



# Vet Integr Sci

## Veterinary Integrative Sciences

ISSN: 2629-9968 (online)

Website: www.vet.cmu.ac.th/cmvj



### Research article

## Correlation between body condition score and body composition in a rat model for obesity research

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### Abstract

The incidences of obesity-associated chronic diseases are increasing worldwide. Research into the causes of obesity as well as potential treatments has highlighted the crucial role of preclinical studies using animal models. Rats are one of the most widely used species in obesity research. However, even with decades of research in both genetically obese rats and diet-induced obese rat models, definitive criteria to practically classify levels of obesity in the rat are not well established. The current study proposes new criteria modified from a 5-point body condition score (BCS) using in an animal health monitoring system and added a half-point scale to extend the range of body weight associated with subcutaneous fat deposition. The modified criteria were tested and compared with body composition from dual energy X-ray absorptiometry scans and selected adipose tissue weights. The results showed that the modified body condition scale was highly correlated with fat deposition in the rat body, particularly the visceral and inguinal fat pads. Both pads were closely related to changes in some specific landmarks used for the scale determination. These findings should extrapolate to obese rats in other models, with the advantage that data classified in BCS can pair the animal data with human body mass index. This will enhance the value of information from preclinical studies to design and predict outcomes of subsequent human clinical trials.

**Keywords:** *Body condition score, body composition, obesity*

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**Article history;** received manuscript: 6 May 2022,  
revised manuscript: 29 June 2022,  
accepted manuscript: 4 July 2022,  
published online: 11 July 2022

**Academic editor;** Korakot Nganvongpanit



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## INTRODUCTION

Animal models play a crucial role in obesity research to unravel the complicated causes of obesity, which include usually polygenic rather than monogenic and include genetics, lifestyle, physiological disorders, aging, menopause and diet (Pradhan, 2007; Ross et al., 2016; Leeners et al., 2017; Archer et al., 2018). They are also a useful tool to demonstrate efficacy of pharmaceutical agents and treatments of obesity. However, there are limited measures to monitor changes in body adiposity in animals. Body weight, body scans and body condition scoring each have both advantages and disadvantages.

Body weight is routinely used as a part of laboratory animal husbandry. It is possible to generate consistent data if the practice has been done accurately. However, weight gain will not correlate with body adiposity when confounding factors such as tumor mass, organ enlargement or intraperitoneal fluid accumulation are present (Hickman and Swan, 2010). In addition, factors such as sex, age, body frame size, and pregnancy status can change the reference weight (Ullman-Cullere and Foltz, 1999). In addition, body weight alone cannot be used as a measurement of obesity because obesity is determined by the ratio of weight and height as body mass index (BMI) in humans. Moreover, body weight cannot be used to compare the degree of obesity across different species of animals.

Dual energy X-ray absorptiometry (DXA) is widely used to assess body composition as well as bone mineral density in human research. By utilizing two levels of X-ray energy the scanner is able to segregate body fat mass from lean mass, thus providing a more accurate adiposity measurement than body weight alone can (Rothney et al., 2009). However, DXA scanners are rarely available in most animal facilities.

Body condition scoring has been successfully used in health monitoring programs of various animal species such as cows (O'Boyle et al., 2006), cats (Teng et al., 2018), sheep (Keinprecht et al., 2016), and non-human primates (Clingerman and Summers, 2012) as well as of laboratory animals. Body condition scoring is a subjective technique of evaluating body fat distribution performed by estimating the body contour and palpating through the bony protuberances along the lumbar spine, hips and tail. For rodents, the criteria of this technique has been established only for mice but it should apply for use in rats as well, based on the fact that both species have fat pad distribution in the same pattern (Chilliard, 1993). Body condition (BC) scales for mice normally range from 1-5 with one score increments (Foltz and Ullman-Cullere, 1999). The midrange score of 3 represents the optimal condition; lower scores represent poor condition and higher scores represent excessive fat deposition.

This 5-level score is appropriate and practical for routine health monitoring but not for obesity classification in research. This is because the experimental animals, which are genetically modified obese rats or rats with diet-induced obesity, tend to put on more weight much more quickly than normal rats. Thus, there is no strong evidence to support the reliability of standard BC scoring for obesity research, and there are no published data describing whether the fat distribution pattern in experimentally obese rats is similar to normal rats. It was speculated that body weight of the rats in this study would be higher than the normative value of rat's body weight. Therefore,

the aim of this study was to establish and optimize a BC scoring system for obese rats by comparing data on the rats' physical condition with the information from DXA scans. To accomplish this, the standard mouse BC scale was modified into a 1-5 scale with half-score increments, and each score was provided with a new description.

