Scoring body condition in the ferret: development of a method based on comparative analysis of condition scoring methods in the cat.

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Abstract

To evaluate the body condition of animals, various methodologies exist, including morphometric methods as BCS, fBMI and MMS and the chemical-analytical methods BIA and DEXA. However, up to this day no method has been developed or validated for the evaluation of the body condition of the ferret. In order to develop a BCS-system for the ferret, first a literature review was performed to compare existing methods in cats, following which a BCS method was developed for the ferret for use in practice. To develop the BCS-chart, 41 ferrets were visually inspected and evaluated on the palpability of different bone processes. In addition, morphometric measurements as body length, belly circumference, ribcage circumference and a leg index measurements were also taken. Using these measurements, a BCS-chart was developed that enables ferrets to be classified as obese, underweight or in optimal condition. Although further research will be needed to validate this BCS-chart, it is expected to serve as a valuable tool for assessing body condition of ferrets for both pet owners and veterinarians.

Keywords

Ferret, body condition, BCS, evaluation of body condition.

Review methodology

For this thesis, the databases Pupmed, Cab abstracts and Scopus have been searched. The keywords used for the search are listed in table 1. Combinations of the keywords were made within categories and between categories, usually combining a keyword for a specific category with an animal species. The keywords for the individual project were combined with the keyword 'ferret'. Google was used while searching the keywords 'ggplot2' and 'glmulti' for data analyses purposes. Articles were selected based upon title and abstract descriptions. Articles validating and/or using different techniques to evaluate body condition were eventually used in the thesis. If possible, reviews were avoided. Only when no regular articles could be found, reviews were used. Some articles were found by reading the references of selected articles or reviews.

Table 1: searched keywords per category

subject researched	Keywords
Cat	Cat, felis
Ferret	Ferret
Rabbit	Rabbit
Horse	Horse, equine
Cow	Cow, cattle
Other species	laboratory animals, pets
General information	Diagnosing obesity, body condition, photograph, body condition score system, scoring system, body condition tool, obesity, body fat, body mass index, measurement of body composition
Introduction	Model, diseases, common diseases, obesity, ferrets as laboratory animals
DEXA	Dual energy x ray absorptiometry, DEXA, DXA, beam hardening, validation, cross calibration, precision, accuracy, radiation dose, validation phantom
Body condition score	BCS, body condition score system
MMS	Estimating lean body mass, muscle mass score, muscle mass, muscle condition score, muscle wasting, prognosis
Morphometric measurements	Morphometric methods, morphometric techniques, zoometric methods, zoometric index, zoometry, body measurements, body fat index
BIA	Bio impedance monitoring, bioelectrical impedance analysis, multifrequency bioelectrical impedance analysis, bioelectrical impedance, bioimpedance phase angle, MFI BIA
ultrasound	ultrasonography, ultrasound, ultrasonic fat meters
Individual project	Anatomy, body weight, weight, body condition (combined with ferret keywords)
Individual project: data analysis	Glmmulti, ggplot2

Abbreviations

Abbreviation	Full meaning
95% CI	95% confidence interval
BC	Belly circumference
BCS	Body condition score
BCS-chart	Body condition score chart
BMC	bone mineral content
BF%	body fat percentages
BIA	Bioelectrical impedance analysis
BW	Body weight
CV	Correlation of variance
DBL	Dorsal body length
DEXA	Dual energy X-ray absorptiometry
ECW	Extracellular water
fBMI	Feline body mass index
FM	fat mass
ICW	Intracellular water
LBM	lean body mass
LIM	Leg index measurement
MF-BIA	Multi frequency – BIA
MMS	Muscle mass score
OR	Odds ratio
PA	Phase angle
R	Resistance
RC	Ribcage circumference
R_e	Extracellular water resistance
R_∞	Total body water resistance
SF-BIA	Single frequency-BIA
SFL	Subcutaneous fat layer
S.H.A.P.E.	Size, Health and Physical Evaluation
TBW	Total body water
VBL	Ventral body length
Xc	Reactance
Z	Impedance

Introduction

The ferret (*Mustela putorius furo*) is a domesticated carnivorous animal that is most likely an descendant of the European Polecat (*Mustela putorius putorius*). It is believed that ferrets have been domesticated for around 2000 years now, even though they were already mentioned by Aristophanes and Aristotle 450 and 350 BC (1).

Over the last century, ferrets have become increasingly popular as pets across the world. Estimations of the number of pet ferrets kept in the US range from 1 million to 7-10 million ferrets (2–4). Although no exact information is available for the general European situation, in the Netherlands 20.000 to 30.000 ferrets are kept as pets and hunting animals (5). These numbers are likely to be similar in the rest of Europe.

Working ferrets have been used for centuries to hunt wild rabbits in a practice known as ferreting (1). Nets are placed over the rabbit holes while the ferrets hunt them out. The ferreter is then able to humanely dispose the ferret (6).

Aside from their popularity for hunting and as companion animals, ferrets are also used as laboratory animals for biomedical research (3,7,8). As laboratory animals, ferrets are being used as a model for human viral pathogens (influenza viruses), cardiovascular research, nutrition research and gastrointestinal disease among other researches (3,8–10). It has been estimated that around 1.1 million ferrets are being used as laboratory animals in the US (3). European numbers, however are not known.

An objective assessment of the body condition of the ferret can be very useful for veterinarians to keep track of ferret health. Just as any other animal, ferrets can develop a great array of diseases. Ferrets especially are prone to the development of tumours, cardiovascular, renal, and endocrine disorders (2,11). Gastrointestinal disease (e.g. Helicobacter associated gastritis) is also common (12). For many of these diseases, weight loss is the most prominent indicator of disease. In laboratory ferrets with experimental infections, this is no different (7). Laboratory ferrets are therefore weighed to assess their change in body condition in studies on, for example, viral disease (13). Although very rare, ferrets can also develop obesity related illnesses (14).

Being able to properly estimate the body condition is thus very important for both veterinarians, researchers and pet owners. The body condition can be scored and evaluated by different methods. For various animal species, e.g. dogs, cats, horses, cows and rabbits, multiple standardized methods have been developed and validated (15–19). These methods include BCS, morphometric measurements, DEXA scans and others. To the author's knowledge, a system to objectively evaluate the body condition of ferrets has not yet been developed or validated. Furthermore, it is known that pet owners often misperceive the body condition of their pets when they are in suboptimal condition (20,21). This will most likely also be the case for pet ferret owners. Therefore, in this pilot study, a first attempt to develop a method to objectively evaluate the body condition of the ferret in a clinical setting will be made.

Although nothing has been made for ferrets, lots of techniques to grade the body condition have been developed for cats, the domestic pet that resemble ferrets the best (17,22). (17,22). Hence, a method to objectively evaluate the body condition of the ferret in a clinical setting will be made, based upon the already existing techniques for the cat.

The first part of this thesis will provide an overview of the currently available techniques for determining body condition in cats. In the second part, the individual project, in which a method to evaluate the body condition is developed, will be described.

Part 1:Current available techniques for scoring the body condition in cats

The body condition of an animal is determined by the amount of body fat and muscle mass the animal possesses. Even though no real reference values are known, the cutoff values between lean and optimal weight are considered 80% lean body mass and 20% body fat (23–25). Obesity is often defined as having more than 25-35% body fat.

Techniques that evaluate an animals body condition can be subdivided in two different categories: techniques that evaluate body condition and techniques that estimate body composition. The golden standard, DEXA, can also be included in this last category, but will be described before the techniques that evaluate body condition, because it is used as a reference method. An overview of all described methods can be seen in Table 6.

The techniques will be compared to each other based on their general principles, reliability and practical availability and applicability. A reliable technique is both precise and accurate. The precision of a technique is subdivided into repeatability (intra-observer variability) and reproducibility (inter-observer variability) (26,27). When an method is precise, an observer will assign the same score to the same animal on separate occasions and another observer will agree with that score. Accuracy is defined as the ability of the method to predict the actual body condition of the animal, as measured by the gold standard (26).

The gold standard: DEXA

Although DEXA estimates the body composition and can thus also be discussed in the third chapter of this introduction, it is considered the gold standard for the evaluation of the body condition in alive humans[1] (29). The method is used as a reference method for validating other techniques in animals, suggesting that DEXA has become a gold standard for body composition measurements in alive animals as well (25,30–32). Therefore, this technique is discussed first. DEXA was originally developed to measure bone density and bone mineral content, but it’s potential to estimate body composition of humans and animals was quickly discovered (33). Since then, the technique has been widely used in humans, but also in laboratory- and companion animals for both purposes (34). The system measures fat mass (FM), body fat percentages (BF%), lean body mass (LBM) and bone mineral content (BMC), making it a three compartment method

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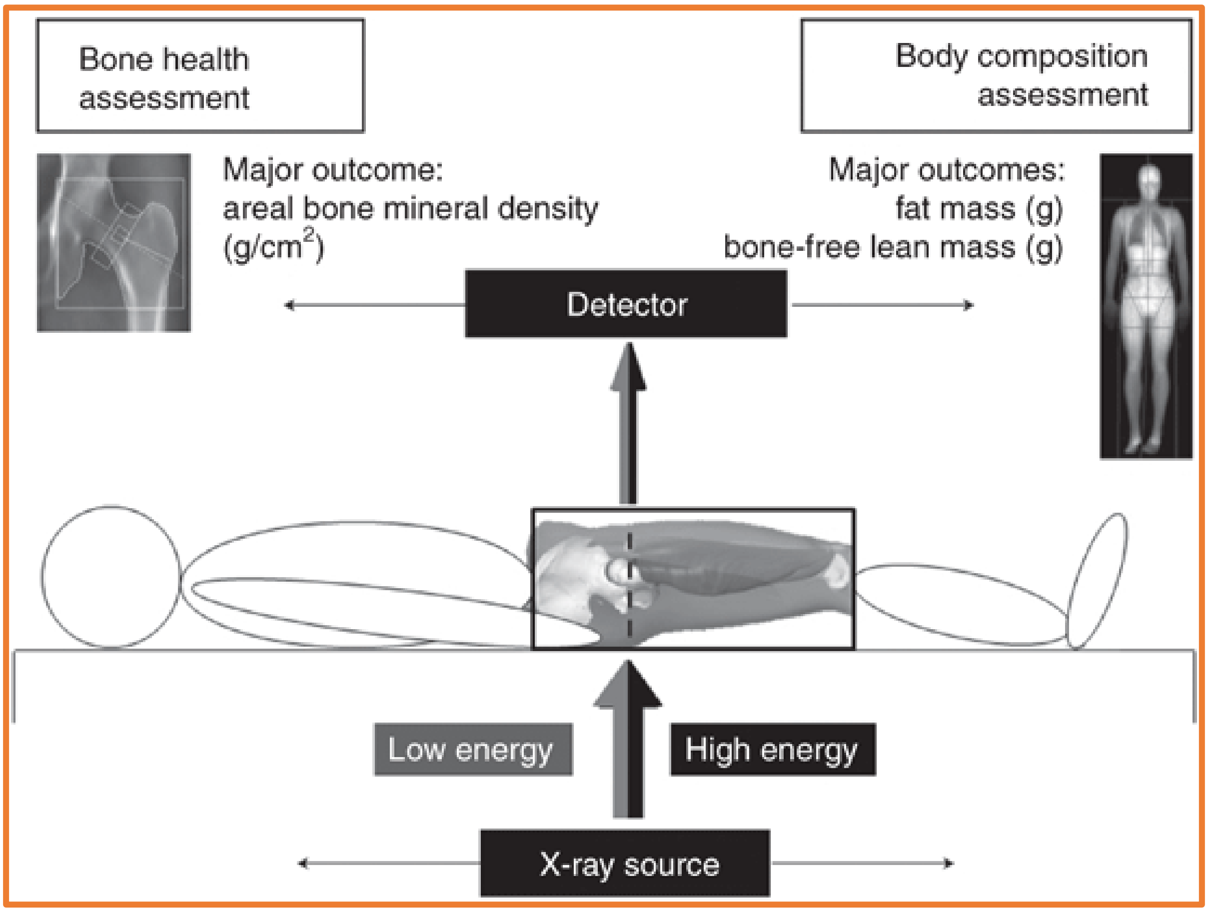
[1] DEXA is not considered to be the true gold standard for body composition measurements in animals. Chemical analysis is (28). However, to execute this technique the animal has to be euthanized, making the applicability of this technique rather limited. For this reason DEXA is discussed here as the gold standard method.

General principle

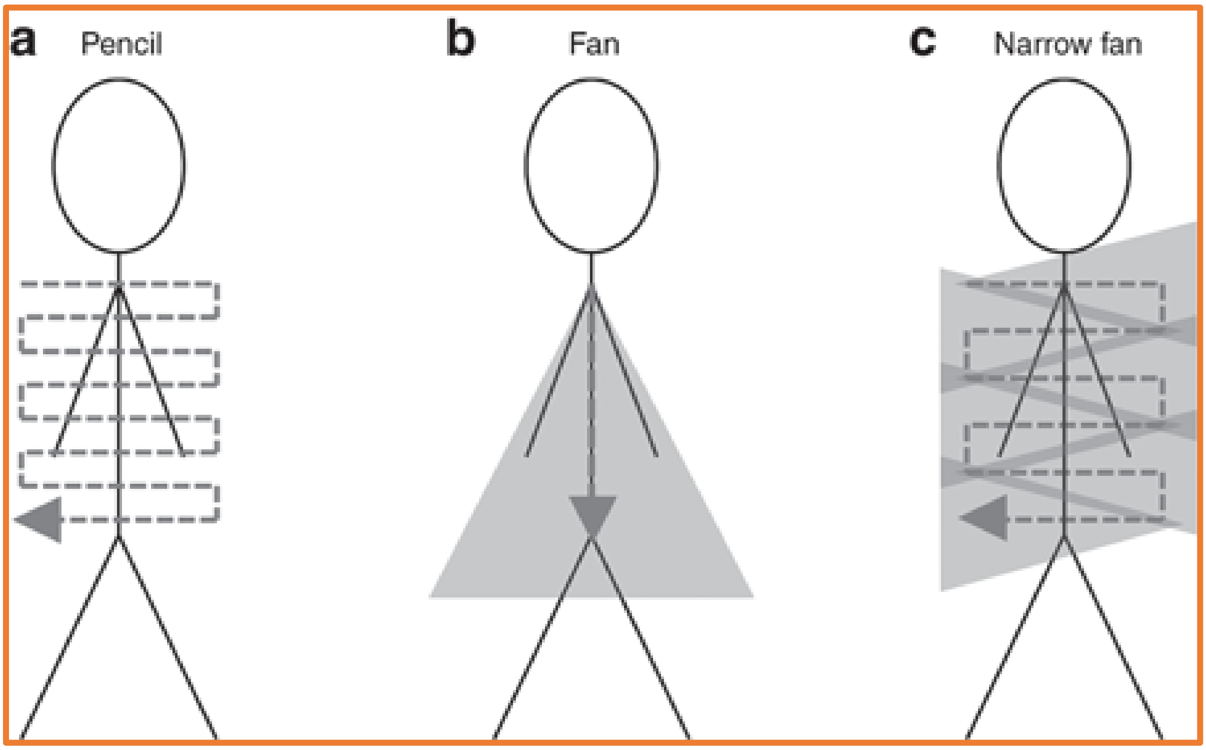
The technique uses an X-ray source (placed under the patient), that produces x-rays with two different photon energies. These X-rays are attenuated when passing through the body tissues, which is then measured by a detector placed above the patient (Figure 1). Each type of tissue (fat mass, lean mass and bone) attenuates the low and high frequency photon energies in varying degrees. Because the attenuation of bone, fat- and lean tissues are known, an estimate can be made from the total attenuation of soft body tissues to determine the amount of LBM and FM (35,36). In pixels containing only soft body tissues, the percentages LBM and FM are directly calculated. In the pixels containing bone and soft tissue, DEXA can only differentiate between the soft tissue and bone mineral content (37).

Figure 1: Principle of action of a DEXA scan. The same principle is used for both bone health assessments as body composition assessments (30).



Over the years, three generations of DEXA scans have been developed, i.e. the pencil-beam, fan-beam and - more recently - the narrow fan-beam densitometers (38,39). Pencil-beam densitometers scan the body in a rectilinear pattern (see Figure 2). However, this results in a relatively long scanning time of approximately 20 minutes (39). To improve scanning speed and resolution, the fan-bean densitometer was developed (see Figure 2). However, the shape of the fan beam causes a significant magnification of structures closer to the x-ray source compared to more peripherally located structures (38). As a result, the modern narrow fan beam densitometer was developed, which uses a combination of the two previously developed tactics to both minimize scanning time and reduce the magnification effect (see Figure 2) (39). Most of the DEXA scans in cats are performed with fan beam scanners (40–44). Only Zangi *et al.* (2013). and Speakman *et al.* (2001) used pencil beam scanners (33,36).

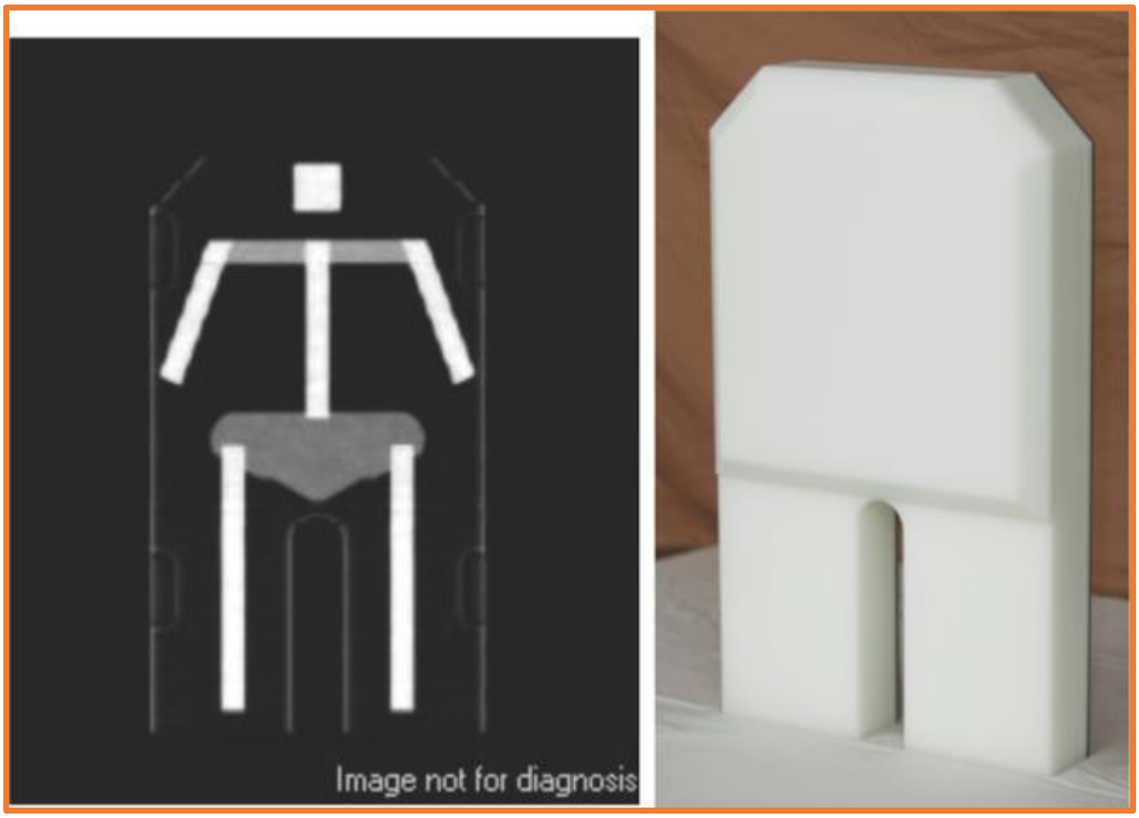
Figure 2: The different type of DEXA scanners and their scanning patterns (37).



Precision

DEXA scans need to be calibrated daily with calibration phantoms to ensure that values measured are repeatable on the same densitometer (45). These phantoms consist of materials that mimic the physiological range of body compositions, tissue thickness or bone density (46). Materials like acrylic and polyethylene are used to imitate fat mass. Adding bars of aluminium or calcium hydroxyapatite will make the phantom mimic bone also (Figure 3) (47). Specific capsuled spine phantoms for measuring BMD are on the marked. The phantoms can also be used for cross calibration, for which they will be scanned 10-30 times per scanner (45,47,48).

