

Use of a Body Condition Score Technique to Assess Health Status in a Rat Model of Polycystic Kidney Disease

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Simple and noninvasive methods of assessing health and wellbeing are valuable when performing clinical evaluation of rodents used in biomedical research. Body condition score (BCS) techniques have been described for a variety of species, including mice. This method can be a sensitive objective assessment of weight loss in animal models where organ enlargement, ascites, or tumor development may mask weight loss. Although deposition of fat is similar in rats and mice, the mouse BCS technique has not been characterized in rats. Here we used the Han:SPRD rat model for polycystic kidney disease to characterize the effectiveness of the mouse BCS scale when applied to rats. This study showed a positive correlation between BCS score and renal function and a negative correlation between weight and renal function, supporting the use of BCS as an effective, noninvasive method of health assessment in this rat model. Our results also demonstrate that the BCS scale described for mice required a slight modification to capture the delay in fat deposition over the lumbar vertebrae in obese animals.

Abbreviation: BCS, body condition score.

Simple, rapid, and noninvasive methods for assessing health status and wellbeing are valuable when monitoring rats used in research studies where anorexia, wasting, dehydration, and death are potential complications. Typically, veterinarians and research staff rely on a panel of clinical observations, such as measurement of body weight, observations of deviations from normal behavioral parameters, and examination of physical appearance, to evaluate the health status and wellbeing of rats used in biomedical research. Depression of response to external stimuli, poor coat condition, nasal or ocular discharge, depressed appetite with associated dehydration and weight loss, and hunched posture are examples of observations that have been proposed as standard indicators of declining health.^{8,10,14,20,24} The preferred clinical indicators are those that can be expressed by using scales that indicate the deviation from normal, minimizing subjective health assessment.^{10,26-28}

Weight loss, measured as a percentage decline from initial weight or as compared with the weight of age-matched conspecifics, is a commonly used criterion of euthanasia.^{4,8,10,18,28} Depending on the study, weight loss may not be a sensitive indicator of animal health. Studies that create physiologic changes, such as intraperitoneal fluid retention or tumor growth, may mask weight loss by interfering with the identification of loss of fat stores and muscle mass.¹⁶ Reference weights can vary according to factors such as sex and age.⁸ Interpretation of collected weights can also be biased due to factors such as equipment error, observer variation, and time of day.¹⁷ The body condition score (BCS) technique in mice is performed by observing and palpating the flesh over the bony protuberances of the hips and lumbar spine.^{6,8,16,28} Similar techniques have been shown to more accurately reflect the condition and nutritional state of the

patient in a number of species, including dairy cows,^{15,21} beef cows,^{11,19} dogs,^{5,13} cats,^{9,25} sheep,²² and nonhuman primates.³ Use of these scales has also been reported to be insensitive to interobserver variability.^{7,16,28}

Although empirical reports use the body condition scoring technique described for mice as part of rodent monitoring programs,¹ the technique has only been validated for mice. Because rodents in the family Muridae are predisposed to carry their fat stores in similar regions (for example intraabdominal and dorsal pelvis),² we expected that the techniques described for mice would be applicable for rats. We used a rat model of genetic polycystic kidney disease, the Han:SPRD strain, to characterize the usefulness of the BCS technique in rats. The results of this study demonstrated that a decline in BCS correlated with an increase in renal function parameters, whereas body weight stayed consistent as the increase in kidney mass masked muscular wasting. The results of this study further showed that fat deposition in rats varied from that expressed in mice, especially in overweight and obese animals. We have used the results of the current study to modify the BCS categories described for mice^{8,28} for application to rats.

Materials and Methods

Rats. This study used a local colony of rats that develop polycystic kidney disease (Han:SPRD). Rats that are homozygous for this trait develop severe disease and typically die within 4 mo of age²³ and were not used for this study. Rats that are heterozygous for this trait have an increased chance of death caused by renal failure between 12 to 18 mo of age.²³ For this study, heterozygous rats were compared with their wildtype littermates, which do not develop polycystic renal disease. All rats were at least 12 mo old.

