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Assessment of body fat in the pony: Part I. Relationships between the anatomical distribution of adipose tissue, body composition and body condition

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Keywords: horse; body composition; body condition; adipose tissues; body fat content; equine obesity

Summary

Reasons for performing study: **Evaluation of equine body fat content is important for nutritional and clinical purposes. However, our understanding of total body fat and its regional distribution in the body is sparse. Currently, body fat evaluation relies on the subjective assessment of body condition score (BCS), which has never been validated against 'gold standard' chemical analysis or dissection measurements in ponies.**

Objectives: **To define the relationships between subjective (BCS), objective (morphometric) indices of body fat and 'gold standard' measurements of actual body composition.**

Hypotheses: **BCS and morphometry offer valid, noninvasive methods for determination of body fat in equids.**

Methods: **Seven mature (mean ± s.e. 13 ± 3 years, 212 ± 14 kg, BCS 1.25–7/9), Welsh Mountain pony mares, destined for euthanasia (for nonresearch purposes), were used. For all ponies, body mass (BM), BCS and various morphometric measurements were recorded. Following euthanasia, all ponies were systematically dissected. Discrete white adipose tissue (WAT) depots were independently described. Gross, body chemical composition was determined by proximate analyses.**

Results: **Total somatic soft tissues increased linearly ($r^2 = 1.00$), whereas body WAT content (1–26% live BM) increased exponentially ($r^2 = 0.96$), with BCS. WAT was equally distributed between internal and external sites in all animals irrespective of BCS. Nuchal fat was a poor predictor of total WAT ($r^2 = 0.66$). Periorbital WAT did not alter with BCS ($r^2 = 0.01$). Heart girth:withers height and ultrasonic retroperitoneal fat depth were closely associated with total, chemically-extracted lipid which comprised 1–29% live BM ($r^2 = 0.91$ and 0.88, respectively).**

Conclusions and potential relevance: **The exponential relationship between BCS and total body WAT/lipid suggests that BCS is unlikely to be a sensitive index of body fat for animals in moderate-obese states. Morphometric measurements (body girths and retroperitonel fat depth) may be useful to augment subjective BCS systems.**

Abbreviations

BCS:	Body condition score
BM:	Body mass
CNS:	Central nervous system
CP:	Crude protein
DM:	Dry matter
GE:	Gross energy
NBL:	Neutral body lipid
recBM:	Recovered body mass
recEBM:	Recovered empty body mass
TBL:	Total body lipid
WAT:	White adipose tissue

Introduction

Growth of the new discipline of adipobiology has been fuelled by the epidemic of human and companion animal obesity in Western civilisations (German 2006; Sillence *et al*. 2006; Wyse *et al*. 2008). White adipose tissue (WAT), traditionally considered to be a passive but important contributor to energy homeostasis, is now understood to be an active secretory tissue with widespread paracrine and endocrine effects throughout the body, which can impact on health (Bastard *et al*. 2006; Vick *et al*. 2007). Obesity has been identified as an important risk factor for morbidity and mortality in domestic horses and ponies (Sillence *et al*. 2006; Geor 2008). Given the importance of adipose tissue to equine health, our understanding of basic fat biology, including its contribution to body composition and its anatomical distribution, remains largely undescribed (Geor 2008; Argo 2009).

Several authors have attempted to quantify the body fat content of living horses objectively using morphometric data, ultrasound measurement of superficial fat or bioelectrical impedance analysis (Westervelt *et al*. 1976; Kane *et al*. 1987; Kearns *et al*. 2002a; Donaldson *et al*. 2004; Frank *et al*. 2006; van der Aa Kuhle *et al*. 2008; Dugdale *et al*. 2010a). However, validation of these indirect methodologies against concurrent 'gold standard' carcase dissection or chemical composition analysis has been sparse, with only 2 studies reporting the associations between regional (rump or

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tailhead) subcutaneous fat depths and chemically-extracted body lipid (Westervelt *et al*. 1976; Kane *et al*. 1987). Inconsistencies between the actual anatomical locations for ultrasound evaluations and the physiological status of animals used, highlights the importance of defining precise anatomical measurement sites (Westervelt *et al*. 1976; Kane *et al*. 1987; Gentry *et al*. 2004). Further, between-breed validation of all these indirect approaches, to confirm method associations with actual body fat content, is lacking.

Currently, the assessment of equine 'body fatness' for management and clinical applications, has focused on the subjective appraisal of body condition score (BCS, Burkholder 2000). However, unlike the successful BCS systems used in agricultural species, current equine BCS methods depend mainly on untested, indirect indices (rump fat thickness) of total body fat mass (Henneke *et al*. 1983; Wright and Russel 1984). Given the current increase in the prevalence of obesity among domestic horses and ponies, a BCS system capable of quantifying body fat content is required (Thatcher *et al*. 2007; Wyse *et al*. 2008).

This study was undertaken to generate fundamental data to accurately describe changes in body composition associated with overall differences in 'body fatness', subjectively appraised as BCS and objectively evaluated by morphometric measurements. A relatively homogeneous group of mature animals was used to optimise the evaluation of body fat-associated change and to minimise differences in body composition related to breed, gender or age in this instance. To facilitate between-study comparisons with future investigations, it was considered important to detail the exact procedures used to determine both the gross anatomical and chemical body composition data presented.

Materials and methods

Animals and husbandry

Seven mature (mean ± s.e. 13 ± 3 years [range 6–20 years], 212 ± 14 kg), Welsh Mountain pony mares destined for euthanasia (for nonresearch reasons) were used. Owner election for euthanasia had been prompted by various chronic conditions in their animals that had proved refractory to treatment (dental disease [P1 and P2], sinusitis [P3], bilateral blindness secondary to uveitis [P4], nonhealing facial fistula following dental extraction [P5], infertility [P6], epilepsy [P7]). Five of the 7 ponies had been fed an identical forage-based diet for 10 days prior to euthanasia; the remaining 2 were at pasture. Four ponies (P1–P4) were presented during winter (February to April) and 3 (P5–P7) were studied in summer (July to August).