Figure 3 An example of a total body phantom. This is the modern BioClinica Body Composition Phantom (BBCP), developed by Bioclinica Inc, Princeton, NJ (47)



Reproducibility (variability between scanners)

The reproducibility of DEXA scans is variable if cross calibration is not applied. As described above, lots of devices and versions of densitometers have been made. When one wants to compare results from different DEXA devices, cross calibration is often necessary, because differences of 0.8% - 8.4% for LBM and FM estimates are seen (47,49–51). A 2% difference between measurements of FM, LBM and BF% and a 1% difference for BMD is deemed acceptable (47). However, even when an device is replaced with an device of the same brand and type, differences in FM, LBM and BF% measurements, before calibration can exceed 2% (47). These differences between results are resolved by recalibrating devices with phantoms or by using cross-calibration equations (45,47,52). Cross calibration equations are developed by scanning human subjects on both scanners. With these results an equation is developed to convert results from one scanner to be comparable with results from the other scanner (45). After calibration with phantoms, difference between scanners can be reduced to <0.05% (47).

Repeatability (variability within one scanner)

Three short-term repeatability studies in cats have been performed in which 4-10 consecutive scans were made (Table 2; (40,42,53). Borges *et al.* (2008) also investigated the effects of repositioning between scans (42). Of the various measurements made, the FM was found to be least repeatable in cats. In total 13 cats were scanned wit fan beam scanners, whereby Munday *et al.* (1994) and Borges *et al.* (2008) found a coefficient of variance (CV) for FM measurements of 5.58% and 7.7%, respectively (Table 2) (40,42). In contrast, Lauten *et al.* (2000) scanned only one cat with a pencil beam scanner for 6 consecutive times, finding a CV for FM of only 1.77% (53). When the cat is repositioned between scans with a fan beam scanner, the precision of BF% measurements decreases even further to a CV of 10.9% (42). LBM measurements in cats have a lower CV, as can be seen in Table 2 These results are comparable with humane literature (48,54).

The CV 's of Lauten *et al.* (1994) were all <1% (except for FM), indicating that the pencil beam densitometer may be more precise. Repositioning of the cat resulted in significantly higher values of CV with a fan beam densitometer, underlining the importance of using the exact same body placement as much as possible. Most cats are placed in dorsal/sternal recumbency on the DEXA densitometer (30,36,42,44,53). Cats are either placed with hind limbs and forelimbs extended caudal (42,53), with hind limbs extended caudal and forelimbs extended cranial (44) or with hind limbs and forelimbs extended cranial (30). A ventral recumbency is sporadically also used (25). It is not known which position produces the most consistent results.

Table 2: CV of DEXA BF% and LBM measurements in cats, as measured by different studies

Research	Number of cats	CV FM	CV LBM	Scanner type
no repositioning between scans: CATS				
Lauten *et al.* (2000) **(53)**	1 cat (6 consecutive scans)	1.77	0.34	**Pencil beam** (Lunar DPX-L)
Munday *et al.* (1994) **(40)**	5 cats (4-10 consecutive scans)	5.58	0.92	**Fan beam** (Hologic QDR 1000/W)
Borges *et al.* (2008) **(42)**	7 cats* (5 consecutive scans)	7.7	3.2	**Fan beam** (Hologic QDR 4500 Elite)
no repositioning between scans: HUMANS				
Bilsborough *et al.* (2014) **(48)**	25 humans † (2 consecutive scans)	5.9	0.5	**Pencil beam** (Lunar DPX-IQ)
	22 humans † (2 consecutive scans)	2.5	0.3	**Fan beam** (Lunar Prodigy)
repositioning between scans: CATS				
Borges *et al.* (2008) **(42)**	7 cats* (5 consecutive scans)	10.9	4.3	**Fan beam** (Hologic QDR 4500 Elite)
repositioning between scans: HUMANS				
Barlow *et al.* (2015) **(54)**	45 humans (2 consecutive scans)	1.6	2.3	**Narrow fan beam** (GE Lunar iDXA)

*The same 7 cats were used. First 5 scans without repositioning were made, after which another 5 scans with repositioning between each scan were made.

† Derived from a pool of 36 humans

Accuracy

Even though DEXA is used as a gold standard to validate methods that evaluate body condition, it has been validated by comparing it to other highly respected techniques as chemical analysis and deuterium dilution. Deuterium dilution is a method in which deuterium, a stable isotope of hydrogen is injected intravascular, after which its concentration is measured in a physiological fluid to estimate TBW (36).

In cats, only the accuracy of pencil beam DEXA scans has been evaluated. Upon comparison of the pencil beam DEXA scanner with chemical analysis of body tissues of cats (and dogs), DEXA correlated well for measurements of LBM, water content, FM and bone mineral concentration (BMC), with correlations between results of the DEXA scan and chemical analysis ranging between 0.909-0.996 and mean errors ranging from 1.6% to 2.6% (33). However, individual errors can be quite large, particularly for the analysis of body fat percentages (ranging from 20.75% to 31.5%), resulting in a low accuracy of the DEXA scan on an individual level (33). Size of the error in fat content as analysed by DEXA appear to be related predominantly to water content of the muscle, with larger errors occurring upon a lager decrease or increase of the water contents of the muscles. This measurement error is largely based on the fact that estimations of LBM are based on the assumption that LBM has a fixed water content of 73%. If the water content of tissue increases or decreases, body fat measurements using DEXA will subsequently overestimate or underestimate the true BF%, respectively (33). Adequate tissue hydration is therefore considered essential for obtaining reliable DEXA results in cats. However, in the humane literature it has been shown that the fat error caused by tissue hydration changes is small when the range of tissue hydration compatible with life is considered (see box 1) (55). Changes in hydration of 1-5% are measured to lead to an error in BF% of <1%. Therefore tissue hydration alone cannot explain the high individual errors for BF% estimates in cats. However, no other explanation was found.

Compared with deuterium dilution, the pencil beam DEXA scanner underestimated LBM in cats by 9.2%. In accordance with Speakman *et al.* (2001) (33), this research also found a high mean error for the estimation of FM (23.3%). However, DEXA results correlated well with the deuterium-dilution estimated LBM (r^2=0.841) and FM (r^2=0.867), demonstrating the high accuracy of DEXA scans (36).

In humans, where the use of DEXA has also been validated using deuterium analysis and chemical analysis of pork carcasses, two sources of error have been identified that have to be considered when using the technique (56–58). They are further explained in box 1 combined with the relevance for cats.

Box 1: Sources of error for DEXA measurements

Tissue hydration

Adequate, constant tissue hydration is considered important for acquiring reliable DEXA results and preventing errors, because the estimations of LBM are based on the assumption that LBM has a fixed water content of 73%. However in the humane literature doubts are expressed if tissue hydration even has a significant effect on DEXA results, with errors <1% in BF measurements caused by 1-5% changes in the hydration status of subjects (50). Carlson and Costello calculated in their book that the error of hydration status in LBM and FM estimates would not exceed 0.5 kg, considering that only 8% of the extra water would be mistaken for FM (54).

Beam hardening

Beam hardening is caused by the preference of tissues to attenuation low energy photons. When the X-ray beam is sent through thicker tissues, more high energy photons will pass the tissue than low energy photons. Because most low energy photons are already attenuated in the first centimetres of tissue, the beam hardens and less attenuation is seen deeper in the tissue. Thus attenuation per cm will be lower than in a thinner tissue. Since this attenuation is used to estimate the FM, the accuracy of FM estimates by DEXA will be dependent on the tissue thickness of an animal (55). When the tissue thickness is under 5 cm, an underestimation of the FM is seen. With a tissue thickness above 20 cm, DEXA is likely to overestimate the FM. Some software packages include corrections for the effects of beam hardening (55), but the effects can also be reduced by filtrating the photons with the lowest energy from the beam before sending it through the patient (56). However, in cats, beam hardening errors are less likely to influence the results since errors are low for tissue thicknesses between 5- 20 cm

Practical applicability & availability

Animals need to lie completely still in order to obtain a successful DEXA scan. As a result, sedation or anaesthesia will usually be required. The necessity to sedate or anesthetize an animal simultaneously limits the use of DEXA for routine measurements of body condition, especially if the animal is considered to have an increased anaesthetic risk (e.g. sick or geriatric animals) (36). Furthermore, the requirement of a constant, adequate tissue hydration for obtaining reliable results inhibits the applicability of DEXA measurements in animals with extreme fluid accumulations (e.g. in case of congestive heart failure) or severe dehydration.

Moreover, DEXA scanners require a lot of space. Also, most primary veterinary clinics do not have the financial funds to acquire and operate a DEXA scan, rendering the technique less suitable for in the first line veterinary practice. However, fan beam DEXA scanners are often successfully used in weight loss studies and for validation of other body condition evaluation techniques in cats (25,44,59,60).

Another perceived disadvantage of routine use of DEXA scan is the potential exposure to radiation. However, radiation doses emitted during DEXA scans are relatively low, and generally far lower than the background radiation in the Netherlands. As a result, DEXA scans should be considered safe (52,61–63).

Techniques that evaluate body condition

The techniques that evaluate body condition evaluate the outer appearance of the animal. Estimations of fat, muscle mass and body size are made. The body condition score (BCS), muscle mass score (MMS) and morphometric measurements will be described.

BCS systems and morphometric measurements evaluate an animals body condition based on the animals body shape and fat covering (43,59,64–66). The technique tries to estimate BF%. Muscle wasting can therefore easily go undetected without a thorough examination of muscle mass when the animal is not underweight. Loss of muscle mass is an important sign of disease that should not be missed during a veterinary examination (64). Therefore a MMS for cats has been developed as described below.

Body Condition Score

The first body condition score (BCS) system for cats was developed in the 90's (59). It comprises a subjective, non-invasive system that is based upon visual inspection and palpation to determine the body condition. A 5-, 6-, 7-, or 9-point scale have been developed, often accompanied with descriptions and lateral and dorsal drawings or photographs of the animal to aid the user in scoring (43,59,64–66). Scoring is done based upon the shape of the body, visibility and palpability of skeletal structures (e.g. ribs and vertebrae) and palpable fat in the abdomen and over the ribs, which indirectly assesses the amount of abdominal- and subcutaneous fat present in the animal.

General principle

The 9-point BCS system was first developed and validated by Laflamme *et al.* (1997) (59). This system (Purine BCS system) currently is one of the most widely accepted and used BCS systems and can be divided into three main categories: 1) animals which are underweight, represented by scores 1 to 4; 2) animals with an ideal weight, represented by score 5; 3) animals that are obese, represented by scores 6-9 (59). Every score is accompanied with a description, whereas images are only provided for scores 1, 3, 5, 7 and 9 (see Appendix 1).

The 5 point system, validated by Shoveller *et al.* (2014), is very similar to the 9-point system (for the score chart see Appendix 2), whereby Points 1 and 2 represent the animals that are underweight; point 3 the animals in ideal body condition and point 4 and 5 the animals that are obese (67). Similar to the 9-point system, visual aids and descriptions of important areas of interest to correctly determine the body condition are given (66). Some veterinarians prefer to grade half points, which basically turns this system into the 9-point system as described above (68).

The 6-point system uses a chart with six cat shapes combined with key-words to describe the body condition of the cat: 1 (cachectic), 2 (lean), 3 (optimal lean), 4 (optimal), 5 (heavy) and 6 (obese) (43,69). No further description of the body conditions are given (Appendix 3).

The 7-point system is one of the more recently developed systems, designed by WALTHAM. The system, which is called S.H.A.P.E (i.e. Size Health And Physical Evaluation), has been developed to both increase usability for non-experienced observers and enhance the reproducibility (70). It is based on an algorithm and uses most of the same visual and manual inspections as the other BCS systems. However, no images or drawings of body shape are given. Instead, this algorithm provides the observers with a set of questions in a flow chart, guiding them through the observations and examinations that have to be made (see Appendix 4). The system uses the letters A (underweight) to G (obese) to describe the different categories.

Precision

Four studies have tested the reproducibility of 5-, 7- and 9- point BCS systems (59,65,67,71). Scores given by trained observers were compared with each other and with scores given by untrained observers (Table 3). For untrained observers owners and other untrained staff were employed. Trained observers were defined as veterinarians, veterinary technicians and other staff trained in evaluating body conditions. Only two studies investigated the repeatability of the BCS systems. In the research of Laflamme *et al*. (1997), six experienced observers each scored the same cats twice with the 9-point purina BCS system (59). The observations were done several days apart, while six observers were blinded for their previously given scores and for the scores given by the other observers. Hawthorne *et al.* (2005), on the other hand, measured scores of eight unexperienced observers for the same BCS system on one occasion (71).

Reproducibility (inter-observer variability)

BCS systems generally show high levels of agreement between skilled observers regardless of the scale used. During various studies that were performed, correlation between trained operators ranged from 0.89 to 0.987 (Table 3;(59,70). Veterinarians and other skilled or trained observers usually have a higher agreement in their assessments than owners or untrained observers and veterinarians (67,70,71). Scores given by untrained observers in a 5-point system without pictures differed significantly from the scores given by trained observers (65). However, when the 9-point system without pictures was used, the difference between trained and untrained observers is not significant. This might be explained by the fact that the 9 point system has a larger scale and allows owners to better nuance their cats body condition even without pictures. The correlation between experienced and unexperienced observers increases vastly in both the 5- and 9-point system when pictures are used (65). Nevertheless, the 7-point system (S.H.A.P.E.), which does not use pictures, also shows high correlations between scores of untrained- and expert observers (70). Most likely because the untrained observer is guided step by step through the process of evaluating the body condition. The 6-point system has, to the authors knowledge, not been tested on reproducibility, making it impossible to know what the correlations are.

The aforementioned data suggest that the 7-point system is the most reproducible method (Table 3). However, the 5-, 7- and 9-point systems can all be used reliably to score the body condition. If images are not used, the 9-point system is preferred above the 5-point system.

Table 3: Correlations between the scores of expert (trained) observers and amateur (untrained) observers when using the different BCS systems

9-point BCS system		**5-point BCS system**		**S.H.AP.E. (7-point sytem) (70)**	
Expert – expert	**Expert –amateur**	**Expert – expert**	**Expert –amateur**	**Expert – expert**	**Expert –amateur**
r^2 = 0.89 **(59)**	No pictures: r^2 = 0.554 (65)	Kappa = 0.752 (67)	No pictures: r^2 = 0.499 (65)	r^2 = 0.987	r^2 = 0.864 & r^2 = 0.867
CV = 15.3% **(71)**	With pictures: r^2 = 0.721 (65)		With pictures: r^2 = 0.736 (65)		
			Kappa = 0.499 (67)		

Repeatability (intra-observer variability)

Laflamme *et al.* (1007) found a high correlation of 0.95 between the scores of the observers, indicating that repeatability with the 9-point BCS system is high (59). Hawthorne *et al.* (2005), on the other hand, measured a moderate repeatability with a CV of 15% (71). However, Laflamme *et al.* (1997) used experienced observers, while Hawthorne *et al.* (2005) employed inexperienced observers, explaining the lower correlation. For the other BCS systems, repeatability is not known in cats nor in dogs, but one can assume that the repeatability would also range between moderate to good, considering they are based on the same principles.

Accuracy

Body condition scoring systems are validated by comparing them with percentages body fat as measured by Dual Energy X-ray Absorptiometry Analysis (DEXA). Besides Laflamme *et al.* (1997), three other validation researches have been conducted by Hawthorne *et al.* (2005). Shoveller *et al.* (2014) and Bjornvad *et al.* (2011) (25,59,67,71). 32, 60, 133 and 72 cats were used in the studies, respectively. Shoveller *et al.* (2014) applied a 5-point scale, while the rest used a 9-point system. All studies compared the cats assigned body condition scores with DEXA results. Borges *et al.* (2012) compared DEXA results with scores of a 9-point BCS system among other methods in 16 cats undergoing a weight loss program on three stages in their weight loss (41).

BCS systems are highly correlated with body fat percentages, as measured with DEXA (5-point: r^2=0.8, 7-point: r^2= 0.83 and 9-point: r^2=0.73-0.92; (59,67,70,71). The highest correlations with BF% are seen when the scores of a trained observer are used (59,67). Each step in the 9-point system, or half step in the 5-point system, was found to correlate with a 5-7% increase in body fat percentage (59,67). However, it should be noted that the mean BF% per BCS category differs between active and relatively inactive cats. Inactive cats have a higher body fat percentage in each category of the BCS than active cats, reflecting their smaller amount of muscle mass (25). BCS systems barely pay attention to muscle mass, and therefore are unable to successfully identify the cut-off point between an ideal and unideal body condition in the right categories (23). This could also explain the high CVs (13.9%-25.8%) between body fat and BCS categories found by Borges *et al.* (2012) (41).

The phenomenon is described as Skinny Fat', a term also used in human literature (67,72). Skinny fat cats have a higher BF% than desirable even though they have an ideal BCS. Skinny fat people have higher health risks, compared to fatter, but fitter people (72). This should be kept in mind when grading the body condition of a cat with a BCS system.

Practical applicability and availability

BCS systems are generally easy to use and non-invasive, requiring no expensive equipment and enabling scoring to take place outside of the veterinary practice without sedation or anaesthesia. The systems are nowadays widely used inside and outside veterinary practices by owners an veterinarians, in order to reliably keep track of the body condition of cats and dogs. Especially in otherwise healthy animals, the BCS is a great way to specify the body condition.

However, the measurements are subjective and training is required to make the observations more reliable. Owners tend to normalize their animals body condition while using the BCS (20,21). The difference in opinion between veterinarian and owner can interfere with owner compliance when suggesting a weight loss program. This should not be overlooked.

Muscle mass score

The muscle mass score (MMS) is a relatively new system for evaluating the body condition of cats and dogs (64,68). In contrast to the BCS and many other body condition scoring methods, this scoring system does not focus on body fat to obtain an impression of the animals general body condition but rather assesses the muscle condition. As such, it can be used to complement the BCS system.

General principle

For the evaluation of the MMS in cats, the muscles mass over the scapula, temporal bones, ilium wings and spine is visually inspected and palpated (see WSAVA score chart, Appendix 5). The amount of muscle wasting is then graded on a 4-point scale ranging from 0 to 3, whereby severe muscle wasting is classified as 0, moderate muscle wasting as 1, mild muscle wasting as 2, and normal muscle mass as 3 (Figure 4; 67).

Precision

To date, only two studies have evaluated the precision and accuracy of this new MMS system (73,74). Linder *et al.* (2013) compared MMS with BCS in 87 dogs. Michel *et al.* (2011) made 10 veterinarians and veterinary technicians score the MMS of 44 cats on three different occasions, after which these results were compared with DEXA scans. Despite the lack of data on the precision and validation of the MMS a short discussion of what is known will follow.

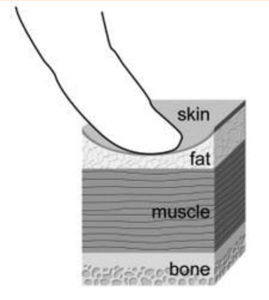
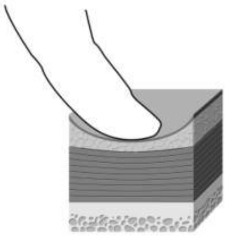
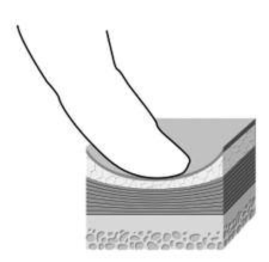
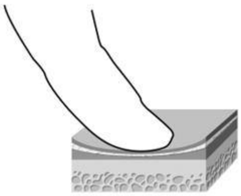
Reproducibility (inter-observer variability)

In the study of Michel *et al.* (2011), the inter-observer variability of the MMS was found to be high. Inter-rater agreement for the MMS system between 10 observers participating in this study was moderate with correlations between the observers for the categories ‘normal’ and ‘severely wasted’ ranging between 0.48 and 0.59 (73). However, little agreement was seen for the intermediate MMS categories 1 and 2, with inter-rater agreement ranging between 0.20 and 0.31. This suggests that fusing the intermediate MMS categories to a 3-point model could increase the reproducibility of this new method. However, further research will be necessary to determine whether this adjustment would result in an acceptable reliability.

