The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.41, No.5, 637-644, 2016

Letter

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

Tadashi Itoh[1], Mifumi Kawabe[2], Takahiko Nagase[1], Katsumi Endo[1], Masafumi Miyoshi[3] and Kazuro Miyahara[3]

[1]Nihon Bioresearch Inc., 6-104, Majima, Fukujucho, Hashima, Gifu 501-6251, Japan
[2]Animal Medical Center, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
[3]Veterinary Medical Center, Obihiro University of Agriculture and Veterinary Medicine, Inadacho, Obihiro, Hokkaido 080-8555, Japan

(Received February 19, 2016; Accepted July 19, 2016)

ABSTRACT — The body surface area (BSA) of an organism is an important parameter 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 a constant by the power of the body weight (BW). Recently, the use of miniature pigs in non-clinical studies for medical drugs or devices has gradually been increasing. However, verification of their BSA is not as yet sufficient. In this study, we measured the BSAs of 40 laboratory miniature pigs (11 males and 9 females of Göttingen minipig and 14 males and 6 females of Nippon Institute for Biological Science [NIBS] miniature pig) by analyzing computed tomography (CT) images, since measurements using a CT scanner were expected to more precisely determine BSA than classical measuring techniques. The measurement results showed the BSAs of the 20 Göttingen minipigs to range from 0.4358 to 0.8356 m^2 (the working BW range: 12.7-37.0 kg) and 20 NIBS miniature pigs to range from 0.2906 to 0.8675 m^2 (the working BW range: 7.9-41.5 kg). Since accuracy and reproducibility were confirmed by measuring the surface area of an acrylic cuboid, we concluded the measurement method employed in this study to be very reliable. We propose the following estimating formula for BSA of laboratory miniature pigs: $100 \times BSA\ [m^2] = 7.98 \times BW\ [kg]^{2/3}$.

Key words: Body surface area, Miniature pig, Computed tomography, CT scanner, CT image

INTRODUCTION

The body surface area (BSA) of an organism is one of the parameters used for evaluating physiological functions. BSA is an essential requirement in calculating cardiac index (Hall, 2011), in assessing basal metabolic rate (Kleiber, 1965; Dale, 1970), or in determining the percentage of burn surface area. BSA has been also used as a criterion for drug dosage determination since the 1950s (Pinkel, 1958). In drug development, the Food and Drug Administration Center for Drug Evaluation and Research (CDER) guidance recommends the use of BSA to estimate the starting dose in the initial clinical trials for therapeutics in volunteer subjects (CDER, 2005). Specifically, the no observed adverse effect levels (NOAELs) in laboratory animal species are converted to human equivalent doses (HEDs) 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. Thus, for most systemically administered therapeutics, NOAELs in the laboratory animal species are divided by the appropriate BSA-conversion factors to calculate HEDs. In addition, the procedures for assessing dermal toxicity are described in the guidelines issued by the Organization for Economic Cooperation and Development (OECD) and the guidelines recommend that the test substance should be applied not less than 10% of the total BSA (OECD, 1981a, 1981b, 1987). Thus, it is apparent that an accurate measurement of the BSA of laboratory animals is extremely important.

The BSA of animals is generally estimated by multiplying a constant by 2/3 of the power of the body weight

Correspondence: Tadashi Itoh (E-mail: ito.tadashi@nisshin.com)

(BW) (Meeh, 1879). The values of $K_{2/3}$ (100 × BSA [m^2] = $K_{2/3}$ × BW [kg]$^{2/3}$) that have been determined in multiple species (Spector, 1956) differ among these subject species, and have been used to extrapolate doses between species (CDER, 2005). Thus, obtaining an accurate BSA is important for determining species-specific factors. Using BSA determined by classical methods (such as skinning, triangulation, surface integrator, and paper cover), the $K_{2/3}$ for domestic pigs was determined to be 7.77-15.3 (Voit, 1901; Rubner, 1902; Hogan and Skouby, 1923; Spector, 1956). In addition to these determinations, several other BSA formulas for domestic pigs have been proposed to date (Hogan and Skouby, 1923; Deighton, 1932; Otsubo, 1957; Kelley *et al.*, 1973).