## MATERIALS AND METHODS

### Animal

The animals examined in this study were also used to investigate the effects of mussel meat on obesity-induced osteoarthritis rats. The groups of the animals were determined by the influential factors on obesity (high fat-high sugar diets and/or ovariectomy). Only data from this project related to the objective of this article are reported here. Ninety six female SD rats with eight-week age were obtained from the small animal production unit of Massey University, Palmerston North, New Zealand and the experiment was carried on in the same animal facility. The animal room environment was set at  $22\pm 1^{\circ}\text{C}$ , 45-55% humidity and 12/12 hour light-dark cycle throughout the study. One week acclimatization period was provided before initiating the study.

The rats were singly housed in conventional cages with heat-treated aspen-chip bedding. To maintain the social behavior of the animals, clear hard-plastic cages (RE Walters, Australia) with high-top wire lids were used so that the rats could stand upright and visualize each other. Food, water, and general health status was checked and recorded daily. Body weights were measured weekly using a 2-digit balance (Sartorius, USA).

The rats received standard chow diets until 12 weeks old before being introduced to the experimental diets. Filtered water in nipple bottles was provided ad lib. Clean water bottles, cages and fresh bedding were provided weekly, with the cages and other equipment cleaned using a mechanical cage washer with the last-step-heating process to eliminate cross contamination. The room and floor were cleaned daily and sanitized weekly with disinfectant. The facility is compliant with Massey University and New Zealand laboratory animal welfare standards. This animal study was approved by Massey University Animal Ethics Committee (protocol number MUAEC approval 16/112), and met or exceeded all laboratory animal care and welfare standards for New Zealand.

### Experimental procedure

Ninety six female rats age 12 weeks were equally allocated ( $n=24$ ) into these test groups: (1) normal diet; (2) normal diet plus mussel meat; (3) High fat-high sugar diet (HFHS); (4) HFHS plus mussel meat. Normal diets contained 5% sucrose, 5% soy oil, and; HFHS diets contained 30% sucrose, 15% soy oil, 15% lard, and 15% casein-based protein (Specialty Feeds, Glen Forrest, Western Australia), and fed the test diet for 36-38 weeks. At the age of 20 weeks old, half of each group (12 of 24 rats) was assigned to ovariectomy procedure as described by Kruger and Morel (Kruger and Morel, 2016). At the end of the study, BC was scored by one examiner prior to performing DXA scans. Rats were deeply anesthetized with 50  $\mu\text{l}/100\text{ g}$  body weight of a cocktail composed of 0.5 ml ketamine + 0.2 ml acepromazine + 0.1 ml xylazine

and 0.2 ml sterile water, administered via intraperitoneal route using a 25 g hypodermic needle and 1ml-syringe. This provided an adequate immobilization for DXA scanning within 30 mins. After humane euthanasia via exsanguination and induction of pneumothorax, all fat pads were harvested separately and weighed during the necropsy process using a 3-digit balance.

### Dual energy X-ray absorptiometry (DXA)

Rat's body composition was measured using high-resolution whole-body scans by a Hologic Discovery, a rat whole body software (Hologic Inc., Bedford, MA, USA). Quality-control checks were performed before scanning the animals, and the acceptance of coefficient of variance was 0.98 to 1.01. Anesthetized rats were placed on an acrylic platform of uniform 1.5-inch (3.75-centimeter) thickness, positioned supine. Each scan took 10-15 min in which the animals remained under the anesthesia effect. Scans were analyzed and values for lean mass, fat mass, total mass, and percent fat were recorded into a spreadsheet.

### Body condition scoring

Data from three rats were excluded on the advice of the attending veterinarian as the consequences of a hernia from an incomplete wound closure, an overgrown tumor and weight loss/failure to thrive. A total of 93 female rats aged 48-50 weeks old were assessed. In accordance with the objective of obesity classification, the scale here is only determined from 2.5-5 (normal condition to obesity). The categories below normal conditions (underweight) are not described here due to the lack of such cases in this study.

Score 2.5 Rat is lean

- Segmentation of vertebral column easily palpable
- Thin flesh cover over dorsal pelvis, small amount of subcutaneous fat
- Sacrum, iliac crest and ischial tuberosity are prominent.
- Segmentation of caudal vertebrae is palpable.

Score 3.0 Rat is well-conditioned

- Segmentation of vertebral column easily palpable
- Moderate subcutaneous fat store over pelvis.
- Sacrum, iliac crest and ischial tuberosity are palpable with pressure
- Spinous process of caudal vertebrae palpable

Score 3.5 Rat is slightly overweight.

- Segmentation of vertebral column palpable with pressure
- Sacrum, iliac crest and ischial tuberosity are not palpable
- Spinous process of caudal vertebrae is not palpable
- Moderate fat store around pubis-tail base (V-shaped bottom)

Score 4.0 Rat is overweight.