On the day prior to euthanasia (09.00–10.00 h), each pony was weighed (±1 kg, Lightweight Intermediate weigh scales[1]; calibration regularly checked) and body condition scored by the same observer (BCS 1 [very poor] to 9 [extremely fat]) in accordance with criteria described by Kohnke (1992, a modification of Henneke *et al*. 1983) and Carroll and Huntingdon (1988, 0 [very poor] to 5 [very fat]). Unless otherwise stated BCS data are reported for only the most commonly used, modified Henneke system (Suagee *et al*. 2008). Heart and belly girths and mid-neck circumference were measured (We-Bo Animal Measure)[2] and the depths of 4 superficially accessible fat deposits were recorded to the nearest 0.01 mm by transcutaneous ultrasonography with a variable frequency (5.5, 7 or 8 MHz) linear array probe (Merlin Ultrasound scanner Type 1101)[3], as previously described (Dugdale *et al*. 2010a,b).

Gross dissection

Body mass (live BM) was recorded prior to euthanasia, which was by intracranial free-bullet (n = 6) or i.v. barbiturate overdose (n = 1). Exsanguination was conducted immediately *post mortem* and blood was collected, weighed (±10 g; Weigh-Tronix, capacity 25 × 0.01 kg)[4] and duplicate samples (~500 g) stored (-20°C) in sealed plastic containers pending chemical analyses.

All cadavers were systematically dissected (detailed description; File SA) to yield 8 discrete tissue categories: collected blood; hide (including pelage); central nervous system (CNS, brain and spinal cord); right side carcase (neck, body wall and upper limbs) bones; right half head and lower limb bones (including hoof capsules); right side carcase skeletally-associated (somatic) soft tissues (white adipose tissue [WAT], skeletal muscle and other); right half head and lower limb soft tissues (WAT and other); viscera (empty) with their associated WAT. WAT (excluding intramuscular WAT) weights from the 3 soft tissue categories were recorded separately from the remaining tissues in those categories. The difference between the pre-euthanasia live weight and the recovered weight (sum of the 8 tissue categories plus: gastrointestinal contents, urine, peritoneal fluid, skinned left body [left carcase, head and lower fore- and hindlimbs] bones and soft tissues) was assumed to be water lost or gained by evaporation and condensation during dissection. Samples (~1 kg) of minced/ground tissue from each category were stored (-20°C) pending chemical (proximate) analyses. A sample (~500 g) of thoroughly mixed total digesta was also stored (-20°C) for later determination of dry matter (DM) content by oven drying.

Samples (~5 g fresh) of muscle (middle gluteal, semimembranosus, diaphragm and cardiac), of viscera (liver and kidney) and of various white adipose tissues (nuchal, lateral withers, tailhead, retroperitoneal, intrapelvic, omental, mesenteric, perirenal, pericardial and epicardial) were taken from the left half carcase (after its weight was determined) for evaluation of DM (lyophilisation) and subsequent evaluation of gross energy (GE) content (isothermal bomb calorimetry; E2K Combustion Calorimeter)[5].

Final preparation of tissues and chemical analyses

Samples from each tissue category were variously minced or ground and the minced/ground samples stored (-20°C) pending proximate analysis (File SB). For proximate analysis, frozen skin samples were diced (~1 mm square) and blood, CNS and all minced soft tissue samples were homogenised separately for each pony (Moulinex Moulinette S)[6]. Bone samples were ground to <1 mm particles (Tecator grinding mill)[7] and mixed thoroughly.

Standard, proximate analytical techniques (Anon 2000) were applied to each sample homogenate in triplicate (DM, lyophilisation; GE, isothermal bomb calorimetry) or in duplicate (lipid extraction, Soxhlet petroleum ether extraction before and after acid hydrolysis; nitrogen, Kjeldahl titration; ash, muffle furnace) sub-samples of the homogenates (File SB). Crude protein (CP) was calculated as nitrogen (N, g or %) × 6.25. Duplicate samples of gastrointestinal contents (digesta, ~20 g) were oven dried (75°C) to constant mass to determine DM content. The mean values derived following replicate analyses were used in each case.

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TABLE 1: Summary data for the 7 Welsh Mountain pony mares ranked in order of increasing body condition scores

Pony	BCS (1–9)	Age (years)	Withers height (m)	Live BM (kg)	Digesta (kg)	RecEBM (kg)	RecEBM + digesta (% live BM)	Unaccounted mass (% live BM)
1	1.25	12	1.15	173.0	34.00	129.74	94.65	5.35
2	2.5	9	1.15	159.0	20.00	141.83	101.78	–1.78
3	4.08	11	1.17	214.0	24.46	179.49	95.30	4.70
4	4.25	17	1.16	238.0	21.00	212.01	97.90	2.10
5	5.9	20	1.08	211.0	21.00	191.48	100.70	–0.70
6	6.8	6	1.07	220.0	16.00	198.26	97.39	2.61
7	7	16	1.17	270.0	21.50	243.21	98.04	1.96
Mean		**13.00**	**1.14**	**212.14**	**22.57**	**185.15**	**97.97**	**2.03**
s.e.		1.85	0.02	14.17	2.13	14.87	0.98	0.98

Age, withers height and live body mass (live BM) immediately prior to euthanasia. The recovered digesta mass and the recovered empty body mass (recEBM) accounted for following the summation of dissected components are presented. The percentage of live BM unaccounted for following dissection (and assumed to be predominantly water loss/gain) is indicated in the final column.

		Major tissues as a percentage of recovered empty BM							
		Musculoskeletal system							
			Soft tissue						
Pony	BCS (1–9)	Bone and cartilage	Total	WAT	Non-WAT	Viscera and vWAT	Hide and pelage	Collected blood	CNS
1	1.25	19.74	49.49	1.18	48.31	15.22	7.98	7.09	0.48
2	2.5	18.90	54.01	5.09	48.91	13.01	7.72	5.92	0.44
3	4.08	15.02	57.87	12.38	45.49	13.21	6.82	6.70	0.38
4	4.25	13.92	58.77	19.37	39.40	13.00	8.16	5.84	0.30
5	5.9	11.07	63.48	30.86	32.61	14.94	5.95	4.21	0.34
6	6.8	9.96	65.70	47.09	18.61	14.08	4.50	5.40	0.36
7	7	10.42	65.95	45.12	20.83	12.23	5.38	5.75	0.27
Mean		**14.15**	**59.32**	**23.01**	**36.31**	**13.67**	**6.65**	**5.85**	**0.37**
s.e.		1.51	2.34	6.99	4.79	0.42	0.53	0.35	0.03

The relative masses of each major tissue category segregated at the time of dissection are presented as percentages of recEBM for each animal. Soft tissues indicate the relative proportion of white adipose tissue (WAT), muscle and connective tissues associated with the skeleton and body wall and exclude the viscera and their associated adipose tissues (vWAT), hide and pelage, collected blood and central nervous system (CNS) tissues, which are listed separately.