Repeatability (intra-observer variability)

In contrast to the reproducibility, Michel *et al.* (2011) found the repeatability of the MMS system to be higher. Correlations between the observers’ scores in three separate evaluations were found to be acceptable (i.e. 0.71 - 0.73; (73). However, the repeatability has been studied in only 10 observers and with at least a weak apart, making the first and last observations a minimal of 2 weeks apart. Since muscle wasting can occur rapidly in diseased animals (73), the muscle mass of some animals could potentially have changed over this period.

Figure 4: A graphic demonstration of the categories from the MMS. AHAA Nutritional Assessment guidelines (75)

Figure	MMS
	MMS = 3 Normal muscle mass.
	MMS = 2 Mild muscle wasting
	MMS = 1 Moderate muscle wasting
	MMS = 0 Severe muscle wasting

Accuracy

When compared with DEXA, the MMS in cats is significantly and positively correlated with LBM. However, correlation between the two parameters is low (r^2=0.62). This can be explained due to the fact that LBM does not only exist out of muscle. For LBM%, MMS had a significant, but low, negative correlation with the LBM% as measured by DEXA (73). This can be explained by the fact that the LBM% increases in a leaner animal, or in an animal losing weight, even though the total LBM in grams decreases (75). This decrease in LBM might be picked up with the MMS system, resulting in the weak negative correlation as seen. The MMS only has a weak correlation with BCS (r^2=0.47-0.76; (73,74). Considering the fact that the BCS mainly focuses on body fat, this is understandable.

Practical applicability & availability

The figures described above show that the MMS system is in its early stages of development and lots can still be improved. The system as it is used right now is not very accurate or precise, making only very broad assessments possible. Just as the BCS, the MMS does not require expensive equipment, is non-invasive and only minimal patient compliance is necessary to perform the evaluation. Therefore the technique can also be used outside the veterinary practice.

In the future the MMS system can be of added value to the BCS in sick, older and obese animals. By assessing muscle mass separately, muscle wasting as a consequence of diets can be earlier detected and addressed, making weight loss regimes safer (64). Also, a more objective assessment of muscle mass in sick animals will allow a veterinarian to keep track of the changes in their body condition.

Besides this, the MMS can help the veterinarian to objectively differentiate between potential animal cruelty cases and severe animal disease by being able to distinguish between stress starvation and simple starvation. Stress starvation is caused by severe clinical disease. Simple starvation, in contrary, is caused by a lack of food intake which could be the result of neglect (64).

The amount and presence of muscle wasting, as described in human literature, has an direct influence on the prognosis of disease (76,77), making the MMS a promising potential prognostic value for the veterinary world, while supplementing the BCS.

Morphometric measurements

Morphometric measurements have been used for at least 25 years to estimate a cat's body condition (28). Besides in cats, the technique has also been applied in dogs and rabbits (15,78). Measurements used among others include height, length, girth, thoracic circumference, pelvic circumference, paw circumferences, head circumference, limb length, leg index measurements. In contrast to humans, measuring skin fold thickness is not considered a reliable method for estimating BF%, because most animals have a rather loose skin and subcutaneous tissue, rendering it difficult to accurately measure the amount of subcutaneous fat present (28).

General principle

Multiple methods have been developed to apply these morphometric measurements. In principle, two gross applications of morphometric measurements can be distinguished. The measurements can be used alone, or to complement other body condition scoring methods. Predictive equations have been developed containing morphometric measurements and other techniques, for example bioelectrical impedance analysis (BIA) and sonography (28,41). By combining the methods in an equation, accuracy is enhanced. Using these equations, BF%, LBM, FM, total body water (TBW) and body weight (BW) (28,31,71,79–81) of cats can be estimated (Table 4). Alternatively, predictive equations have been developed containing only morphometric measurements

Methods only applying morphometric measurements include the feline body mass index (fBMI) and other unnamed systems developed by Stanton *et al.* (1992) and Witzel *et al.* (2014; (28,31). Mixed equations have been developed by Stanton *et al.* (1992) and Borges *et al.* (2012; (28,41).

fBMI

At least three different fBMI systems have been developed over the years (71,80,82,83). All use different equations and definitions of BMI.

The oldest BMI for cats has been described by Nelson *et al.* (1990; (83), and Hoenig *et al.* (2013; (82). It uses a BMI equation based on a measurement of the amount of body weight per body surface area (Kg/m^2; Table 4). For this purpose, body length, body height and body weight are measured, whereby body length is defined as the distance between the scapula point and the tuber ischium, and body height is defined as the distance between the point of the scapula through the elbow and the proximal boundary of the central metacarpal pad.

A different fBMI system estimates the BF% of cats. It was originally developed by Hawthorne *et al.* (2000) and patented in 2005 (71,81). This fBMI method uses two morphometric measurements, i.e. the ribcage circumference, which is highly correlated with BF%, and the leg index measurement (LIM) that shows little correlation with BF%. Thoracic circumference is measured at the 9th rib with a tape measure. The LIM is measured as the distance between the patella and the calcaneal tuber a standing cat. It is used to measure the stature of the animal to correct the thoracic circumference to the animal's size, making it possible to estimate BF% based upon the thoracic circumference of an animal. The predictive equation can be seen in Table 4, where a BF% of 25% is considered an ideal body condition, as described by Hawthorne *et al.* (2005) based on data collected at the Waltham Centre for Pet Nutrition (WPCN) (71).

Kawasumi *et al.* (2016) developed the most recent fBMI system, to improve accuracy and lower the complexity of the method. It is a combination of the methods as described above, using body weight and PCL (i.e. the distance between the patella and the top of the calcaneus in the standing cat). It is comparable to the LIM in the fBMI of Hawthorne *et al.* (2000 & 2005). The fBMI is in this system expressed in kg/m (Table 4), with values ≥ 28 are considered overweight.

Table 4: Different equations containing morphometric measurements and their correlations with DEXA. References of the equations are displayed in the 'estimate' column.

Estimate	Morphometric equations: fBMI	Correlation with DEXA
Body height and weight-derived fBMI (82,83)	$fBMI\ (kg/m^2) = \frac{body\ weight\ (kg)}{body\ length\ (m)\ X\ height\ (m)}$	unknown
Thoracic circumference-derived fBMI **(71,81)**	$BF\% = \left[\frac{(\frac{thoracic\ circumference}{0.7067} - LIM)}{0.9156}\right] - LIM$	0.85
PCL-derived fBMI (80)	$fBMI\ (kg/m) = \frac{body\ weight\ (kg)}{PCL\ (m)}$	Unknown
	Other morphometric equations	
TBW (28)	$TBW(kg) = (0.65 * \ body\ weight) - (0.03 *$ $pelvic\ circumference) + (0.04\ * right\ hind\ limb\ length) - 0.031$	0.98*
LBM (31)	$LBM\ = 30.3(head\ diameter * hind\ limb\ length$ $+316.9\ (forelimb\ circumference) + 2.55$ $*\ 0.85(thoracic\ diameter\ \ * forelimb\ length)$ $+14.4(body\ length) - 3.0587$	0.85
FM (31)	$FM\ = 436.9(body\ weight)$ $-24.0(head\ diameter\ \ * forelimb\ length)$ $-309.2(forelimb\ circumference) + 2.5227$	0.98
BW (84)	$BW = -4.53 + 0.11(Wither\ height) + 0.13\ (Body\ Length)$ (79)	0.57
	Mixed equations	
FM (41)	$FM\ = 0.4(Body\ weight) + 0.006R(\ BIA) + 9.67SFL - 0.69$	0.94
FM (41)	$FM\ = -0.005(body\ length) + 0.7(body\ weight)$ $+0.007R(BIA) - 0.60$	0.98
FM (28)	$FM\ = 0.04(pelvic\ circumference) - 0.004(length^2/R(BIA)$ $-0.08(right\ forelibm\ length) + 1.11$	0.93*
LBM (28)	$LBM\ = 0.74(body\ weight) + 0.11(right\ forelibm\ length)$ $+0.02(body\ length) - 0.03(pelvic\ circumference)$ $-0.001R(BIA) - 1.50$	0.98*
BF% (28)	$BF\% = -0.02(length^2/R(BIA)) - 4012(right\ forelibm\ length)$ $+1.48(pelvic\ circumference)$ $-1.16(cranial\ thoracic\ circumference) + 92.93$	0.82

* Equations are compared with chemical analysis as reference method, instead of DEXA

Other morphometrical methods:

Apart from the BMI, other morphometric equations have been developed (able 4). For example, Stanton *et al.* (1992) developed an equation to estimate TBW using body weight, pelvic circumference and right hind limb length (28). Furthermore, Witzel *et al.* (2014) developed two equations that estimate LBM and FM in overweight or obese cats, by comparing morphometric measurements with DEXA results (31). Equations have also been made to estimate body weight in cats, using a variety of morphometric methods (84,85).

Examples of mixed equations combining morphometric measurements with DEXA-, sonography- or BIA values, estimating FM, FM%, LBM and body weight, can also be seen in Table 4 (28,41). The mixed equations are developed with stepwise-regression analysis, while choosing the dependent variables from reference methods as DEXA and chemical analysis.

Precision

Witzel *et al.* (2014a & b) have, in two separate studies of dogs and cats, tested the reproducibility and repeatability of single morphometric measurements in dogs and cats (31,78). Four investigators took each measurement twice. Repeatability was tested by Hawthorne *et al.* (2005) by 8 investigators who took each measurement in duplicate (71).

Reproducibility (inter-observer variability)

Reproducibility of independent morphometric measurements and full equations is generally high, dependent on the measurements used. Inter-observer variation ranges between <2% and 5% for most independent measurements, for example body length, thoracic circumference and limb length (31). In dogs inter-observer variation was actually found to account for <1% of the total variation of the developed equations estimating LBM, FM and BF% (78). However, some measurements in cats show greater variations than 10%, with metacarpal and metatarsal pad width & length and forelimb circumferences having variations between 16.4%-19.5% (31).

The reproducibility of the full fBMI equation by Hawthorne *et al.* (2005) is, just as independent measurements, high with a CV of around 10% (81). For the other equations and systems, no reproducibility data is known.

Repeatability (intra-observer variability)

Morphometric measurements will generally have a high repeatability. Intra-observer variations are lower than 10% for most individual measurements (71,81), but variances as low as <2% have been reported (31), comparable with reproducibility results. Independent measurements are thus comparable within and between investigators.

Accuracy

Most studies compare the morphometric measurements with DEXA results, making predictive equations using multiple regression analysis (28,31,41,71). Chemical analysis, however, can also be used as a reference method (28).

Thoracic circumference and girth measurements have been found to correlate well with DEXA BF%, with correlations of 0.83 and 0.77, respectively (81). When considering the full equations, in which multiple techniques or measurements are included, correlations with DEXA tend to increase to 0.85 and 0.98 as can be seen in Table 4 (31,41). By adding more explanatory variables, the accuracy increases, because more of the observed variation in the animals can be explained by the model.

All fBMI systems correlate with DEXA- and BCS-determined BF%. However, the PCL-derived fBMI of cats with BCS 5/5 overlap the fBMI values found in previous BCS categories, with values ranging from 29.9 to 40.3 (80). For BCS 5, the correlation with PCL-derived fBMI values is thus low. Values of the body height- and weight derived fBMI system for obese cats were significantly higher than values in lean cats in the body height- and weight-determined fBMI system (86). The same significant difference was seen for DEXA BF% measurements, suggesting that the fBMI values are correlated with BF%. However, to the author's knowledge, no further validation studies have been performed, limiting the ability to draw definitive conclusions about the validity of this system.

By adding the LIM to the thoracic circumference measurements, the fBMI of Hawthorne *et al.* (2005) achieved a correlation of 0.85 with DEXA BF% (81). This is higher than the correlation between DEXA BF% and the 9-point BCS system, rendering this fBMI method more reliable than BCS systems and other fBMI methods.

The equations made by Witzel *et al.* (2014) for the estimation of LBM and FM only obtain morphometric measurements, but nevertheless these were found to be highly correlated with DEXA results (31). The LBM equation correlates well with DEXA LBM with an correlation of 0.85, whereas the estimated FM had an even higher correlation of 0.98. Another equation only using morphometric measurements, estimating TBW, showed a correlation of 0.98 with TBW as estimated by chemical analysis (28). These high correlations combined with low standard errors show that morphometric measurement equations can be highly accurate in predicting body condition parameters . However, even though Witzel *et al.* (2014) included 76 cats in their study, all of them were overweight or obese. The equations estimating LBM and FM are only valid in overweight or obese cats and cannot be extrapolated to the entire cat population.

Mixed equations show high correlations for estimated FM, LBM and BF% (0.82-0.98) with low standard errors (28,41). However, it is doubtful if these equations are representative for the entire cat population. For example, the number of cats used in the studies by Borges *et al.* (2012) and Stanton *et al.* (1992) was low (n=16 and n=22; (28,41). Moreover, the equations of Borges *et al.* (2012) have solely been based on measurements in obese cats that underwent a weight loss programme, rendering the accuracy of these equations in lean or underweight cats questionable and necessitating. further research to confirm the findings and validate the techniques.

Practical applicability and availability

Predictions of BF% can be used to determine the ideal body weight and energy requirements of an animal (81). This can aid the veterinarian in establishing a diet plan and target weight for overweight animals. The morphometric measurements can thus be used to treat pet obesity without the need for expensive weight control programmes that include DEXA scans.

Similar to BCS and MMS, the use of morphometric measurements to estimate body condition parameters is a cheap method that can be applied virtually anywhere. For most equations, only a tape-measure and a scale are required. Moreover, little training is necessary to apply the techniques, making it possible for measurements to be performed by untrained owners or technicians (71).

Because of the non-invasiveness of the techniques, no sedation is necessary, making it possible for old and sick animals to be evaluated without extra risks. However, patient compliance can become a problem when many measurements need to be taken or when faced with an hyperactive animal, which can particularly be challenging in cats.

However, the equations consist of 2 up to 6 different measurements (31,41,87), taking on average 5 minutes to perform (31). In practice, where clinic consultations usually only last 10 minutes, these measurements will thus take up half of the consult, rendering it impossible to include this in the standard examination and necessitating extra time to be scheduled and charged for enabling these measurements to be performed. As it is unlikely that an owner is willing to pay extra for the time needed to accurately determine the body condition of their cat, this could limit the application of these techniques in a practical setting. However, solutions to this problem are certainly conceivable, e.g. by training veterinary technicians to take the measurements.

Lastly, as described above, study populations used to develop these equations need to be kept in mind. For example, the equations that estimate LBM and FM (Table 4), can only be used for overweight cats, considering the equations was made based solely on overweight animals. These type of study errors can greatly limit the applicability of an equation.

Thus, when developed properly, equations based on morphometric measurements can give highly accurate and precise estimations of parameters as LBM, FM, BF% and fBMI with a better reliability than the BCS. Combined with the absence of highly invasive and expensive techniques, these equations are very interesting for application in veterinary practice.

Techniques that estimate body composition

The techniques that estimate body composition make an attempt to measure the exact amount of fat and/or lean body mass. The composition of the body is analysed. In this category bioelectrical impedance analysis (BIA) and ultrasonography will be considered.

Bioelectrical impedance analysis

Bioelectrical impedance analysis (BIA) was originally developed to estimate bone density. However, nowadays it is also used to estimate the LBM and the FM of humans and multiple different animal species (23,28,88–90).

General principle

BIA is a technique that estimates total body water (TBW) and extracellular water (ECW) by measuring the resistance (*R*) and reactance (Xc) of a small electrical, alternating current that is send through the body between 2 or more electrodes, usually needles (32,91). The electrodes can be placed in different configurations on the cats body, as described by Elliot *et al.* (2002) and Stanton *et al.* (1992; (87,92). With the TBW, estimates of FM and LBM can subsequently be made. Estimates can also be made with the use of predictive equations formulated from multiple regression analysis (41,87). The fundamentals and basic principles of BIA will be explained briefly below. For more information the author refers the reader to the reviews of Khalil *et al.* (2014), Jaffrin *et al.* (2008) and Kyle *et al.* (2004; (90,91,93).

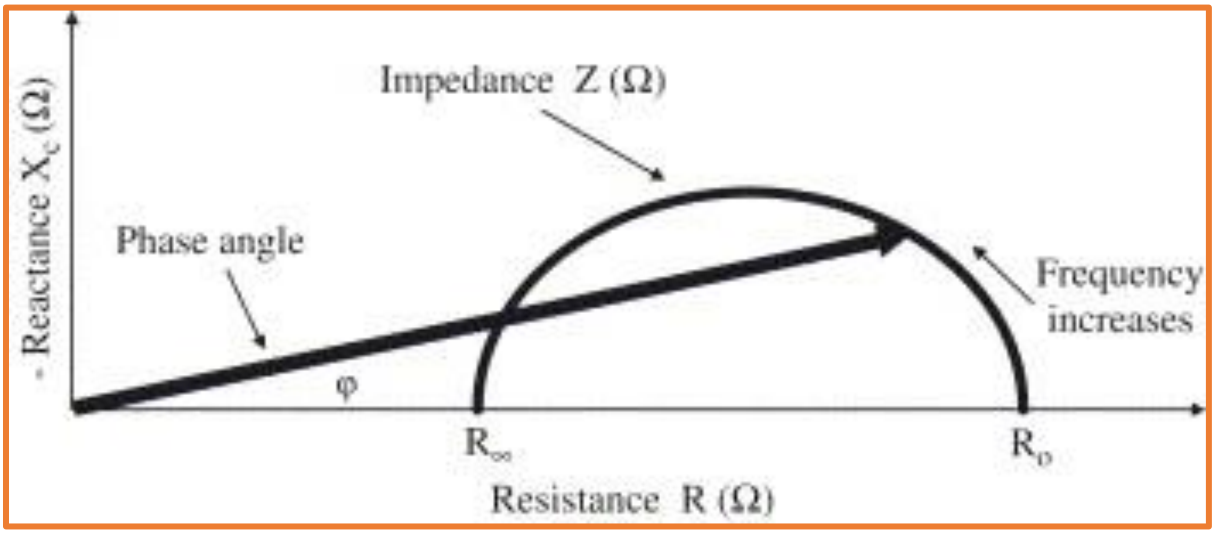
In order to be able to understand the principles of BIA, the terms impedance, reactance, capacitance and resistance need to be explained further: The impedance consists of a combination of *R* to the electrical current (caused by the ECW and intracellular water (ICW)) and Xc (Figure 5; (93). *R* and Xc are the two variables measured by BIA. Reactance is the reciprocal of capacitance formed by cellular membranes at low frequencies (94). Cellular membranes act as condensers. If subjected to an alternating current they are continuously charged and discharged when the current changes its direction (95). Capacitance is the brief storage of voltage by the condenser, while reactance is the release of this stored voltage (94).

The relation between the Xc and *R* is measured by calculating the linear phase angle (PA) (32). The PA ranges between 0 and 90 degrees (Figure 5). A PA of 0 degrees represents a resistive circuit with no cell membranes. At 90 degrees a capacitive circuit is present, no fluids and only cell membranes would be seen. An electrical circuit with a PA of 45 degrees possesses and equivalent amount of *R* and Xc. The PA can be calculated directly from the measured values with the following equation: $PA = (Xc/R) * 180°/\pi$. It is an indicator of membrane stability and can be used as a prognostic value to predict survival of humans with certain diseases, for example liver cirrhosis and cancer (96–98).

The *R* is, often combined with morphometric measurements and analysed in multiple mathematical equations and models to estimate the LBM and FM (41,87). The measured reactance is usually not implemented in these equations, but used for calculating the PA. Different types of BIA can be distinguished as reviewed in the humane literature (93). However, only single frequency- and multifrequency BIA have been used in the cat and will be discussed here.

