (before weaning): BSA (cm^2)=8.58 × BW $(g)^{2/3}$.
Key words: body surface area, computed tomography, CT image, CT scanner, juvenile miniature pig

Introduction

Laboratory miniature pigs have increasingly been used as a non-rodent species for safety testing of chemicals and drugs [1]. Furthermore, the use of miniature pigs in non-clinical studies for medical devices has also been increasing. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for human use has been deliberating on the S11 guideline designated "Nonclinical Safety Testing in Support of Development of Pediatric Medicines". Therefore, in the near future, it is expected that juvenile animals will be used more often in laboratory practice. Despite infants, because juvenile miniature pigs have advantages including being large enough to serve as a laboratory animal model, the demand for these animals is anticipated to increase. For this reason, it is important to investigate various parameters of juvenile miniature pigs.

The body surface area (BSA) of an organism is one of the parameters used for evaluating physiological functions. It has been an essential requirement in calculating the cardiac index [6], assessing the basal metabolic rate [3, 10], and determining the burn surface area as a percentage of the total. BSA has also been used as a criterion for drug dosage determination since the 1950s [18]. In drug development, the no observed adverse effect

levels in laboratory animal species have been converted to human equivalent doses using scaling factors. Normalization by BSA (i.e., conversion of a dosage from mg/kg to mg/m^2) is an appropriate method for extrapolating doses between species. The Food and Drug Administration Center for Drug Evaluation and Research guidance recommends the use of BSA to estimate starting doses in the initial clinical trials for therapeutics in volunteer subjects [2]. In addition, the procedures for assessing dermal toxicity are described in the guidelines issued by the Organization for Economic Cooperation and Development and these guidelines recommend that the test substance be applied to not less than 10% of the total BSA [14–16]. Thus, accurately determining the BSA of laboratory animals is extremely important.

The BSA of animals has generally been estimated by multiplying a constant by 2/3 of the power of the measured body weight (BW) [11]. Using BSA values which had been determined by classical methods such as skinning, triangulation, surface integrator, paper cover, the mold method, and the perimeter method, the values of k(BSA [cm^2]=$k \times$ BW [g]$^{2/3}$, i.e., Meeh's formula) in various species were compiled by Spector [20]. The k values differed among the species examined and were used for dose extrapolations for each species [2]. The k values for domestic pigs reportedly range from 7.77 to 15.3 [7, 19–21]. In addition to these determinations, several other BSA formulas for domestic pigs and miniature pigs have been proposed [1, 4, 7, 9, 17, 22]. In neonatal domestic pigs, DeRoth and Bisaillon measured the BSA 6–24 h after birth employing a skinning method, and reported the following first-degree formula for obtaining their estimates: BSA (cm^2)=337.2 + 0.533 × BW (g) (hereafter DeRoth's formula) [5]. Myers *et al* introduced this formula for neonatal pigs weighing less than 2 kg [12]. However, BSA is difficult to measure because of the complex structural features of these animals. Therefore, the accuracy and reproducibility of the classical methods have apparent limitations.

The computed tomography (CT) scanner can obtain detailed 3-dimensional (3D) images of an object, and analysis of these CT images is expected to determine BSA more precisely than the classical measuring techniques. In our previous report [8], we measured the BSA of peripubertal and mature miniature pigs (ranging in age from 3 to 22 months) using a CT scanner and 3D analysis software. Analysis of the CT images was based on the computer graphics algorithm known as Marching Cubes. Applying our measurement results, we calculated the k value for peripubertal or mature miniature pigs to be 7.98. Furthermore, the k values of Göttingen minipigs and NIBS miniature pigs were approximately equal. Because the BSA of juvenile miniature pigs has not as yet been reported, we employed the previous measurement method and calculated the k value in this study.

Materials and Methods

Animals

The NIBS miniature pig was derived by mating three distinct porcine breeds, i.e. Pitman-Moore, Chinese native (Short-ear pig of Taiwan) and Göttingen, starting in 1993, and is now a breed of laboratory miniature pig available in Japan [13]. The bodies of 13 NIBS miniature pigs less than 1 month old (before weaning) were obtained from Nippon Institute for Biological Science, Tokyo, Japan. They appeared to have been stillborn or to have died accidentally after birth (for example, crushing death by the mother pig, death due to change in temperature, or sudden death).