For laboratory miniature pigs, Wachtel's formula is as follows: BSA [m^2] = 0.121 × BW [kg]$^{0.575}$. This formula has been defined employing a geometric design, *i.e.*, by a classical method (Wachtel *et al.*, 1972). Myers *et al.* (2016) described the most applicable formula for miniature pigs in current research as probably being Wachtel's formula. Furthermore, the $K_{3/4}$ (1000 × BSA [m^2] = $K_{3/4}$ × BW [kg]$^{3/4}$) was calculated to be 70 based on the theory that animals have a basal metabolism of 70 kcal/metabolic BW: $BW^{3/4}$ and produce 1000 kcal of heat per square meter (Bollen *et al.*, 2010). Furthermore, the HED was calculated with the standard k_m (k_m = 100/$K_{2/3}$ × BW [kg]$^{1/3}$= BW [kg]/BSA [m^2]) to convert the dose in mg/kg to the dose in mg/m^2 for each species including laboratory miniature pigs (CDER, 2005). The k_m for micropigs (working BW ranges: 10-33 kg) was determined to be 27 and the k_m for mini-pigs (working BW ranges: 25-64 kg) was determined to be 35 in the guidance issued by the CDER. However BSA is difficult to measure because of the complex architecture of the animals. Therefore, it appears that the accuracy and reproducibility of the classical methods have limitations. Although laboratory miniature pigs are increasingly being used as non-rodent species for safety testing of chemicals and drugs (Bollen *et al.*, 2010), verification of the BSA of laboratory miniature pigs is not as yet sufficient.

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 classical measuring techniques. In this study, we measured the BSA of laboratory miniature pigs using a CT scanner and then analyzed the CT images. Because the CT images, unlike classical measuring techniques, do not depend on the skills of the person performing the measurement, the method used in this study is expected to be a reproducible method. Additionally, we calculated the values of $K_{2/3}$, $K_{0.575}$ (BSA [m^2] = $K_{0.575}$ × BW [kg]$^{0.575}$), $K_{3/4}$, and k_m for laboratory miniature pigs from the BSA and the BW.

MATERIALS AND METHODS

Animals

Göttingen minipigs were obtained from Oriental Yeast Company Ltd, Tokyo, Japan. The Göttingen minipig is mainly used for regulatory toxicity studies, and is available worldwide (Köhn, 2012).

Nippon Institute for Biological Science miniature pigs (NIBS miniature pigs) were obtained from Nippon Institute for Biological Science, Tokyo, Japan. The NIBS miniature pig represents the results of mating three distinct porcine breeds, Pitman-Moore, Chinese native (Short-ear pig of Taiwan) and Göttingen since 1993, and is a breed of laboratory miniature pig available in Japan (Nunoya *et al.*, 2007).

The BSA of 40 miniature pigs (11 males and 9 females of Göttingen minipig and 14 males and 6 females of NIBS miniature pig) that had been used in other non-clinical studies and euthanized by anesthesia with sodium pentobarbital according to the protocols of the previous studies were measured. No critical abnormalities in clinical signs or BW changes had been noted in these animals during the survival period. All of the studies were conducted in compliance with the guidelines for the management and welfare of experimental animals of Nihon Bioresearch Inc.

Experimental procedures

The measuring method using a CT scanner is shown in Fig. 1. The body of each miniature pig was set in the prone position for whole-body CT scanning on the day of euthanasia. Images were obtained using a Multislice CT scanner (Alexion TSX 033A, Toshiba Medical Systems Co., Ltd., Tochigi, Japan) with a slice thickness of 5 mm (tube voltage: 120 kV, tube current: 150 mA, helical pitch: 5.5). The BSA was determined from the CT images (Fig. 2) using high-speed 3D analysis software (TRI-

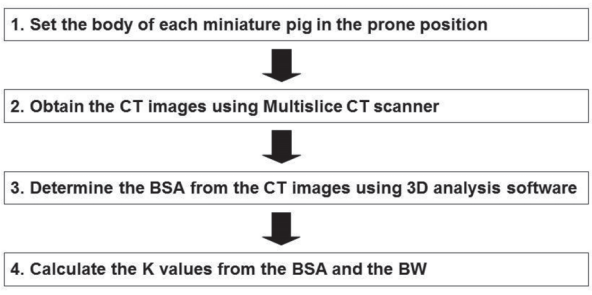

Fig. 1. The measuring method using a CT scanner.