Data describing the chemical composition and total mass of each of the 8 tissue categories enabled the 'chemical reconstruction' of each pony in terms of overall water, DM, ash, CP, neutral lipid, total lipid and GE contents.

Data analyses

All data were initially entered into Excel spreadsheets (Microsoft Office Professional Edition 2003)[8] and statistical analyses were performed using Excel, Minitab version 15.1.0[9] and STATA 10[10]. Normality of all data sets for body mass, body water, ash, CP, body lipid (total and neutral) and dissected WAT (individual depots and total WAT), whether expressed as actual weights or as percentages of live or recovered empty body mass, was confirmed by both visual assessment of their frequency distributions and by Anderson-Darling normality tests. F tests were used to check for equal variance of data prior to least squares linear regression analyses. In addition, scatterplots of the data were performed to ensure that data distributions were suitable for application of linear regression; where scatterplots revealed nonlinear data distribution, alternative line fits were explored in Excel. For linear regression, the outcome variables tested were: proximate analysis-derived results for body lipid, dissection-derived results for body WAT content and body condition score. Predictor variables included: body condition score, ultrasonic measurements of subcutaneous and retroperitoneal fat depth, neck and body girths and all cadaver-derived measurements (gut contents, bone mass, individual organ mass, GE content, dissected depot WAT). Following linear regression, normality of the distribution of residuals was confirmed using the Anderson-Darling test. Coefficients of determination (r^2) are reported for the results of linear regression analyses. Statistical significance was assumed if $P<0.05$. Summary statistics, unless otherwise stated, are reported as mean ± s.e.

Results

By using mature animals of a single breed and sex, between-animal differences in the scale of the underlying skeletal framework, most readily evaluated as withers height (1.14 ± 0.02 m, Table 1a) and total skeletal mass (25.0 ± 1.3 kg, Table 1b), were minimised. Changes in these markers of skeletal dimension were independent of changes in BCS (Table 1). Body mass varied widely across the group and increased exponentially with increasing BCS ($r^2 = 0.64$). When the individual weights for all the components of each pony, following euthanasia and gross segregation of the cadaver into digesta and the major tissue categories (Table 1b), were re-combined, recovered body mass (recBM = recovered empty body mass [recEBM] + digesta) was strongly associated with live BM ($r^2 = 0.98$, $P<0.001$, Table 1a). Mass deficits or excesses in recBM were minimal (~2% of live BM) and were attributed to water lost or gained by evaporation or condensation. Net water loss during dissection tended to increase with decreasing BCS and body WAT content (Table 1). Although the water content of digesta was almost constant between animals (89.5 ± 0.9%, range 86–92%),

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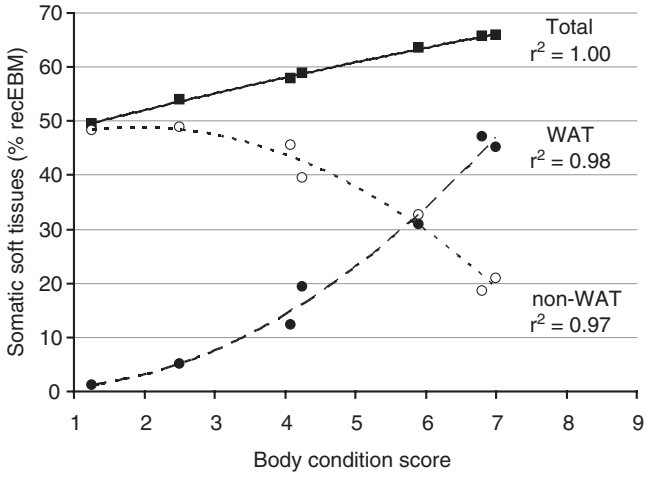

Fig 1: The recovered mass of total somatic soft tissues (skeletally-associated, ■) and the components of this soft tissue mass comprised of white adipose tissue (WAT, ●) and other, nonWAT (largely skeletal muscle) tissues (○), for the 7, Welsh Mountain pony mares are illustrated against their individual, ante mortem *body condition scores. Coefficients of determination (r^2) are presented.*

total digesta mass comprised 11 ± 2% (range 7–20%) of live BM and decreased logarithmically with BCS ($r^2 = 0.91$, Table 1a).

Gross anatomical evaluation

Skeletally-associated (somatic) soft tissue was both the largest component of each cadaver and the most variable between animals when considered as a proportion of recEBM (Fig 1, Table 1b). Subjective measurements of BCS, almost perfectly, linearly, described ($r^2 = 1.00$, $P<0.001$) total somatic soft tissue (WAT + non-WAT) contributions to recEBM (Fig 1, Table 1b). However, as BCS increased, the relative contribution of WAT and non-WAT (skeletal muscle and connective tissues) within the somatic soft tissues, changed in a reciprocally curvilinear manner (Fig 1, Table 1b).

The absolute mass of collected blood (10.7 ± 0.8 kg) and CNS tissues (655 ± 12 g) was relatively constant between ponies. Although hide (including pelage) mass varied little between ponies (12.0 ± 1.0 kg), a seasonal effect was apparent when hide was expressed as a percentage of recEBM ($P = 0.03$; winter, 6.5 ± 0.4 kg; summer, 4.8 ± 0.4 kg, Table 1b).

When the relatively small proportion of total WAT associated with the head and lower limbs (2.3 ± 0.8%) was excluded, WAT was essentially equally distributed between the internal and external carcase sites in all animals irrespective of BCS (Table 2). Although anatomically-regional WAT depots contributed variously to the total WAT mass in individual ponies, the most profound changes associated with increased total body WAT content were attributed to increased mass in 3 principal regional deposits: intra-abdominal belly wall-associated (retroperitoneal), subcutaneous and intermuscular (Table 2).