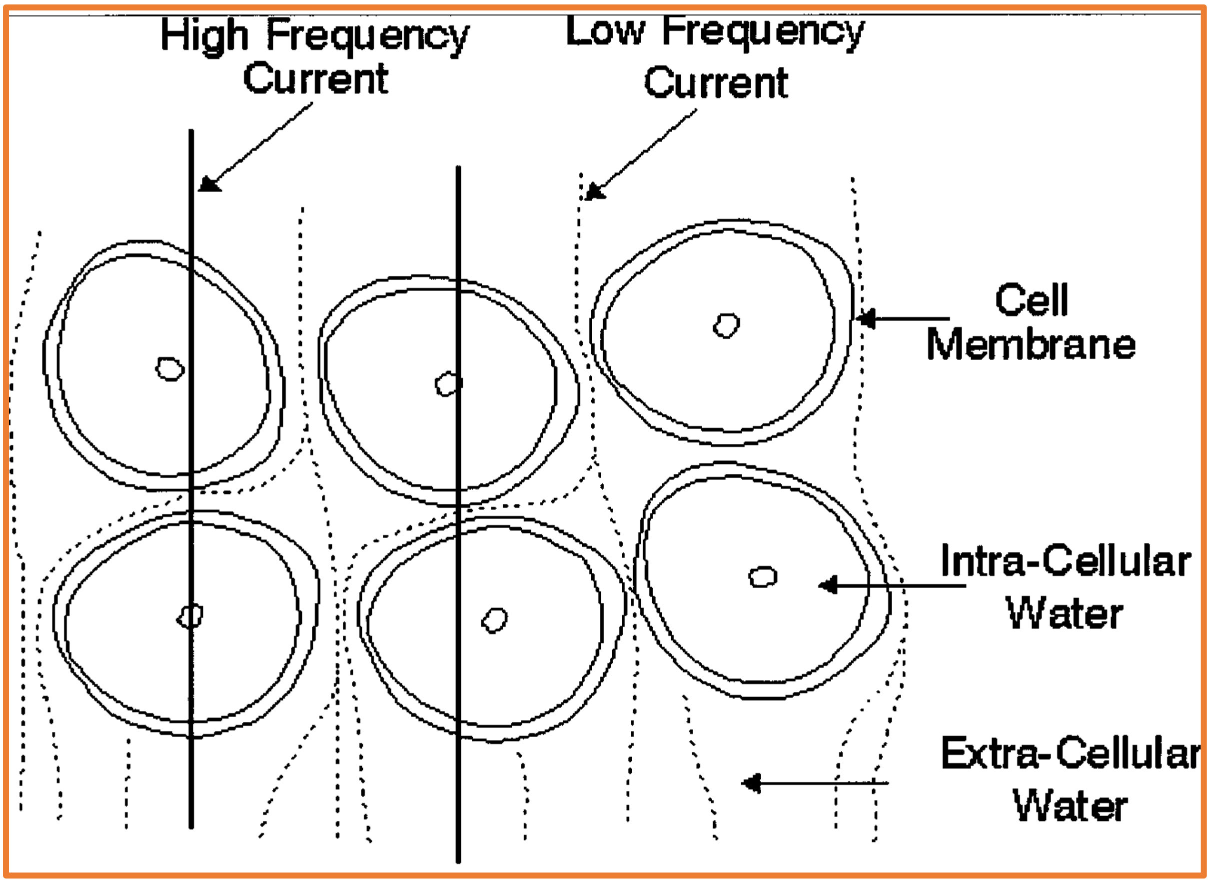
Single frequency-BIA (SF-BIA) is the method in which the alternating current is sent with a fixed frequency, usually 50 kHz, through the body (32,87). The obtained values of *R* and Xc are then analysed using mathematical equations. These equations are empirically made with multiple regression analysis and mixture theories, often including other body parameters as weight (in cats) or morphometric measurements or length (in humans) (41,87). With SF-BIA equations estimating TWB and LBM can be developed by comparing the measurements with DEXA results (41).

Figure 5: Graph displaying the relationship between the Impedance (z), Phase angle (PA), resistance (R) and reactance (Xc). R_0 represents the resistance of ICW, and R_∞ represents resistance of ICW and ECW (TBW; (92).



Multifrequency-BIA (MF-BIA) measures the impedance (existing of Xc and *R*) at multiple frequencies. Because the cellular membranes act as capacitators, very little conduction through the cells is possible at low frequencies (93,99). Therefore properties of the ECW predominantly determine conductivity. Cells and their ICW at low frequencies are nonconductive materials. The resistance of ICW (R_0) is thus ideally measured at frequencies lower than 1 kHz (Figure 5 & 6; (99). Conversely, the influence of the capacitance of the cellular membranes on the impedance (Z) is very small at high frequencies, causing the electrical current to pass through both the ICW and ECW (99). The TBW resistance (R_∞) is thus best measured at frequencies higher than 5000 kHz (Figure 6; (99). For technical reasons, measuring values at these extreme frequencies is not possible for impedance meters, as described in humane literature (90). In cats measurements are therefore usually taken at 50 frequencies between 5 and 1000 kHz (23,92,100). Values of R_0 and R_∞ are subsequently extrapolated in an enhanced Cole-Cole model (23,100), as described in a humane article in 1997 (99). By measuring *R,* TBW, ICW and ECW can be calculated using an equation from the Hanai mixture theory (92). This theory describes the conductivity of an electrical current in a suspension of nonconducting materials (99). Equations to estimate FM, LBM and BF% can also be developed, using measured *R* and some morphometric measurements (41,87).

Figure 6: Conductivity of an alternating electric current through tissues at high and low frequencies. At high frequencies the capacitance on the cellular membranes on the impedance is small and therefore the electric current passes through both the intracellular water (ICW) and extracellular water (ECW). At low frequencies, the cell membranes act as capacitators and inhibit conduction through the cells. All electrical current will be conducted through the ECW (98).



Precision

Repeatability of BIA has been tested by three studies. Cintra *et al.* (2010) measured for each electrode SF-BIA values three consecutive times on 20 animals (32). Center *et al.* (2011) took 9 and 5 MF-BIA measurements in two cats over a period of three days. Center *et al.* (2013) tested repeatability of MF-BIA LBM measurements in 11 cats by measuring on two different days. Reproducibility is difficult to determine, as described below.

Reproducibility (inter-observer variability)

The reproducibility of BIA in cats is questionable. Not only is there is no standardized method to conduct the measurements, but the technique uses multiple systems (SF-BIA, MF-BIA) and different types of electrodes (32,41,87,92), which poses a challenge for comparing results to reference values as well as comparing findings of different studies. The use of different types of electrodes, for example, causes significant differences in the measured values of Xc and *R* (32). Moreover, different body postures and BIA configurations can alter the accuracy of the method (87,92), although differences are not always significant (100).

Repeatability (intra-observer variability)

The repeatability of BIA has been tested for both the SF- and MF-BIA methods and can be considered as good to moderate. Repeatability of measurements is highly dependent on the value that is estimated and the type of needle that is used. For example, the CV for TBW, EWC, LBM and FM estimates is reported to be between 2.8% and 16.6% (23,32,101). ICW gives the lowest CV (2.8%-6.9%), while ECW and FM produces the highest CVs (8.7%-16.6%; (23). Similarly, the type of needle used as electrode affects the repeatability, with acupuncture needles providing the most stable results, with a CV of only 0.62% for *R* measurements (Table 5; (32).

Tabel 5: Repeatability of different BIA measurements for various types of electrodes (32,72)

	CV *R*	CV Xc	CV PA	CV LBM	CV FM
Adhesive electrodes*	0.62%	0.69%	5.93%	11%	-
Acupuncture needle*	0.66%	1.69%	9.99%	6%	-
Hypodermic needle*	7.34%	19.83%	29.22%	6%	-
Tetrapolar platinum electrode†	-	-	-	6.6-10.1%	6.2-16.6%

*Electrodes tested with a SF-BIA method

† Electrode tested with a MF-BIA method

Accuracy

In order to develop reliable mathematical equations that estimate LBM and FM, a few assumptions have to be made, i.e. 1) the shape of the body is accurately portrayed as 5 cylinders; 2) the relationship between trunk and leg lengths are constant; 3) the body is euhydrated; and 4) the fat fraction has a lower water content than the LBM (102). Because of these assumptions, differences in electrolyte concentrations can influence the BIA results, even without a change in body fluids (93). Changed electrolyte concentrations will interfere with the ICW-ECW balance, which is exactly what BIA indirectly measures. However, an abnormal hydration status also changes BIA results (32), making it difficult to apply BIA in diseased animals, as also seen in humans (103). In human populations large variations in BIA results can be seen between different populations, because of differences in body proportions among other things, making wide application of BIA equations difficult (93). This should be taken into consideration when evaluation achondroplasitic cat breeds.

Both the SF- as the MF-BIA methods have been validated in the cat (32,87,92,100). The reference methods of choice for validating BIA estimates are chemical analysis, or deuterium dilution (TBW) and Sodium-bromide dilution (ECW), but the DEXA scan has also been used for validating or developing FM and LBM estimates (32).

Estimated TBW and LBM by SF-BIA showed excellent correlation with the results from chemical analysis (r^2=0.98 for both; (87). FM and BF% estimated using BIA and multiple morphometric measurements also resulted in high correlations of 0.93 and 0.82, respectively (87). Equations derived from SF-BIA results combined with some morphometric measurements can thus accurately predict TBW, LBM, FM and BF%. However, in the humane literature it is inconclusive if fluctuations in the ICW can be detected with SF- BIA (91). Also, when compared with DEXA, the equations for LBM estimates significantly overestimate the LBM (32,87)

With MF-BIA, no significant differences were found for TBW and ECW estimates compared to the reference methods sodium-bromide dilution and deuterium dilution (r^2=0.84-0.86 and r^2= 0.74-0.93, respectively; (23). LBM estimates are also highly correlated with reference methods (r^2=0.89; (23) All 6 different configurations for electrode placement, as described by Elliot *et al.* (2002) were validated for MF-BIA (92,100). However, some configurations correlate better with reference methods (92,100). The 'sternal contralateral path-length' configuration showed to be best correlated with deuterium dilution determined TBW (r^2=0.84). ECW, as measured by the bromide dilution method, was best correlated with the 'sternal body head to tail configuration' (r^2=0.91).

Compared to the BCS, the MF-BIA method appeared better at diagnosing underweight cats than the BCS system, even though the BCS-system was applied by highly trained observers (23). In a study that took multiple variables into account, MF-BIA proved to be more useful to estimate lean body mass in obese animals, while morphometric measurements were found to be more important in leaner animals (41).

Practical applicability and availability

The BIA system is portable, non-invasive and easy to use. Cats generally do not have to be sedated for measurements, but application of the needles can cause some discomfort and therefore reduce compliance (23).

Practical applicability of BIA is encountered by certain limitations, because assumptions have to be made that do not include all breeds. Predictive equations cannot simply be extrapolated to an entire cat population without taking the assumptions that go along with it into account. As a result, this restricts the applicability of BIA in the general population, and only enables the equation to be valid in a population that is similar to the population that was studied.

Moreover, BIA equipment is rather expensive with prices ranging between \$3000 and \$4000 (104). Accessories needed and software packages are not included and have to be bought separately which increases the costs even more. This renders the technique less attractive for use in practice.

Ultrasonography

Ultrasonography has been used in a single study in cats to estimate total BF% (41). However, in farm animals, horses and donkeys a lot of research has been conducted into the use of this method to predict quality of meat and to determine overall body condition (105–108). In dogs few studies have been performed as well (41,109–111).

General principle

With ultrasonography, depth of the subcutaneous fat layer (SFL) can be measured and used to subsequently estimate the total BF% of an animal. A great array of different transducers can be used. Using a high frequency transducer (e.g. 20 MHz) makes it possible to detect smaller variations in the subcutaneous fat layer (109), but 10 MHz (in dogs) and even 6-8 MHz (in cats) appear sufficient for this application (41,109). The sole criterion for successful measurements is the use of a linear instead of a curved transducer. To select the right location for the measurements, the following aspects need to be taken into consideration: 1) at the location, the SFL has to correlate well with the body condition; 2) the anatomical location has to be readily identifiable; and 3) the location has to be easy to reach (110). In dogs, multiple anatomic locations have been tested for predicting the BF% with the SFL measurements. The lumbar region seems to be the best location. In cats therefore the subcutaneous fat layer over the 7th lumbar vertebra is measured (41).

Precision

In companion animals, only one research has tested repeatability of ultrasonography measurements in dogs by taking three measurements of each sonographic image (109). Reproducibility has been tested in humans, with three unexperienced sonography operators (112). However in the humane literature concerns about the repeatability and reproducibility of the relatively unknown technique have been reviewed (113). The interpretation of ultrasonography is more difficult than with different body condition scoring methods and experience is essential for reliable results.

Reproducibility (Inter-observer variability)

Reproducibility of sonographic SFL measurements is not described for companion animals. However, supraspinal measurements in humans show great inter-observer correlations with only a CV of 3% (112). When other measurement locations are considered, CVs range between 1 and 7%. Thus even with inexperienced operators, reproducibility is high. Although cats do have fur, it can be expected that CVs will be high, just as in humans.

Despite these promising results, standardization of measurement methods still remains necessary to enhance the reproducibility of the results in both animals and humans (113). The differences in normal body composition between cat breeds can also, if not taken into account, increase the variability of the measurements.

Repeatability (Intra-observer variability)

The experience of the operator plays an important role in the precision, and thus the variability, of the measurements (113). In dogs, the intra-observer variability of SFL measurements appears to be high, with CV's between 33.2% and 51.2% found for three measurements taken of a single ultrasonography image (109). Measurements on the chest were found to be the most precise (CV=33.2%), whereas measurement of the SFL on the flank and lumbar region resulted in higher CVs of 40,8% and 39.6%, respectively.

Accuracy

Sonographic measurement of the subcutaneous fat and body condition in dogs was found to be feasible, whereby the lumbar region, and in particular the L6, L7 and S1 region, was found to be the preferred anatomical location to measure the SFL for determining body condition or BF%. This was irrespective of the transducer used (109–111). Estimations of BF% using this technique correlated well with chemical analysis (r^2=0.87) (111). SFL measurements also directly correlated with a 5-point BCS (r^2=0.708; (109). However, the correlation between SFL and BW thus far was found to be inconclusive, with correlations ranging between 0.60 and 0.80 (109,110).

Since no studies have been performed to validate the use of the technique in cats, the accuracy of this technique remains unknown. However, one study looked at weight loss in cats, while measuring their change in body condition with different techniques, among which ultrasonography (41). Weight loss resulted in an significant reduction of the SFL. Moreover, a good correlation between the estimates of BF with SFL in the lumbar area and FM as measured by DEXA is recorded.

Practical applicability and availability

As ultrasonography is a non-invasive procedure and can be performed without clipping of the fur, it can generally be performed in the awake animal. Highly motile cats or those that are difficult to handle might need sedation or anaesthesia in order to complete the measurements.

Since a great number of first line veterinary clinics have an ultrasound machine readily available in their practice, the threshold of using this technique once properly validated could be low. However, at this moment in time, in cats but also in dogs, there is insufficient knowledge and research available to make this technique applicable in clinical practice. First the precision has to be improved and methods need to be standardized to make the technique reliable.

However, for the owner, the costs of a routine ultrasound may be a reason to decline the use of this technique to evaluate the body condition of their animal. Moreover, adequate training of the operator has to be arranged in order to enhance the precision of the methodology.

Discussion

When comparing the different methods with each other to evaluate their possibilities for application in the ferret DEXA and morphometric measurements appear to be the most reliable (Table 6). Other techniques, such as the relatively new MMS and ultrasonography are promising for scoring the body condition of cats. However, since both techniques are still under development and not fully validated yet, their reliability remains uncertain and therefore limit their use in practice for the current time being. Moreover, each of the techniques has its own additional limitations related to the accuracy of measurements (i.e. MMS only focuses on the amount of muscle mass of an animal (114,115), and does not evaluate fat reserves, which comprise an important part of the animal's total body condition whereas ultrasound only evaluates the subcutaneous fat reserves and not the muscle mass), costs and time associated with the measurements (particularly for ultrasonography), and necessity for sedation (particularly non-compliant animals). Sonography might pose extra difficulties in the ferret related to their small body size and active character. Sedation will therefore often be necessary. The MMS, in contrary, should be easily applicable in ferrets also. But as described above, no full validation is present for either techniques.

Of the various methodologies that exist, BIA is the only method that in its present form does not appear useable in the veterinary practice. Although BIA can have great accuracy, it's precision is often too variable for reliable application in practice. For ferrets, this would be no different.

DEXA and morphometric measurements appear to be the most reliable methods for estimating LBM, FM and BF%. Both methods are very precise and show high correlations with their reference methods (33,36). DEXA is considered the gold standard in alive animals, even though errors in the estimation of BF% for the individual can be high (29,33). DEXA results are also less dependent on different body morphologies, in contrast to morphometric measurements, which necessitate separate equations to be made for specific subpopulations to enable accurate estimations to be made for breeds with distinct body morphologies. As such, this system is less easily applied in practice, especially because of time constraints on the consultation, which do not allow much time to be spent on the different measurements. In ferrets, being very active animals, these time constraints will make it difficult for tape measurements to be taken precisely. However, great reproducibility of body measurements in ferrets has been reported (116), suggesting this method should be applicable.

Although accuracy is lower, BCS systems in general are considered reliable as well and because of their practical applicability, these are used most commonly in practice. For use in the ferret this technique is very promising. Veterinarians are already accustomed to the technique and because of low time requirements, compliance in the ferrets can be more easily assured. However, precision and accuracy differ between BCS systems, with the 9-point system showing the highest correlations with DEXA BF% estimates (59). Since training and experience have been found to significantly influence the reproducibility of results, this system is best applied by an experienced veterinarian. For less experienced staff and owners, the S.H.A.P.E. BCS system, which was also found to have good reliability, can provide a suitable alternative (70). However, even though a 5-7% increase in BF% per step (9-point system) or half-step (5-point system) is reported, making predictions of BF% based solely on an animals BCS is highly inaccurate (59,67). The differences between BF% in the various categories are often nonsignificant and overlap between categories is frequently seen (25,59,67). In addition, BF% within BCS categories have been found to differ greatly between study populations, activity level and sex (25,59,67). Thus, although the BCS systems can be very useful for estimating the body condition of an animal, specific estimates of BF% or total LBM cannot be made with this technique.

Conclusion

Based on the findings of this literature study, the objective and easily reproducible morphometric measurements seems to be the perfect combination between quick, cheap assessments and objective and reliable results. Moreover, with morphometric measurements estimates of LBM and FM% can also be made, which is not possible when using a BCS system. However, BCS systems can be used as a good substitution for morphometric measurements, especially when inadequate data is available for developing reliable morphometric equations. Morphometric measurements and BCS systems are thus the best options for evaluating the body condition of ferrets in the basic veterinary practice. Therefore, in the individual project, a combination of these techniques will be use to developed a system for the evaluation of a ferret’s body condition.

Table 6: An overview of the body condition scoring methods reviewed

Technique	Reliability	Advantages	Disadvantages	Possibilities for the ferret
DEXA (golden standard)	DEXA results are repeatable on the same densitometer, but reproducibility is low. Normal, constant tissue hydration is very important for acquiring reliable results. However, DEXA is very accurate and considered a golden standard for measuring BF%.	This technique is considered the golden standard for measuring LBM, FM and BF%. It gives an precise and accurate estimation of an cats body composition.	Cross calibration is necessary for comparing results between different densitometers. Sedation is needed to perform the scan and the scans are relatively expensive.	When using paediatric or small animal software, the DEXA scan can be easily used for ferrets.
BCS	Training is needed to make the BCS more reliable, but correlations with other observers and DEXA are generally high.	These systems are cheap, non-invasive and easy to use.	The measurements are subjective and training is required.	A body condition score system for the ferret can be developed and is easily usable.
MMS	The MMS is still under development and only moderately reproducibility is seen. It’s reliability is still uncertain.	This tool can help diagnose muscle wasting, an important sign of disease. It is non-invasive and aids in the documentation of visible changes in muscle mass.	The accuracy of this method is only moderate and observations are subjective.	When, after more research, the reliability of this technique is proven, it could be developed to supplement the BCS in ferrets.
Morphometric measurements	Morphometric measurements are in general really reliable, but care must be taken when applying the equations to a population different from the study population.	The measurements are non-invasive and little training is needed to make them reliable. No expensive equipment or lots of space are required.	Equations made are only reliable for a population similar to the study population. Multiple measurements are usually necessary and animals can become intolerant to handling.	Ferrets are very active animals, making the measurements a challenge. However, it is not impossible and the technique is very reliable.
BIA	BIA has been validated in dogs and cats, but no standardized methods are available and the reproducibility is questionable. Including morphometric measurements makes BIA more accurate.	The LBM and FM can be estimated without the need of anaesthesia, and the devices are portable.	As with DEXA, differences in electrolyte concentrations, or hydration (oedema) interfere with the results. Also, application of the needles can cause some discomfort.	Fixation without sedation of the ferret can be difficult when executing this technique.
ultrasonography	The reliability for this technique in cats is for the biggest part unknown, but the experience of the operator plays an important role in the precision of the measurements.	Ultrasound machines are readily available in veterinary clinics and ultrasonography is a non-invasive procedure.	The ultrasound takes, compared to the other techniques, longer and is relatively expensive. The SFL measurements are not validated in cats, and repeatability is currently low.	Patient compliance can become an issue, but it should be possible to use this technique, if properly validated.