Husbandry. The Veterinary Medical Unit is operated and maintained by the Research and Development Service of the VA Medical Center (Portland, OR). The animal care facilities meet the requirements of all applicable federal regulations, includ-

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ing the US Department of Agriculture, National Institutes of Health Office of Laboratory Animal Welfare, and Department of Veterans Affairs. In addition, the animal facilities have been fully AAALAC-accredited since 1973. All rats in this study were pair-housed with a conspecific of the same sex in polycarbonate shoebox cages with heat-treated hardwood bedding (Sani-Chips, PJ Murphy Forest Products, Montville, NJ). The cages were topped with filter tops and kept on ventilated racks (Ancare, Belmore, NY). Standard operating procedures for the animal facility required that all cages be changed at least twice weekly in a laminar flow changing station (Lab Products, Seaford, DE). The animal caretakers wore gloves while changing the cages and disinfected their hands with diluted bleach solution between cages. Soiled cages were sanitized in a mechanical cage washer with a final rinse temperature of 180 °F (82 °C) and autoclaved prior to reuse. The rooms were kept on a 12:12-h light:dark cycle, and animals were provided rodent chow (LabDiet 5010, Purina Mills International, St Louis, MO) and tap water ad libitum. For the final 6 mo of the study, female wild-type rats at least 12 mo old were fed a high-fat breeder chow (LabDiet 5021, Purina) to encourage obesity. Temperature and humidity were maintained at 72 °F (22 °C) and at least 30%, respectively. Rat colonies were screened quarterly for rat coronavirus, Sendai virus, pneumonia virus of mice, parvovirus, *Mycoplasma pulmonis*, encephalomyelitis virus, lymphocytic choriomeningitis virus, pinworms, and mites by exposing sentinels to dirty bedding. At the time of this study, the facility was free of all of the listed pathogens.

Experimental procedure. To ensure evaluation of sufficient rats during this project while reducing the overall use of animals, a 2-tiered system of assessment was used. For the first tier, an observer scored and weighed all of the heterozygous Han:SPRD rats and wild-type rats once each week. In consultation with the investigator, the observer assigned individual rats for terminal assessment (second tier) so that each BCS category was populated with at least 4, but no more than 6, rats of each sex.

To assign a rat for terminal assessment, the observer notified the terminal assessment technicians that a rat was ready for evaluation. To remove potential bias from their assessment, these technicians were blinded to the observer scores of individual animals and the collected data. If multiple technicians assessed a rat, the assessments occurred within 2 h of each other, and each technician was blinded to the observations of the other. To perform the terminal collection, all technicians used the same set of scales to weigh rats; the data were recorded. The technicians also palpated the hips and lumbar spine of the rat and recorded a BCS, as described later. For euthanasia, the technician anesthetized the rat with isoflurane (2% to 5% inhaled to effect; Flurane, Fort Dodge, Ames, IA) and performed cardiocentesis to collect samples for CBC and serum chemistry panels. The carcass was weighed again and the information recorded. The kidneys and liver were removed and weighed separately. All data collected by the technicians during the terminal collection were recorded on a form that was returned to the observer, who compiled the data for analysis. In cases where the assessment of BCS score assigned by the observer differed from that assigned by the technician(s), the value assigned by the technician was used for data analysis. However, interobserver variability between the observers and technicians was not statistically significant (data not shown).

The CBC and serum chemistry panels for the male rats primarily were submitted to an outside laboratory (Antech, Portland, OR), whereas those for the female rats predominantly were performed inhouse (Abaxis, Union City, CA). During the transition from the outside laboratory to the inhouse analysis,



Figure 1. To obtain the body condition score, the rat is allowed to rest on the wire top. The vertebrae are assessed by palpation of the lumbar spine. The pelvic bones are assessed by palpation of the hips (illustrated).

samples from individual animals were run by using both methods to ensure consistency. Comparisons of results from individual animals by using both methods failed to reveal any statistically significant differences (data not shown).