- Segmentation of vertebral column palpable with pressure
- Sacrum, iliac crest and ischial tuberosity are not palpable
- Spinous process of caudal vertebrae is not palpable
- Overwhelming fat store around pubis-tail base (U-shaped bottom)
- Moderate subcutaneous fat around flanks

Score 4.5 Rat is obese

- Segmentation of vertebral column is not palpable
- Sacrum, iliac crest and ischial tuberosity are not palpable
- Spinous process of caudal vertebrae is not palpable
- Overwhelming fat store around pubis-tail base (U-shaped bottom)
- Overwhelming subcutaneous fat around flanks

Score 5.0 Rat is extremely obese

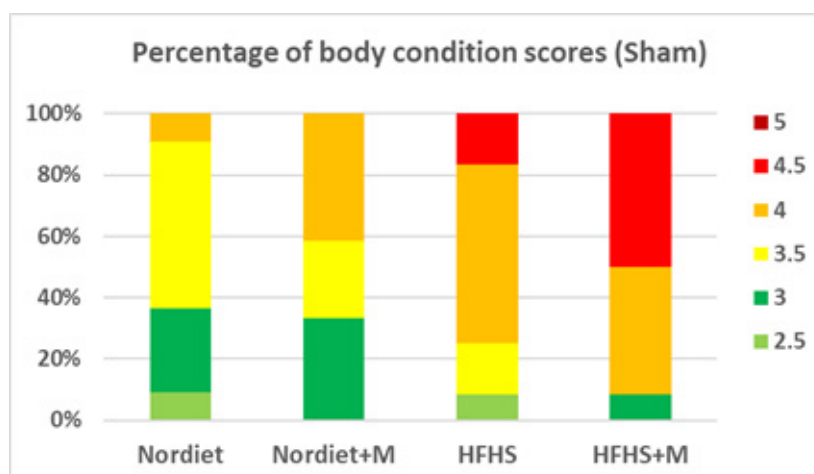
- Segmentation of vertebral column is not palpable
- Sacrum, iliac crest and ischial tuberosity are not palpable
- Spinous process of caudal vertebrae is not palpable
- Overwhelming fat store around pubis-tail base (U-shaped bottom)
- Overwhelming subcutaneous fat covers all scapular region

### Data analysis

Body weight, fat depots and body composition measurements from DXA scan were pooled across all experimental groups and categorized into each BCS (2.5, 3.0, 3.5, 4.0, 4.5 and 5.0). Pearson correlation was used to evaluate the relationship between body weight or BCS with the body composition from DXA scans. Means were calculated and plotted into graphs for visualizing the fitness of curves. The statistics software IBM SPSS (version 24) was used to analyze all data in this study. A p-value <0.5 was considered statistically significant.

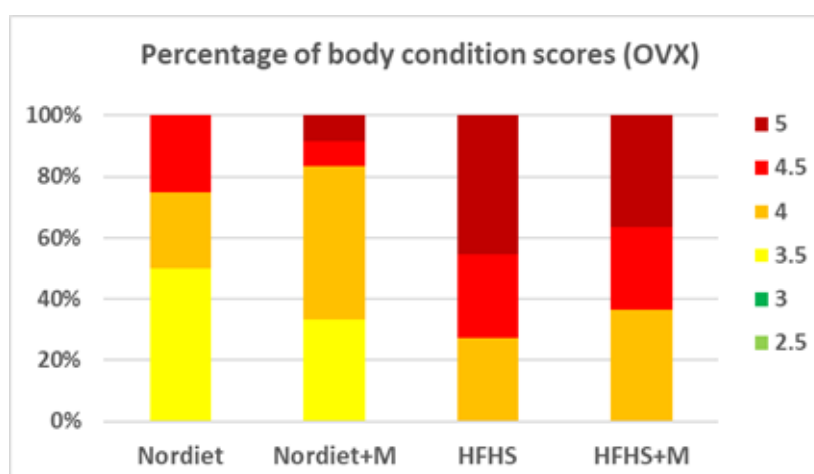
## RESULTS

The purpose of the main experiment was to investigate the effects of various factors driving obesity. The data were obtained to determine how the information from BCS may be of benefit in animal studies of obesity research. As can be seen in [Figure 1](#), >50% of the sham rats on the normal formula diet group were scored 3.5 (slightly overweight). Including mussel meat in the normal diet shifted the proportion of animals in the overweight category with a score of 4.0 from <10% to >40%, although the proportion of rats in the well-conditioned category (score of 3.0) did not change as this is known that outbred stock rats are composed of diet resistant group and obesity prone group ([Levin et al., 1989](#); [Collins et al., 2016](#)). A HFHS diet, as expected, induced obesity as the almost 60% expansion of the score 4.0 and introduced another one-upper score (4.5) into this group; we have previously shown that a HFHS diet is more effective than high sugar diet alone on inducing obesity with metabolic syndrome ([Pranprawit et al., 2013](#)). Once again, adding mussel meat into HFHS diet caused a further increase in obese rats (score 4.5) to 50%. This information shows that both a HFHS diet and the inclusion of mussel meat have an influence on weight gain.



