1 **Original research paper**

2

3 **Ultrasonographic Assessment of Regional Fat Distribution and its Relationship with**

4 **Body Condition in an Easy Keeper-Horse Breed**

5

6 Tamara Martin-Gimenez[a*]; Carla N. Aguirre-Pascasio[b]; Ignacio de Blas[a]

7

8 [a]Department of Animal Pathology, Faculty of Veterinary Medicine, Universidad de Zaragoza,

9 Miguel Servet 177, 50013 Zaragoza, Spain.

10 [b]Veterinary Teaching Hospital, Faculty of Veterinary Sciences, University of Murcia,

11 Campus de Espinardo, 30100 Murcia, Spain.

12

13 *Corresponding author: tamara.martin.gimenez@gmail.com

14 **Abstract**

15 Body scoring system is a functional method to monitor overall adiposity. However, scorer

16 experience, horse breed, gender or local fat deposits are confounding factors, primarily in

17 breeds with noticeable phenotype and tendency to subcutaneous fat deposition. Therefore, the

18 ultrasonographic evaluation of subcutaneous fat thickness (SFT) could be an objective

19 alternative method to assess fat distribution. The aims of this study were to characterize SFT

20 at seven anatomical locations (over 25%, 50% and 75% of neck-length, behind the shoulder,

21 over the ribs, over the rump and over the tailhead), to evaluate the relationship between the

22 body condition score (BCS) and SFT and to determine the influence of gender and age on

23 BCS and SFT measurements. A sample of 127 Andalusian horses (78 stallions and 49 barren

24 mares) was included. Body condition score was estimated and SFT was measured by

25 ultrasonography.

26 The results showed that in the neck area, significant differences in fat accumulation over 25%

27 and 50% of neck length were observed according to the gender and the age however,

28 regarding the body condition no significant differences were found. Subcutaneous fat

29 thickness at tailhead was the largest deposit and the most positively correlated parameter with

30 BCS with influence of gender and age. However, SFT at the rump was the only measurement

31 related to the body condition, independent of gender and age. Therefore, the assessment of

32 localized fat deposits by ultrasonography suggests that preferred anatomical sites for fat

33 deposition are evident and BCS systems should be adjusted according to breed specific

34 criteria.

35

2

1. Introduction

The adipose tissue is a key component for equine health and despite our understanding of its contribution to body composition and its anatomical distribution remains largely unrecognized [1]. In the clinical equine practice, the most commonly used methods to assess adiposity are the body condition score (BCS) systems based on a numerical scale. Several BCS scales have been specifically developed for use in horses [2–6]. Although, condition scoring systems include some objective criteria; also require some subjective assessment of physical attributes [7] influenced by scorer experience, horse breed [8] or gender; which may result in confounding factors. In addition, recent studies have shown an exponential relationship between BCS and total body white adipose tissue (WAT) suggesting that BCS is unlikely to be a sensitive index of body fat for equids in moderate-obese states [8–11]. Although, references to Andalusian horses in the literature are scarce, body condition evaluations by equine practitioners suggest that this breed has a tendency to accumulate large fat deposits. In addition, a recent study [12] where three breeds were compared, showed that Andalusians tended to retain their body condition and were relatively resistant to body fat loss, suggesting that this breed could be considered as an example of an easy keeper horse [13]. Due to the fact that these horses commonly exhibit obesity or increased regional adiposity [14,15] it is speculated that this type of horses are in the group of breeds predisposed to the development of endocrinopathic disorders [13]. Then, considering the tendency towards obesity of Andalusians [16] and other easy keeper horses [14] and the fact that BCS systems lose their sensitivity when the BCS exceeds 7/9, [8,11] it is essential to establish objective measurements of body fat to avoid the obesity overestimation in these breeds.

In addition, a greater understanding of regional fat deposition could help to find some pieces of the lipidic metabolism in the horse. Consequently, more objective body fat descriptors are

63 needed to increase the precision of current BCS systems to highlight animals at risk of

64 obesity-related diseases [8,10,11]. In this context, the measurement of subcutaneous fat

65 thickness (SFT) by ultrasonography should be considered as a method to discern regional

66 subcutaneous fat distribution among different body locations in conventional breeds or a

67 method to monitor increased fat pads in horses with phenotypically established fat deposition

68 patterns such is the case of many Andalusian-related breeds (Spanish Mustangs, Paso Fino)

69 considered as easy keeper horses [14].

70 Hitherto, ultrasonographic assessment of fat thickness has been proposed as the most

71 accurate, objective and non-invasive method for measuring subcutaneous fat distribution in

72 live horses [17]. Heretofore, there are published studies including only one breed [18], both

73 genders [18–21], age influence [21] and evaluating SFT measurements at different fat

74 deposition areas [19,20,22–24]. However, to our knowledge, there are no studies about SFT

75 including large numbers of only one breed horses and considering gender and age at the same

76 time. The main hypothesis was that Andalusians, due to their breed phenotypic

77 characteristics, have a tendency to accumulate large fat deposits in specific areas that can vary

78 with gender and age, confounding a correct BCS evaluation.

79 Therefore, the aims to address this hypothesis were to characterize the fat thickness

80 measurements at different adipose deposits; to evaluate whether or not BCS and

81 ultrasonographic measurements are influenced by gender and age in Andalusian horses and;

82 to establish accurate correlations between BCS and ultrasonographic measurements.

