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Body Condition Scoring: A Rapid and Accurate Method for Assessing Health Status in Mice

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Practical, rapid, noninvasive methods for assessing health status and establishing endpoints are needed in mouse experiments in which wasting and death are a potential endpoint, including aging and toxicology studies, ascites production, and phenotype analysis in mutant mouse colonies. Current methods for assessing the health status of a mouse and establishing endpoints might include observation of behavior, assessment of physical appearance, and measurement of body weight (BW). Behavioral parameters include observation of unprovoked behavior and responses to external stimuli. Classic changes in physical appearance include exophthalmia or enophthalmia (bulging or sunken eyes, respectively), nasal or ocular discharge, rough coat, and hunched back. These observations, as well as additional ones particular to an experimental procedure or to the genetic makeup of an animal, have been suggested as standard indicators of ill health (1–4). These clinical indicators can be scored as degree-of-deviation-from-normal, thereby allowing an animal to be monitored over time as health declines (1–4). Decreased food and water consumption is an important sign of deteriorating health (4), which generally results in loss of BW; support for using weight loss as an indicator of poor health in rodents comes from the study by Redgate et al. (5). They determined that 7 or more consecutive days of weight loss in central nervous system tumor-bearing rats correlated well with irreversible progression to death. However, Beynen et al. (2) found that observation of behavior and physical appearance was largely ineffective for discriminating between gallstone-bearing mice and healthy controls, and weight loss was significantly different between the two groups of mice for the males but not the females. They concluded that response to palpation of the right hypochondrium (i.e., signs of a painful response) was the best indicator of gallstones for males and females.

Twenty percent loss of rodent BW or prolonged weight loss (progressing to an emaciated state) are generally established criteria for euthanasia (3, 6). However, there are practical problems with the measurement of BW, which may not yield an accurate measure of fat stores and muscle mass, because reduction of fat stores and muscle mass (as measured by BW) is masked if weight loss is displaced by tumor growth, organ enlargement, or intraperitoneal fluid accumulation. Furthermore, the reference weight of a healthy mouse will vary according to sex, age, body frame size, and in females, pregnancy status. Scoring body condition (BC) by observing the amount of flesh covering bony protuberances is largely independent of the aforementioned confounding variables. The technique of BC scoring as a method for evaluating animal condition and nutritional state has already been validated for use in dairy cows (7–9), beef cows (10), goats (11), sheep (12), and horses (13). In cows, BC score correlates with the amount of subcutaneous fat stores (7, 10) and nutritional status (14). Additionally, abnormal loss of BC was found to be an indicator of mastitis in dairy cows (15).

Our goals were to evaluate the accuracy of the BC scoring technique in assessing the health of mice that have organ enlargement concurrent with declining health; compare the accuracy of this method with that of using BW for assessing health status in these animals; and determine the interobserver reliability of the BC scoring technique. P- and E-Selectin double deficient ($P/E^{-/-}$) mice were chosen for this study because they are susceptible to opportunistic bacterial infections and as their health status declines, their salivary glands, mandibular and superficial cervical lymph nodes, and spleen markedly enlarge (16). Additionally, $P/E^{-/-}$ mice have defects in leukocyte extravasation at sites of inflammation, and their white blood cell (WBC) count is known to increase with declining health (16, 17). For evaluating the health status of these mice, we used the techniques of BC scoring and BW. White blood cell count and adjusted body weight (ABW: BW minus the mass of tissues prone to enlargement with declining health in P/E-/- mice, the salivary glands, mandibular and superficial cervical lymph nodes, and spleen) were selected as additional indices against which to evaluate the accuracy of BW and BC scoring for assessing health.

Animals: Female and male $P/E^{-/-}$ mice with a mixed 129/Sv x C57BL/6 background were evaluated. This genetically altered line was created and maintained at the laboratory of Richard O. Hynes at the Massachusetts Institute of Technology. These mice were housed in polycarbonate Micro-Isolator™ cages (Lab Products, Inc., Seaford, Del.) containing a heat-treated hardwood chip bedding (Sani-Chips; P.J. Murphy Forest Products Corp., Montville, N.J.). Mice were viral antibody free, and free of *Helicobacter hepaticus* and ecto- and endoparasites, as determined by vendor health reports and sentinel monitoring by examination of skin scrapings, fecal flotation samples, and anal tape impressions. All mice were given ad libitum access to the same commercial pelleted mouse diet

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(PROLAB 3000; Purina Mills Inc., St. Louis, Mo.) and filtered city water. Prior to the experiment, mice of the same sex were housed in groups of one to five. The cages were located in a room with controlled lighting (light, 0630 to 1830 h; dark, 0630 to 1830 h; temperature 21±1°C and relative humidity (50 ± 10%). Animal procedures were done with approval of the Institutional Animal Care and Use Committee.