Part 2: Individual project. Evaluating the body condition of the ferret

The BCS-system is very popular and is used with great success in dogs and cats. The ease of use and the lack of tools needed, makes it a very attractive method. Therefore the aim of this research is to develop a body condition scoring method for ferrets resembling a BCS-system, if needed supplemented with morphometric measurements. To validate the techniques, DEXA scans would ideally need to be performed as these are considered the gold standard. Within the time frame of this study, this was not found feasible, and therefore considered to be beyond the scope of the project.

Material and Methods

The animals:

41 ferrets, 19 males, 22 females, from different ferret shelters[2] (37 animals) and private owners[3] (4 animals) were enrolled in the study. In addition, 3 ferret patients from the 'Veterinary Ferret Clinic' (Reygerboslaan 32, Giessen, Netherlands) were examined. Females weighed 759g ± 219 g (515-1385g) and males 1317 ± 339 g (828-2390g). Animals were aged 3.5 years ± 1.8 (6 months – 7 years)[4]. Among other characteristics collected were age, gender, body weight (BW) and castration status. All ferrets, except three, were surgically neutered. Of these three animals (one male and two females), two animals (the females) had received an implant, resulting in only one intact animal (a male ferret) included in the study. Most ferrets were reported to be generally healthy, but an enlarged spleen, chronic bronchitis, kidney tumours, stomach problems and middle ear infections were seen.

There were no specific inclusion criteria's for ferrets entering the study; however severely ill animals or animals that appeared to be in pain were not included in this study. In order to increase the ferrets' compliance with the measurements, ferrets were provided with treats, vitamin paste or convalescence support (Royal Canin, Poort van Veghel 4930, Veghel, Netherlands).

Dependent on de shelter, the ferrets were housed together in groups of two or three (foundation 'Frettig Gestoord') or individually (foundation 'De Fret', foundation 'Fret & Welzijn'). In most cases, ferrets were housed indoors in a ferret room where most of the ferret cages and play areas were stationed to enable visual, auditory and/or olfactory contact. In one shelter, ferrets were housed outside in the garden.

Data was collected in March and April 2017. The measurements performed were non-invasive and caused no discomfort to the animals. All caregivers gave informed consent before animals were measured.

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[2] three Ferret shelters of foundation "Frettig Gestoord": Ilonka van Lieshout, Rine Oddens and Stephenie Baas, a Ferret shelter of foundation "De Fret": Marianne Boymans and a ferret shelter from Foundation "Fret & Welzijn": Chaimel Lerou were visited.

[3] Of these four privately owned ferrets, three were housed together in one cage, while the other ferret was housed alone with a different owner.

[4] For many ferrets, the age has been estimated by the shelters.

Study design

To develop the body condition scoring system, all ferrets were photographed, visually inspected, palpated, and weighed. Moreover, morphometric measurements were taken to evaluate the animals body condition. All procedures were carried out by one researcher (I.B.), whereby results were noted on two separate forms: one for the inspection and palpation of bodily structures and one for the morphometric measurements (Appendix 6 and 7).

Because of their experience with ferrets , the ferret owners, caregivers of the shelter ferrets in the shelters, and veterinarian were also questioned about their opinion of the animals' body condition. Based on their evaluations, the ferrets were divided into three categories: underweight – optimal weight – overweight. Next, the body condition was evaluated by the researcher (I.B.) based on the visibility and palpability of specific anatomic structures. Similarly, morphometric measurements and body weight were collected. These aforementioned measurements were subsequently statistically 36nalysed to determine whether and which variables correlated best with the body condition as identified by the experts. For the variables that correlated well with the body condition, it was also determined which description was most often chosen for a particular body condition. These description were taken up into the BCS-system chart to complement the photographs.

Photographs

To develop the BCS-chart, lateral and dorsal photographs were taken of 44 ferrets in different body conditions using a 14.0 megapixels Nikon Coolpix s3100 camera. To even out influence of the background, a green screen cloth was used as background.

Weight

Bodyweight was collected from all ferrets. The type of scale that was used depended on the type that was available in the shelter or home of the ferret. As a result, a mixture of , one analogous- and four digital kitchen scales, as well as two advanced baby scales were used. All scales could at least weigh accurately in grams and were tarred before use. Therefore, weight measurements were collected in grams.

Visual inspection

Ferrets were visually inspected for visibility of the cervical and lumbar vertebrae, the tuber ischium, ribs and waist, as described for de BCS in cats (59,67,70). The visibility of each of these bony structures and presence of a waist were scored on a three point scale, i.e. clearly visible – partially visible – not visible.

Palpation of anatomical structures

The following bony structures were evaluated for their palpability: cervical and lumbar vertebrae, tuber ischium, ribs, waist and paws (see also Table 7 for a detailed description, methodology and grading) Ribs were additionally evaluated for an estimation of the thickness of the fat layer on the ribs. Moreover paws were graded for the amount of muscle and/or fat present, whereas the abdomen was palpated on visceral fat content and alignment with the thorax, whereby the amount of visceral fat present was estimated in centimetres. Last, muscle mass was graded by palpating the m. longissimus and m. transversospinalis of the lumbar region of the back and the m. biceps femoris of the hind limb (Table 7).

Morphometric measurements

Five morphometric measurements were taken of each ferret, i.e. ventral body length (VBL), dorsal body length (DBL), the leg index measurement (LIM), ribcage circumference (RC) and belly circumference (BC) (71,116). To obtain the measurements, a tailor's measuring tape with a centimetre scale (accurate up to 1mm) was used. All measurements were collected once from each ferret by the same researcher (I.B.), while the ferret was being scruffed, held in a vertical or horizontal position by the caregiver as depicted in Figure 7 (Figure 7, Table 7).

Data analysis

Data analysis was performed using the program R-studio (open source desktop version 0.98.1062) [5]. Univariate linear regression models were used to test morphometric measurements for correlations with each other, BW, body condition and gender. Moreover, body condition was tested for correlations with age, BW and gender.

In order to make preselection of the variables to be tested for collinearity, a Fisher's exact test for each of the variables was performed. This test was chosen because the relatively small dataset in combination with the large number of variables resulted in small numbers of animals per category. To facilitate interpretation of the Fisher's exact test and logistic regression, the continuous variables (i.e. BW and morphometric measurements) were converted into categorical variables by dividing these into three groups whereby the lower third of ferrets with the lowest score received the label small/light, the middle third of ferrets received the label average and the top third received the label large/heavy. For all the variables with $p<0.2$ in the Fisher exact test, the Odds-ratio (OR) and 95% confidence interval (95% CI) were determined using univariate analysis. Hereby making a selection for which variables can be used in an logistic regression. For some of the variables no animals fulfilled the criteria for a specific category. In order to still enable a reasonable estimation to be made of the OR and its 95% CI, the OR was manually calculated by adding 0,5 to all four fields of the table, after which the 95% CI was determined (see Box 2).

The dependent variable 'body condition' was coded into dummy variables: obese, optimal weight and underweight. With these three dummy variables and preselected variables from the Fisher exact test, the five best logistic regression models were found by fitting all the possible models, using the package glmulti (117). From these five best models, the most suitable model for each dummy variable was chosen. These three final models were subsequently implemented in the BCS-chart.

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[5] www/rstudio.com

Figure 7: Photographs representing the way the ferrets were hold during morphometric measurements. The photographs are not owned by the author and have been taken from the internet (117–119).

Box 2: Formulas used for the manual calculation of OR and its 95% CI

$$OR = \frac{c/d}{a/b}$$

ln(CI) = ln(OR)±1,96 * $LN(CI) = LN(OR) \pm 1.96 * \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$

with

	obese/optimal/underweight	The rest
Variable level 1 (ref)	a	b
Variable level 2	c	d
Etc.		

Table 7: Detailed description of the various structures that were visually inspected and/or palpated as well as the morphometric measurements that were obtained during this study. Accidental missing data is reported as 'unknown'.

Variable	Description of the measurement	Grading
Visual inspection	Visually inspection took place of the following structures: cervical and lumbar vertebrae, the tuber ischium, ribs and waist.	• Clearly visible • Partially visible • Not visible
Palpability of the cervical and thoracic vertebrae	Palpability of the cervical and thoracic vertebrae was performed while holding the ferret on one hand and palpating with the other.	• Very easily palpable • Easily palpable • Palpable with some pressure • Hardly/not palpable
Palpability of the ischial tuberosity	The ischial tuberosity was palpated while the ferret was standing on the table.	• Easily palpable • Palpable with some pressure
Rib fat coverage	With the ferret standing on a table, the rib fat coverage was estimated by sliding both hands simultaneously across both sides of the ribcage and estimating the amount of fat covering the ribs.	• No fat coverage • Scant fat coverage • Little fat coverage • Moderate fat coverage • Substantial fat coverage • Unknown
Palpability of the ribs	Palpability of the ribs was determined with the ferret standing on a table while the observer slided both hands simultaneously over the ribcage. Note: In contrast to rib fat coverage this variable provided an estimation of the palpability of the ribs rather than an estimation of the fat coverage over it.	• Very easily palpable • Easily palpable • Palpable with some pressure • Hardly/not palpable

Palpability of the waist	The waist was palpated by sliding both hands from the ribs dorsocaudal to feel the waist.	• Very easily palpable • Easily palpable • Palpable with some pressure • Hardly/not palpable
Palpability of the lumbar vertebrae	The lumbar vertebrae were palpated by taking the ferret up in one hand and feeling along the spine with the other.	• Very easily palpable • Easily palpable • Palpable with some pressure • Hardly/not palpable • unknown
Shape of the abdomen	The shape of the abdomen was determined primarily based on palpating the transition from ribcage to abdomen. I	• Tucked in abdomen, whereby the abdomen is of a smaller height than the thorax • Normal abdominal shape, whereby the abdomen is of similar height as the thorax • Distended abdomen, whereby the height of the abdomen is larger than the height of the thorax
Estimation of abdominal fat content	An estimation of abdominal fat content was made by holding the ferret in one hand and palpating the subcutaneous fat at the abdominal level with the other hand.	• < 1 cm • ≥ 1cm • Unknown
Palpability of the bones in the front paws	With the ferret standing on the table, the palpability of the radius and ulna was determined by feeling both front paws simultaneously whereby the front leg was palpated between thumb and index/middle finger.	• Easily palpable • Palpable with some pressure • Unknown
Estimation of muscle and fat tissue in the front paws	The amount of muscle and fat covering the radius and ulna was determined similar to the palpability of the bony structures of the front leg	• No fat present • Scant fat/muscle coverage • Little fat/muscle coverage • Moderate fat/muscle coverage • Substantial fat/muscle layer • Unknown
Muscle evaluation	The muscle mass of the ferrets was evaluated by palpating the m. longissimus and m. transversospinalis in the lumbar region and by palpating the m. biceps femoris of both hindlegs.	• Normal • Slight muscle wasting • Severe muscle wasting
LIM*	The leg index measurement was determined by measuring the distance between the patella and the calcaneus tuber of one of the hindlimbs. Which hind limb was measured, was dependent on the way the owner presented the ferret to the caregiver (Figure 7A & B).	• Small ○ female<6 cm ○ male<7.5 cm • Average ○ female: 6-7 cm, ○ male: 7.5-8.4 cm • Large ○ female>7 cm • male>8.4 cm)

DBL*	Dorsal body length was measured from the tip of the nose to the tale base, while the ferret was being scuffed or held in a vertical position by the caregiver (Fig7 A & B).	• Small o female<34.5 cm o male<42.9 cm • Average o female: 34.5-37 cm o Male: 42.9-44.9 cm • Large o female>37 cm • male>44.9 cm
VBL*	Ventral body length was measured from the tip of the nose to the anus, across the ventral side of the animal, while the caregiver holds the animal in vertical position.	• Small o female<33.0 cm o male<39 cm • Average o female: 33-4 cm o male: 39-42.9 cm • Large o female>36 cm • male >42.9 cm
RC*	The ribcage circumference was measured with the ferret held in vertical or horizontal position by the caregiver. The measurements were taken at the xyphoid process.	Corrected for gender: • Small o female <17.0 cm o male <20 cm • Average o female: 17.0-18.2 cm o male: 20-21.9 cm • Large o female:> 18,3 cm o male >21.9 cm
BC*	The belly circumference was measured at the widest point of the belly, with the ferret being hold in a vertical or horizontal position by the caregiver.	• Small o female<18.5 cm o male<23.9 cm • Average o female: 18.5-20.9 cm o male: 23.78-25.5 cm • Large o female>20.9 cm • male>25.5 cm
BW*	The body weight of the ferrets was measured in grams using a kitchen scale (analogous or digital) or a professional baby scale.	• Light o female<637 g o male<1248 g • Average o female: 637-716 g o male: 1248-1404 g • Heavy o female>716g o male>1404 g

*These variables received a correction for the gender influence on body size. See results for more explanation.

Results

Of the 41 ferrets, 9 were classified as overweight, 20 as having an optimal body condition and 12 as being underweight.

Visual inspections

The extensive amount of fur that the ferrets possessed hindered visual inspections of the body conditions. Only in one animal that was partially bald because of an endocrine disorder, the lumbar vertebrae, waist and the ischial tuberosity were partially visible. As a result, this data was not further analysed.

Palpations

All ferrets were compliant with the palpations and in most ferrets all variables were evaluated. However, in some animals a variable was forgotten or not registered. These missing values were assigned to the category 'unknown' (Table 7). For all anatomical structures that were palpated, a significant correlation with body condition score was found (Fisher's exact test; p<0.2; Table 8).

The palpability of the cervical and thoracic vertebrae showed a clear distinction between obese, optimal and underweight animals (p=0.002). Overweight animals were most likely to be scored 'hardly/not palpable' (OR=1.29; CI=0.04-38)), while ferrets in optimal body condition were 9 times more likely to be scored 'easily palpable' than 'very easily palpable' (OR=9; CI=0.2-363). Underweight animals, in contrast, were most likely to be scored 'very easily palpable'. Similarly, in most overweight ferrets and those in optimal condition, the ischial tuberosity was palpable with some pressure (OR=1.87; CI=0.2-23 and OR=2.22; CI=0.2-27, respectively). In underweight ferrets, the ischial tuberosity was 3.3 times more likely to be easily palpated than to be only palpable with some pressure (p=6.90E-07, OR=0.3; CI=0.01-6).

Overweight animals were most likely to have a considerable (moderate) amount of fat covering their ribs (p<0.002, OR=7.61; CI=0.3-175, compared with no fat coverage), whereas ferrets in optimal body condition had the highest odds for a small amount of fat coverage (OR=9; CI=0.8-108) and underweight ferrets most often had no fat covering their ribs (all OR<1, compared to no fat covering).

Upon evaluation of the palpability of the ribs themselves, overweight animals were found 21 times more likely to have hardly or non-palpable ribs (p=0.18, OR=21; CI=0.2-2860), whereas the ribs of ferrets in optimal condition were generally easily palpable (OR=2.67; CI=0.2-34) and very easily palpable in underweight ferrets (all OR<1, compared to 'very easily palpable'). Similar findings were observed for palpability of the lumbar vertebrae (p=1.43E-05, $\text{OR}_{\text{overweight}}$=12; CI=0.-422 and $\text{OR}_{\text{optimal weight}}$=5; CI=0.4-72), with the only exception that the lumbar vertebrae of animals in optimal body condition were most likely to be palpable with some pressure (Table 8).

In contrast to the other parameters, the palpability of the waist revealed no obvious distinction between the different body condition groups, i.e. ferrets in all body conditions were found just as likely to have a very easily palpable waist as a hardly/not palpable waist (p=5.31E-07). Nevertheless, overweight animals were found 7 times more likely to have their waist palpable with some pressure rather than very easily palpable (OR=7; CI=0.2-291) while in underweight ferrets the waist was most likely to be easily palpable (OR=1.6; CI=0.1-42, compared to very easily palpable)). Abdominal shape, on the other hand, was found to be more informative, with obese ferrets being 6.3 times more likely to have an extended abdominal and 0.3 times more likely to have an normal abdominal shape than an tucked in abdomen (p=3.44E-11). For ferrets in optimal body condition, the OR for these categories were 12.8 (CI=8-17) and 7.9 (CI=-1-5), respectively. In contrast, underweight ferrets were 7.7 times more likely to have a tucked in abdomen than a normal-sized abdomen (CI=0.4-167), compared to ferrets that are not underweight.

The estimation of abdominal fat content and palpability of the bones in the front paws show similar patterns. Obese ferrets and ferrets in optimal condition are 12 times and 1.9 times more likely to have >1cm abdominal fat, respectively (p=0.001, CI=3.3-43 and CI=0.4-8.6). Underweight animals most often have <1 cm of abdominal fat (Table 8). However, the bones in the front paws are only palpable with some pressure in the group of obese ferrets (p=9.92E-08, OR=18; CI=3-115).

For the front legs, the amount of muscle and fat tissue were divided into 5 categories resulting in very few ferrets being classified in each category. Nevertheless, a clear shift between the ORs in the different body condition groups could be seen, with the obese group being 15 times more likely to have a substantial fat/muscle layer and the underweight animals 9 times more likely to have scant fat/muscle coverage (p=0.045).