83 **2. Materials and methods**

84 *2.1 Study design*

85 As part of a larger study investigating insulin resistance (IR) and obesity, a census of

86 Andalusian horses located in the South-East of Spain was carried out. A cross-sectional study

87 was developed from June to August of 2012. Owners were contacted by telephone and asked

4

88   if they were willing to take part in the study. The study population included 1722 Andalusian

89   horses between 2 and 15 years.

90   Based on previously reported IR prevalence data for equine population, a sample with

91   randomly generated numbers and with representative proportion of both genders and different

92   ages was selected. Sample size to estimate a proportion were calculated with WinEpi 2.0

93   (http://www.winepi.net/winepi2) assuming a 34.1% of expected prevalence [25] with an

94   accepted error of 7.5% and a confidence level of 95% (n=149), with an additional correction

95   of +5% (n=157). Selected final sample size after rounding-up applied during stratification

96   included 166 animals. Considering the bibliography that establishes that the tendency to

97   become obese increases with age [26–28], two age groups were defined: young horses (2-5

98   years) and adult horses (6-15 years). The evaluated horses were selected from 44 breeding

99   farms, horse riding schools and private stables. Every owner or person in charge signed an

100 informed consent before the non-invasive procedures were performed. According to Spanish

101 regulations, an Animal Care and Ethics Committee approval was not necessary.

102 The inclusion criteria were that all included animals should be healthy based on the results of

103 physical examination, complete blood count and serum biochemical analysis. Only horses on

104 a normal herd health schedule, adequate management and fed with a proper diet (0.5-1kg

105 concentrate/100 kg BW and at least, double quantity of forage) [29] were included.

106 For the current study, additional exclusion criteria were applied: pregnant mares, lactating

107 mares and horses undergoing medical treatment. In the random selection, only one gelding

108 horse was selected. Considering its low representativeness for the statistics this was excluded.

109 Finally, 127 horses were included in this study.

110 *2.2 Body Condition Score and ultrasonographic measurements*

5

111   Two independent and trained evaluators (TM and FM) determined BCS [3] and the average of

112   both scores was used. Based on their BCS, obese horses were defined as BCS≥7 and, non-

113   obese as BCS<7 [26,30,31].

114   All measurements of SFT were performed via B-mode with commercial ultrasonographic

115   imaging system (Honda Electronics HS-1500V, Aichi, Japan) equipped with a 7.5 MHz linear

116   transducer [24,32]. All images were taken on the left side, assuming that bilateral variation

117   errors were small enough to consider only one side necessary to be measured [33]. The horses

118   were standing in a normal position and individually restrained to minimize movements. Due

119   to the time of year during which the study was conducted, it was not necessary to clip the hair

120   and alcohol was used as a coupling medium. The scanning and the interpretation of the

121   images were performed by the same researcher (TM) to avoid variability in the measurement

122   technique.

123   The depth of seven local fat deposits, three of them along the neck-length and four over the

124   trunk were measured to the nearest 0.01 mm by transcutaneous ultrasonography. The probe

125   was positioned perpendicular to the floor. Anatomic landmarks used to guide the transducer

126   placement in the neck area were located at the interface between the crest and neck

127   musculature, identified by palpation and visual assessment at: 25% (SFT-N25%), 50% (SFT-

128   N50%), and 75% (SFT-N75%) of neck-length [30]. Regarding the measurements on the

129   trunk, the probe was situated as described [23,24] at: the area just caudal to the shoulder

130   (SFT-S), the area between 12-13th ribs (SFT-R12-13), the rump (SFT-R), and the tailhead

131   (SFT-TH) (Fig. 1). Coefficients of variation for SFT measurements were determined by using

132   triplicate measurements in each location of 127 horses, the values being between 3.4 and

133   7.9%. Furthermore intraclass correlation coefficients for the reliability of repeated

134   measurements were significant (p<0.001) and varied between 96.3 and 99.5%.

135   *2.3 Statistical analysis*

6

136 Quantitative variables were described using mean, standard deviation, coefficients of

137 variation and range (minimum and maximum). Normality was checked using Kolmogorov-

138 Smirnov test. The mean comparisons between groups were carried out with Student's t test

139 for independent samples, when variables were normally distributed or otherwise Mann-

140 Whitney U test was applied. In order to analyse the relationship of SFT measurements with

141 different factors considered as fixed effects (anatomical site, gender, age group and obesity

142 status according to BCS), a general linear model for repeated measures was applied.

143 Pearson's and Spearman's correlation coefficients and its significance were calculated using

144 the average from triplicate measurements. The association between categorical variables was

145 assessed using the Pearson's Chi-square test. Statistical analyses were performed using a

146 commercial statistical software (IBM SPSS 19.0 for Windows IBM Corp., Armonk, NY,

147 USA). In all cases confidence level was set at 95% and power at 80%.

148

149 **3. Results**

150 Of the 127 horses evaluated, 61.4% were stallions and 38.6% mares. Age groups included

151 young horses (n=68) with a mean age of 3.59 ± 1.10 years and adult horses (n=59) with a

152 mean age of 9.56 ± 2.99 years. There was no association between either gender and age

153 (p=0.317), or gender and age groups (p=0.519).

154 Overall BCS was 6.06 ± 1.04 without significant differences by gender (p=0.252) or by age

155 groups (p=0.102). The overall distribution of BCS in the studied sample and, corresponding

156 frequencies according to gender and age within each BCS category are presented in Table 1.