Experimental procedure: Twenty-four male and twenty-nine female P/E$^{-/-}$ mice, aged 6.5 to 8 months, were selected from a larger population according to BC score (BCS) so that an even distribution of BC scores would be represented in the male and female experimental groups. For uniformity, only virgin females were used in the study.

Mice were housed singly for the period of evaluation; the cages were arranged randomly (using a random numbers table) and scored, without knowledge of any health measure, for body condition. Within 2 days of BC scoring, mice were euthanized by inhalation of CO_2. Once respiration had ceased, mice were removed from the chamber, and blood was collected via cardiac puncture for complete blood count. Body weight was measured, then salivary glands and mandibular and superficial cervical lymph nodes were removed as a unit and weighed. Finally, the spleen was removed and weighed. White blood cell counts were determined by use of an automatic cell counter (Hemavet 800; CDC Technologies, Oxford, Conn.).

Body-condition score: Body-condition scores ranged from 1 to 5 (Figure 1); in this experiment, each numerical score was further subdivided into positive (+) and negative (-) categories that represent the gradations of BCS characteristics. Thus, scores were 1, 1+, 2-, 2, 2+, 3-, 3, 3+, 4-, 4, 4+, 5-, and 5. In a mouse of BC1 status, muscular wasting was advanced (the gluteal and biceps femoris were severely atrophied) and fat deposits were gone, resulting in extremely prominent skeletal structure with sharp-edged protuberances (the wing of the ileum, the sacrum, and the spinous processes) and deep indentations between vertebral processes. A mouse of BC1+ status had the beginnings of flesh cover, making bony protuberances less sharp. A mouse of BC2- status had distinct (not sharp) bony protuberances, and the indentations between vertebra were shallower. A mouse with BC2 status was still underconditioned; the segmentation of the vertebral column and the dorsal pelvic bones remained distinct with slightly rounded ridges; the indentation between the vertebral processes had filled in by half. In a mouse with BC2+ status the bony ridges were round, and the indentation between the vertebra was less pronounced. In a mouse with BC3- status, only the ends of the bony processes were distinct (flesh had filled the once deep indentations between vertebra). A mouse of BC3 status was in optimal condition, and the segmented vertebral column was readily palpable with slight pressure; however, only the edges of two to three sacral vertebra were distinct. In a mouse with BC3+ status these sacral vertebra were less noticeable, and only one was distinct in the mouse with BC4- status. A mouse of BC4 status was well-fleshed, and the spine was a continuous column (unless palpated with firm pressure). In the mouse of BC4+ status, only a few sacral vertebra could be palpated with firm pressure, and in the mouse of BC5- status, the individual vertebrae were not palpable, but the vertebral column could be palpated with slight pressure. For status BC5, the mouse was obese (smooth and bulky); the continuous column prominent at BC4 status blended with the hindquarters of the mouse due to overlying fat deposits and could be palpated only with firm pressure.

A veterinarian and two veterinary technicians evaluated the mice; these individuals were experienced in the clinical application of BC scoring, but did not practice body condition scoring as a group to confer agreement on BC assessment prior to the experiment or to establish a uniform palpation technique. Body condition score was assessed by placing the mouse on a flat surface (i.e., wire bar lid) and holding the base of the tail with the thumb and index finger of one hand, and scoring the degree of flesh and fat cover either by running the little finger of the same hand over the sacroiliac region or by palpating the sacroiliac region with the fingers of the opposite hand. The assessment of BC by a veterinarian/technician using BC scoring in their daily routine for 1 month or more takes less than 30 sec.

Statistical analysis: Each BCS was assigned a numerical value. For a BCS with minus value, 0.33 was subtracted from the whole number value of the score, and for BCS with a positive value, 0.33 was added to the whole number score. Thus, scores were 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.33, 3.67, 4.00, 4.33, 4.67, and 5.00. The BCS for each mouse was calculated by adding the BCSs from three observers and dividing by three. Spearman's rank correlation coefficients (r_{ranks}) were calculated as described by Glass and Hopkins (18). Methods for inter-observer analysis were adapted from the work of Ferguson et al. (8).