Experimental procedures

The measuring methods followed those described in our previous report [8] except for the slice thickness and the reconstruction interval (previously, the intervals were 5mm and 2.5 mm, but for this study 2 and 1 mm, respectively), which were modified according to body size. The body of each juvenile miniature pig, which had been refrigerated since discovery of death, was set in the prone position within a few days postmortem. Images were obtained using a Multislice CT scanner (Alexion TSX 033A, Toshiba Medical Systems Co., Ltd., Tochigi, Japan). The BSA of each animal was determined from the CT images (Fig. 1) using high-speed 3D analysis software (TRI-3D/VOL, Ratoc System Engineering Co., Ltd., Tokyo, Japan). After the BSA had been determined, the k value was calculated from the BSA and the BW.

Accuracy confirmation

To confirm the accuracy of the method used in this study, the surface areas of 2 acrylic cuboids (d=15 cm, w=15 cm, and h=15.5 cm; calculated surface area=1,380 cm^2 and d=27 cm, w=27 cm, and h=30 cm; calculated surface area=4,698 cm^2) were measured. Measurements were repeated 5 times employing the same method as that used for the juvenile miniature pigs.
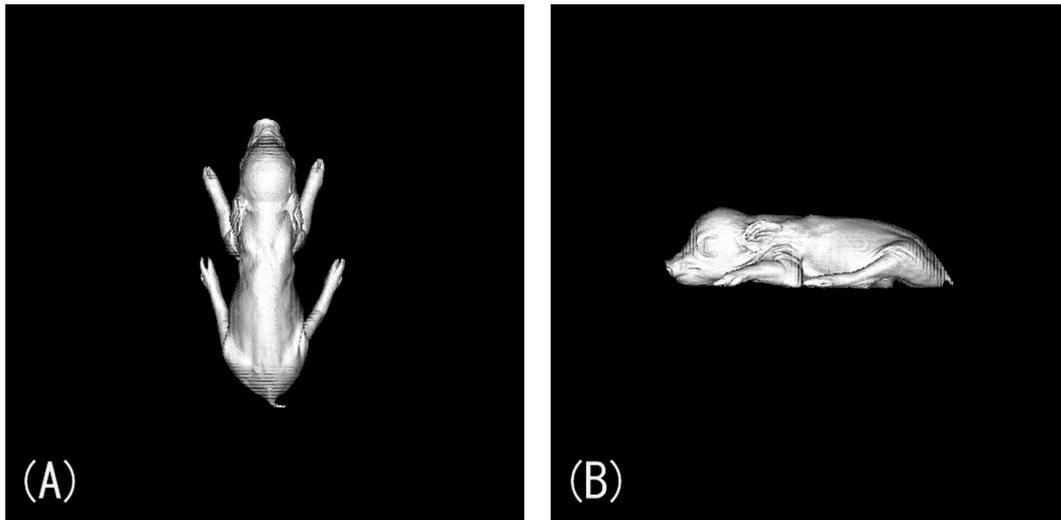
Fig. 1. CT images of juvenile miniature pigs. (A) Juvenile miniature pig in dorsal aspect. (B) In lateral side.

Results and Discussion

BSA and k value

As shown in Table 1, the BWs of the 13 juvenile miniature pigs ranged from 278 to 3,200 g (mean: 783 g), and their ages ranged from 0 to 25 days (mean: 6.5 days). There were no obvious difference between the BWs and the background data of the breeder (Data not shown). The BSA values ranged from 386 to 1,672 cm^2.

The calculated mean value ± SD and coefficient of variation (CV) of the k value were 8.58 ± 0.40 and 4.70%, respectively. The mean k value for the juvenile miniature pigs was larger than that of peripubertal or mature miniature pigs (7.98) [8]. The k values of male and female juvenile miniature pigs were approximately equal (p value of Aspin-Welch's t-test was 0.62).

Surface area of the cuboid

The surface area of the smaller cuboid yielded values of 1,393 ± 0.3 cm^2 and 0.02% (mean ± SD and CV). The average value of the measured surface area was 101.0% of the calculated value (1,380 cm^2). The surface area of the larger cuboid yielded values of 4,759 ± 5.4 cm^2 and 0.11% (mean ± SD and CV). The average value was 101.3% of the calculated value (4,698 cm^2).