157 Agreement between the two BCS evaluators was moderate (weighted Cohen's Kappa = 0.490;

158 p<0.001). Thirty-three of sampled horses (26.0%) were classified as obese. The proportion of

159 obese horses was similar (p=0.761) in males (26.9%) and females (24.5%). Furthermore,

160 obesity status according to BCS was not associated with age groups (p=0.137).

161 In the global sample, the subcutaneous fat deposit with higher values of fat thickness was the

162 SFT-TH, followed by SFT-R and SFT-N50% (Table 2). The SFT-TH area was maintained as

163 the highest point of fat deposition in all categories of gender, age groups and obesity status

164 (Table 3).

165 When the sample was stratified by gender (Table 3), stallions showed significant higher

166 values in the following locations: SFT-N50%, SFT-N25%, SFT-S and SFT-R12-13.

167 However, mares showed greater SFT measurements at tailhead area.

168 Four SFT measurements (SFT-N25%, SFT-N50%, SFT-S and SFT-TH) were significantly

169 higher in adult horses compared with young horses (Table 3).

170 The stratification by obesity status showed significant differences in all sites at trunk level,

171 especially at SFT-R and SFT-TH. Several significant interactions (p<0.050 and power>0.800)

172 between obesity status and age were observed at SFT-N25% and SFT-S with higher values in

173 obese adults (Table 3).

174 In the neck area, SFT-N75% was significantly correlated with BCS in the total of horses.

175 When the gender and age were considered, this deposit was correlated with BCS in mares and

176 both age groups. In the trunk area, SFT-S was correlated with BCS in the global sample, in

177 the mares and adult horses. However, the rest of measurements assessed over the trunk, were

178 significantly correlated with BCS globally and in both genders and age groups. The best

179 correlation value was obtained for STF-TH (Table 4).

180 **4. Discussion**

181 Andalusian horses are classified as a light breed with a natural tendency to increased adiposity

182 and considered as an example of easy keeper horse [13]. In many studies, BCS≥7 has been

183 used as a cut-off value to define equine obesity [26,30,31]. Our results show that over a

184 quarter of the sample presented a BCS≥7, the global mean of BCS being higher than values

185 reported previously in ponies [34,35] and horses [23,31,36,37]. Similarly, the BCS in stallions

186 was higher than values obtained in other males [19] however; the BCS in females was lower

187 than in other light mares [19,38]. Pregnant and lactating mares were excluded, due to different

188 condition changes suffered during pregnancy and lactating period; giving the possibility to

189 compare our results with other studies made in other breed mares in similar conditions

190 [19,38]. According to what has been previously observed [21,36,39], no significant

191 differences were found in BCS between both genders.

192 Ultrasonography has gained importance as an objective method to assess regional fat

193 distribution [40] because it allows for the evaluation of subcutaneous fat tissue independently

194 of muscle mass [41]. Additionally, this method is technically limited in areas containing large

195 amounts of connective tissue within the adipose tissue, such as the cresty neck, because a

196 discrete limit separates adipose tissue and muscle not being easily discernible [17]. However,

197 in the present study, the repeatability for the ultrasonographic measurements in selected body

198 regions, indicated an excellent reliability [42] supporting the applicability of this method.

199 In obese horses, the fat is frequently deposited around the neck, caudal to the triceps, over the

200 ribs, back, flank and tailhead [43]. In this study, SFT was evaluated in seven anatomical areas,

201 which coincide with the usual fat pads in easy keeper horses. The selected landmarks for the

202 ultrasonographic measurements were determined based on previous studies in equids

203 [8,11,22–24,44]. It has long been recognized that adipose reserves associated with the nuchal

204 ligament are increased in obese horses [1,14,37,45,46]. Conversely, in our study, significant

205 differences on fat deposits between obese and non-obese horses were only located at trunk

206 level, and no significant differences were observed in fat accumulation in the neck area

207 suggesting that neck adiposity is not completely related to general obesity in Andalusians.

208 This difference could be related to the neck phenotypic conformation of Andalusians, which

209 are characterized, by medium size and thick necks [47].

9

210 Regarding gender influence, this study has one limitation because gelding horses were

211 excluded due to its low representativeness in the population of Andalusian horses. Generally

212 Andalusian stallions are not gelded because they are honest and have a good behaviour, so

213 differences between stallions and gelding horses could not be assessed in a study with a

214 randomly selected sample. However, clear differences were found between stallions and

215 mares at the sites studied. First of all, in the neck area stallions presented higher SFT

216 measurements over the first and second third of the neck-length. This may be explained by

217 Dugdale et al.[10] which suggested that nuchal fat could have a functional and sexually

218 dichotomous role, evidenced by a higher content of connective tissue. The deposition of

219 subcutaneous fat in these two neck areas continues to increase until 5 years of age being the

220 area at mid-neck length, which tended to accumulate higher fat deposits and had less variation

221 in the neck. These results cannot be compared because to the authors' knowledge, there are no

222 studies evaluating ultrasonographically subcutaneous fat in the neck area at three different

223 locations.

224 In relation to the evaluated areas over the trunk, SFT-S was in the range of values reported in

225 other breeds [23,48]. Furthermore, Westervelt et al. [48] did not find differences in this area

226 between subjects classified according to live weights. However, in our case, SFT-S was

227 significantly greater in obese adults. In contrast with other works [20,24], SFT-S was the

228 evaluated measurement which exhibited the highest variation.