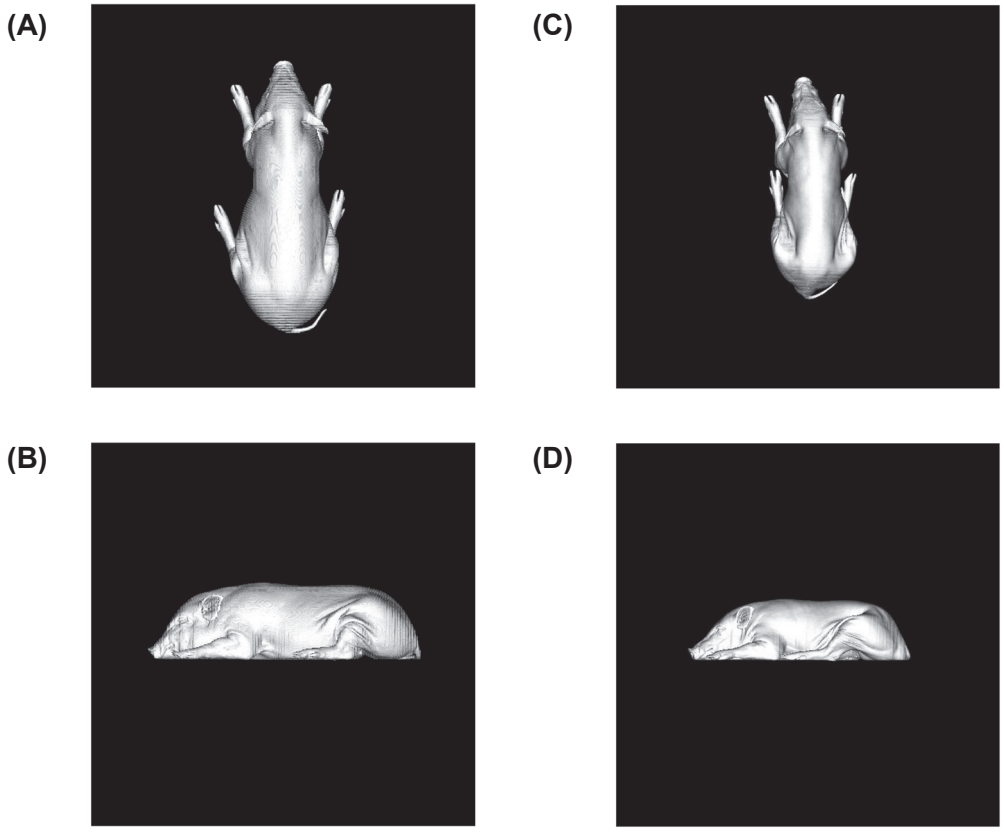

Fig. 2. CT images of laboratory miniature pigs. BSA was determined from CT images using high-speed 3D analysis software. (A) Göttingen minipig in dorsal aspect. (B) Göttingen minipig in lateral side. (C) NIBS miniature pig in dorsal aspect. (D) NIBS miniature pig in lateral side.

3D/VOL, Ratoc System Engineering Co., Ltd., Tokyo, Japan). Analysis of the CT images was based on the computer graphics algorithm known as Marching Cubes. After the BSA had been determined, the K values ($K_{2/3}$, $K_{0.575}$, $K_{3/4}$, and k_m) were calculated.

Accuracy confirmation of the method

To confirm the accuracy of the method used in this study, the surface area of an acrylic cuboid (d = 27 cm, w = 27 cm, and h = 30 cm; calculated surface area = 0.4698 m^2) was measured from 2 directions (Fig. 3). For the first direction, 2 sides of the bottom of the cuboid form were set parallel to a moving direction of the bed of the CT scanner, and for the second direction, a diagonal of the bottom was set parallel to a moving direction. Measurements were repeated 5 times employing the same method, as in the case of the miniature pigs.

RESULTS AND DISCUSSION

BSA and K values of the Göttingen minipig

As shown in Table 1, the BWs of the 20 Göttingen minipigs ranged from 12.7 to 37.0 kg (mean: 19.9 kg), and their ages ranged from 6 to 22 months (mean: 10.4 months). The BSA values ranged from 0.4358 to 0.8356 m^2.

The mean value ± S.D., C.V., and the correlation coefficient between the BW (r) of the $K_{2/3}$ value were calculated to be 7.94 ± 0.21, 2.65%, and -0.163, respectively, and those of the $K_{0.575}$ value were 0.104 ± 0.004, 3.74%, and 0.667, and those of the $K_{3/4}$ value were 62.2 ± 2.5, 3.99%, and -0.771. Additionally, the k_m values ranged from 29.1 to 44.3 (mean ± S.D.; 33.8 ± 4.0, C.V.; 11.92%, r; 0.976).