**Figure 1** Percentage of body condition scores (sham) The effects of high-fat-high sugar diet and of mussel meat on obesity incidence in ovary intact rats. Normal diet (Nordiet), normal diet plus mussel meat (Nordiet+M), High-fat-high-sugar diet (HFHS), and high-fat-high-sugar diet plus mussel meat (HFHS+M).

Data from the ovariectomized rats (Figure 2) showed a similar pattern between normal and HFHS diets but with an even more intensified obese condition. The intact rats in both normal diet groups showed no scores above 4.0 but up to 20% of the ovariectomized rats on the same diets were in two upper scores. The combination of ovariectomy and HFHS diet further exacerbated the condition, with all animals falling into the overweight, obese, or extremely obese categories. Interestingly, the inclusion of mussel meat in the diets of ovariectomized rats slightly reduced, rather than increasing, the proportion of obese (4.5-5 score) rats.



**Figure 2** Percentage of body condition scores (OVX) The effects of high-fat-high sugar diet and of mussel meat on obesity incidence in ovariectomized rats. Normal diet (Nordiet), normal diet plus mussel meat (Nordiet+M), High-fat-high-sugar diet (HFHS), and high-fat-high-sugar diet plus mussel meat (HFHS+M).



The lowest body weight of the rats was 320 g and the highest was 843.4 g. Only 10 rats (11%) were scored 2.5 and 3, which would be defined as the optimal condition, with mean weights of 322.20 g  $\pm$  3.11 and 356.02 g  $\pm$  13.41 respectively (Table 1). The next two categories (3.5 and 4.0) accounted for 59% of the population and covered the body weight range of 409.3-615.00 g. These two categories indicate the rats were overweight. The remaining 30% of the population occupied the two uppermost categories (4.5-5.0) and were obese (weight range=559.40-843.40 g).

**Table 1** Distribution of body weights and fat deposits by body condition score

Body condition score		Body and fat pad weight (3-digit balance)				
		Body weight (g)	Visceral fat (g)	Inguinal fat (g)	Scapular brown fat (g)	Visceral: inguinal ratio
<b>2.5</b> (n=2)	mean $\pm$ SD	322.20 $\pm$ 3.11	22.83 $\pm$ 4.67	4.34 $\pm$ 0.22	0.67 $\pm$ 0.05	5.26
	min-max	320.00-324.40	19.53-26.14	4.19-4.51	0.64-0.71	
<b>3</b> (n=8)	mean $\pm$ SD	356.02 $\pm$ 13.41	24.07 $\pm$ 4.10	7.04 $\pm$ 1.73	0.83 $\pm$ 0.15	3.41
	min-max	331.00-375.40	20.07-33.32	4.16-9.90	0.57-1.08	
<b>3.5</b> (n=21)	mean $\pm$ SD	444.98 $\pm$ 25.81	35.64 $\pm$ 7.97	12.35 $\pm$ 4.57	0.92 $\pm$ 0.16	2.88
	min-max	409.30-493.00	24.79-51.65	5.95-20.69	0.69-1.31	
<b>4</b> (n=34)	mean $\pm$ SD	523.84 $\pm$ 53.33	48.52 $\pm$ 10.46	20.37 $\pm$ 5.30	1.14 $\pm$ 0.22	2.38
	min-max	448.00-615.00	29.71-67.31	3.96-28.23	0.84-1.83	
<b>4.5</b> (n=18)	mean $\pm$ SD	623.11 $\pm$ 37.62	63.15 $\pm$ 10.29	29.66 $\pm$ 8.99	1.10 $\pm$ 0.23	2.13
	min-max	559.40-685.00	50.25-83.87	4.00-46.74	0.72-1.63	
<b>5.0</b> (n=10)	mean $\pm$ SD	725.21 $\pm$ 69.74	73.95 $\pm$ 12.81	46.87 $\pm$ 7.82	1.14 $\pm$ 0.22	1.57
	min-max	628.30-843.40	62.37-96.17	35.39-64.30	0.79-1.47	

The visceral fat including retroperitoneal and epididymal fat pad was harvested from the abdominal cavity while inguinal and subscapular brown fat pad were dissected from subcutaneous fat tissue. Inguinal fat covers the dorsolumbar region to the gluteal region (Chusyd et al., 2016). Subscapular brown fat, a butterfly-like shaped pad brown in color, is fitted in between the scapulae and underneath the subscapular white fat tissue. The visceral fat pad, which was the major white fat pad examined, accounted for 7-10% of total body weight in rats across BC 2.5-5.0 (Table 1). Inguinal fat was smaller and more variable, contributing 1.34-6.45% of body weight across BC 2.5-5.0. Subscapular brown fat pads were very small by comparison. The ratio of visceral fat to inguinal fat varied, with a mean ratio of 5.26:1 in BC 2.5 and 1.57:1 in BC 5.0.