229 Likewise, the values described for SFT-R12-13 were higher than the values described in non-

230 obese ponies [8] but lower than values observed in mature horses [20] and donkeys [24]. In

231 contrast with Cartmill et al. [19], in Andalusian stallions higher accretion of fat over the ribs

232 was found with respect to females.

233 Regarding the SFT-R, it was considerably higher than the measurements previously reported

234 in other equids [8,19,24,48]. However, SFT-R was the second highest value in our study,

235 followed by the area at mid-neck length. These results are in contrast to the findings described

236 by Gentry et al. [22] in a study carried out in light broodmares where the area over the ribs

237 presented higher SFT than the rump area. Similar results have been shown in another study

238 developed in female donkeys [24]. These discrepancies should be interpreted with caution

239 since the studied populations cannot be compared because, the cited studies were performed

240 just considering one gender as influence factor, and only females were included in both.

241 Otherwise, the existence of breed, gender and age influence in the rump deposit has only been

242 evaluated in Thoroughbreds, Standardbreds and Italian saddler horses [21], being independent

243 in all cases. These results were in agreement with our findings where no gender or age

244 differences were found, although, the rump deposit was influenced by the condition in

245 Andalusians. This means that STF-R was the only measurement exclusively related to obesity

246 status, and independent of gender and age. These outcomes highlight the importance of

247 defining and validating precise anatomical landmarks for different breeds considering gender

248 and age as influence factors.

249 With respect to tailhead area, the results of the present study are in accordance with data

250 previously reported [22,24] where SFT-TH showed a higher value than any other evaluated

251 body location. In addition, Andalusian horses showed higher SFT measurements than values

252 described in other equids [19,24]. In human beings, it has been demonstrated that males have

253 greater muscle mass and less fat than females [49], similar results have been reported in

254 horses [50,51]. Similarly, when localized fat deposits have been assessed [19], females have

255 shown higher SFT in each area than stallions. However, in contrast with the bibliography

256 [19], Andalusian mares tended only to accumulate significantly higher amounts of fat over the

257 tailhead area. In obese Andalusians, large deposits were found at SFT-TH with no possibility

258 of comparison with the literature, because the most recent study where similar evaluations

259 were considered was made in mare ponies with *ad libitum* diet [8].

260 In humans, fat increases with age and is redistributed, resulting in a greater intra-abdominal

261 and WAT deposition [52]. Similarly, it has been described that subcutaneous fat tissue was

262 one of the last in developing in young horses [9]. In our case, our results show that two (SFT-

263 25% and SFT-N50%) of the three evaluated sites in the neck area and two of the four areas

264 assessed at the trunk level (SFT-S and SFT-TH) increased with age.

265 In agreement with other studies [53], our results show that the measurement of SFT has the

266 advantage of detecting slight variations in fat reserves, difficult to detect with subjective body

267 scoring systems and also demonstrates, significant differences in fat deposition according to

268 demographic variables such as gender and age.

269 In horses, it has been reported that not all SFT points equally reflect fat reserve changes [22].

270 Our results showed that not all the areas evaluated were correlated with BCS and the

271 correlation coefficients obtained in some ultrasonographic measurements were lower than

272 values reported in the bibliography [20,22,24]. In accordance with previous studies [22,54],

273 SFT-TH was closely associated with BCS being the best correlation. Coinciding with the

274 literature we suggest that SFT-TH [8,22], but also SFT-R are reliable in the body fat

275 appraisal, because they are firmly correlated with BCS in both genders and all ages.

276 **5. Conclusions**

277 In the present study, our findings suggest that in Andalusian horses there are anatomical

278 variations in fat deposition that could potentially confound the estimation of body fatness

279 derived from conventional BCS systems. Therefore, the use of ultrasonography to assess

280 subcutaneous fat deposits may aid clinicians in the accurate evaluation of body fat avoiding

281 overestimation in easy keeper-like horses. Moreover, these results can contribute to a better

282 understanding of regional fat distribution, helping to objectively monitor body condition in

283 breeds with a tendency to particular fat deposition patterns. Further studies are needed to

284 adjust BCS systems, according to breed specific criteria.

Authorship

T. Martin-Gimenez, contributed to the conception and study design, acquisition of the data, execution, data analysis and interpretation, and preparation of the manuscript. I. de Blas contributed to the study design, data analysis, interpretation, and preparation of the manuscript. C.N Aguirre-Pascasio contributed to the conception and study design and preparation of the manuscript. All authors approved the final version of this manuscript.

Conflicts of interest Statement

None

References

[1]   Argo CM. Appraising the portly pony: Body condition and adiposity. Vet J 2009;179:158–60.

[2]   Webb AI, Weaver BM. Body composition of the horse. Equine Vet J 1979;11:39–47.

[3]   Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983;15:371–2.

[4]   Carroll CL, Huntington PJ. Body condition scoring and weight estimation of horses. Equine Vet J 1988;20:41–5.

[5]   Kohnke JR. Feeding and nutrition: the making of a champion. Australia: Birubi Pacific; 1992.

309   [6]   Kienzle E, Schramme SC. Body condition scoring and prediction of body weight in

310   adult warm blooded horses. Pferdeheilkunde 2004;20:517–24.

311   [7]   Vecchi I, Sabbioni A, Bigliardi E, Morini G, Ferrari L, Di Ciommo F, et al.