Successive, linearly-ordinal increments in BCS were associated with unexponential increase ($r^2 = 0.96$) in the proportions of WAT within the recEBM. A similar exponential increment in total body WAT (1–26% live BM) was observed when changes in body condition were independently appraised using the 0–5 system of Carroll and Huntingdon (1988, $r^2 = 0.90$; data not presented). Internal and external WAT, inclusive of their component depots, contributed equally to BCS-associated changes in total WAT (Fig 2; Table 2). Further, the 2 major categories that comprised internal WAT, body wall-associated and organ-associated, also appeared to contribute equally to changes in total internal WAT. The animal for which the lowest BCS was recorded (P1) was the exception to this general trend. For this individual, mesenteric WAT, the largest organ-associated depot in all ponies, comprised 68% of total internal WAT, in the absence of any dissectable retroperitoneal WAT (Table 2; File SC). In addition, all dissectable WAT in this animal was of a gelatinous nature. This was particularly notable between the dorsal spinous processes of the vertebrae.

As BCS increased, increasing quantities of intermuscular WAT were evident between the major muscle bellies (Table 2). Intermuscular WAT recorded for the animal in poorest BCS (P1) consisted mainly of periarticular WAT and WAT associated with neurovascular and lymphoid tissues. Intramuscular WAT was not amenable to dissection and remained unquantified but was grossly visible in only one animal (P7). In this individual, intramuscular WAT was apparent as 'marbling' between individual muscle fascicles throughout the *quadriceps femoris* alone. Although digital cushions were not removed from hoof capsules, the remaining lower limb WAT was negligible (<5 g) in all ponies, was independent of BCS and was mainly periarticular or associated with neurovascular and lymphatic tissue.

Regression of each WAT depot on total WAT mass demonstrated strong, positive associations for most depots. Body wall-associated intra-abdominal (retroperitoneal, $r^2 = 0.89$, $P = 0.001$) and intrathoracic (retropleural, $r^2 = 0.92$, $P<0.001$) depots were strongly associated with total WAT mass, whereas the intrapelvic depot was not ($r^2 = 0.05$, $P = 0.6$). Of the organ-associated WAT depots, mesenteric ($r^2 = 0.88$, $P = 0.002$), uterine broad ligament ($r^2 = 0.83$, $P = 0.004$) and perirenal ($r^2 = 0.78$, $P = 0.008$) depots demonstrated the strongest associations with total WAT mass. Conversely, omental ($r^2 = 0.51$, $P = 0.07$) and heart-associated WAT (pericardial and epicardial depots, $r^2<0.3$, $P>0.2$) were poorly associated with total WAT mass. Intermuscular ($r^2 = 0.91$, $P = 0.001$) and subcutaneous ($r^2 = 0.87$, $P = 0.002$) depots were more strongly associated with total WAT than the other 2 depots of external WAT (udder-associated, $r^2 = 0.77$, $P = 0.009$; and nuchal, $r^2 = 0.66$, $P = 0.03$). Nuchal fat was grossly different, firmer and paler than other WAT deposits. Periorbital ($r^2 = 0.01$, $P = 0.9$) and other head WAT ($r^2 = 0.3$; $P = 0.2$) were independent of total WAT mass and BCS.

Total mass of 'WAT-denuded' viscera (thoracic and abdominal) increased linearly (slope <0.1) as WAT-free, recEBM increased ($r^2 = 0.63$, $P = 0.03$; File SD). However, total organ mass (WAT-denuded) was more usefully described as a proportion of WAT-free recEBM. On this basis, the proportion of WAT-free recEBM comprised of WAT-denuded viscera decreased linearly as BCS increased ($r^2 = 0.84$, $P = 0.004$) but the contribution of specific viscera to this overall trend differed. The proportion of WAT-free recEBM comprised of heart and liver were independent of changes in BCS ($r^2<0.1$, $P>0.5$). Conversely, the proportion of WAT-free recEBM comprised of gastrointestinal ($r^2 = 0.89$, $P = 0.001$) and respiratory ($r^2 = 0.67$, $P = 0.03$) tracts, decreased markedly as BCS increased.

The major body organs, once denuded of their WAT, contributed variously to live BM (CNS, 0.32 ± 0.02%; heart, 0.62 ± 0.02%; respiratory tract, 0.98 ± 0.06%; empty gastrointestinal tract, 5.33 ± 0.48%; liver, 1.20 ± 0.11%; pancreas, 0.11 ± 0.01%;

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TABLE 2: Data from the 7 Welsh Mountain pony mares are presented in order of increasing body condition score

Pony	1	2	3	4	5	6	7		
BCS	1.25	2.5	4.08	4.25	5.9	6.8	7	Mean	s.e.
a) Internal carcase (body wall associated)									
Intrathoracic	0.06	0.23	0.20	0.27	0.43	0.48	0.48	0.31	0.06
Intraabdominal	0.00	0.36	3.07	1.15	4.12	7.38	3.98	2.87	0.98
Intrapelvic	0.05	0.15	0.09	1.50	0.82	0.23	0.51	0.48	0.20
Total (a)	0.11	0.74	3.36	2.92	5.37	8.09	4.97	3.65	1.05
b) Internal carcase (organ associated)									
Omental	0.05	0.06	0.28	0.05	1.33	0.74	0.36	0.41	0.18
Mesenteric	0.68	0.44	1.44	0.58	4.26	4.53	2.70	2.09	0.66
Perirenal	0.03	0.07	0.16	0.17	0.25	0.22	0.17	0.15	0.03
Pericardial	0.08	0.11	0.09	0.14	0.16	0.05	0.23	0.12	0.02
Epicardial	0.04	0.07	0.05	0.12	0.09	0.06	0.12	0.08	0.01
Broad ligament	0.00	0.16	0.22	0.22	0.34	0.54	0.25	0.25	0.06
Total (b)	0.88	0.91	2.24	1.28	6.43	6.14	3.83	3.1	0.91
Total internal (a + b)	0.99	1.65	5.6	4.2	11.8	14.23	8.8	6.75	1.9
c) External carcase (palpable)									
Subcutaneous	0.03	0.74	1.31	0.85	2.08	5.69	4.42	2.16	0.79
Udder-associated	0.01	0.01	0.11	0.00	0.11	0.37	0.34	0.14	0.06
Nuchal	0.01	0.81	0.4	0.32	0.32	1.55	1.15	0.65	0.21
Inter-muscular	0.64	1.06	1.58	4.85	8.08	7.88	7.47	4.51	1.27
Total external (c)	0.69	2.62	3.4	6.02	10.59	15.49	13.38	7.46	2.16
d) Head/lower limb									
Periorbital	0.07	0.09	0.05	0.06	0.06	0.04	0.05	0.06	0.01
Other	0.02	0.16	0.09	0.15	0.09	0.12	0.15	0.11	0.02
Total head (d)	0.09	0.25	0.14	0.21	0.15	0.16	0.20	0.17	0.02
Grand total (a + b + c + d)	1.77	4.52	9.14	10.43	22.54	29.88	22.38	14.38	3.99

Regional white adipose tissue (WAT) depots from 4 main sites (a–d) are presented as percentages of recovered empty body mass.