DXA scans (Table 2) showed that mean percent body fat increased consecutively from 36.56% in BC 2.5 to 64.35% in BC 5.0. Table 3 shows the correlations of body weight or BCS with body composition. Generally, it was highly correlated with all parameters except lean mass and subscapular fat. Body weight measured using a standard balance matched the total mass measured by DXA scan (Pearson correlation value 0.999).

**Table 2** Distribution of body composition by DXA scans into body condition score

Body condition score		DXA scan measurement			
		Fat mass (g)	Lean mass (g)	Total mass (g)	% fat
<b>2.5</b> (n=2)	mean±SD	115.26±0.23	200.00±6.89	315.26±6.65	36.56±0.84
	min-max	115.09-115.43	195.13-204.88	310.56-319.97	35.97-37.14
<b>3</b> (n=8)	mean±SD	131.67±14.05	220.38±12.52	352.05±13.24	37.36±3.27
	min-max	118.15-163.34	202.64-238.72	330.89-374.31	33.65-44.63
<b>3.5</b> (n=21)	mean±SD	195.14±33.04	243.11±18.97	438.25±26.53	44.32±5.58
	min-max	139.04-246.01	210.38-278.70	394.95-483.73	33.33-53.90
<b>4</b> (n=34)	mean±SD	271.46±43.42	248.73±28.02	520.20±54.03	52.00±4.80
	min-max	187.65-346.07	200.35-306.19	438.39-614.51	40.26-59.91
<b>4.5</b> (n=18)	mean±SD	375.39±32.46	245.94±21.71	621.33±38.18	60.38±3.01
	min-max	326.37-426.25	212.57-289.02	558.75-683.23	55.61-65.67
<b>5</b> (n=10)	mean±SD	462.67±75.51	252.85±23.81	715.53±74.55	64.35±4.93
	min-max	325.63-584.36	217.94-296.20	621.83-842.20	52.37-69.38

**Table 3** Correlations of BCS and body weight with body composition and fat pad weight

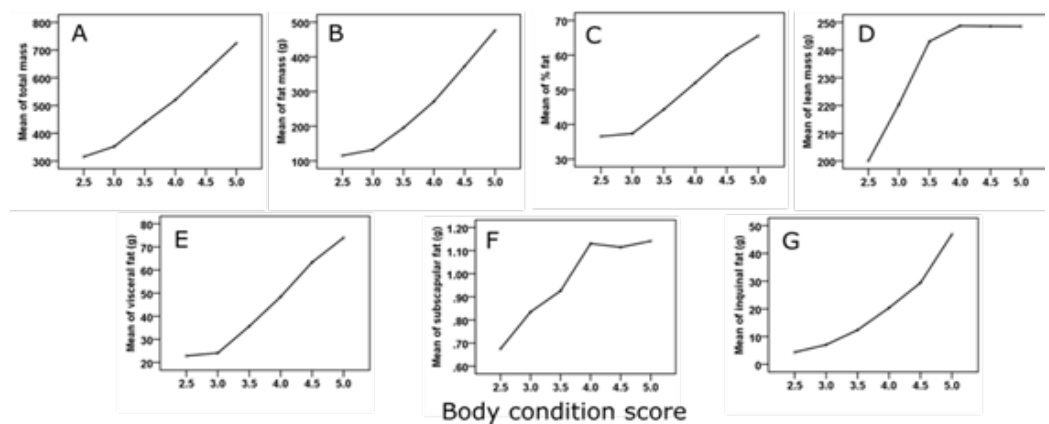
	BC score		Body weight	
	Pearson Correlation	Significance	Pearson Correlation	Significance
BC score	1		0.921	< 0.001
Body weight	0.921	< 0.001	1	
Fat mass	0.919	< 0.001	0.974	< 0.001
Lean mass	0.316	0.002	0.447	< 0.001
Total mass	0.921	< 0.001	0.999	< 0.001
% fat	0.882	< 0.001	0.877	< 0.001
Visceral fat	0.84	< 0.001	0.945	< 0.001
Inguinal fat	0.851	< 0.001	0.876	< 0.001
Subscapular brown fat	0.443	< 0.001	0.509	< 0.001

**Table 4** Correlations of fat mass and % fat from DXA scans with fat pad weight

	Fat mass		Percent fat	
	Pearson Correlation	Significance	Pearson Correlation	Significance
Visceral fat	0.924	< 0.001	0.844	< 0.001
Inguinal fat	0.898	< 0.001	0.833	< 0.001
Subscapular brown fat	0.477	< 0.001	0.477	< 0.001