Univariate analysis of the muscle evaluation revealed that underweight animals are more prone to be suffering from muscle wasting than other animals, with them having 5.06 times more change of having slight muscle (CI=-0.3-3.58) wasting and being 9,71 more likely to have strong muscle wasting compared with underweight ferrets with normal muscle mass (CI=-1-5.6), while the obese animals and ferrets in optimal condition have very low ORs for muscle wasting compared to normal muscle mass (OR=0.003-0.93)

Table 8: Correlations of BCS variables with body condition, Fisher exact test

variables	category	Overweigt (n=9)	optimal body condition (n=20)	underweight (n=12)	p-value Fishers exact test
		% (n)	% (n)	% (n)	
palpability of the cervical and thoracal vertebrae	very easily palpable	0	0	8.3 (1)	0.002
	easily palpable	0	20 (4)	8.3 (1)	
	palpable with some pressure	55.5 (5)	45 (9)	58.3 (7)	
	hardly/not palpable	44.4 (4)	35 (7)	25 (3)	
palpability of the ischial tuberosity	easily palpable	88.9 (8)	90 (18)	100 (12)	6.90E-07
	palpable with some pressure	11.1 (1)	10 (2)	0 (0)	
rib fat coverage	no fat present	0 (0)	5 (1)	33.3 (4)	0.002
	scant fat/muscle coverage	11,1 (1)	10 (2)	33.3 (4)	
	little fat/muscle coverage	22.2 (2)	45 (9)	16.7 (2)	
	moderate fat/muscle coverage	44.4 (4)	20 (4)	16.7 (2)	
	substantial fat/muscle layer	11.1 (1)	5 (1)	0	
	unknown	11.1 (1)	15 (3)	0	
palpability of the ribs	very easily palpable	0 (0)	5 (1)	16.7 (2)	0,18
	easily palpable	22.2 (2)	60 (12)	58.3 (7)	
	palpable with some pressure	66.7 (6)	35 (7)	25 (3)	
	hardly/not palpable	11.1 (1)	0 (0)	0 (0)	
palpability of the waist	very easily palpable	0 (0)	5 (1)	0 (0)	5.3E-07
	easily palpable	66.7 (6)	85 (17)	100 (12)	
	palpable with some pressure	33.3 (3)	5 (1)	0 (0)	
	hardly/not palpable	0 (0)	5 (1)	0 (0)	
palpability of the lumbar vertebrae	very easily palpable	0 (0)	5 (1)	16.7 (2)	1.4E-05
	easily palpable	33.3 (3)	40 (8)	75 (9)	
	palpable with some pressure	33.3 (3)	50 (10)	8.3 (1)	
	hardly/not palpable	22.2 (2)	5 (1)	0 (0)	
	unknown	11.1 (1)	0 (0)	0 (0)	
Shape of the abdomen	tucked in abdomen	0 (0)	0 (0)	33.3 (4)	3.4E-11
	Normal abdomen	0 (0)	35 (7)	66.7 (8)	
	Distended abdomen	100 (9)	65 (13)	0 (0)	
estimation of adominal fat content	<1 cm	0 (0)	20 (4)	58.3 (7)	0.001
	≥ 1 cm	77.8 (7)	55 (11)	25 (3)	
	unknown	22.2 (2)	25 (5)	16.7 (2)	
palpability of the bones in the front paws	easily palpable	77.8 (7)	20 (4)	8.3 (1)	9.9E-08

	palpable with some pressure	22.2 (2)	75 (15)	91.6 (11)	
	unknown	0 (0)	5 (1)	0 (0)	
estimation of muscle and fat tissue in the front paws	no fat present	0 (0)	5 (1)	0 (0)	0.045
	scant fat/muscle coverage	0 (0)	5 (1)	33.3 (4)	
	little fat/muscle coverage	11.1 (1)	35 (7)	41.7 (5)	
	moderate fat/muscle coverage	22.2 (2)	5 (1)	0 (0)	
	substantial fat/muscle layer	22.2 (2)	0 (0)	0 (0)	
	unknown	44.4 (4)	50 (10)	25 (3)	
muscle evaluation	normal	100 (9)	90 (18)	66.7 (8)	9.1E-03
	slight muscle wasting	0 (0)	10 (2)	25 (3)	
	strong muscle wasting	0 (0)	0 (0)	8.3 (1)	

Table 9: Correlations of morphometric measurements with body condition, Fisher exact test. All measurements are corrected for the gender influence on body size.

variables	category	Overweight (n=9)	optimal body condition (n=20)	underweight (n=12)	p-value Fishers exact test
LIM	small	22.2(2)	30 (6)	8.3 (1)	
	average	33.3 (3)	25 (5)	66.7 (8)	
	large	44.4 (4)	45 (9)	25 (3)	0.23
DBL	small	11.1(1)	45(9)	25 (3)	
	average	22.2(2)	25(5)	41.7 (5)	
	large	66.7(6)	30(6)	33.3 (4)	0.28
RC	small	11.1 (1)	20 (4)	58.3 (7)	0.01
	average	11.1 (1)	50 (10)	25 (3)	
	large	77.8 (7)	30 (6)	16.7 (2)	
BC	small	0 (0)	25 (5)	66.7 (8)	0.02
	average	33.3 (3)	35 (7)	16.7 (2)	
	large	66.7 (6)	40 (8)	16.7 (2)	
BW	light	44.4 (4	25 (5)	58.3 (7)	0.05
	average	44.4 (4)	15 (3)	25 (3)	
	heavy	11.1 (1)	60(12)	16.7(2)	

Table 10: Univariate analysis of the BCS variables with p<0.2 in the Fisher exact test

variables	categories	overweigt vs the rest		optimal vs the rest		underweight vs the rest	
		OR	95% CI	OR	95% CI	OR	95% CI
palpability of the cervical and thoracal vertebrae	very easily palpable	1	ref	1	ref	1	ref
	easily palpable	0.3*	0.004-20.4*	9*	0.2-362.5*	0.2	0.01-7.4
	palpable with some pressure	1*	0.04-28.3*	2.3*	0.08-62.4*	0.5	0.09-2.7
	hardly/not palpable	1.3*	0.04-8.0*	3*	0.1-86.1*	0.3	0.01-5.8
palpability of the ischial tuberosity	easily palpable	1	Ref	1	Ref	1	Ref
	palpable with some pressure	1.9	0.15-23.4	2.2	0.2-26.6	0.3*	0.01-6.3*
rib fat coverage	no fat present	1	Ref	1	Ref	1	Ref
	scant fat/muscle coverage	2.5*	0.09-75.8*	1.6	0.1-24.7	0.3	0.02-4.7
	little fat/muscle coverage	2.4*	0.1-58.8*	9	0.8-108.3	0.05	0.003-0.7
	moderate fat/muscle coverage	7.6*	0.3-175.0*	2.7	0.2-33.5	0.06	0.004-0,9
	substantial fat/muscle layer	11*	0.3-433.8*	4	0.1-137.0	0.07*	0.002-2.3*
	unknown	4.7*	0.15-151.5*	12	0.5-280.1	0.04*	0.001-1.2*
palpability of the ribs	very easily palpable	1	Ref	1	Ref	1	Ref
	easily palpable	0.9*	0.4-23.0*	2.7	0.2-34.2	0.3	0.02-3.3
	palpable with some pressure	4.3*	0.2-98.2*	1.6	0.1-20.9	0.1	0.01-1.7
	hardly/not palpable	21*	0.2-2859.8*	0.6*	0.02-34.2*	0.2*	0.01-8.8*
palpability of the waist	very easily palpable	1	Ref	1	Ref	1	Ref
	easily palpable	0.6*	0.02-18.1*	0.3*	0.10-1.0*	1.6*	0.06-42.1*
	palpable with some pressure	7*	0.2-291.4*	0.1*	-5.7-1.8*	0.3*	0.004-25.4*
	hardly/not palpable	1*	0.01-92.4*	1*	0.01-92.4*	1*	0.01-92.4*
palpability of the lumbar vertebrae	very easily palpable	1	Ref	1	Ref	1	ref
	easily palpable	1.4*	0.06-33.6*	1.3	0.1-17.3	0.4	0.03-5.3
	palpable with some pressure	2.1*	0.09-52.0*	5	0.4-71.9	0.04	0.002-0.9
	hardly/not palpable	11.7*	0.32-422.2*	1	0.03-29.8	0.1*	0.002-3.1*
	unknown	21*	0.15-2859.8*	0.6*	0.01-24.5*	0.2*	0.005-8.8*
abdominal shape	tucked in abdomen	1	Ref	1	Ref	1	Ref
	normal abdominal shape	0.2*	0.1-0.8*	7.9*	-1.0-5.2*	0.1*	0.006-2.8*
	distended abdomen	6.3*	0.3-132.1*	12.8*	9.8-15.8*	0.002*	0.00004-0.1*
estimation of adominal fat content	<1 cm	1	Ref	1	Ref	1	Ref
	≥ 1 cm	11.9*	3.3-43.5*	1.9	0.4-8.6	0.01	0.02-0.5
	unknown	7.7*	0.3-183.0*	2.2	0.4-13.2	0.2	0.02-0.2
palpability of	easily palpable	1	ref	1	Ref	1	Ref

the bones in the front paws	palpable with some pressure	18.2	2.9-114.6	004	0.1-1.8	0.1	0.02-1.3
	unknown	0.2*	0.004-14.3*	5.7*	0.1-336.2*	2.6*	-2.7-4.6*
estimation of muscle and fat tissue in the front paws	no fat present	1	Ref	1	Ref	1	Ref
	scant fat/muscle coverage	0.3*	0.004-2.4*	0.1*	0.003-4.5*	9*	0.22-362.5*
	little fat/muscle coverage	0.4*	0.005-25.8*	0.4*	0.01-11.2*	1.9*	3..4*
	moderate fat/muscle coverage	5*	0.1-220.6*	0.2*	0.005-8.8*	0.4*	0.01-33.6*
	substantial fat/muscle layer	15*	0.2-1236.3*	0.07*	0.0008-5.5*	0.6*	0.01-49.5*
	unknown	1*	0.03-29.2*	0.5*	0.02-13.1*	0.7*	0.02-21.9*
muscle evaluation	normal	1	Ref	1	Ref	1	Ref
	slight muscle wasting	0.003*	0.0001-0.1*	0.6	-2.4-1.5	5.1	-0.3-.6
	strong muscle wasting	0.9*	0.03-24.8*	0.3*	0.01-8.3*	9.7*	-1.0-5.6*

*values that are manually calculated with the formulas shown in box 2

Morphometric measurements

VBL, DBL and LIM were found to be moderately correlated with each other and BW in univariable regression analysis (r^2=0.34-0.72; p<0.05). However, low, insignificant correlations between these measurements and the body condition of the ferrets were found (r^2=0.01-0.10). The Fishers exact test showed similar results, with p-values found between 0.28 and 0.76 (Table 9 & 11).

Gender and body size (VBL, DBL and LIM) showed an obvious, but moderate, correlation (Figure 9), with males clearly having higher values for these measurements (r^2=0.44-0.61, p<2.06E-06 for all three body size measurements).

Similarly, RC, BC and BW significantly correlated with gender with correlations of 0.40, 0.45 and 0.51 found, respectively. As a result, a correction took place to separate out any gender influences before performing the Fishers exact test (Table 9). The Fishers exact test shows that RC and BC are correlated with the body condition of the ferret. A large RC and BC classification resulted in high odds for a ferret to be classified as obese (OR=9.5 and 16.7, respectively; Table 11). Similar findings were observed for BW, where obese animals were more likely to be classified as average or heavy (OR=8.0 and 3.4., respectively, Table 11) Alternatively, an underweight animal was found 9.1 (RC & BW) and 11.1 (BC) times more likely to be classified as a small/light animal than a large/heavy animal.

Moreover, obese ferrets tended to be younger than ferrets assigned an optimal or too lean body condition (p=0.09; Figure 8). However, a Fisher exact test showed that, age, just as gender, was not significantly correlated with the body condition of the ferret (p=0.57 and p=0.84, respectively).

Table 11: Univariate analysis of the morphometric measurements with p<0.2 in the Fisher exact test. RC=ribcage circumference, BC=belly circumference and BW=bodyweight

Variables	Categories	Overweight vs the rst		Optimal vs the rest		Underweight vs the rest	
		OR	95% CI	OR	95% CI	OR	95% CI
RC†	Small	1	Ref	1	Ref	1	Ref
	average	0.9	0.05-15.2	5	0.94-26.5	0.2	0.04-1.1
	big	9.6	1.0-95.0	1.3	0.3-6.5	0.1	0.02-0.7
BC†	Small	1	Ref	1	Ref	1	Ref
	average	10.0*	0.5-215.9*	2.2	0.5-11.1	0.1	0.02-0.8
	big	16.7*	0.8-331.5*	1.6	0.4-7.1	0.1	0.01-0.6
BW†	light	1	Ref	1	Ref	1	Ref
	average	8.0	0.7-88.2	0.7	0.1-4.0	0.4	0.1-2.1
	heavy	3.4	0.3-35.0*	3.2	0.7-14.2	0.1	0.02-0.7

† corrected for gender influence on body size

Figure 8: Correlation between Age and Body condition. Obese animals tend to be younger (2.6 ± 2.0 years, p=0.09) than animals in optimal condition (3.7 ± 1.7 years) and underweight animals (3.9 ± 1.6 years).

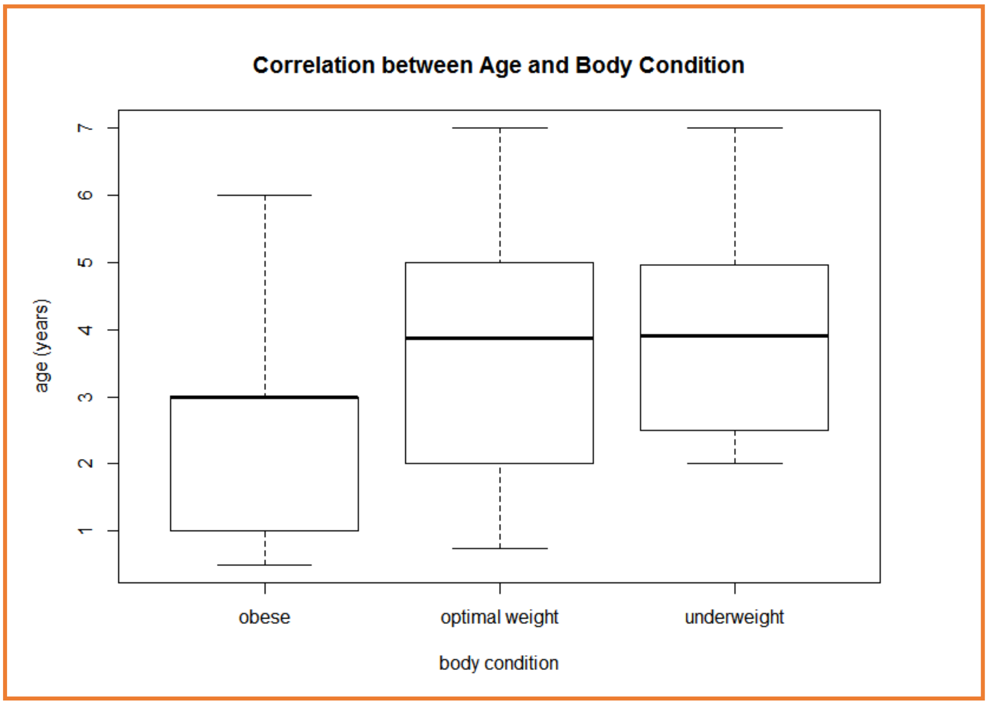
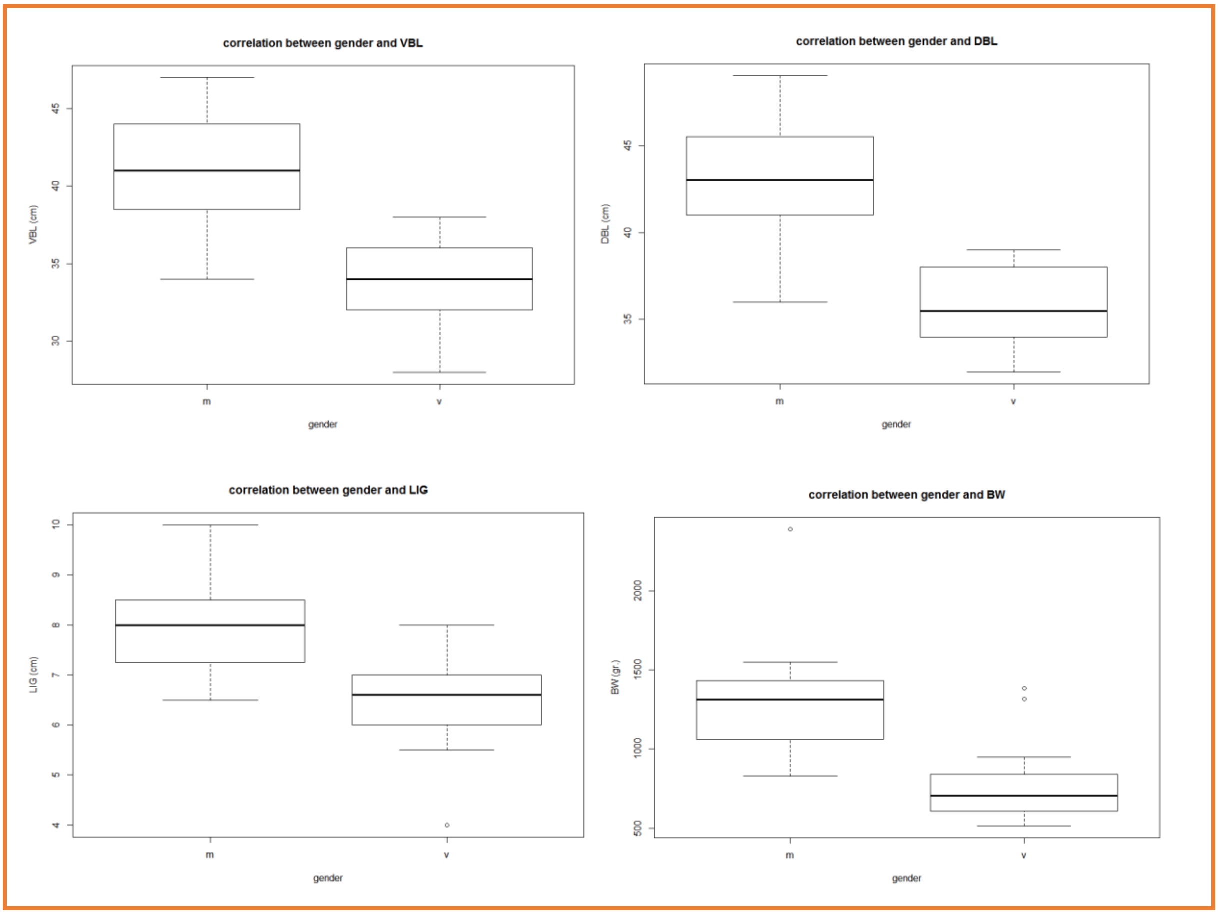


Figure 9: Relationships of VBL, DBL, LIM and BW with gender in 41 ferrets. Males have a significantly higher VBL (41.2 ± 3.7) than females (33.9 ± 2.7; p=9.62E-09). The same is observed for DBL and LIG measurements where male DBL and LIG are on average 43.1 ± 3.6 and 8.0 ± 0.9 and female DBL and LIG measurements are 35.7 ± 2.3 and 6.4 ± .9, respectively. The gender differences are for both DBL and LIG measurements significant (p=1.38E-09 and 2.05E-06, respectively). Similarly, male body weights (1317.16 ± 339) are higher than female body weights (759 ± 220; p=2E-16). M=male, F=female. Scales on the y-axis are in cm and for BW in grams.



The BCS-model:

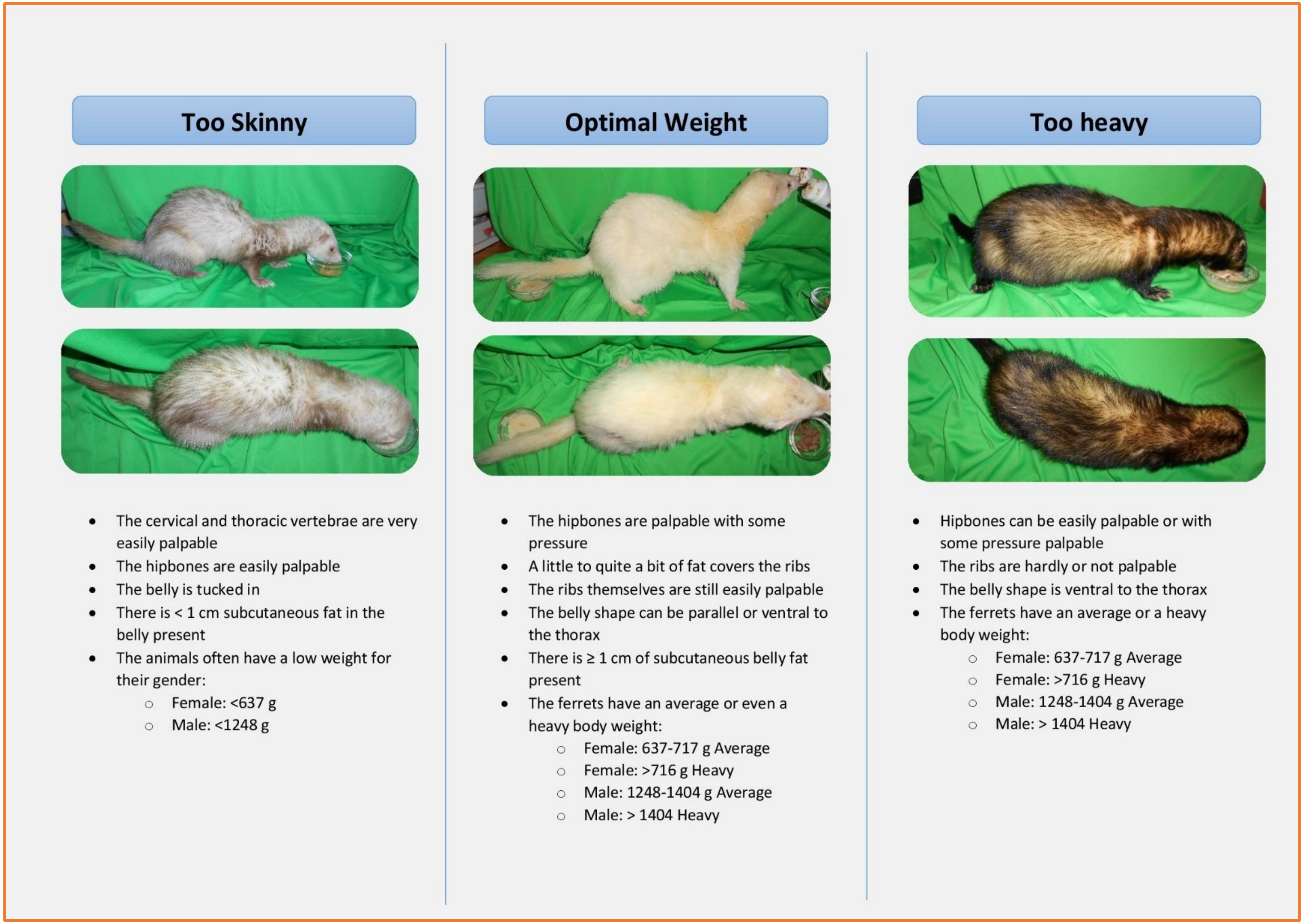
For each body condition category, a multivariable logistic regression was performed, giving the 5 best models per body condition. Of these 5 models per body condition, the best model for developing a BCS-chart was chosen and can be seen in Table 12. The models were selected based on the number of variables included and their p-values in the model. From these best models, models 2 and 3 each have two variables with one or more non-significant categories, but because these models were found to result in the best fit, these variables remained in the model. In contrast to the other selected models, model 1 only included one significant category. However, since other models resulted in a lower quality of fit, this model was rendered the best for the analysed data.
With these three final models a BCS-chart was developed (Figure 10). For each variable, the most common description per body condition (based on OR values) was implemented on the chart, after which photographs of two ferrets per body condition were added.