Body condition scoring. Each rat was palpated over the lumbar spine and pelvic bones (Figure 1). The whole-integer BCS criteria previously established for mice^{8,28} were used initially to assign a BCS to each rat (Figure 2); scores were not qualified by addition of + or – because our recent studies with mice have shown greater interobservational variability when these gradations are used.^{16,17} As an internal control, analysis of the BCS assigned by the observer was compared with that assigned by the technician; interobserver variability was not statistically significant (data not shown).

Early in the study, identifying a rat that had a BCS of 4 was difficult because they appeared to develop considerable fat deposition over the pelvic bones that eventually spread to the lumbar vertebrae instead of first developing fat deposition over the lumbar vertebrae, as reported in mice.^{8,28} The BCS scale subsequently was modified slightly to capture this delay in fat deposition over the lumbar vertebrae (Figure 2), the observer and technicians together scored additional rats from the larger group to reach consensus on the appropriate score assignment, and the 4 rats that had been assessed by using the previous method were excluded from the data set.

Data analysis. The average BCS and weight were calculated for each rat by summing the results recorded by each technician and dividing it by the number of technicians who assessed the rat. The adjusted carcass weight was calculated by subtracting the weight of the liver and kidneys from the carcass weight of each rat. The data for male rats were analyzed separately from data for female rats. Single-factor ANOVA (Excel, Microsoft, Seattle, WA) was used to compare the averages of carcass weight, adjusted carcass weight, BUN, and creatinine to BCS. Statistical significance was defined as a *P* value of 0.05.

Results

A total of 31 male and 24 female rats were assessed as part of this study. The BCS 1 and 2 groups each comprised 6 male and 5 female rats. The BCS 3 group consisted of 6 male and 6 female rats, the BCS 4 group contained 6 male and 4 female

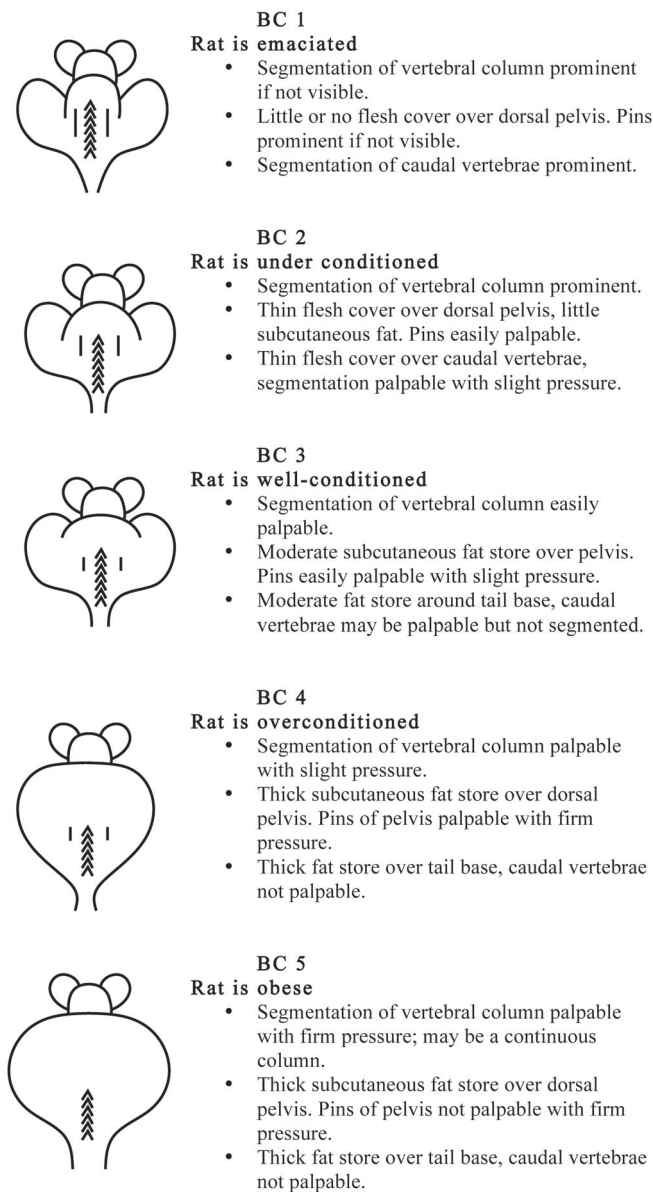


Figure 2. Demonstration of palpation findings for the assessment of rat BCS. This chart was developed as a comparison of previously published evaluation criteria for mouse BCS.^{8,28} Fat deposition in the rats was more reliably assessed through the palpation of the fat overlying the dorsal pelvic protuberances instead of that overlying the vertebral column, as is recommended for mice.