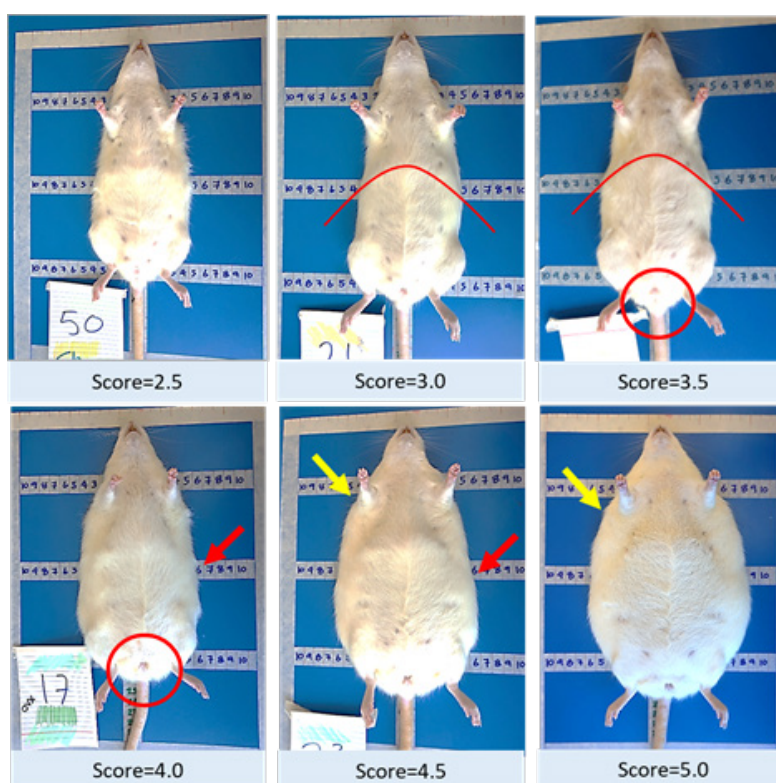


Similarly, BCS showed a high correlation with total mass (0.921) as well as other parameters. The five most highly correlated parameters were fat mass, total mass, percent fat, visceral fat and inguinal fat. Only lean mass and subscapular fat were less well correlated. Furthermore, the correlation of fat compositions analyzed from DXA scans with the fat pad harvested at necropsy is also highly correlated especially, visceral fat and inguinal fat (Table 4). However, the subscapular brown fat, a small portion of fat when compared with the whole body fat, has a low correlation coefficient with fat mass or percent fat. As shown in Figures 3A, 3B and 3C, the correlation curves of BCS with total mass or fat mass or percent fat fitted a near-perfect linear regression, except at the lowest score of 2.5. While this may indicate a poor correlation between the parameters at this score it is more likely due to insufficient sample size and thus requires further investigation. Conversely, lean mass and BCS showed a steep slope in the lower scores (2.5, 3.0 and 3.5) but a horizontal line in the three uppermost scores, indicating that lean mass proportionally increased under conditions of normal weight but not obesity.

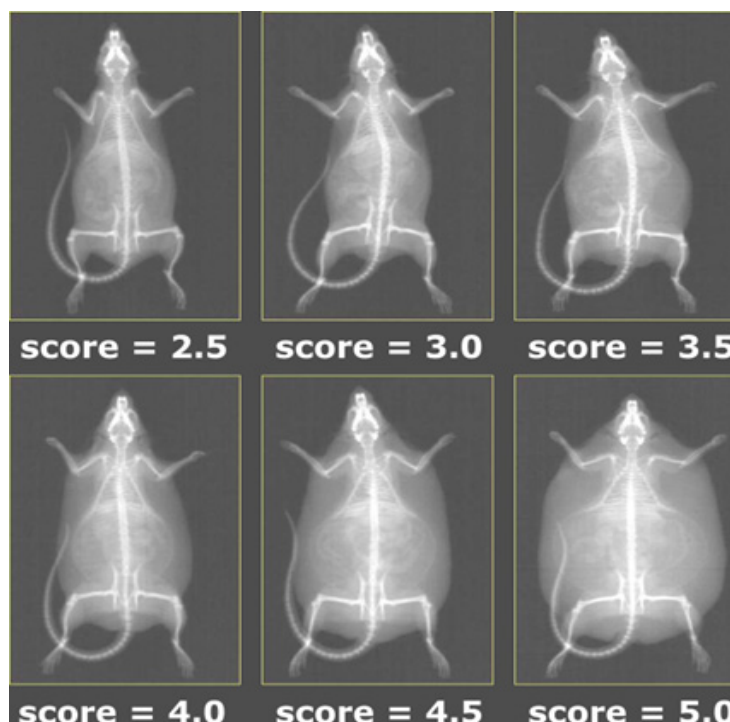


**Figure 3** Relationship between body composition and body condition score. Curves show relative trends of body condition score with various parameters of body composition from DXA scans (A) total mass, (B) fat mass, (C) percent fat and (D) lean mass and with individual fat pads (E) visceral fat, (F) subscapular brown fat and (G) inguinal fat.