Table 12: The best logistic regression models for each body condition, selected based on the number of variables included and their p-values. These models were implemented in the BCS-chart.

Model 1: obese animals vs not obese animals				
Variables	category	p-value	OR	95% CI
palpability of the ischial tuberosity	easily palpable		1	Ref
	palpable with some pressure	0.13	0.67	0.67-2.11
palpability of the ribs	very easily palpable		1	Ref
	easily palpable	1.0	0.99	0.41-1.11
	palpable with some pressure	0.37	1.23	0.65-1.51
	hardly/not palpable	0.02	3.45	1.33-8.96
abdominal shape	tucked in abdomen		1	Ref
	normal abdominal shape	0.54	0.89	0.61-1.30
	distended abdomen	0.29	1.23	0.84-1.80
BW	Light		1	Ref
	average	0.40	1.17	0.81-1.72
	heavy	0.23	0.8	0.56-1.14
Model 2: animals with an optimal weight vs obese and underweight ferrets				
palpability of the ischial tuberosity	easily palpable		1	Ref
	palpable with some pressure	0.04	1.94	1.07-5.51
rib fat coverage	no fat present		1	Ref
	scant fat/muscle coverage	0.05	1.91	1.03-3.54
	little fat/muscle coverage	0.001	3.12	1.66—5.86
	moderate fat/muscle coverage	0.03	2.40	1.17-4.92
	substantial fat/muscle layer	0.02	3.89	1.40-10.82
	unknown	0.004	3.06	1.50-6.21
palpability of the ribs	very easily palpable		1	Ref
	easily palpable	0.03	0.44	0.22-0.90
	palpable with some pressure	0.01	0.32	0.14-0.69
	hardly/not palpable	0.001	0.06	0.01-0.25
abdominal shape	tucked in abdomen		1	Ref
	normal abdominal shape	0.003	2.08	1.33-3.28
	distended abdomen	0.008	2.06	1.26-3.37
estimation of abdominal fat content	<1 cm		1	Ref

	≥ 1 cm	0.67	1.09	0.73-1.64
	unknown	0.33	1.22	0.82-1.81
BW	Light		1	Ref
	average	0.23	0.73	0.44-1.20
	Heavy	0.10	1,45	0.94-2.23
Model 3: underweight animals vs obese animals and animals with an optimal weight				
palpability of the cervical and thoracic vertebrae	very easily palpable		1	Ref
	easily palpable	0.003	0.33	0.17-0.64
	palpable with some pressure	0.009	0.43	0.24-0.78
	hardly/not palpable	0.007	0.39	0.21-0.74
palpability of the ischial tuberosity	easily palpable		1	Ref
	palpable with some pressure	0.26	0.82	0.58-1.15
abdominal shape	tucked in abdomen		1	Ref
	normal abdominal shape	0.002	0.56	0.41-0.78
	distended abdomen	2.29E-06	0.36	0.25-0.51
estimation of abdominal fat content	<1 cm		1	Ref
	≥ 1 cm	0.36	0.88	0.67-1.15
	unknown	0.003	0.61	0.46-0.82
BW	Light		1	Ref
	average	0.57	1.10	0.80-1.52
	heavy	0.41	0.88	0.66-1.18

Figure 10: The BCS-chart for ferrets (pilot version)



Too Skinny

- The cervical and thoracic vertebrae are very easily palpable
- The hipbones are easily palpable
- The belly is tucked in
- There is < 1 cm subcutaneous fat in the belly present
- The animals often have a low weight for their gender:
    - Female: <637 g
    - Male: <1248 g

Optimal Weight

- The hipbones are palpable with some pressure
- A little to quite a bit of fat covers the ribs
- The ribs themselves are still easily palpable
- The belly shape can be parallel or ventral to the thorax
- There is ≥ 1 cm of subcutaneous belly fat present
- The ferrets have an average or even a heavy body weight:
    - Female: 637-717 g Average
    - Female: >716 g Heavy
    - Male: 1248-1404 g Average
    - Male: > 1404 Heavy

Too heavy

- Hipbones can be easily palpable or with some pressure palpable
- The ribs are hardly or not palpable
- The belly shape is ventral to the thorax
- The ferrets have an average or a heavy body weight:
    - Female: 637-717 g Average
    - Female: >716 g Heavy
    - Male: 1248-1404 g Average
    - Male: > 1404 Heavy

Discussion

It is important to be able to objectively evaluate the body condition of the ferret, because losing weight in ferrets is often a primary indicator of disease. For cats, lots of different body condition scoring methods have already been tested and validated. Therefore, in this study a pilot body condition scoring system for the ferret was developed, based on what is already known and used in cats.

All palpations executed in this study were significantly correlated with the body condition. However, in correspondence with the best fit models, only palpations of the ischial tuberosity, ribs and cervical and thoracic vertebrae, rib fat coverage, abdominal shape, estimation of abdominal fat content and BW were included in the BCS-chart. All of these palpations have been used in previous BCS-charts for cats (59,67,70). The pilot BCS-chart for ferrets is thus comparable with other systems. Peron *et al.* (2016) determined that adding pictures to the BCS-chart enhanced the owners performance in correctly estimating the body condition (65). Even though a specific visual inspection proved unachievable because of the ferrets fur, a general impression of body condition can be created based on body posture. For this reason pictures are included in the BCS-chart (appendix 8).

In order to determine the size of the ferret, VBL, DBL and LIM, as described by Jones *et al.* (2016) and Hawthorne *et al.* (2005), were measured (71,116). VBL, DBL and LIM in ferrets were not correlated with body condition, corresponding with cats, where LIM shows little correlation with BF% (r^2<0,15), thus rendering it an adequate parameter to be used for estimating body size in cats as well as ferrets (71). Since the RC was found to be highly correlated with BF% in cats (71), it was assumed that similar findings would be observed in ferrets. In addition, BC was also considered as a parameter that would be highly dependent on the ferrets body condition, although no evidence was found in the literature to demonstrate this relationship in ferrets or other animals. The found relation of RC and BC with body condition confirmed these assumptions even though correlations in univariate analysis were low. Splenomegaly is frequently seen in ferrets that are 2 years or older (118), which can potentially interfere with the BC, making the results less reliable and correlations lower. The low correlation between RC and BW can be explained by breathing of the animal, which makes an objective assessment of the RC difficult.

Moreover, ferret size was found to be depended on gender. Male ferrets were significantly larger (VBL, DBL, LIM, RC, BC and BW) than female ferrets. In this study population female ferrets weighed between 515 and 1385g, males between 828 and 2390g. These gender differences in BW have been included in the BCS-chart. Even though body measurements per gender are unreported, gender difference in BW is well reported and similar values have been described (male 1-2 kg, females 0.6-1 kg; (119).

However the small sample size limits the reliability of this study. Low sample sizes per body condition lead to zero animals being assigned to certain categories in the contingency table (Table 8 & 9), making manual calculation of OR, necessary. It is unknown if these 0s are 'true zeros' or accidents of sampling, making more than a rough estimation by manual calculation impossible. Further research is therefore necessary to better understand the possibilities and restrictions of the pilot BCS-chart for ferrets. The variables tested could be narrowed down by excluding variables that evaluate the same body components or are proven not to correlate with the body condition. For example BW might not be necessary for body condition evaluation. Also a choice could be made between estimating the palpability of the ribs and palpating rib fat coverage. By developing standard protocols for the evaluation of the ferrets and by using one scale for BW, the consistency of the data could be improved. Also, using a small scale indicator (like a ruler) or a different, scaled, background as described by Gant *et al.* (2016) for the photographs could help in interpreting and comparing the photographs (120). The scale indicator would make it possible to use software like Coach to analyse the photographs.

Moreover, in the study as performed, seasonal changes in BCS were not taken into account. However, it is well known that in ferrets, large seasonal weight changes can occur. In fall, ferrets usually gain weight and store additional fat for the upcoming winter, whereas they will often start to lose weight in the spring again. As a result, a ferret's BW can fluctuate as much as 40% (119), which may subsequently have an effect on the animal's BCS as well. It may thus be possible that optimal body condition of a ferret differs dependent on the time of year, rendering it necessary to create BCS-charts and reference values for the winter and summer seasons. Further research will be necessary to determine whether this would indeed be needed. These seasonal weight influences can also have influenced this study population. Data were collected in spring season, causing a low number of obese ferrets (n=9) to be encountered.

In addition to a repetition of the study in the winter months to evaluate the effect of season on BCS, validation studies are necessary. For this purpose, repeatability and reproducibility of all the measurements and estimates need to be determined. In addition, comparative studies of BCS-results with BF% estimated by DEXA scans will be needed to determine the accuracy of the developed BCS-chart. Moreover these DEXA-scans could be useful to help further divide the BCS in additional classes (i.e. minimum of 5 rather than 3), which was currently impossible due to the small number of ferrets analysed. However, in order to be able to make this distinction, a sufficient number of ferrets with a high range in body conditions (i.e. covering the range from cachectic to severely obese) would need to be evaluated using both methods.

However it has to be taken into account that these evaluations of animals based on a body condition score take practice, as is known from feline literature. Higher interrater-agreements (reproducibility) is seen between veterinarians and trained observers than between veterinarians and untrained observers (67,70). The researcher (I.B.) had no training or previous experience in evaluating body conditions, which could have influenced the results of this study. Further research in which multiple observers, experienced and inexperienced, evaluate ferrets with the same palpations is therefore necessary to determine reproducibility.

The BCS-chart is able to distinguish ferrets in optimal body condition from obese or underweight ferrets. It is a promising, easily applicable tool that can aid owners and veterinarians in the estimation of body condition.

Conclusion

In this study, a new body condition score system based on a BCS-chart was developed and found to be a promising method for evaluation of a ferret's body condition. However, further research will be necessary in order to validate the system and determine its reliability.

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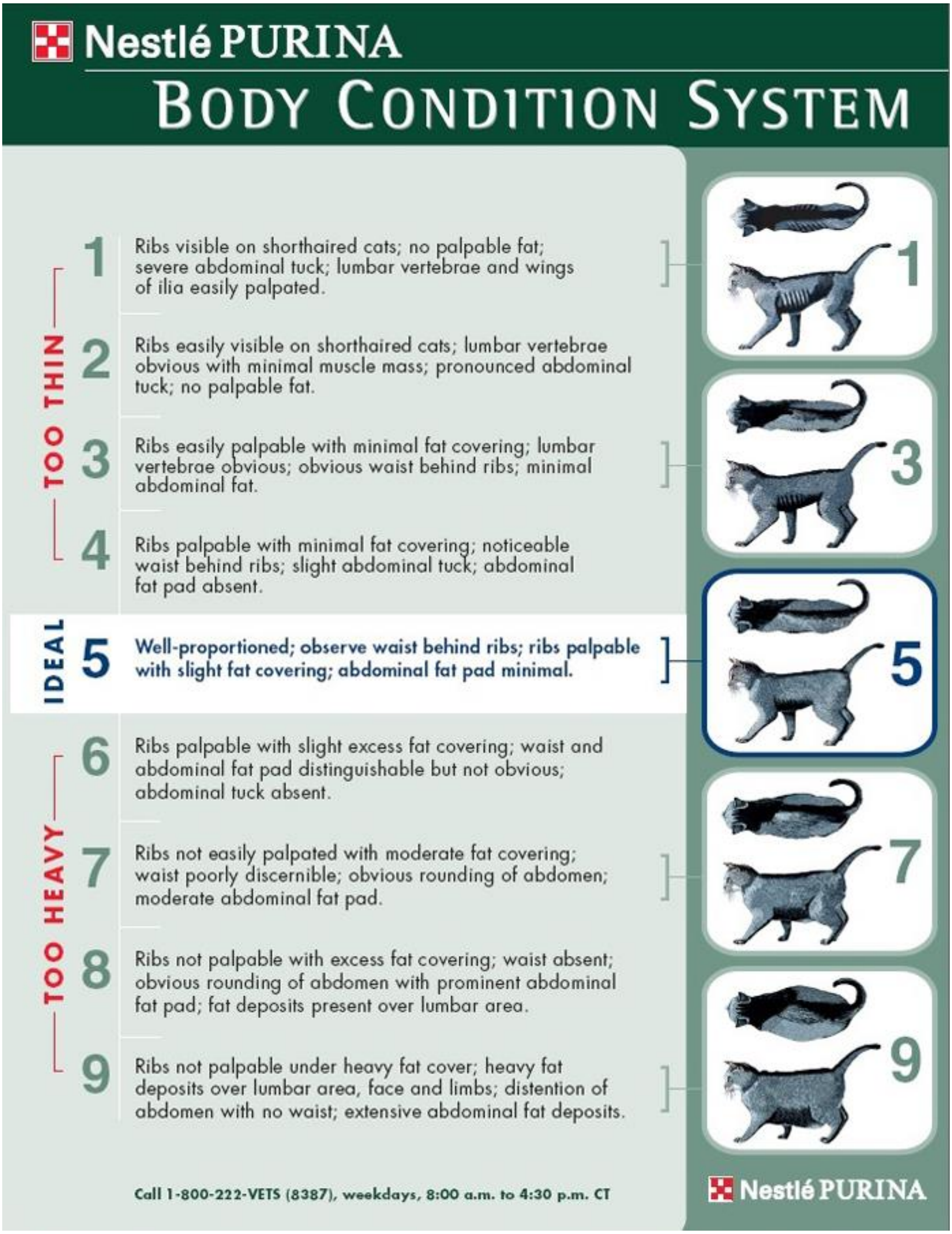
Appendices

Appendix 1 The 9-point BCS system

The purine BCS system was developed by Laflamme *et al.* in 1997. It is a 9-point scale that with the aid of pictures, helps the observer choose the right body condition for their cat.



Nestlé PURINA

BODY CONDITION SYSTEM

TOO THIN	1	Ribs visible on shorthaired cats; no palpable fat; severe abdominal tuck; lumbar vertebrae and wings of ilia easily palpated.
	2	Ribs easily visible on shorthaired cats; lumbar vertebrae obvious with minimal muscle mass; pronounced abdominal tuck; no palpable fat.
	3	Ribs easily palpable with minimal fat covering; lumbar vertebrae obvious; obvious waist behind ribs; minimal abdominal fat.
	4	Ribs palpable with minimal fat covering; noticeable waist behind ribs; slight abdominal tuck; abdominal fat pad absent.
IDEAL	5	**Well-proportioned; observe waist behind ribs; ribs palpable with slight fat covering; abdominal fat pad minimal.**
TOO HEAVY	6	Ribs palpable with slight excess fat covering; waist and abdominal fat pad distinguishable but not obvious; abdominal tuck absent.
	7	Ribs not easily palpated with moderate fat covering; waist poorly discernible; obvious rounding of abdomen; moderate abdominal fat pad.
	8	Ribs not palpable with excess fat covering; waist absent; obvious rounding of abdomen with prominent abdominal fat pad; fat deposits present over lumbar area.
	9	Ribs not palpable under heavy fat cover; heavy fat deposits over lumbar area, face and limbs; distention of abdomen with no waist; extensive abdominal fat deposits.

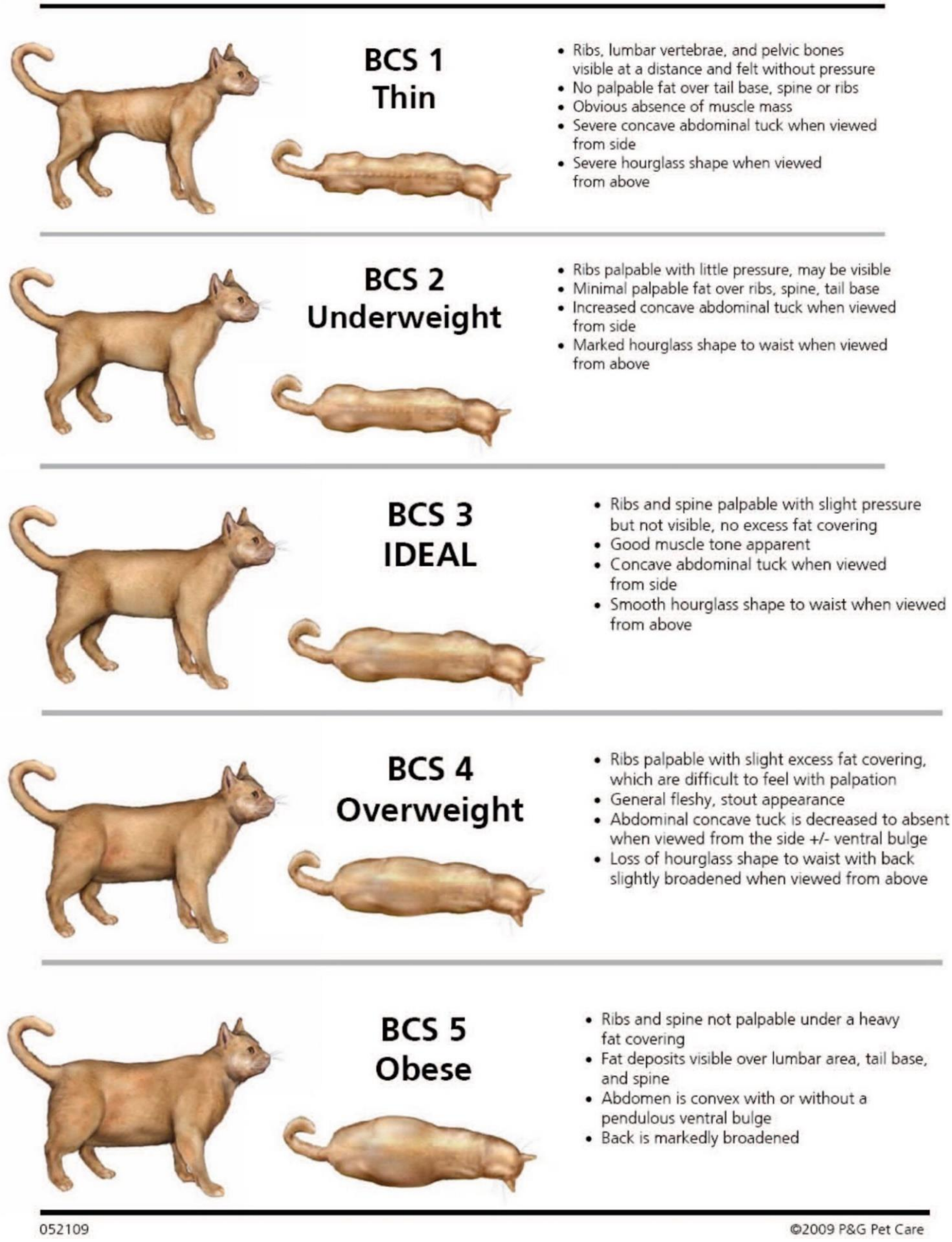
Call 1-800-222-VETS (8387), weekdays, 8:00 a.m. to 4:30 p.m. CT

Nestlé PURINA

Source: webpage "Fit or Fat: Your Pet's Body Condition Score (BCS)" (121)

Appendix 2 The 5-point BCS system

The 5-point BCS system is very much similar to the 9-point system. If the animals are scored in half-steps, it works on the same scale as the 9-point system.