BSA and K values of the NIBS miniature pig

As shown in Table 2, the BWs of the 20 NIBS min-

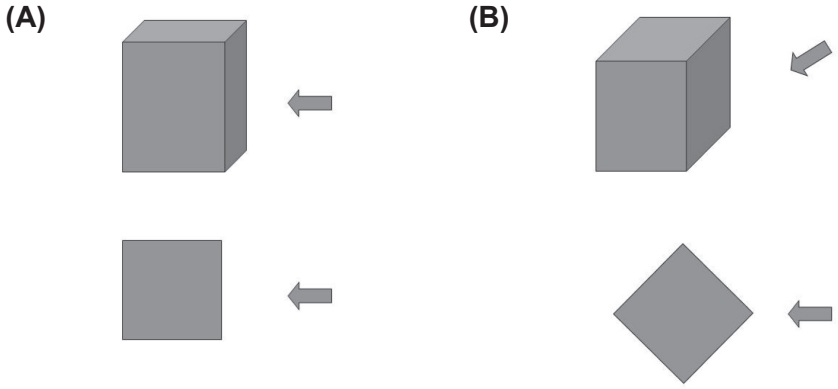

Fig. 3. Direction of the CT scanning of an acrylic cuboid. From 2 directions the surface area of an acrylic cuboid (d = 27 cm, w = 27 cm, and h = 30 cm) was measured to confirm the accuracy of the method used in this study. (A) The first direction. 2 sides of the bottom of the cuboid form were set parallel to a moving direction of the bed of the CT scanner. (B) The second direction. A diagonal of the bottom was set parallel to a moving direction. Cuboid (upper); measured acrylic cuboid, square (lower); bottom of the cuboid, arrow; moving direction of the bed of the CT scanner.

Table 1. BW, age, BSA, and K values in Göttingen minipigs.

Sex	BW (kg)	Age (month)	BSA (m^2)	$K_{2/3}$	$K_{0.575}$	$K_{3/4}$	k_m
Male	12.7	6	0.4358	8.01	0.101	64.8	29.1
Female	13.1	6	0.4417	7.95	0.101	64.1	29.7
Male	13.3	6	0.4497	8.01	0.102	64.6	29.6
Female	14.3	6	0.4691	7.96	0.102	63.8	30.5
Female	14.4	6	0.4583	7.74	0.099	62.0	31.4
Male	14.5	6	0.4714	7.93	0.101	63.4	30.8
Female	14.8	6	0.4639	7.70	0.099	61.5	31.9
Male	15.0	6	0.4826	7.93	0.102	63.3	31.1
Male	15.7	11	0.5202	8.30	0.107	66.0	30.2
Female	16.2	10	0.5008	7.82	0.101	62.0	32.3
Male	17.1	11	0.5516	8.31	0.108	65.6	31.0
Female	20.8	10	0.5761	7.62	0.101	59.1	36.1
Male	21.7	12	0.6249	8.03	0.106	62.2	34.7
Female	22.2	10	0.6142	7.78	0.103	60.1	36.1
Male	23.0	13	0.6389	7.90	0.105	60.8	36.0
Male	26.3	12	0.7080	8.01	0.108	61.0	37.1
Male	26.6	14	0.7092	7.96	0.108	60.5	37.5
Male	27.3	12	0.7536	8.31	0.113	63.1	36.2
Female	32.0	22	0.8051	7.99	0.110	59.8	39.7
Female	37.0	22	0.8356	7.53	0.105	55.7	44.3
Mean	19.9	10.4	0.5755	7.94	0.104	62.2	33.8
S.D.	7.0	4.9	0.1287	0.21	0.004	2.5	4.0
C.V.				2.65%	3.74%	3.99%	11.92%
r				-0.163	0.667	-0.771	0.976

r: the correlation coefficient between the BW

$100 \times BSA\ (m^2) = K_{2/3} \times BW\ (kg)^{2/3}$

$BSA\ (m^2) = K_{0.575} \times BW\ (kg)^{0.575}$

$1000 \times BSA\ (m^2) = K_{3/4} \times BW\ (kg)^{3/4}$

$k_m = 100/K_{2/3} \times BW\ (kg)^{1/3} = BW\ (kg)/BSA\ (m^2)$

Table 2. BW, age, BSA, and K values in NIBS miniature pigs.