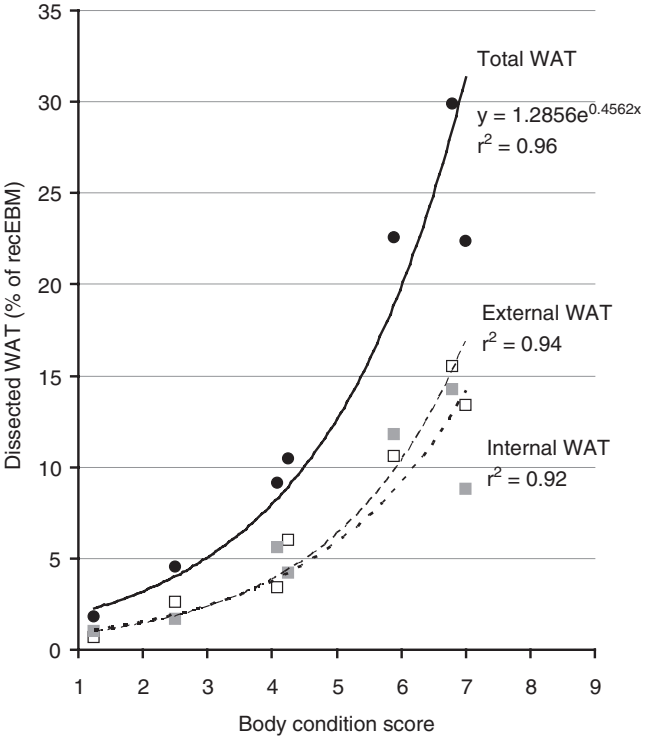

Fig 2: Total white adipose tissue mass (WAT, black circles) and its distribution between the 'external' (subcutaneous and intermuscular, white squares) and 'internal' (body wall and organ associated, grey squares) body compartments for the 7 Welsh Mountain ponies are regressed on their respective ante mortem *body condition scores (BCS). The coefficients of determination (r^2) for each data set and the exponential equation describing the overall relationship between BCS and total WAT as a percentage of recovered body mass are presented.*

spleen, 0.25 ± 0.02%; kidneys, 0.34 ± 0.03%; empty urogenital tract, 0.37 ± 0.06%).

Gross chemical evaluation

Chemical analyses of all cadaver parts allowed the gross chemical reconstitution of each pony (Fig 3; File SE). Chemically-reconstituted empty body mass (Fig 3a) overestimated recEBM by 2.5 ± 1.2%, and comprised: water, 60.4 ± 3.2% (range 48.2–73.9%); crude protein, 18.4 ± 0.9% (range 15.0–21.6%); total (neutral plus polar) lipids, 15.3 ± 4.1% (range 1.3–31.1%); and ash, 4.6 ± 0.4% (range 3.4–6.2%). Carbohydrates (assumed mainly glycogen) were not analysed but calculation by difference would suggest that they accounted for no more than 1.4 ± 0.2% of chemically-reconstituted empty body mass.

Body lipid mass, whether expressed as neutral body lipid (NBL, mainly 'storage' triglycerides) or total body lipid (TBL = NBL plus 'structural', polar lipids), was strongly associated with the mass of total dissected WAT (r^2 = 0.99, P<0.001). NBL comprised 0.8–31.1% recEBM (0.6–28% live BM) and TBL comprised 1.3–32.0% recEBM (1–29% live BM). Structural lipids (calculated by difference, TBL - NBL), comprised 9 ± 5% (range 1.5–38.5%) of TBL and their relative contribution to TBL was inversely associated with BCS. However, structural lipids comprised a relatively constant 0.6 ± 0.1% of recEBM, regardless of BCS.

Body water comprised 63.3 ± 3.2% (range 51.4–77.1%) of live BM, 62.0 ± 3.7% (range 49.6–79.2%) of recEBM and varied inversely with the percentage of total and neutral body lipid (r^2 = 0.94, P<0.001; Fig 3a; File SE). This relationship was also evident at tissue level. The water content of 4 different muscles (r^2 = 0.81, P = 0.015) and up to 10 different adipose tissues (r^2 = 0.80, P = 0.016) varied inversely with BCS (File SF). Total

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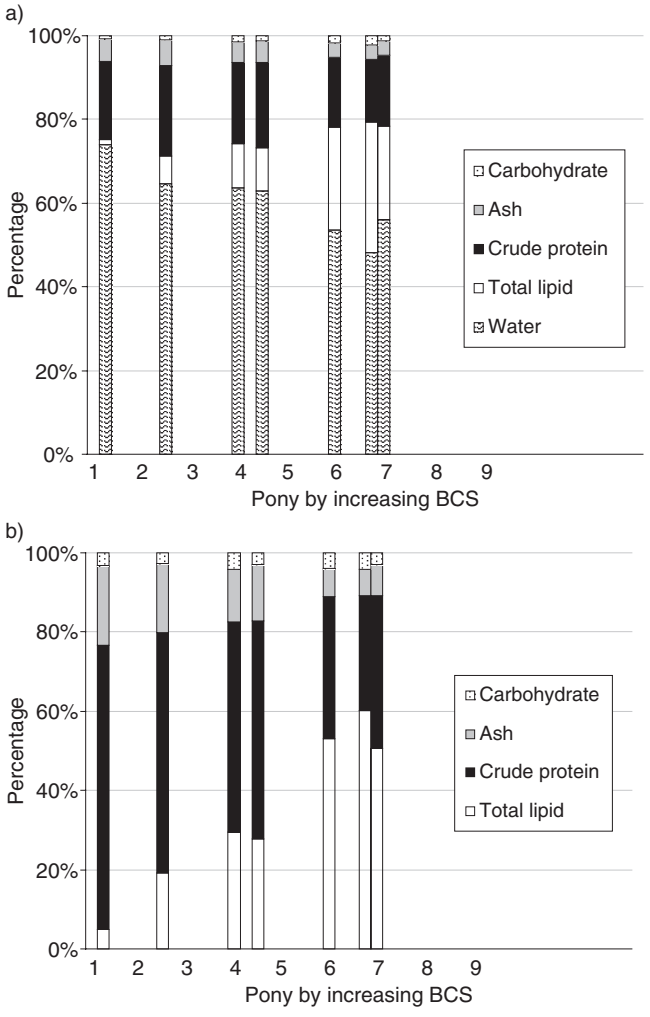