rats, and the BCS 5 group comprised 7 male and 4 female rats. The results of the statistical analysis are summarized in Table 1.

For male rats, BCS and carcass weight ($P = 0.100$) did not differ significantly, but there was significant difference between BCS and adjusted carcass weight ($P < 0.05$). For the females, the differences between BCS and both CW and ACW were significant ($P < 0.05$). Male rats also showed significant ($P < 0.05$) differences between BCS and BUN and creatinine levels. In contrast, BCS and creatinine did not differ ($P = 0.290$) in female rats, but there was significant ($P < 0.05$) difference between BCS and BUN.

Discussion

The data from the male rats supported the conclusion that the use of BCS to assess the health status of rats that develop polycystic kidney disease is more sensitive than weight and

Table 1. Results of the single-factor ANOVA of each variable compared with BCS

	<i>P</i>	
	Male rats	Female rats
Carcass weight	0.100233	0.000837 ^a
Adjusted carcass weight	0.026372 ^a	0.000487 ^a
BUN	4.06×10^{-7a}	0.021064 ^a
Creatinine	5.29×10^{-6a}	0.290222

^a $P < 0.05$

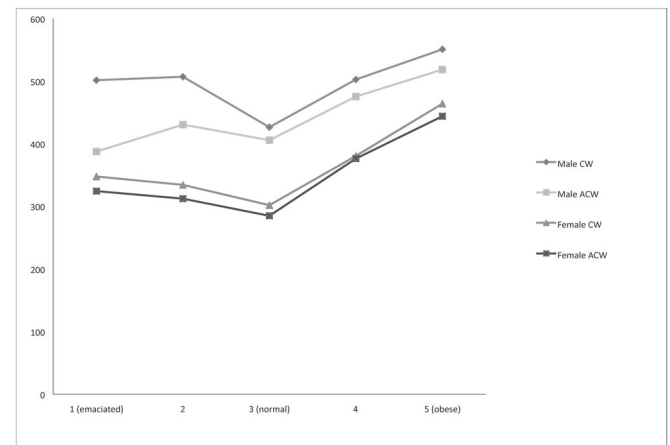


Figure 3. Comparison of carcass weights (CW) and adjusted carcass weights (ACW) of male and female rats. Male carcass weight stays consistent regardless of BCS until the large, polycystic kidneys are removed. Therefore, mild weight gain associated with decline in BCS may assist in the diagnosis of polycystic kidneys. Because the female rats do not develop large renal cysts, the difference between carcass weight and adjusted carcass weight is not reflected in their data.

accurately reflects the health status of the individual animal as assessed by serum chemistry analysis. Rats that were developing polycystic kidney disease increased in weight until comparable to obese animals (Figure 3). However, when the weights of the kidneys and liver were subtracted from the carcass weight, the muscle loss and emaciation expressed by the rats with polycystic kidney disease was apparent (Figure 3). The decrease in BCS also correlated well with the increases in BUN and creatinine (Figure 4), demonstrating that monitoring of BCS could be used to reduce the need for frequent serial blood samples when assessing the health of these animals. For example, daily assessment of BCS would be a noninvasive way to regularly monitor animal health, especially when coupled with blood analysis weekly or every 2 wk to confirm the findings from palpation.