Sex	BW (kg)	Age (month)	BSA (m^2)	$K_{2/3}$	$K_{0.575}$	$K_{3/4}$	k_m
Female	7.9	3	0.2906	7.33	0.089	61.7	27.2
Female	13.1	5	0.4350	7.83	0.099	63.2	30.1
Female	14.0	5	0.4608	7.93	0.101	63.7	30.4
Female	17.4	5	0.5372	8.00	0.104	63.1	32.4
Male	18.2	7	0.5617	8.12	0.106	63.7	32.4
Male	18.4	7	0.5682	8.15	0.106	64.0	32.4
Male	19.0	6	0.5752	8.08	0.106	63.2	33.0
Male	19.8	7	0.5841	7.98	0.105	62.2	33.9
Male	20.0	7	0.5991	8.13	0.107	63.3	33.4
Male	20.1	6	0.5949	8.05	0.106	62.7	33.8
Male	20.3	7	0.5937	7.98	0.105	62.1	34.2
Male	20.9	16	0.6590	8.69	0.115	67.4	31.7
Male	22.5	7	0.6464	8.11	0.108	62.6	34.8
Male	23.2	16	0.6597	8.11	0.108	62.4	35.2
Male	24.5	10	0.7345	8.71	0.117	66.7	33.4
Male	25.6	9	0.6851	7.89	0.106	60.2	37.4
Male	27.2	9	0.7239	8.00	0.108	60.8	37.6
Male	27.2	9	0.7538	8.33	0.113	63.3	36.1
Female	33.4	13	0.7841	7.56	0.104	56.4	42.6
Female	41.5	20	0.8675	7.24	0.102	53.1	47.8
Mean	21.7	8.7	0.6157	8.01	0.106	62.3	34.5
S.D.	7.3	4.4	0.1305	0.36	0.006	3.1	4.5
C.V.				4.47%	5.54%	4.99%	12.96%
r				-0.147	0.395	-0.659	0.970

r: the correlation coefficient between the BW

$100 \times BSA\ (m^2) = K_{2/3} \times BW\ (kg)^{2/3}$

$BSA\ (m^2) = K_{0.575} \times BW\ (kg)^{0.575}$

$1000 \times BSA\ (m^2) = K_{3/4} \times BW\ (kg)^{3/4}$

$k_m = 100/K_{2/3} \times BW\ (kg)^{1/3} = BW\ (kg)/BSA\ (m^2)$

iature pigs ranged from 7.9 to 41.5 kg (mean: 21.7 kg), and their ages ranged from 3 to 20 months (mean: 8.7 months). The BSA values ranged from 0.2906 to 0.8675 m^2.

The mean value ± S.D., C.V., and r of the $K_{2/3}$ value were calculated to be 8.01 ± 0.36, 4.47%, and -0.147, respectively, and those of the $K_{0.575}$ value were 0.106 ± 0.006, 5.54%, and 0.395, and those of the $K_{3/4}$ value were 62.3 ± 3.1, 4.99%, and -0.659. Additionally, the k_m values ranged from 27.2 to 47.8 (mean ± S.D.; 34.5 ± 4.5, C.V.; 12.96%, r; 0.970).

Surface area of the cuboid

The surface area of the cuboid measured in the first direction yielded a value of 0.4737 ± 0.0016 m^2, 0.34% (mean ± S.D., C.V.), and that in the second direction a value of 0.4775 ± 0.0001 m^2, 0.03%.

The average values of the surface area of the cuboid measured in the first and the second directions were 100.8% and 101.6% of the calculated value (0.4698 m^2), respectively. In addition, the respective values of their C.V. were 0.34% and 0.03%. Since sufficient accuracy and reproducibility were confirmed, we concluded this measurement method to be very reliable.

Estimating formula for BSA of laboratory miniature pigs

According to the present results, the values of the C.V. of the $K_{2/3}$ were smaller than those of the other K values ($K_{0.575}$, $K_{3/4}$, and k_m). The $K_{2/3}$ values were not the variable most affected by the BW among the K values calculated in this study. In fact, the absolute values of r of the $K_{2/3}$ were smaller than those of the other parameters determined. The $K_{2/3}$ is also useful for extrapolating doses between species. Furthermore, the $K_{2/3}$ values of Göttingen minipigs and NIBS miniature pigs were approx-

Table 3. BW, measured BSA, and calculated BSAs in Göttingen minipigs.