Fig 3: Gross chemical body compositions (water, crude protein, total [neutral and polar] lipid, ash and by difference, carbohydrate) as determined by proximate analyses, are presented as percentages of the total body in composite histogram bars for each of the 7 animals. Data for body composition a) as fresh tissues and b) as dry matter are ranked for each animal against their ante mortem *body condition scores.*

body lipid constituted 13.9 ± 3.8% (range 1.0–28.8%) of live BM (15.6 ± 4.2% of recEBM; range 1.3–32.0%), of which neutral lipids were the majority component (13.4 ± 3.8% [range 0.6–28.0%] of live BM; 15.0 ± 4.1% recEBM [range 0.8–31.1%]). Crude protein comprised 16.32 ± 0.73% live BM (18.84 ± 0.89% recEBM), carbohydrate 1.23 ± 0.17% live BM (1.41 ± 0.8% recEBM) and ash 4.03 ± 0.34% live BM (4.67 ± 0.43% recEBM). As body lipid content increased, the nonlipid components of body dry matter (protein and ash) decreased (Fig 3b; File SE).

Correlation and regression analyses of all analytes for each of the 8 dissected tissue categories indicated that the chemical composition of CNS and hide were conserved in the face of changing BCS (File SE). For all other tissue categories, water and lipid contents were inversely associated. This was exemplified by changes in the chemical composition of somatic soft tissues and viscera (File SE). Blood lipid contents were greatest for 2 animals, one of low BCS (P2) and one with the highest BCS (P7).

Total body GE content increased exponentially with BCS (Fig 4). For ponies of moderate and obese BCS, the GE content of WAT DM was similar, both between animals and between anatomically distinct WAT deposits (42.0 ± 0.2 MJ/kg DM), although there was a trend for nuchal WAT to contain least energy while retroperitoneal WAT was the most calorific reserve (File SG). Insufficient WAT was harvested from lean animals (P1 and P2) to allow replicate evaluations of adipose tissue-specific GE for each lean individual, such that only a single determination was possible which suggested a lower GE content than WAT from moderate and obese animals (27.6 MJ/kg DM).

Evaluation of morphometric data indicated that belly girth was more strongly correlated with total chemically-extracted lipid (r^2 = 0.73, P = 0.014) than mid-neck circumference (r^2 = 0.64, P = 0.02) and heart girth (r^2 = 0.56, P = 0.54). When normalised for withers height, heart girth was more strongly associated with total chemically-extracted lipid (r^2 = 0.91, P = 0.001) than belly girth (r^2 = 0.82, P = 0.005) and mid-neck circumference (r^2 = 0.75, P = 0.01). In addition, retroperitoneal fat depth, measured by transcutaneous ultrasonography, demonstrated the strongest correlation with chemically-extracted body lipid (r^2 = 0.88, P = 0.002). Tailhead (r^2 = 0.51, P>0.05), 12th rib-eye (r^2 = 0.19, P = 0.3) and gluteal (r^2 = 0.15, P = 0.4) sites were not useful predictors of body lipid content in these 7 pony mares.

Discussion

In companion animal management, BCS has become accepted as a useful monitor of body 'fatness' (La Flamme 1997a,b). However, in this relatively homogeneous group of animals, while BCS offered an almost perfect index of total somatic (skeletally-associated) soft tissues, it was markedly less useful in predicting total body fat. This finding serves as an important reminder that BCS systems were originally developed by the food animal industry for the evaluation of superficial 'flesh' in general and were not intended for the evaluation of fat alone (Jeffries 1961). To date, data validating the use of BCS systems against 'gold standard' cadaver dissection or chemical composition analysis are sparse for all species and only a small number have addressed the ability of BCS to discriminate between body fat and muscle (Russel *et al*. 1969; Wright and Russel 1984; Otto *et al*. 1991; Gregory *et al*. 1998; Martin-Rosset *et al*. 2008).

An understanding of the nonlinear relationship between current equine BCS systems and body fat content was gained by exploration of the relative contribution of WAT to the total somatic soft tissues. In the horse and other animals, quantitatively, WAT comprises the most variable of all body tissues (Lohman 1971; Webb and Weaver 1979). In contrast to the precise linear relationship between subjective BCS and total somatic soft tissue, total body WAT content increased exponentially with BCS irrespective of the specific BCS system used. Current data, although restricted to animals of BCS 7/9 and below, suggest that the sensitivity of BCS for the quantification of body WAT or lipid content, is decreased when BCS exceeds 5–6/9 (Dugdale *et al*. 2010a). This loss of sensitivity is associated with the increasing slope of the relationship, coincident with a region of the BCS scale where subjective descriptors of body condition inadequately distinguish between animals for which bony anatomical landmarks are already obscured by soft tissue (Dugdale *et al*. 2010a). Similar exponential or curvilinear relationships between total body fat and BCS have been demonstrated for horses and cattle (Gregory *et al*. 1998; Martin-Rosset *et al*. 2008). These data suggest the need to identify more precise descriptors or objective measurements of