Results

Descriptive statistics for the male and female experimental populations are given in Table 1. Comparison between the use of BCS and BW as methods for assessing the health status of P/E$^{-/-}$ mice was made by calculating r_{ranks} between WBC count and BCS, BW, and ABW (adjusted body weight); these results are presented in Table 2. In the male mice, BCS, BW, and ABW correlated strongly with WBC count, although a noticeably higher correlation was apparent for BCS. Additionally, in the males, there was a strong correlation between BCS and BW ($r_{ranks} = 0.93$; $P < 0.05$). For the female mice of this study, BCS was significantly correlated with WBC count. In contrast, BW was not significantly correlated with WBC count. However, when the mass of salivary glands, mandibular and superficial cervical lymph nodes, and spleen was subtracted from the mouse's overall BW (represented as ABW), there was a significant correlation with WBC count. Furthermore, the correlation between BCS and BW ($r_{ranks} = 0.61$; $P < 0.05$) increased markedly when weight of these tissues was subtracted from BW ($r_{ranks} = 0.73$; $P < 0.05$). In the female mice, the combined weight of salivary glands, mandibular and superficial cervical lymph nodes, and spleen ranged from 0.9 to 9.8% of the mouse's total BW. In the male mice, the combined weight of these tissues ranged from 0.9 to 8.2% of BW (data not shown).

Finally, the correlation among observer BCSs was high, ranging from $r_{ranks} = 0.90$ to 0.94 ($P < 0.05$) for male and from $r_{ranks} = 0.84$ to 0.87 ($P < 0.05$) for female mice.

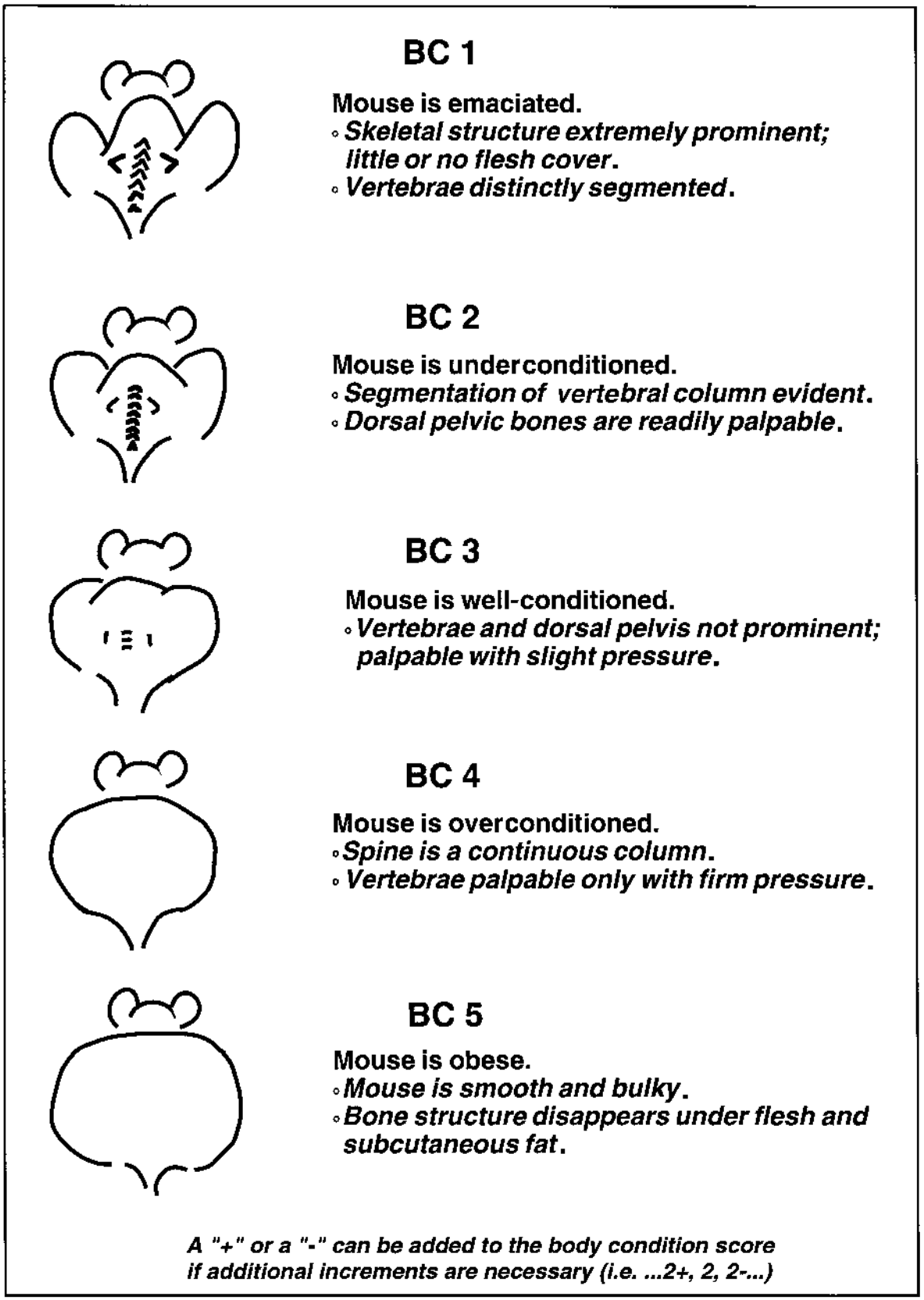

Figure 1. Line drawings and descriptions of body-condition (BC) scoring.