BW (kg)	measured	present formula	Wachtel	Bollen	$k_m = 27$	$k_m = 35$
12.7	0.4358	0.4344	0.5218	0.4709	0.4704	NC
		(99.7%)	(119.7%)	(108.1%)	(107.9%)	(-)
13.1	0.4417	0.4435	0.5311	0.4820	0.4852	NC
		(100.4%)	(120.2%)	(109.1%)	(109.8%)	(-)
13.3	0.4497	0.4480	0.5358	0.4875	0.4926	NC
		(99.6%)	(119.1%)	(108.4%)	(109.5%)	(-)
14.3	0.4691	0.4701	0.5586	0.5148	0.5296	NC
		(100.2%)	(119.1%)	(109.7%)	(112.9%)	(-)
14.4	0.4583	0.4723	0.5608	0.5175	0.5333	NC
		(103.1%)	(122.4%)	(112.9%)	(116.4%)	(-)
14.5	0.4714	0.4745	0.5631	0.5201	0.5370	NC
		(100.7%)	(119.5%)	(110.3%)	(113.9%)	(-)
14.8	0.4639	0.4810	0.5698	0.5282	0.5481	NC
		(103.7%)	(122.8%)	(113.9%)	(118.2%)	(-)
15.0	0.4826	0.4854	0.5742	0.5335	0.5556	NC
		(100.6%)	(119.0%)	(110.5%)	(115.1%)	(-)
15.7	0.5202	0.5003	0.5894	0.5521	0.5815	NC
		(96.2%)	(113.3%)	(106.1%)	(111.8%)	(-)
16.2	0.5008	0.5109	0.6001	0.5652	0.6000	NC
		(102.0%)	(119.8%)	(112.9%)	(119.8%)	(-)
17.1	0.5516	0.5297	0.6191	0.5886	0.6333	NC
		(96.0%)	(112.2%)	(106.7%)	(114.8%)	(-)
20.8	0.5761	0.6035	0.6929	0.6818	0.7704	NC
		(104.8%)	(120.3%)	(118.3%)	(133.7%)	(-)
21.7	0.6249	0.6208	0.7100	0.7038	0.8037	NC
		(99.3%)	(113.6%)	(112.6%)	(128.6%)	(-)
22.2	0.6142	0.6303	0.7193	0.7159	0.8222	NC
		(102.6%)	(117.1%)	(116.6%)	(133.9%)	(-)
23.0	0.6389	0.6454	0.7341	0.7352	0.8519	NC
		(101.0%)	(114.9%)	(115.1%)	(133.3%)	(-)
26.3	0.7080	0.7057	0.7930	0.8130	0.9741	0.7514
		(99.7%)	(112.0%)	(114.8%)	(137.6%)	(106.1%)
26.6	0.7092	0.7111	0.7982	0.8199	0.9852	0.7600
		(100.3%)	(112.5%)	(115.6%)	(138.9%)	(107.2%)
27.3	0.7536	0.7235	0.8102	0.8360	1.0111	0.7800
		(96.0%)	(107.5%)	(110.9%)	(134.2%)	(103.5%)
32.0	0.8051	0.8043	0.8877	0.9418	1.1852	0.9143
		(99.9%)	(110.3%)	(117.0%)	(147.2%)	(113.6%)
37.0	0.8356	0.8861	0.9649	1.0501	NC	1.0571
		(106.0%)	(115.5%)	(125.7%)	(-)	(126.5%)

measured: The measured BSA using a CT scanner (m^2)
present formula: The calculated BSA (m^2) by present formula ($100 \times BSA\ [m^2] = 7.98 \times BW\ [kg]^{2/3}$)
Wachtel: The calculated BSA (m^2) by Wachtel's formula ($BSA\ [m^2] = 0.121 \times BW\ [kg]^{0.575}$)
Bollen: The calculated BSA (m^2) by Bollen's formula ($1000 \times BSA\ [m^2] = 70 \times BW\ [kg]^{3/4}$)
$k_m = 27$: The calculated BSA (m^2) by BW (kg)/27 (working BW range: 10-33 kg)
$k_m = 35$: The calculated BSA (m^2) by BW (kg)/35 (working BW range: 25-64 kg)
NC: Not calculated because of out of the working BW range (kg)
Percentage in parentheses is 100 × the calculated BSA (m^2)/the measured BSA using a CT scanner (m^2)

Table 4. BW, measured BSA, and calculated BSAs in NIBS miniature pigs.