312   Relationship between body fat and body condition score and their effects on estrous

313   cycles of the Standardbred maiden mare. Vet Res Commun 2010;34:41–5.

314   [8]   Dugdale AHA, Curtis GC, Cripps PJ, Harris PA, Argo CM. Effects of season and body

315   condition on appetite, body mass and body composition in ad libitum fed pony mares.

316   Vet J 2011;190:329–37.

317   [9]   Martin-Rosset W, Vernet J, Dubroeucq H, Arnaud G, Picard A, Vermorel M. Variation

318   of fatness and energy content of the body with body condition score in sport horses and

319   its prediction. In: Saastamoinen M, Martin-Rosset W, editors. Nutrition of the

320   Exercising Horse. European Association for Animal Production Publication. Number

321   125, Wageningen, the Netherlanders: Wageningen Academic Publishers; 2008, p. 167–

322   76.

323   [10]   Dugdale AHA, Curtis GC, Harris PA, Argo CM. Assessment of body fat in the pony:

324   Part I. Relationships between the anatomical distribution of adipose tissue, body

325   composition and body condition. Equine Vet J 2011;43:552–61.

326   [11]   Dugdale AHA, Grove-White D, Curtis GC, Harris PA, Argo CM. Body condition

327   scoring as a predictor of body fat in horses and ponies. Vet J 2012;194:173–8.

328   [12]   Potter SJ, Bamford NJ, Harris PA, Bailey SR. Comparison of weight loss, with or

329   without dietary restriction and exercise, in Standardbreds, Andalusians and mixed

330   breed ponies. J Equine Vet Sci 2013;33:339.

331   [13]   Bamford NJ, Potter SJ, Harris PA, Bailey SR. Breed differences in insulin sensitivity

332   and insulinemic responses to oral glucose in horses and ponies of moderate body

333   condition score. Domest Anim Endocrinol 2013;47:101–7.

334 [14] Johnson PJ. The equine metabolic syndrome Peripheral Cushing´s syndrome. Vet Clin

335   North Am - Equine Pract 2002;18:271–93.

336 [15] Frank N, Geor RJ, Bailey SR, Johnson PJ, Durham AE, et al. Equine metabolic

337   syndrome. J Vet Intern Med 2010;24:467–75.

338 [16] Mendoza F, Diez E, Lopez I, Estepa J, Guerrero F, Aguilera-Tejero E. Prevalence of

339   Insulin Resistance in Andalusian Horses. J Vet Intern Med 2009:426.

340 [17] Carter RA, Dugdale AHA. Assessment of body condition and bodyweight. In: Geor R,

341   Harris PA, Coenen M, editors. Equine Applied Clinical Nutrition, Edinburgh, UK:

342   Elsevier Ltd; 2013, p. 393–404.

343 [18] Gee EK, Fennessy PF, Morel PCH, Grace ND, Firth EC, Mogg TD. Chemical body

344   composition of 20 Thoroughbred foals at 160 days of age, and preliminary

345   investigation of techniques used to predict body fatness. N Z Vet J 2003;51:125–31.

346 [19] Cartmill JA, Thompson DL, Del Vecchio RP, Storer WA, Crowley JC. Leptin

347   secretion in horses: Effects of dexamethasone, gender, and testosterone. Domest Anim

348   Endocrinol 2006;31:197–210.

349 [20] Silva SR, Guedes CM, Couto P, Santos AS, Melo-Pinto P. Relationship between body

350   condition and neck crest score systems and subcutaneous fat, tissue and muscle

351   ultrasonic measurements in horses. In: Saastamoinen M, Fradinho MJ, Santos AS,

352   Miraglia N, editors. Forages grazing horse Nutrition, Wageningen, the Netherlanders:

353   Springer; 2012, p. 381–6.

354 [21] Superchi P, Vecchi I, Beretti V, Sabbioni A. Relationship among BCS and Fat

355   Thickness in Horses of Different Breed , Gender and Age. Annu Res Rev Biol

356   2014;4:354–65.

357 [22] Gentry LR, Thompson DL, Gentry GT, Del Vecchio RP, Davis KA, Del Vecchio PM.

358 The relationship between body condition score and ultrasonic fat measurements in

359 mares of high versus low body condition. J Equine Vet Sci 2004;24:198–203.

360 [23] Carter RA, McCutcheon LJ, George LA, Smith TL, Frank N, Geor RJ. Effects of diet-

361 induced weight gain on insulin sensitivity and plasma hormone and lipid

362 concentrations in horses. Am J Vet Res 2009;70:1250–8.

363 [24] Quaresma M, Payan-Carreira R, Silva SR. Relationship between ultrasound

364 measurements of body fat reserves and body condition score in female donkeys. Vet J

365 2013;197:329–34.

366 [25] McGowan CM, Geor R, McGowan TW. Prevalence and risk factors for

367 hyperinsulinemia in ponies. J Vet Intern Med 2008;22:734.

368 [26] Giles SL, Rands SA, Nicol CJ, Harris PA. Obesity prevalence and associated risk

369 factors in outdoor living domestic horses and ponies. PeerJ 2014;2:e299.

370 [27] Robin CA, Ireland JL, Wylie CE, Collins SN, Verheyen KLP, Newton JR. Prevalence

371 of and risk factors for equine obesity in Great Britain based on owner-reported body

372 condition scores. Equine Vet J 2014:1–6.