Visual evidence of the correlation between subcutaneous fat and obesity are shown in a set of photographs (Figure 4), in which the red line demarcates the junction of rat's costal arch and upper abdomen. In BC 2.5, this line was obviously prominent with the abrupt recession of the abdomen. The line was less dominant, with smooth recession of the abdomen, in BC 3.0. From BC 3.5 onward, the recession of abdomen was replaced with abdominal contents due to expansion of visceral fat. A second anatomical landmark, used to discriminate BC 3.5 from 4.0, was at the tail base. This region was covered with subcutaneous fat tissue, which was connected to the inguinal fat pad. As fat volume increased, the contour of this region changed from a V shape to a U shape. The third landmark was the lateral abdominal line, visible as a straight line and running parallel along the body frame in BC 4.0. This line shifted outward at BC 4.5. The final landmark, near the scapular region, was used to identify BC 5 as the subcutaneous fat at this site overwhelmed the normal body contours. The images of rat's body surface also corroborated with the whole body DXA scans in Figure 5.



**Figure 4** Characterization of the rat body to match body condition score. Changing body contours spanning BC 2.5 - 5.0 by specific landmark: Red arcing lines labels the costal arch; red circles show the tail base area; red arrows indicate the lateral abdominal line; yellow arrows indicate subcutaneous fat around the scapular area.



**Figure 5** Rat's whole-body DXA scans on the series of modified body condition score.

## DISCUSSION

Body condition scoring has conventionally been using in laboratory rat health monitoring, with the grading protocol standardized from normal rats. According to the laboratory animal guideline, the normative value of adult rat body weight is 250-300 g in females and 300-500 g in males (Otto et al., 2015). However, the body weight of obese rats in the current study far exceeded those values. Therefore, the previous criteria do not cover the upper limit of obese rat weight. Indeed, if the BCS for routine health monitoring had been applied in to the animals in the current study, all of those with a body weight >500 g would have been scored in category 5. These data would be too skewed to represent a normal distribution. Therefore, a new set of body scoring criteria are needed for obese rat models.

One possible solution is to stratify body weight into many levels of interval scales; however, body weight alone is still not an ideal measure to explain obesity due to numerous confounding factors. We found that the higher body weight did not always result in a higher score using the BCS criteria described above, as obesity is a factor of fat distribution. The aim of this study was to modifying the BCS criteria to better fit obesity rat research. These findings may extrapolate to and be used with current BCS in animal health monitoring programs, which have a reliable justification in normal-weight animals.

The approach used in the current study was to adjust and modify the standard scores to be proportionally dependent on body weight, body composition or fat distribution pattern of rat body. Our findings demonstrated that body weight measured using conventional animal scales and body mass calculated from DXA scans (total mass) were highly correlated (Pearson correlation =0.999). Thus, as the two methods are comparable in term of accuracy and precision, interpretation of other tissue fractions from DXA scans in vivo should be reliable as those made by physical weighing ex vivo. DXA scans provides the information of whole body fat mass, lean mass, total mass and percent fat while weighing on scales gives more detail about the different fractions of fat tissue. Each method can stand alone, but each also complements the other.

The visceral fat harvested in the current study consisted of the combination of epididymal fat and retroperitoneal fat from the abdominal cavity, whereas the two subcutaneous fat pads harvested and assessed separately were the inguinal white fat and subscapular brown fat. The result showed that both BCS and body weight had an equivalent correlation with those parameters; body weight had a slightly higher correlation coefficient with most parameters, except for percent fat (BCS=0.882 vs body weight=0.877).

Comparison of visceral, inguinal, and subscapular fat pads showed that visceral and inguinal fat increased at the same rate as total fat mass and percent fat. In particular, the curve correlating inguinal fat and BCS fitted the trend line across the spectrum, from the lowest point (BC 2.5) to extreme obesity (BC 5.0). Interestingly, the ratio of visceral/inguinal fat was reduced as BCS increased. Moreover, visceral fat only increased ~4 fold from BC 2.5-5.0 while inguinal fat increased ~10 fold. This suggests that subcutaneous fat bore a greater responsibility than visceral fat in the weight gain observed in the obese rats, and verified the study hypothesis that the distribution pattern of

subcutaneous fat in particular could be used fat to classify the levels of obesity in rats and other animals. Inguinal fat, a combined characteristic between white and brown fat, showed that it has more influence on body condition scoring than the white visceral fat. However, the key player of obese animal models still remains on the visceral fat which is mainly responsible for the pathogenic factors in metabolic disorders (Minihane et al., 2015; Todendi et al., 2016; Ellulu et al., 2017)