BW (kg)	measured	present formula	Wachtel	Bollen	$k_m = 27$	$k_m = 35$
7.9	0.2906	0.3165	0.3971	0.3299	NC	NC
		(108.9%)	(136.6%)	(113.5%)	(-)	(-)
13.1	0.4350	0.4435	0.5311	0.4820	0.4852	NC
		(102.0%)	(122.1%)	(110.8%)	(111.5%)	(-)
14.0	0.4608	0.4635	0.5518	0.5066	0.5185	NC
		(100.6%)	(119.7%)	(109.9%)	(112.5%)	(-)
17.4	0.5372	0.5358	0.6253	0.5964	0.6444	NC
		(99.7%)	(116.4%)	(111.0%)	(120.0%)	(-)
18.2	0.5617	0.5521	0.6417	0.6168	0.6741	NC
		(98.3%)	(114.2%)	(109.8%)	(120.0%)	(-)
18.4	0.5682	0.5562	0.6457	0.6219	0.6815	NC
		(97.9%)	(113.6%)	(109.5%)	(119.9%)	(-)
19.0	0.5752	0.5682	0.6578	0.6370	0.7037	NC
		(98.8%)	(114.4%)	(110.7%)	(122.3%)	(-)
19.8	0.5841	0.5840	0.6735	0.6570	0.7333	NC
		(100.0%)	(115.3%)	(112.5%)	(125.5%)	(-)
20.0	0.5991	0.5880	0.6775	0.6620	0.7407	NC
		(98.1%)	(113.1%)	(110.5%)	(123.6%)	(-)
20.1	0.5949	0.5899	0.6794	0.6645	0.7444	NC
		(99.2%)	(114.2%)	(111.7%)	(125.1%)	(-)
20.3	0.5937	0.5938	0.6833	0.6695	0.7519	NC
		(100.0%)	(115.1%)	(112.8%)	(126.6%)	(-)
20.9	0.6590	0.6055	0.6948	0.6842	0.7741	NC
		(91.9%)	(105.4%)	(103.8%)	(117.5%)	(-)
22.5	0.6464	0.6360	0.7249	0.7232	0.8333	NC
		(98.4%)	(112.1%)	(111.9%)	(128.9%)	(-)
23.2	0.6597	0.6491	0.7378	0.7400	0.8593	NC
		(98.4%)	(111.8%)	(112.2%)	(130.3%)	(-)
24.5	0.7345	0.6732	0.7613	0.7709	0.9074	NC
		(91.7%)	(103.6%)	(105.0%)	(123.5%)	(-)
25.6	0.6851	0.6932	0.7808	0.7967	0.9481	0.7314
		(101.2%)	(114.0%)	(116.3%)	(138.4%)	(106.8%)
27.2	0.7239	0.7217	0.8085	0.8337	1.0074	0.7771
		(99.7%)	(111.7%)	(115.2%)	(139.2%)	(107.3%)
27.2	0.7538	0.7217	0.8085	0.8337	1.0074	0.7771
		(95.7%)	(107.3%)	(110.6%)	(133.6%)	(103.1%)
33.4	0.7841	0.8276	0.9098	0.9725	NC	0.9543
		(105.5%)	(116.0%)	(124.0%)	(-)	(121.7%)
41.5	0.8675	0.9565	1.0308	1.1445	NC	1.1857
		(110.3%)	(118.8%)	(131.9%)	(-)	(136.7%)

measured: The measured BSA using a CT scanner (m^2)
present formula: The calculated BSA (m^2) by present formula ($100 \times BSA\ [m^2] = 7.98 \times BW\ [kg]^{2/3}$)
Wachtel: The calculated BSA (m^2) by Wachtel's formula ($BSA\ [m^2] = 0.121 \times BW\ [kg]^{0.575}$)
Bollen: The calculated BSA (m^2) by Bollen's formula ($1000 \times BSA\ [m^2] = 70 \times BW\ [kg]^{3/4}$)
$k_m = 27$: The calculated BSA (m^2) by BW (kg)/27 (working BW range: 10-33 kg)
$k_m = 35$: The calculated BSA (m^2) by BW (kg)/35 (working BW range: 25-64 kg)
NC: Not calculated because of out of the working BW range (kg)
Percentage in parentheses is 100 × the calculated BSA (m^2)/the measured BSA using a CT scanner (m^2)

imately equal (p value of Aspin-Welch's t-test was 0.45). The mean value of $K_{2/3}$ of 40 laboratory miniature pigs (20 Göttingen minipigs and 20 NIBS miniature pigs) was 7.98. We propose the following estimating formula for BSA of laboratory miniature pigs: $100 \times BSA\ [m^2] = 7.98 \times BW\ [kg]^{2/3}$