BCS 1
Thin

- Ribs, lumbar vertebrae, and pelvic bones visible at a distance and felt without pressure
- No palpable fat over tail base, spine or ribs
- Obvious absence of muscle mass
- Severe concave abdominal tuck when viewed from side
- Severe hourglass shape when viewed from above

BCS 2
Underweight

- Ribs palpable with little pressure, may be visible
- Minimal palpable fat over ribs, spine, tail base
- Increased concave abdominal tuck when viewed from side
- Marked hourglass shape to waist when viewed from above

BCS 3
IDEAL

- Ribs and spine palpable with slight pressure but not visible, no excess fat covering
- Good muscle tone apparent
- Concave abdominal tuck when viewed from side
- Smooth hourglass shape to waist when viewed from above

BCS 4
Overweight

- Ribs palpable with slight excess fat covering, which are difficult to feel with palpation
- General fleshy, stout appearance
- Abdominal concave tuck is decreased to absent when viewed from the side +/- ventral bulge
- Loss of hourglass shape to waist with back slightly broadened when viewed from above

BCS 5
Obese

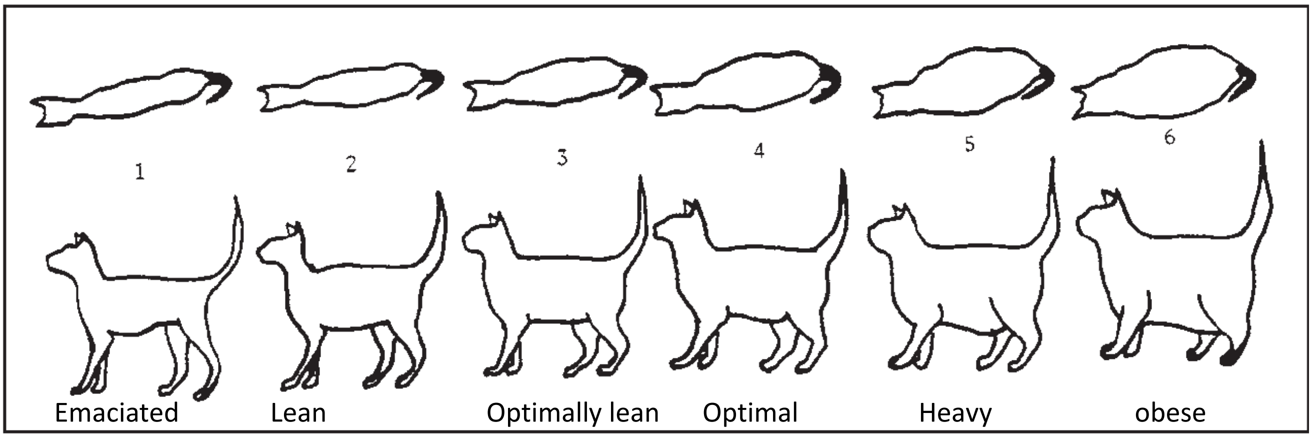
- Ribs and spine not palpable under a heavy fat covering
- Fat deposits visible over lumbar area, tail base, and spine
- Abdomen is convex with or without a pendulous ventral bulge
- Back is markedly broadened

052109

Source: Shovellar e.a. (2014) (67)

Appendix 3 The 6-point BCS system

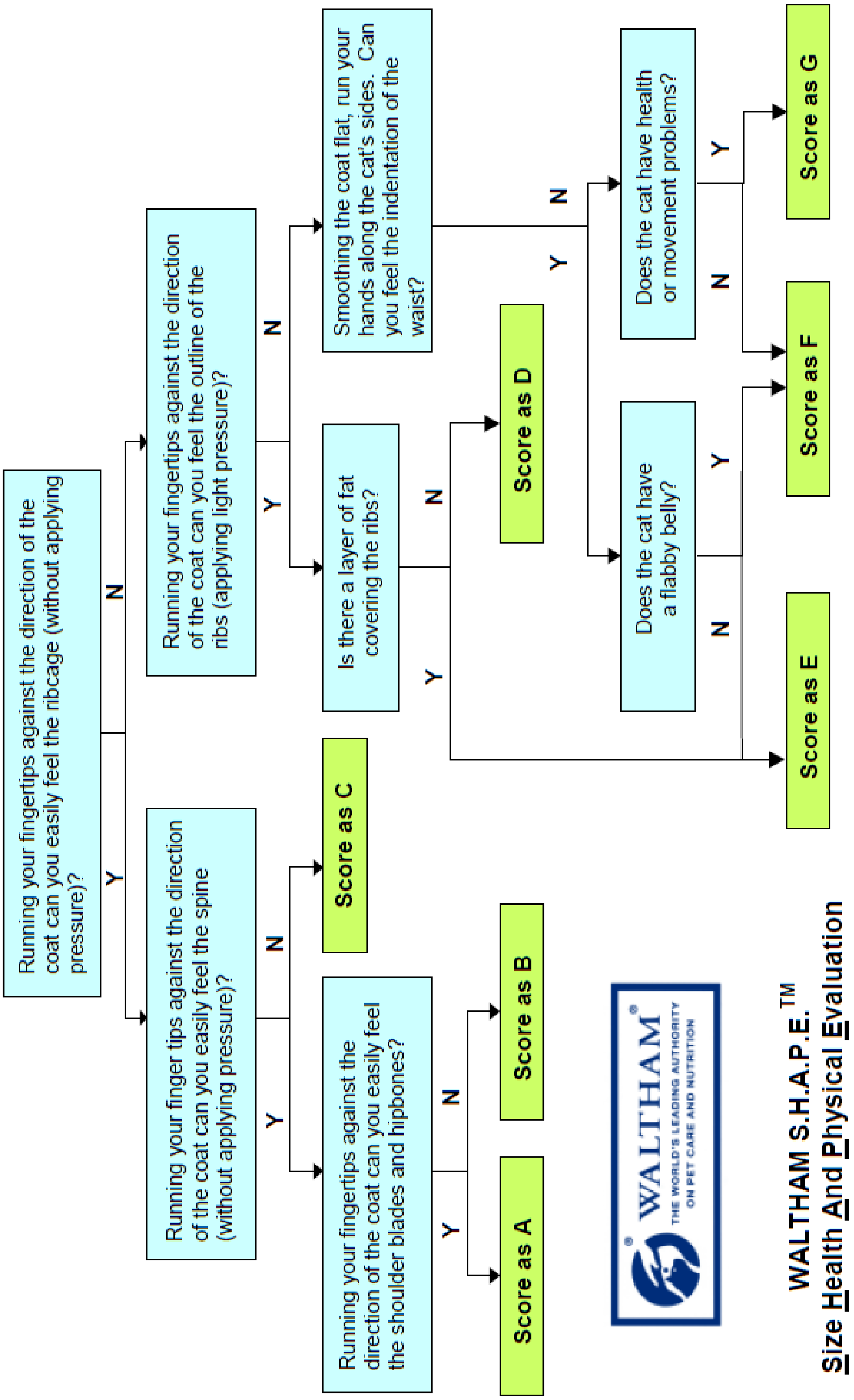
This 6-point BCS system only relies on the body shape of the animal. No descriptions of the palpability of skeletal components or fat estimates are given.

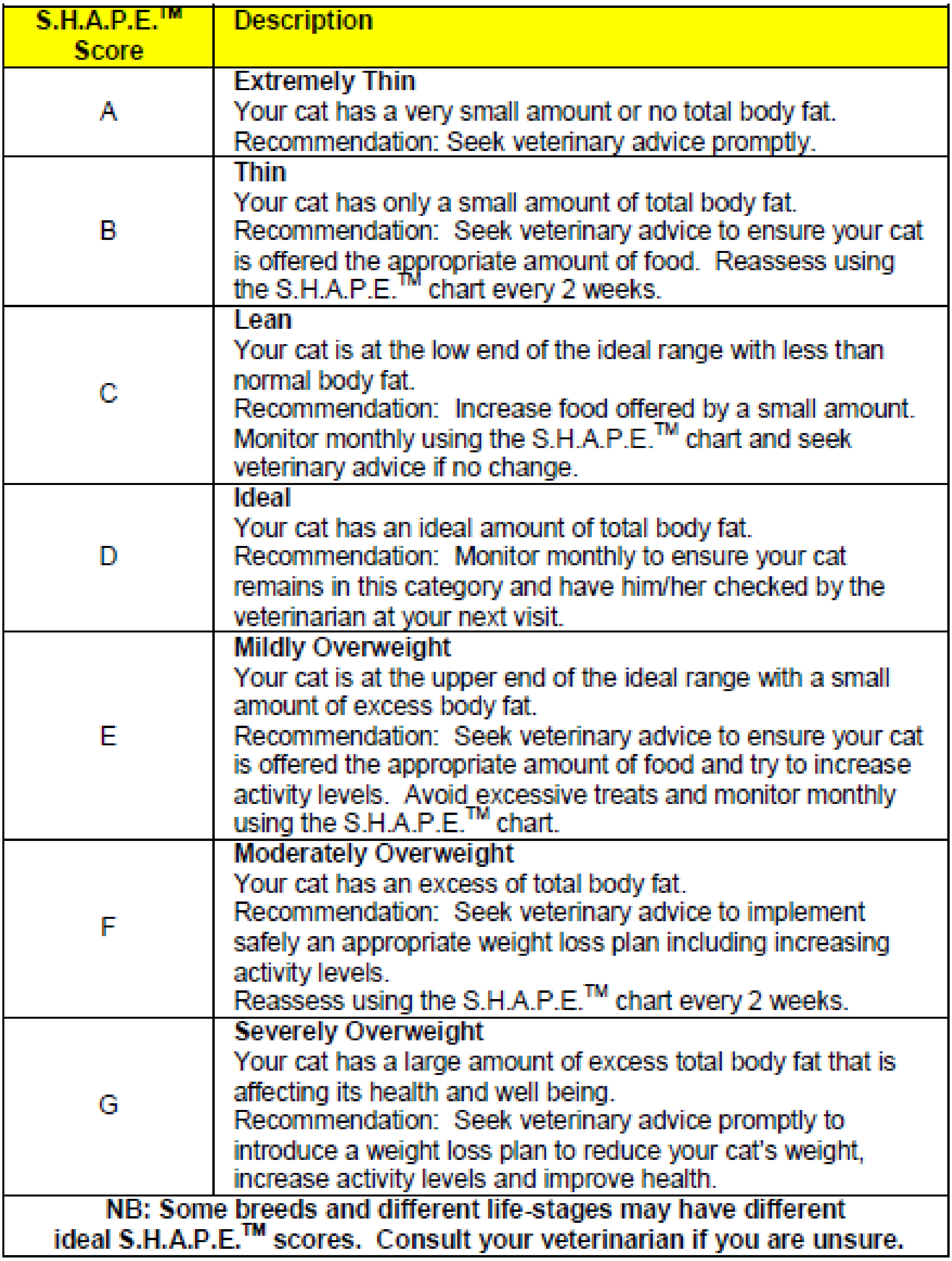


Source: Doria-Rose and Scarlott (2000) (69)

Appendix 4 S.H.A.P.E. flow chart and table

S.H.A.P.E., Size Health And Physical Evaluation, does not use pictures to aid the observer. With the help of the flow chart, the observer guided step by step through the process of evaluating the body condition. Eventually a score from A-G is given, whereby D is considered ideal.



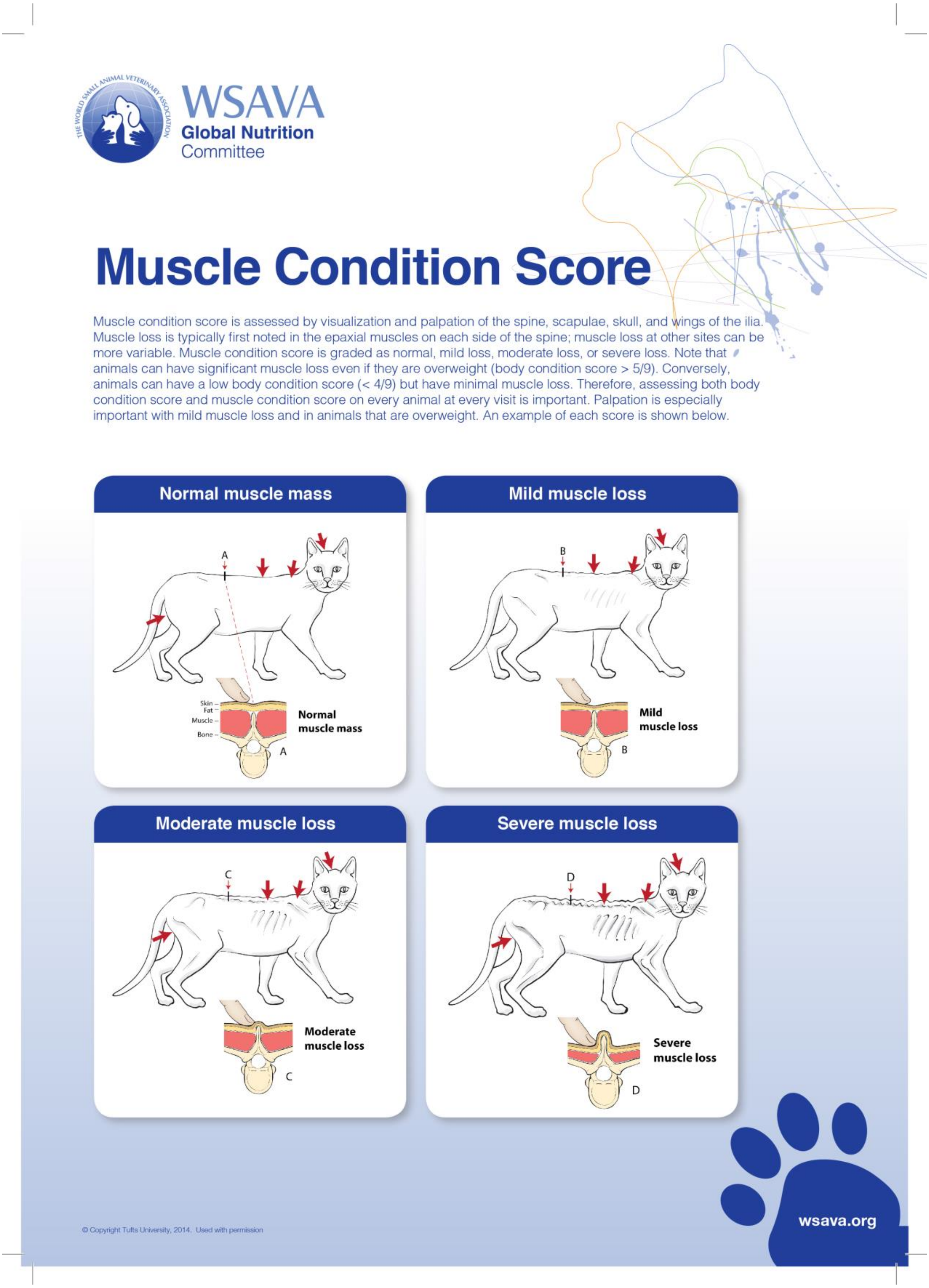


S.H.A.P.E.^{TM} Score	**Description**
A	**Extremely Thin** Your cat has a very small amount or no total body fat. Recommendation: Seek veterinary advice promptly.
B	**Thin** Your cat has only a small amount of total body fat. Recommendation: Seek veterinary advice to ensure your cat is offered the appropriate amount of food. Reassess using the S.H.A.P.E.^{TM} chart every 2 weeks.
C	**Lean** Your cat is at the low end of the ideal range with less than normal body fat. Recommendation: Increase food offered by a small amount. Monitor monthly using the S.H.A.P.E.^{TM} chart and seek veterinary advice if no change.
D	**Ideal** Your cat has an ideal amount of total body fat. Recommendation: Monitor monthly to ensure your cat remains in this category and have him/her checked by the veterinarian at your next visit.
E	**Mildly Overweight** Your cat is at the upper end of the ideal range with a small amount of excess body fat. Recommendation: Seek veterinary advice to ensure your cat is offered the appropriate amount of food and try to increase activity levels. Avoid excessive treats and monitor monthly using the S.H.A.P.E.^{TM} chart.
F	**Moderately Overweight** Your cat has an excess of total body fat. Recommendation: Seek veterinary advice to implement safely an appropriate weight loss plan including increasing activity levels. Reassess using the S.H.A.P.E.^{TM} chart every 2 weeks.
G	**Severely Overweight** Your cat has a large amount of excess total body fat that is affecting its health and well being. Recommendation: Seek veterinary advice promptly to introduce a weight loss plan to reduce your cat's weight, increase activity levels and improve health.
NB: Some breeds and different life-stages may have different ideal S.H.A.P.E.^{TM} scores. Consult your veterinarian if you are unsure.	

Source: WALTHAM (122)

Appendix 5: Muscle mass score chart cat

The muscle mass is scored in 4 categories based on palpations of muscle over the spine, and head.



WSAVA
Global Nutrition
Committee

Muscle Condition Score

Muscle condition score is assessed by visualization and palpation of the spine, scapulae, skull, and wings of the ilia. Muscle loss is typically first noted in the epaxial muscles on each side of the spine; muscle loss at other sites can be more variable. Muscle condition score is graded as normal, mild loss, moderate loss, or severe loss. Note that animals can have significant muscle loss even if they are overweight (body condition score > 5/9). Conversely, animals can have a low body condition score (< 4/9) but have minimal muscle loss. Therefore, assessing both body condition score and muscle condition score on every animal at every visit is important. Palpation is especially important with mild muscle loss and in animals that are overweight. An example of each score is shown below.

Normal muscle mass

Normal muscle mass

Mild muscle loss

Mild muscle loss

Moderate muscle loss

Moderate muscle loss

Severe muscle loss

Severe muscle loss

wsava.org

Source: Webpage: WSAVA Nutritional toolkit (123)

Appendix 6: Phase 1 registration table

This Dutch form, was used during the study to register the palpability and visibility of the different variables quickly and in an organised way.

Naam: geslacht: m/v dik/goed op gewicht/ te mager
leeftijd: implantaat: ja/nee intact/geneutraliseerd/gecastreerd

ruggenwervels (nek)	zichtbaarheid	goed	deels	niet		
	voelbaarheid	heel makkelijk	makkelijk	met enige druk	nauwelijks/niet	
heupbotten	zichtbaarheid	goed	deels	niet		
	voelbaarheid	makkelijk	met enige druk	nauwelijks/niet		
ribben	zichtbaarheid	goed	deels	niet		geen / weinig / beetje / redelijk wat / dikke vetlaag
	voelbaarheid	makkelijk	met enige druk	nauwelijks/niet	heel makkelijk	
taille	zichtbaarheid	goed	deels	niet	vetlaag?	
	voelbaarheid	makkelijk	met enige druk	nauwelijks/niet		
lumbale rugwervels	zichtbaarheid	goed	deels	niet	vetlaag?	
	voelbaarheid	makkelijk	met enige druk	nauwelijks/niet	heel makkelijk	
buik	vorm/grootte	opgetrokken	parellel aan thorax	ventraal van thorax		
	vet	0.5	1	1.5	2	
pootjes	voelbaarheid beenderen	goed	deels	niet		
	vet/spier	weinig	beetje	redelijk wat	veel	
spieren		normaal	iets verminderd	sterk verminderd		

Appendix 7: Phase 1 registration table, morphometric measurements

The second Dutch form used in the research. Morphometric measurements and BW were registered in this table. Names of the ferret and the shelter were written above the table.

morphometrische metingen

	fret 1	fret 2	fret 3	fret 4	fret 5	fret 6	fret 7	fret 8	fret 9	fret 10
LIG (cm)										
dorsal body length (cm)										
ribcage circumference (cm)										
belly circumference (cm)										
ventral body length (cm)										
Body weight										

aantekeningen