Compared with the findings from male rats, the data from the female rats were not as clear because they did not develop the severe extremes of emaciation and obesity as easily as did the male rats. Necropsy of the BCS1 and 2 female rats demonstrated gross evidence of polycystic kidney disease, but instead of the large fluid-filled cysts that were common in heterozygous male Han:SPRD rats (Figure 2), the cysts were small and did not cause marked organ enlargement with associated muscle wasting. In addition, the female rats did not clinically appear to be as ill (as demonstrated by subjective criteria such as hunched posture, decreased responsiveness) as the male rats. This difference in clinical presentation has been described.²³ However, the serum chemistry values demonstrated that the rats that were scored at BCS 2 and 1 were more likely to be expressing evidence of renal disease according to the BUN level than were those that

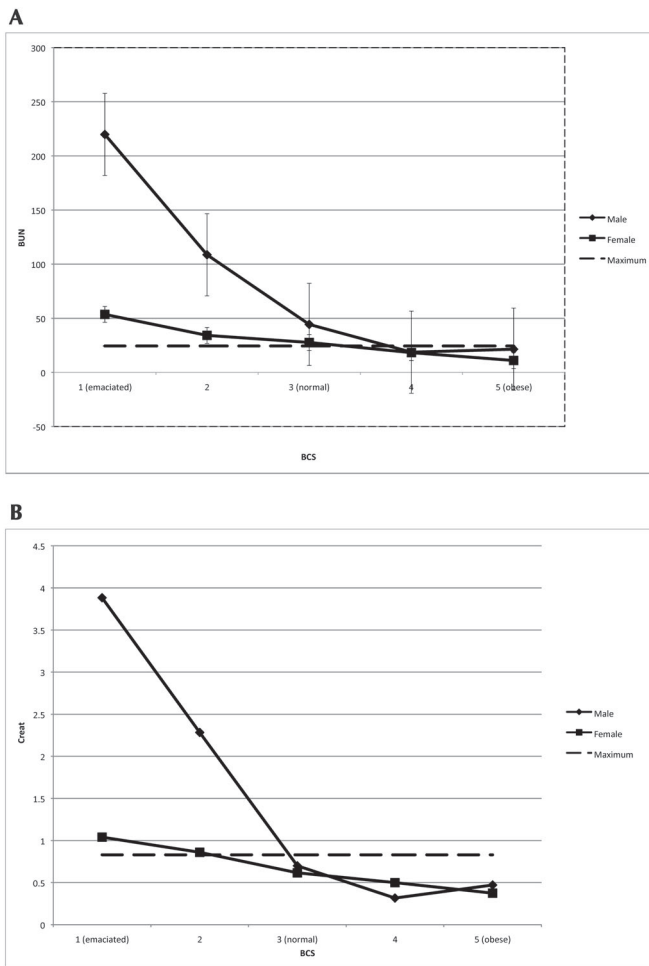


Figure 4. As BCS declined, the rats expressed increases in BUN and creatinine, 2 factors used to indicate compromise of renal function. (A) Average BUN levels for male and female rats at each BCS as compared with the published maximum.¹² (B) Average creatinine levels for male and female rats at each BCS as compared with the published maximum.¹²

were scored at BCS 4 or 5 (Table 1). This pattern supports our conclusion that the use of BCS would still be valuable for the health assessment of female heterozygous Han:SPRD rats.

Although the male rats readily developed obesity on the standard rodent chow, the majority of the female rats remained at a normal BCS. Study completion was delayed 6 mo, and the female rats were placed on a high-fat breeder chow in an attempt to encourage obesity. However, although more than 20 rats were maintained on the high-calorie feed for this time, only 8 developed sufficient fat stores to be characterized as BCS 4 or 5. Why the female rats were resistant to the development of obesity is unknown.

This study showed that BCS is a sensitive indicator of progressive illness in the rat polycystic kidney disease (Han:SPRD) model. Although fat deposition is reported to be similar between mice and rats, this study revealed that the specific deposition of fat over the lumbar vertebrae was delayed in this stock of rats as compared with mice. In light of this finding, we have modified the mouse BCS evaluation criteria to reflect the characteristics displayed by this stock of rats. Further study should be pursued to determine whether the rat BCS scale presented here is applicable to other rat stocks and models of disease, such as carcinogen studies.

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