Since sufficient accuracy and reproducibility were confirmed, we concluded this measurement method to be highly reliable.

Table 1. BW, age, BSA, and k value in juvenile miniature pigs (NIBS miniature pigs)

Sex	BW (g)	Age (day)	BSA (cm^2)	k value
Female	278	0	386	9.06
Female	287	0	390	8.96
Female	309	0	389	8.51
Male	309	0	398	8.71
Male	348	0	433	8.75
Male	363	0	423	8.31
Male	422	0	493	8.76
Female	580	6	597	8.58
Female	630	6	616	8.38
Female	916	13	798	8.46
Male	1,240	17	1,061	9.19
Male	1,300	18	966	8.11
Female	3,200	25	1,672	7.70
Mean	783	6.5	663	8.58
S.D.	809	8.8	380	0.40

BSA (cm^2)=k × BW (g)$^{2/3}$. The mean value ± SD of the k values of male juvenile miniature pigs: 8.64 ± 0.38. The mean value ± SD of the k values of female juvenile miniature pigs: 8.52 ± 0.44.

Comparison with DeRoth's formula

As shown in Table 2, the percentages of the BSAs calculated by Meeh's formula (k=8.58) and DeRoth's formula relative to the BSAs measured employing a CT scanner were as follows; those determined by Meeh's formula ranged from 93.3 to 111.4%, and those by DeRoth's formula (working BW range: less than 2 kg) from 96.4 to 130.6%. With the exception of just one animal, Meeh's formula (k=8.58) was more accurate than DeRoth's formula. Especially in miniature pigs at postnatal day 0, the difference between the BSA calculated by DeR-

Table 2. BW, age, measured BSA, and calculated BSA in juvenile miniature pigs (NIBS miniature pigs)

BW (g)	Age (day)	Measured	Meeh (%)	DeRoth (%)
278	0	386	365 (94.6)	491 (127.2)
287	0	390	373 (95.6)	496 (127.2)
309	0	389	392 (100.8)	508 (130.6)
309	0	398	392 (98.5)	508 (127.6)
348	0	433	424 (97.9)	530 (122.4)
363	0	423	437 (103.3)	538 (127.2)
422	0	493	483 (98.0)	571 (115.8)
580	6	597	597 (100.0)	658 (110.2)
630	6	616	631 (102.4)	686 (111.4)
916	13	798	809 (101.4)	844 (105.8)
1,240	17	1,061	990 (93.3)	1,023 (96.4)
1,300	18	966	1,022 (105.8)	1,056 (109.3)
3,200	25	1,672	1,863 (111.4)	NC (-)

measured: The measured BSA using a CT scanner (cm^2). Meeh: The calculated BSA (cm^2) by Meeh's formula (BSA [cm^2]=8.53 × BW [g]$^{2/3}$). DeRoth: The calculated BSA (cm^2) by DeRoth's formula (BSA [cm^2]=337.2 + 0.553 × BW [g]). NC: Not calculated because of out of the working BW range (kg). Percentage in parentheses is the calculated BSA (cm^2) / the measured BSA using a CT scanner (cm^2) .

oth's formula and the actual size was not insignificant. This is at least in part attributable to miniature pigs at postnatal day 0 being smaller than neonatal domestic pigs. The BW of the neonatal domestic pigs actually measured by DeRoth and Bisaillon ranged from 445 to 1,976 g (mean: 1,008.4 g) [5]. In other words, the BW of miniature pigs at postnatal day 0 is less than the coverage provided by DeRoth's formula. Thus, the intercept of the first-degree formula exerts an excessive influence and the BSA of miniature pigs at postnatal day 0 calculated by this first-degree formula therefore exceeds the actual size.

In mathematics, if it is assumed that the body shape and density of the animals being studied are essentially constant, the BSA would be proportional to 2/3 of the power of the BW. Meeh's formula is also useful for extrapolating doses between species. We propose using Meeh's formula, as follows, for estimating the BSA of juvenile miniature pigs less than 1 month old (before weaning): BSA (cm^2)=8.58 × BW (g)$^{2/3}$.

Conflict of Interest

The authors declare that there is no conflict of interest.