373 [28] Thatcher CD, Pleasant RS, Geor RJ, Elvinger F. Prevalence of Overconditioning in

374 Mature Horses in Southwest Virginia during the Summer. J Vet Intern Med

375 2012;26:1413–8.

376 [29] Kapper D. Applied nutrition. In: Reed S, Bayly W, Sellon D, editors. Equine Internal

377 Medicine 2nd ed., Missouri: Elsevier Co; 2004, p. 1543–55.

378 [30] Frank N, Elliott SB, Brandt LE, Keisler DH. Physical characteristics, blood hormone

379 concentrations, and plasma lipid concentrations in obese horses with insulin resistance.

380 J Am Vet Med Assoc 2006;228:1383–90.

381 [31] Pratt-Phillips SE, Owens KM, Dowler LE, Cloninger MT. Assessment of Resting

382 Insulin and Leptin Concentrations and Their Association With Managerial and Innate

383 Factors in Horses. J Equine Vet Sci 2010;30:127–33.

384 [32] Argo CM, Curtis GC, Grove-White D, Dugdale AHA, Barfoot CF, Harris PA. Weight

385 loss resistance: A further consideration for the nutritional management of obese

386 Equidae. Vet J 2012;194:179–88.

387 [33] Domecq JJ, Skidmore AL, Lloyd JW, Kaneene JB. Validation of body condition scores

388 with ultrasound measurements of subcutaneous fat of dairy cows. J Dairy Sci

389 1994;78:2308–13.

390 [34] Bailey SR, Habershon-Butcher JL, Ransom KJ, Elliott J, Menzies-Gow NJ.

391 Hypertension and insulin resistance in a mixed-breed population of ponies predisposed

392 to laminitis. Am J Vet Res 2008;69:122–9.

393 [35] Wray H, Elliott J, Bailey SR, Harris PA, Menzies-Gow NJ. Plasma concentrations of

394 inflammatory markers in previously laminitic ponies. Equine Vet J 2013;45:546–51.

395 [36] Donaldson MT, McFarlane D, Jorgensen AJR, Beech J. Correlation between plasma α-

396 melanocyte-stimulating hormone concentration and body mass index in healthy horses.

397 Am J Vet Res 2004;65:1469–73.

398 [37] Carter RA, Geor RJ, Staniar WB, Cubitt TA, Harris PA, Burton Staniar W, et al.

399 Apparent adiposity assessed by standardised scoring systems and morphometric

400 measurements in horses and ponies. Vet J 2009;179:204–10.

401 [38] Vick MM, Adams AA, Murphy BA, Sessions DR, Horohov DW, Cook RF, et al.

402 Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the

403 horse. J Anim Sci 2007;85:1144–55.

404 [39] Treiber KH, Kronfeld DS, Hess TM, Byrd BM, Splan RK, Staniar WB. Evaluation of

405   genetic and metabolic predispositions and nutritional risk factors for pasture-associated

406   laminitis in ponies. J Am Vet Med Assoc 2006;228:1538–45.

407   [40]   Stouffer JR. Ultrasound technology: live animal and carcass evaluation. In: Ullrey DE,

408   Baer CK, Pond WG, editors. Encyclopedia of Animal Science, New York: CRC Press;

409   2011, p. 853–5.

410   [41]   Maes DGD, Janssens GPJ, Delputte P, Lammertyn A, De Kruif A. Back fat

411   measurements in sows from three commercial pig herds: Relationship with

412   reproductive efficiency and correlation with visual body condition scores. Livest Prod

413   Sci 2004;91:57–67.

414   [42]   Fleiss J. The design and analysis of clinical experiments. New York: John Wiley and

415   Sons; 1986.

416   [43]   Donaldson MT, Jorgensen AJR, Beech J. Evaluation of suspected pituitary pars

417   intermedia dysfunction in horses with laminitis. J Am Vet Med Assoc 2004;224:1123–

418   7.

419   [44]   Dugdale AHA, Curtis GC, Cripps P, Harris PA, Argo CM. Effect of dietary restriction

420   on body condition, composition and welfare of overweight and obese pony mares.

421   Equine Vet J 2010;42:600–10.

422   [45]   Carter RA, Treiber KH, Geor RJ, Douglass L, Harris PA. Prediction of incipient

423   pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised

424   and localised obesity in a cohort of ponies. Equine Vet J 2009;41:171–8.

425   [46]   Frank N. Equine metabolic syndrome. Vet Clin North Am Equine Pract 2011;27:73–

426   92.

427   [47]   Stud breeding book of Andalusians, Orden APA/3319/2002, de 23 de diciembre, por la

428   que se establecen las normas zootécnicas del caballo de Pura Raza Española. Boletín

429   Oficial del Estado (Spain), 31th december 2002, 313; 46330-46333.

430   [48] Westervelt RG, Stouffer JR, Hintz HF, Schryver HF. Estimating fatness in horses and

431   ponies. J Anim Sci 1976;43:781–5.

432   [49] Abe T, Brechue WF, Fujita S, Brown JB. Gender differences in FFM accumulation and

433   architectural characteristics of muscle. Med Sci Sports Exerc 1998;30:1066–70.

434   [50] Kearns CF, Mckeever KH, Kumagai K, Abe T. Fat-free mass is related to one-Mile

435   race performance in elite standardbred horses. Vet J 2002;163:260–6.

436   [51] Fonseca RG, Kenny DA, Hill EW, Katz LM. The relationship between body

437   composition, training and race performance in a group of Thoroughbred flat

438   racehorses. Equine Vet J 2013;45:552–7.