Table 1. Descriptive statistics for male and female P/E$^{-/-}$ mice

	Males (n = 24)[a]			Females (n = 29)		
Variable	Mean	SD	Range	Mean	SD	Range
WBC (x mm^3)	60.4	43.9	15.4–151.0	51.6	34.1	14.7–133.0
BW (g)	38.7	8.12	7.4–53.6	29.6	4.6	22.9–43.5
ABW (g)	37.6	8.6	25.8–52.9	28.6	4.7	22.7–42.8
BCS[b]	3.2	1.1	1.6–5.0	2.7	0.5	1.9–3.5

[a]One male mouse with a BCS = 1.0 and moribund at the time of blood sampling was eliminated from the data set. WBC were abnormally low.
[b]Average body condition score (BCS: 1 = emaciated to 5 = obese).
WBC = white blood cells; BW = body weight; ABW = adjusted body weight (body weight minus the combined mass of salivary glands, mandibular and superficial cervical lymph nodes, and spleen); BCS = average of three observers' body condition scores.

Table 2. Spearman's rank correlation coefficients between WBC count and selected health indices for male and female P/E$^{-/-}$ mice

WBC (x mm^3)	Males (n = 24)	Females (n = 29)
BCS	-0.78[a]	-0.76[a]
BW (g)	-0.65[a]	-0.35[b]
ABW (g)	-0.67[a]	-0.45[a]

[a]$P < 0.05$, significant.
[b]Not significant at $P < 0.05$.
See Table 1 for key.

In this study, BCS was documented to correlate to the selected index of the health status (WBC count) in the male and female experimental groups, proving BC scoring an accurate indicator of health status. In the male mice, WBC count was also documented to correlate well with BW. In contrast, for the female mice, a nonsignificant correlation between WBC count and BW indicated that BW was uninformative for monitoring the health status of female mice with enlarged organs or distended tissues. However, when the weight of these tissues (salivary glands, mandibular and superficial cervical lymph nodes, and spleen) was subtracted from BW, represented by ABW, the mouse's adjusted body weight was documented to correlate significantly with WBC count. This serves to illustrate the ability of enlarged or distended tissues and organs to mask loss of fat stores and muscle mass as measured by body weight. Furthermore, the strong correlation between BCS and BW in the male mice verifies that BC scoring accurately assesses the health status of these mice by assessing fat stores and muscle mass, as measured by BW, in a system where BW correlates with health status.

Scoring for BC has numerous advantages over the measure of BW for assessing the health status of mice, including minimizing the potential for the spread of disease and maximizing use of health-monitoring techniques, because BCS is more rapidly and practically assessed than is measurement of BW, when mice must be moved to a common procedure room or a shared scale must to be brought for body weight measure. Additionally, a reference weight is not needed to calculate the percentage of weight loss for assessment of the mouse's health status. Furthermore, BC scoring should prove useful on clinical rounds or in the definition of a novel phenotype where the possibility of tumor growth, intraperitoneal fluid accumulation, or enlargement/distention of tissues or organs remains a possibility (or is known to develop) in a sick mouse. Guidelines for the clinical use of BCS and investigator training are outlined by Foltz and Ullman-Culleré (19).

The differences in the ability of BW to assess the health status of male and female P/E$^{-/-}$ mice is likely due to higher percentage of BW represented by enlarged/distended organs for a given BCS in the females. Two parameters likely contribute to this: smaller frame size and lower average BW of female mice, and slightly greater increase in tissue mass in response to disease, compared with male mice. Variables in these parameters contribute to the masking of losses in fat stores and muscle mass as measured by BW. It should also be noted that WBC count proved an accurate linear index of health status due to the homogeneity of the population studied and the characteristics of the P/E$^{-/-}$ phenotype (relatedness of WBC count and health status may vary for other knockout or transgenic mice).

The BC scoring technique needs to be formally evaluated for application in tumor and aging studies as well as ascites production and the variety of phenotypes found in mutant mouse colonies. In addition, further studies developing and evaluating the accuracy of health assessment tools need to be made, with particular attention of potential sex differences in the general response to deteriorating health. The greater the number of well-developed health-assessment tools available, the more effectively one can follow progression of disease in the mouse. This will aid in defining transgenic phenotypes, monitoring experiments, and establishing meaningful endpoints; it will also reduce the number of experimental animals necessary due to unexpected data loss because of ineffective methods for identifying and monitoring ill mice. Body-condition scoring should prove to be an important technique in assessing the health status of mice.

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