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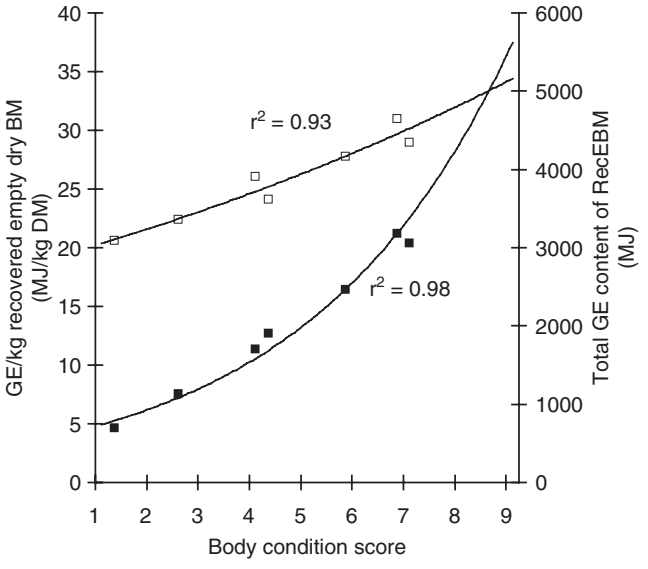

Fig 4: Body condition score for each of the 7 Welsh Mountain pony mares regressed on total gross energy (GE) content of the recovered empty body mass (black squares) and the GE content of each kg of dry matter (white squares). Coefficients of determination (r^2) are presented.

body fat in animals in moderate to obese condition to augment the precision of current BCS systems.

In this study, changes in BCS provided a valid, linear method for the determination of the highly variable, total somatic soft tissue content (2.8% of recEBM/BCS point; or 3.5% of live BM/BCS point). For lean animals, non-WAT soft tissues (largely skeletal muscle) comprised ~40% live BM (~50% recEBM), less than reported for racing breeds (53–57% live BM, Kearns *et al*. 2002b), which probably reflected differences in breed and/or fitness. In contrast to data for human subjects, particularly women, and unfit Standardbred mares, a reciprocal relationship was evident between WAT and non-WAT soft tissues with increased BCS, suggesting that lean tissue loss may accompany WAT deposition (Forbes 1987; Kearns *et al*. 2002b; Dugdale *et al*. 2010a).

The relative distribution of WAT across different anatomical regions was largely independent of BCS in these ponies. This agreed with comparable reports for cattle and sheep (Russel *et al*. 1971; Wright and Russel 1984). In the current study, WAT was equally distributed between 'internal' (body wall/organ-associated) and 'external' (intermuscular/subcutaneous) deposits. Uniformity of WAT distribution between the 'internal, covert' and 'external, potentially palpable' reserves, would lend credence to the view that the quantitative appraisal of external reserves by clear measurements or descriptors (BCS) should provide a useful index of total WAT. However, animals in the current trial had been in their final BCS for a prolonged period before study, whereas animals undergoing active weight change may favour different regional deposits during fat deposition or mobilisation (Dugdale *et al*. 2010a,b). This observation is supported by work in horses and other mammalian species that suggests that WAT accretion and depletion follows a preset order, 'fat patterning', which may be species, breed, age and individual specific (Riney 1955; Russel *et al*. 1971; Westervelt *et al*. 1976; Butler-Hogg 1984; Butler-Hogg *et al*. 1985; Pond 1998; Gentry *et al*. 2004; Carter *et al*. 2009). On this basis, the quantitative appraisal of BCS (external WAT) has the potential to misinform when used to monitor changes in total body WAT.

Of special interest, while at the low end of the BCS spectrum, 'structural' periorbital adipose tissue was conserved (P1, BCS 1.25), at the higher end of the studied BCS range (P6 and P7, BCS 6.8 and 7.0), palpably and quantitatively enlarged deposits of WAT were associated with the udder. Evaluation of udder and/or preputial fat, considered a 'structural depot', may therefore offer a useful aid to BCS assessment in overweight animals (Pond 1998).

Although the relative mass of the majority of superficially accessible fat depots, which contributed to both BCS systems, were strongly associated with total body WAT, nuchal ligament fat was the least dependable variable in the current study. This observation reiterates the importance of appraising fat deposition for BCS estimation at several discrete anatomical sites (Burkholder 2000). Crest fat thickness has previously been reported as a good indicator of total empty carcase fat (r^2 = 0.77); however, these data were derived from 107 horses grouped towards the lower end of the expected range of total body fat contents (7.85 ± 4.87%; range 1.2–18.5%), where the sensitivity of BCS descriptors for the determination of body fat would have been greatest (Znamirowska 2005). Nuchal fat may differ from other fat deposits as it also has a functional and sexually dichotomous role, evidenced by a higher content of connective tissue, which would also account for the lower energy concentrations determined in this study and previously reported for this reserve (Korzeniowski *et al*. 1994; Pond 1998). The GE contents of different pony tissues were almost identical to those reported for a limited range of bovine tissues (Blaxter and Rook 1953). Notably, the GE content of adipose tissues for ponies in higher BCS exceeded published values for cattle (41–43 MJ/kg DM vs. 39 MJ/kg DM, Blaxter and Rook 1953). However, recent reports may support the hypothesis that different regional WAT depots are metabolically distinct but the physiological relevance of these differences has yet to be determined (Burns *et al*. 2009; Suagee *et al*. 2010).

The relative contributions of the gross tissue categories (hide, collected blood, bones, etc) to the recovered empty (digesta excluded) body of the mature pony mares were consistent with data published for horses, cattle, sheep and pigs (Webb and Weaver 1979; Ockerman and Hansen 2000). Similarly, central nervous system and individual visceral organ weights were in agreement with ranges published for horses (Bradley 1896; Webb and Weaver 1979). Seasonal differences in hide weights were most probably associated with differences in pelage mass. Of note, in this study and that of Webb and Weaver (1979), was that the equine heart (0.6% live BM) and CNS (0.3% live BM) appeared to be relatively larger than those of pigs and ruminants (0.3–0.5% and 0.1–0.15% live BM, respectively; Ockerman and Hansen 2000).