439   [52] Villareal DT, Apovian CM, Kushner RF, Klein S. Obesity in older adults: technical

440   review and position statement of the American Society for Nutrition and NAASO, The

441   Obesity Society. Am J Clin Nutr 2005;82:923–34.

442   [53] Mottet R, Onan G, Hiney K. Revisiting the Henneke body condition scoring system: 25

443   years later. J Equine Vet Sci 2009;29:417–8.

444   [54] Gobesso AAO, Françoso R, Toledo RAD, Centini TN, Gonzaga IVF, Gil PCN, et al.

445   Evaluation of body condition score in horses by ultrasonography. In: Saastamoinen M,

446   Fradinho MJ, Santos AS, Miraglia N, editors. Forages in grazing horse nutrition,

447   Wageningen, the Netherlanders: Springer; 2012, p. 387–90.

448

449

450 **Figure captions**

451 Fig.1. Anatomic landmarks used for ultrasonographic measurements.

452 In the neck area, equidistant measurements of subcutaneous fat thickness (SFT) at 25% (SFT-

453 N25%), 50% (SFT-N50%) and 75% (SFT-N75%) of neck-length were assessed over the

454 interface between the crest and neck musculature. Over the trunk, SFT was evaluated over the

455 area just caudal to the triceps at three quarters of the distance from the dorsal midline to one

456 third of the distance from the point of the shoulder to the point of the tuber coxae (SFT-S);

457 over the area between 12-13th ribs at two thirds of the distance from the point of the shoulder

458 to the point of the tuber coxae (SFT-R12-13); over the rump at 5 cm lateral from the dorsal

459 midline at the centre of the iliac wing (SFT-R) and on the flat area of tailhead and parallel to

460 the midline (SFT-TH).

461

462 **Table 1.** Distribution of body condition score (BCS) in the sample studied of 127 horses and

463 corresponding proportions according to gender and age within each body condition score

464 category.

465

BCS		Gender		Age	
	Global	Stallions	Mares	2-5 years	6-15 years
n	127	78	49	68	59
3.0	2.4%	1.3%	4.1%	2.9%	1.7%
3.5	0.0%	0.0%	0.0%	0.0%	0.0%
4.0	2.4%	2.6%	2.0%	2.9%	1.7%
4.5	4.7%	3.8%	6.1%	4.4%	5.1%
5.0	8.7%	6.4%	12.2%	7.4%	10.2%
5.5	20.5%	20.5%	20.4%	26.5%	13.6%
6.0	14.2%	14.1%	14.3%	14.7%	13.6%
6.5	21.3%	24.4%	16.3%	20.6%	22.0%
7.0	16.5%	17.9%	14.3%	17.6%	15.3%
7.5	6.3%	5.1%	8.2%	0.0%	13.6%
8.0	2.4%	3.8%	0.0%	2.9%	1.7%
8.5	0.0%	0.0%	0.0%	0.0%	0.0%
9.0	0.8%	0.0%	2.0%	0.0%	1.7%

466

467 **Table 2**

468 Comparison of subcutaneous fat thickness (SFT) measurements (mm) among different

469 anatomical sites (n = 127).

470

Variable	Mean ± Standard Deviation	Coefficient of Variation (%)	Range Minimum	Maximum
SFT-N25%	6.59 ± 2.07^{a}	31.4	2.80	12.20
SFT-N50%	11.09 ± 3.43^{c}	30.9	3.50	19.60
SFT-N75%	10.11 ± 3.62^{b}	35.8	2.40	24.2
SFT-S	7.15 ± 3.20^{a}	44.7	1.60	21.6
SFT-R12-13	7.60 ± 2.87^{a}	37.8	2.60	26.3
SFT-R	14.80 ± 5.42^{d}	36.6	3.40	30.2
SFT-TH	25.61 ± 8.05^{e}	31.4	4.70	51.2

471 Significance of general linear model for repeated measures using anatomical site as fixed

472 effect, $p<0.001$. Different super indexes in the same column indicates significant differences

473 using the Duncan post hoc test ($p<0.050$)

22

474 **Table 3**

475 Association of subcutaneous fat thickness (SFT) measurements (mm) in different anatomical sites with sex, age group and obesity status

476 according to body condition score (BCS).

Variable	Gender			Age groups			Obesity status		
	Stallions	Mares	p-value	2-5 years	6-15 years	p-value	BCS < 7	BCS ≥ 7	p-value
n	78	49		68	59		94	33	
SFT-N25%	7.55 ± 2.48	5.64 ± 3.27	<0.001*	6.23 ± 3.04	6.96 ± 2.82	0.047	6.39 ± 2.14	6.79 ± 3.61	0.283*
SFT-N50%	12.00 ± 4.17	10.19 ± 5.41	0.003*	10.43 ± 4.96	11.76 ± 4.62	0.030	10.97 ± 3.49	11.22 ± 5.86	0.691
SFT-N75%	10.24 ± 4.40	9.98 ± 5.75	0.682	10.01 ± 5.30	10.20 ± 4.96	0.765	9.49 ± 3.72	10.72 ± 6.20	0.058
SFT-S	8.00 ± 3.83	6.30 ± 5.07	0.003*	6.26 ± 4.62	8.04 ± 4.40	0.002*	6.58 ± 3.27	7.73 ± 5.52	0.047*
SFT-R12-13	8.16 ± 3.49	7.03 ± 4.51	0.027	7.68 ± 4.17	7.51 ± 3.94	0.737	6.95 ± 2.93	8.24 ± 4.96	0.013
SFT-R	14.79 ± 6.54	14.81 ± 8.56	0.988	14.33 ± 7.89	15.27 ± 7.44	0.327	12.54 ± 5.52	17.06 ± 9.35	<0.001
SFT-TH	24.06 ± 9.69	27.16 ± 12.73	0.030	23.85 ± 11.72	27.37 ± 10.93	0.014	21.27 ± 8.11	29.95 ± 13.86	<0.001