In contrast to the white adipose tissue deposits, the change in subscapular brown fat pad weight was irregular and did not demonstrate a proportional increase at the higher scores (4.0-5.0). This corroborates other published studies that show subscapular brown fat is not linked with overall body fat deposition (Tatsuhiro et al., 2002; Reed et al., 2011). This is due to the fact that brown adipose tissue's function is to metabolize fatty acid to generate heat (Ràfols, 2014; Warner et al., 2016)

Weight ranges in humans are defined by body mass index (BMI), which is a value derived from the relation of weight and height. The World Health Organization has divided human BMI into four major categories; underweight (<18.5), normal weight (18.5-24.9), over weight (25-29.9) and obese (>30) (World Health Organization, 2004), with variations based on ethnicity or country. For example, Hong Kong defines BMI 25-30 as "overweight-moderate obese" (World Health Organization, 2004) while Japan categorizes four levels of obesity (Shiwaku et al., 2004). This demonstrates the necessity of adjusting BMI ranges to fit individual populations.

BMI categories are associated with defined health risks. Overweight and underweight people have higher mortality rates than those of normal weight (Whitlock et al., 2009). Similarly, high BMI subjects have a greater incidence of type 2 diabetes (Lim et al., 2007). These examples support the importance of including BMI in public health data. Further, information from preclinical studies that mimic the general human population is useful in epidemiological prediction modelling.

For research purposes, obesity can manipulate in animals by three main methods which are monogenic, polygenic, and surgical models. Single mutation of ob gene or db gene leading to leptin deficiency in "ob/ob mice" or the leptin receptor deficiency in "db/db mice" results in early-onset obesity with hyperphagia, low energy expenditure, and insulin resistance (Nilsson et al., 2012). Many more details on this monogenic obesity model were described by Lutz and Woods (2012). Polygenic models, on the contrary associated with many genes to affect body metabolism are demonstrated in high energy diet feeding rats. As the result of polygenic factors and heterogeneity of outbred stock rats, one out of three rats can display obesity resistant phenotype (Collins et al., 2016). This model has been frequently used in obesity research as the convenience of the protocol and representing the public health problem. The surgical model using ovariectomy in female subjects can mimic the menopause condition in women as estrogen loss induces increased food consumption at the transitional period (Siriarchavatana et al., 2022) and worsens metabolic and lipid profiles (Siriarchavatana et al., 2020; Abshirini et al., 2021). Boonyapakorn et al. (2019) demonstrated that lacks of testosterone in castrated dogs resulted in significant weight gain due to the reduction of physical activities but this manipulation did not affect metabolic profiles or cardiac function.



The HFHS diet in the current study increased the obese population. Similarly, ovariectomy in rats, mimicking the post-menopausal period in women, dramatically impacted obesity frequency. The combination of both factors demonstrated an additive effect. The BCS of the current study runs from BC 2.5 to 5.0, which spans the spectrum from normal weight to obese. These BCS scales could be stratified into three categories to match the human body ranges: normal weight (BCS 2.5-3.0), overweight (3.5-4.0) and obese (4.5-5.0). This would provide a visual and measurable prediction about the independent and combined consequences of consuming HFHS diet and of estrogen loss, which drive the healthy normal weight population (light-dark green zone) into obese population (light-dark red zone).

In conclusion, this study demonstrates the relationship between a HFHS diet and menopause on obesity development. The modified BC scales with clear landmark scoring parameters correlated significantly with visceral and inguinal fat pads weights; the only exception was the poor correlation between the score and the visceral fat pad normal weight animals, possibly due to insufficient sample size. The limitation of this study is that the range of BCS did not cover underweight populations. However, for rat obesity studies, the BCS presented in this study present an alternative method to identify and categorize overweight and obese animals that is both cost-effective and non-invasive.

## ACKNOWLEDGEMENTS

This study was partially funded by a grant from the New Zealand Ministry of Primary Industries High Value Nutrition research program and was carried out as a collaboration between Massey University, Cawthron Institute, and Sanford Ltd. Massey University also provided financial support through a doctoral scholarship. The authors thank Anne Broomfield and Corrin Hulls for the main responsibility in surgical procedure and DXA scanning; Gabrielle Plimmer and Shampa De for supporting in necropsy.

## AUTHOR CONTRIBUTIONS

Conceptualization, F.M.W., M.C.K., P.S.; methodology, F.M.W., M.C.K. and P.S.; formal analysis, P.S.; investigation, P.S., and F.M.W.; resources, F.M.W. and M.C.K.; data curation, P.S.; writing—original draft preparation, P.S.; writing—review and editing, F.M.W., M.C.K., and P.S.; visualization, P.S.; supervision, F.M.W. and M.C.K.; project administration, F.M.W.; funding acquisition, M.C.K.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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#### How to cite this article;

Parkpoom Siriarchavatana, Marlana C.Kruger and Frances M.Wolber. Correlation between body condition score and body composition in a rat model for obesity research. *Veterinary Integrative Sciences.* 2022; 20(3): 531- 545.

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