The observation that gut fill varied inversely with BCS in these ponies for which forage was available *ad libitum* reinforces an important issue. It has previously been demonstrated that both appetite and maintenance energy requirements are decreased in obese ponies and cattle (Bines *et al*. 1969; Dugdale *et al*. 2010a). For cattle, it has been suggested that decreased appetite is secondary to physical constraints on gut capacity as a result of increased intra-abdominal fat (Bines *et al*. 1969). Conversely, it could be argued that decreased appetites are secondary to decreased metabolic requirements in obese subjects (Woods and D'Alessio 2008). However, total gastrointestinal tract mass also decreased with increasing BCS. Atrophy of gut tissues has previously been reported for other herbivorous mammals when voluntary food intake decreased (Gross *et al*. 1985; Rhind *et al*. 2002). Further study is required to unravel the relationships between obesity,

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appetite and gastrointestinal function. Despite the decreased gut fill of obese animals, belly girth retained its strong association with BCS, a relationship that has been demonstrated in previous studies (Dugdale *et al*. 2010a). Retroperitoneal fat depth and body girths provided reasonably objective measurements of whole body adiposity in this study and may offer practical tools for monitoring body fat in ponies in a manner comparable to that of waist circumference measurements in humans (Lean *et al*. 1996). The sheer bulk of intrathoracic WAT in the overweight ponies was alarming and may have serious implications for respiratory function for health and athletic performance, especially since none was scored higher than BCS 7/9.

In gross chemical terms, the composition of the empty pony body was comparable (water, ~62%; CP, ~19%; lipid, ~16%; ash, ~5%) to that of beef and dairy cattle and horses (Reid *et al*. 1955; Kane *et al*. 1987). Carbohydrates, mainly muscle glycogen, have been reported to comprise <0.5% of the human and bovine body (Reid *et al*. 1955; Sheng and Huggins 1979). However, current estimates of carbohydrate content exceeded those for cattle, which would agree with previous reports which indicate that the glycogen content of flight-adapted horse muscle is greater than that of the bovine (Lindholm and Piehl 1974; Immonen *et al*. 2000). The actual nitrogen content of equine tissue protein has not been determined. Application of the generic 6.25 conversion factor used to estimate CP content from tissue nitrogen concentrations may have contributed to the 2.5% overestimation when body mass was re-calculated as the sum of its chemical components. One cattle study proposed an alternative correction factor (5.8) which more correctly accounted for the nitrogen content of bovine tissue proteins (Odwongo *et al*. 1984). The nitrogen content of equine-specific tissue proteins has yet to be determined.

The chemical compositions of hide, bone and, to all intents and purposes, blood were consistent between ponies and similar to values reported for cattle (Ferrell and Jenkins 1984; Nour and Thonney 1987; Ockerman and Hansen 2000).

The reciprocal relationship between body water and body lipid content may have accounted for the greater dissection-associated water losses recorded for lean animals (Siri 1956). The range of total lipid content of these ponies was greater (up to 29% live BM or 32% recEBM) than has been suggested but previous reports tended to use leaner animals (6.6–18.9% of empty BM, Robb *et al*. 1972; 15.9 ± 2.0% of empty BM, Westervelt *et al*. 1976; <1–>11% of live BM, Webb and Weaver 1979; 1–2% live BM, Gunn 1986; 10–24% of empty BM, Kane *et al*. 1987; 2.6–14.7% of empty BM, Martin-Rosset *et al*. 2008). The range of neutral body lipid contents recorded for these ponies, however, was within the wide range (1–60%) reported for various animal species (Siri 1956; Lohman 1971). In the present study, structural (polar) lipids comprised a constant <1% of recEBM, a value within the published range for man (Siri 1956).

By using animals of common breed and gender, these data demonstrated the exponential relationship between linearly-ordinal BCS points and body fat content and suggested a loss of sensitivity of subjective BCS systems in overweight subjects. Objective measurements of whole body adiposity, including measurements of neck and body girths and ventral abdominal, retroperitoneal fat depth may provide useful supplements to BCS for monitoring live obese ponies. As for other species, further study is warranted to increase animal numbers before data can be confidently extrapolated to animals of differing breeds, ages and genders and to define 'healthy' ranges in body fat content (Russel *et al*. 1969; Wright and Russel 1984). The complex interactions between body fat, appetite and the gastrointestinal tract are also of future interest.

Authors' declaration of interests

The authors declare no conflict of interest.

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[2]Danish Agricultural Association, Copenhagen, Denmark.
[3]BK Medical, Herlev, Denmark.
[4]Avery Weigh-Tronix Ltd, West Bromwich, West Midlands, UK.
[5]Digital Data Systems (Pty), Ltd, Northcliff, South Africa.
[6]Group SEB, Ecully, France.
[7]Tecator AB, Perstorp, Sweden.
[8]Microsoft Corp., Redmond, Washington, USA.
[9]Minitab Inc., State College, Pennsylvania, USA.
[10]Stata Corp., College Station, Texas, USA.

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Author contributions P.A.H. and C.Mc.G.A. designed the project (modifications to technique by A.H.A.D.). A.H.A.D. and G.C.C. conducted the research A.H.A.D. analysed the data. A.H.A.D. and C.Mc.G.A. wrote the paper with input from P.A.H.

Supporting information

Additional Supporting Information may be found in the online version of this article:

File SA: Systematic cadaver dissection and sampling.
File SB: Tissue homogenisation and proximate analyses.
File SC: The mass (g) of each white adipose tissue (WAT) depot is presented, for 7 mature pony mares of increasing body condition score (BCS system: 1 [very poor] to 9 [extremely fat]).
File SD: Organ/tissue masses (g) from 7 pony mares ranked according to increasing body condition score. Values presented in brackets below each mass represent that mass as a proportion of WAT-free recovered empty body mass.
File SE: Results of proximate analysis for the 8 tissue categories from each of 7 mature pony mares, ranked by increasing body condition score.
File SF: Individual muscle, visceral and white adipose tissue depot water contents, presented as percentages for 6 of 7 mature pony mares of differing body condition scores.
File SG: Gross energy contents (MJ/kg DM) of muscle, visceral and white adipose tissues from 6 of 7 mature pony mares of differing body condition scores. *Only one sample could be tested for tailhead fat of Pony 1; the result was excluded from calculation of the mean value shown in the table.

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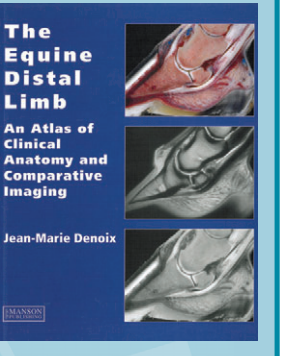

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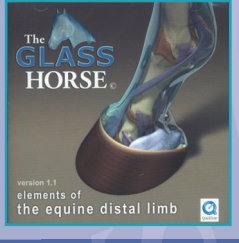

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