477 * Power value greater than 80%

23

478 **Table 4**

479 Correlation coefficients of body condition score (BCS) with the subcutaneous fat thickness

480 (STF) measurements (mm), in the global sample and stratified by gender and age groups.

481

| Variable | Global | Gender | | Age | |
		Stallions	Mares	2-5 years	6-15 years
n	127	78	49	68	59
SFT-N25%	0.149	0.201	-0.036	-0.011	0.268*
SFT-N50%	0.153	0.171	0.069	0.074	0.224
SFT-N75%	0.210*	0.126	0.303*	0.259*	0.266*
SFT-S	0.196*	0.100	0.309*	0.056	0.377**
SFT-R12-13	0.335**	0.284*	0.359*	0.257*	0.456**
SFT-R	0.428**	0.542**	0.298*	0.413**	0.445**
SFT-TH	0.627**	0.654**	0.641**	0.606**	0.734**

482

483 Significant values of correlation coefficients * $p < 0.050$; ** $p < 0.010$

484 In all cases values correspond with Spearman's coefficient (rho) except in case of SFT-R12-

485 13 for ages 2-5 and 6-15 years where Pearson's coefficient (r) was calculated.
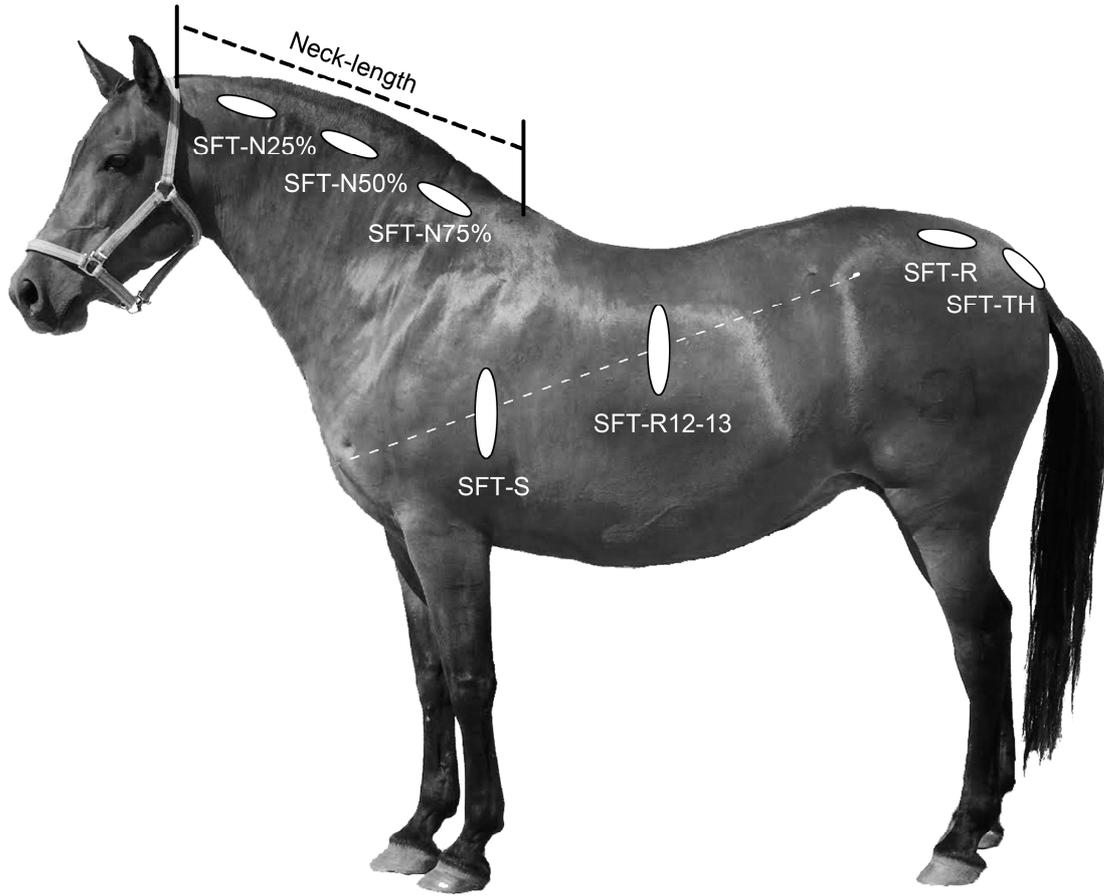
1. This study expands our knowledge about the distribution of regional fat deposition

2. The influence of gender and age on BCS and SFT measurements was observed

3. Ultrasonography may avoid overestimation of obesity in easy keeper-like horses

4. SFT at the rump was the only site related to BCS, independent of gender and age

5. SFT ultrasnonography is a suitable technique to objetify body condition evaluation

.