Comparison with the conventional formulas

As shown in Tables 3 and 4, the percentage of the calculated BSAs by several formulas to the measured BSAs by a CT scanner were as follows; the present formula ranged from 91.7 to 110.3%, Wachtel's formula from 103.6 to 136.6%, Bollen's formula from 103.8 to 131.9%, $k_m = 27$ (working BW range: 10-33 kg) from 107.9 to 147.2%, and $k_m = 35$ (working BW range: 25-64 kg) from 103.1 to 136.7%. Thus, if employing conventional formulas, the BSA of laboratory miniature pigs have been calculated as larger than the actual size. In other words, some values based on BSAs calculated by the conventional formulas might have not a little error. Based on the 130% of the magnitude of the actual BSA value, the calculated values of cardiac index, the percentage of burn surface area, and HED are only 77% of the magnitude of the actual values. We propose that the BSA of laboratory miniature pigs calculated by the present formula will be used for future studies in various fields. Furthermore, we expect that BSA of other laboratory animals will be verified by this measuring method.

ACKNOWLEDGMENTS

The authors wish to thank all members of Nihon Bioresearch Inc., for their help and support during this study.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Bollen, P.J.A., Hansen, A.K. and Olsen Alstrup, A.K. (2010): Experimental techniques. In The Laboratory Swine, pp.86-87, CRC press, New York.

CDER (2005): Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. In guidance for industry.

Dale, H.E. (1970): Energy metabolism. In Duke's physiology of domestic animals eighth edition (Swenson, M. J., ed.), pp.619-633, Cornel University Press, Ithaca.

Deighton, T. (1932): The determination of the surface area of swine and other animals. J. Agric. Sci., **22**, 418-449.

Hall, J.E. (2011): Cardiac output, venous return, and their regulation. In textbook of medical physiology, pp.229-241, Saunders, Philadelphia.

Hogan, A.G. and Skouby, C.I. (1923): Determination of the surface area of cattle and swine. J. Agric. Res., **25**, 419-430.

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.

Kleiber, M. (1965): Metabolic body size. In energy metabolism (Blaxter, K.L., ed.), pp.427-435, Academic Press, London.

Köhn, F. (2012): History and development of miniature, micro- and minipigs. In the minipig in biomedical research (McAnulty, P. A., Dayan, A. D., Ganderup N. C. and Hasting K. L., eds.), pp.3-15, CRC press, New York.

Meeh, K. (1879): Oberflächenmessungen des menschlichen Körpers. Z. Biol., **15**, 425-458.

Myers Jr., D.D., Lester, P., Conte, M.L. and Swindle, M.M. (2016): Cardiovascular catheterization, electrophysiology, and imaging laboratory procedures. In swine in the laboratory third edition (Swindle, M. M. and Smith A. C., eds.), pp.337-382, CRC press, New York.

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.

OECD (1981a): Test No. 410: Repeated Dose Dermal Toxicity: 21/28-day Study. In OECD Guidelines for the Testing of Chemicals, Section 4.

OECD (1981b): Test No. 411: Subchronic Dermal Toxicity: 90-day Study. In OECD Guidelines for the Testing of Chemicals, Section 4.

OECD (1987): Test No. 402: Acute Dermal Toxicity. In OECD Guidelines for the Testing of Chemicals, Section 4.

Otsubo, T. (1957): Studies on the body surface area in the swine. Bull. Fac. Agric. Kagoshima Univ., **6**, 137-140.

Pinkel, D. (1958): The use of body surface area as a criterion of drug dosage in cancer chemotherapy. Cancer Res., **18**, 853-856.

Rubner, M. (1902): Gesetze des Energieverbrauches bei der Ernährung, pp.280-281, F. Deuticke, Leipzig, Wien.

Spector, W.S. (1956): Constants for estimating surface area: mammals. In handbook of biological data, p.175, W. B. Saunders Company, Philadelphia.

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

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, 73-5, Fort Rucker, Alabama.