Acknowledgments

References

1. Bollen, P.J.A., Hansen, A.K., and Olsen Alstrup, A.K. 2010. Experimental techniques. pp. 86–87. *In*: The laboratory swine., CRC press. New York.
2. Center for Drug Evaluation and Research. 2005. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. *In*: Guidance for industry.
3. Dale, H.E.1970. Energy metabolism. pp. 619–633. *In*: Duke's physiology of domestic animals, 8th ed. (Swenson, M.J., ed.), Cornel University Press, Ithaca.
4. Deighton, T. 1932. The determination of the surface area of swine and other animals. *J. Agric. Sci.* 22: 418–449. [CrossRef]
5. DeRoth, L. and Bisaillon, A. 1979. Determination of body surface area in neonatal swine. *Lab. Anim. Sci.* 29: 249–250. [Medline]
6. Hall, J.E. 2011. Cardiac output, venous return, and their regulation. pp. 229–241. *In*: Textbook of medical physiology, Saunders, Philadelphia.
7. Hogan, A.G. and Skouby, C.I. 1923. Determination of the surface area of cattle and swine. *J. Agric. Res.* 25: 419–430.
8. Itoh, T., Kawabe, M., Nagase, T., Endo, K., Miyoshi, M., and Miyahara, K. 2016. Body surface area measurement in laboratory miniature pigs using a computed tomography scanner. *J. Toxicol. Sci.* 41: 637–644. [Medline] [CrossRef]
9. Kelley, K.W., Curtis, S.E., Marzan, G.T., Karara, H.M., and Anderson, C.R. 1973. Body surface area of female swine. *J. Anim. Sci.* 36: 927–930. [Medline] [CrossRef]
10. Kleiber, M.1965. Metabolic body size. pp. 427–435 *In*: Energy metabolism (Blaxter, K.L., ed.), Academic Press, London.
11. Meeh, K. 1879. Oberflächenmessungen des menschlichen Körpers. *Z. Biol.* 15: 425–458 (in German).
12. Myers, D.D. Jr., Lester, P., Conte, M.L., and Swindle, M.M.2016. Cardiovascular catheterization, electrophysiology, and imaging laboratory procedures. pp. 337–382. *In*: Swine in the laboratory, 3rd ed. (Swindle, M.M. and Smith A.C., eds.), CRC press, New York.
13. Nunoya, T., Shibuya, K., Saitoh, T., Yazawa, H., Nakamura, K., Baba, Y., and Hirai, T. 2007. Use of miniature pig for biomedical research, with reference to toxicologic studies. *J. Toxicol. Pathol.* 20: 125–132. [CrossRef]
14. OECD. 1981. Test No. 410: Repeated Dose Dermal Toxicity: 21/28-day Study. *In*: OECD Guidelines for the Testing of Chemicals, Section 4.
15. OECD. 1981. Test No. 411: Subchronic Dermal Toxicity: 90-day Study. *In*: OECD Guidelines for the Testing of Chemicals, Section 4.
16. OECD. 1987. Test No. 402: Acute Dermal Toxicity. *In*: OECD Guidelines for the Testing of Chemicals, Section 4.
17. Otsubo, T. 1957. Studies on the body surface area in the swine. *Bull. Fac. Agric. Kagoshima Univ.* 6: 137–140 (in Japanese).
18. Pinkel, D. 1958. The use of body surface area as a criterion of drug dosage in cancer chemotherapy. *Cancer Res.* 18: 853–856. [Medline]

19. Rubner, M.1902. Gesetze des Energieverbrauches bei der Ernährung. pp.280–281. F. Deuticke, Leipzig, Wien (in German).

20. Spector, W.S.1956. Constants for estimating surface area: mammals. p. 175. *In*: Handbook of biological data. W. B. Saunders Company, Philadelphia.

21. Voit, E. 1901. Über die Grösse des Energiebedarfs der Tiere in Hungerzustande. *Z. Biol.* 41: 113–154 (in German).

22. Wachtel, T.L., McCahan, G.R. Jr., Watson, W.I., and Gorman, M.1972. Determining the surface areas of miniature swine and domestic swine by geometric design–A comparative study. USAARL Report. No. 73–5. Fort Rucker, Alabama.