



UNIVERSITY OF HELSINKI



<https://helda.helsinki.fi>

Helda

Insulin dysregulation in a population of Finnhorses and associated phenotypic markers of obesity

Box, Justin Ryan

2019

Box, J R, Raekallio, M, Mykkänen, A & Karikoski, N 2019, 'Insulin dysregulation in a population of Finnhorses and associated phenotypic markers of obesity', Eläinlääkäripäivät 2019, Helsinki, Finland, 11/12/2019 - 13/12/2019.

<http://hdl.handle.net/10138/316725>

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

Insulin dysregulation in a population of Finnhorses and associated phenotypic markers of obesity

Justin R. Box¹, Cathy M. McGowan², Marja R. Raekallio¹, Anna K. Mykkänen¹, Harry Carslake²,
Ninja P. Karikoski¹

¹Department of Equine and Small Animal Sciences, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland

²Institute of Veterinary Science, University of Liverpool, Neston, United Kingdom

Keywords: OST, equine, laminitis, EMS

Abbreviations:

AUC, area under the curve; BCS, body condition score; CNS, cresty neck score; EMS, equine metabolic syndrome; ID, insulin dysregulation; IS, insulin sensitivity; OST, oral sugar test

Correspondence:

Justin R. Box, University of Helsinki

PL 57

00014 University of Helsinki

Email: justin.box@helsinki.fi

Acknowledgements:

The authors would like to thank Heidi Tanskanen for her help with sample collection. Partial salary funding was paid with an EDUFI Fellowship.

Conflicts of Interest:

Authors declare no conflict of interest.

Off-label Antimicrobial Use:

Authors declare no off-label use of antimicrobials.

Institutional Animal Care and Use Committee or Other Approval:

The study protocol was approved by the National Animal Experimentation Board of Finland (ESAVI/6728/04.10.07/2017).

**Insulin dysregulation in a population of Finnhorses and associated phenotypic markers of
obesity**

Abstract

Background: Obesity and insulin dysregulation (ID) predispose horses to laminitis.

Determination of management practices or phenotypic markers associated with ID may benefit animal welfare.

Objectives: Determine ID status of a population of Finnhorses using an oral sugar test (OST) and compare phenotypes and management factors between ID and non-ID Finnhorses.

Animals: One-hundred twenty-eight purebred Finnhorses ≥ 3 years of age.

Methods: Owners were recruited using an online questionnaire regarding signalment, history, feeding and exercise of their horses. Selected contributing stables within the pre-defined area (150 km from the institution) were visited. Phenotypic markers of obesity and the weight of each horse were recorded. After an overnight fast, horses received 0.45 ml/kg corn syrup PO. Serum samples before and at 60 and 90 minutes after syrup administration were analyzed for insulin by chemiluminescent assay. Horses met ID criteria if insulin concentrations were ≥ 33 $\mu\text{IU/ml}$ at T0, ≥ 66 $\mu\text{IU/ml}$ at T60 or T90 or some combination of these. Associations between phenotypic markers, feeding and exercise variables and ID were examined using mixed effects logistic regression modeling.

Results: Several phenotypic markers of obesity were significant on univariable analysis but in the final multivariable model, only obesity (body condition score [BCS] ≥ 8) was associated with ID ($p = .043$). Over half of the horses (60% [95% confidence interval (CI), 51-68%]) were considered overweight or obese whereas 16 % (95% CI, 10-23%) were classified as having ID.

Conclusions and Clinical Importance: Because obesity is associated with ID in cold-blooded type horses, objective monitoring of phenotypic markers by owners may be beneficial for health outcomes.

1 **Introduction**

2 Obesity is a major risk factor for insulin dysregulation (ID) and a substantial health problem among
3 horse populations worldwide^{1,2,3}. Insulin dysregulation is defined as any combination of basal
4 hyperinsulinemia, post-prandial hyperinsulinemia (in response to dynamic testing), or insulin
5 resistance⁴, and is an important predisposing factor for laminitis, a painful hoof condition in horses
6 that can lead to loss of use, chronic lameness, and even death⁵. Insulin dysregulation and
7 generalized or regional adiposity are features of equine metabolic syndrome (EMS) and can be
8 used as predictors of laminitis^{6,7}. Although not all obese animals have ID (and vice versa),
9 dynamic endocrine testing and appropriate weight management of overweight animals are
10 recommended to decrease the possible risk for laminitis^{6,8}.

11 Dynamic testing is the preferred method of determining ID in horses and is more sensitive than
12 basal testing alone⁶. The oral sugar test (OST) has been described in several studies^{9,10,11} as an
13 ideal, replicable method for dynamic ID testing at different doses (0.15-0.45 ml/kg). It has been
14 shown to be repeatable using binary outcomes¹⁰ and is comparable to more invasive tests⁹;
15 therefore, it is a practical approach for on-farm testing.

16 Prevalence of ID in horses has been shown to vary from 18-27% depending on the specified
17 population^{2,12,13}. Breed differences in ID have been identified. For example, ponies and Andalusian
18 horses had significantly lower insulin sensitivity (IS) than did Standardbred horses¹⁴. Additionally,
19 many of the published cases of EMS have occurred in native British breeds. One study found that
20 cases of primary endocrinopathic laminitis (induced by ID with or without pituitary pars

21 intermedia dysfunction) were more likely to occur in native British ponies compared to native
22 Nordic ponies, cold-, warm-, and hot-blooded horses¹⁵. However, in another study, cold-blooded
23 type ponies had increased risk of laminitis compared to warm-blooded type ponies¹⁶. The ID status
24 of certain cold-blooded horse populations, such as Finnhorses, has not been investigated.

25 The Finnhorse is a cold-blooded horse originating from Northern European domestic horses. They
26 are the only horse breed native to Finland and have been bred as a pure breed since 1907, when
27 the studbook was founded. The registry recognizes 4 types of Finnhorse: racing trotter, riding and
28 pleasure, working, and pony-sized (www.hippos.fi).

29 Factors associated with increased obesity risk include management and exercise, primarily
30 resulting from decreased physical activity and excess energy intake, although genetic and
31 epigenetic factors also may play a role^{17,18}. In humans, physical activity has been shown to improve
32 IS, even in the absence of apparent weight loss¹⁹. In studies of horses, exercise has been shown to
33 decrease serum concentrations of inflammatory markers (serum amyloid A and haptoglobin)²⁰ and
34 improve ID, particularly when exercise was of moderate intensity^{21,22}.

35 In this study, we evaluated the ID status of a population of Finnhorses in southern Finland by
36 determining insulin response to corn syrup OST. Our aim was to compare phenotypic markers of
37 obesity and management factors between ID and non-ID Finnhorses.

38 **Materials and methods**

39 *Animals*

40 The study protocol was approved by the National Animal Experimentation Board of Finland
41 (ESAVI/6728/04.10.07/2017). Horses met study inclusion criteria if they were located within 150
42 km of Helsinki, were ≥ 3 years old, and had no clinical evidence or history of systemic
43 inflammatory disease. A physical examination was performed on all horses by a veterinarian and

44 any animals with fever (≥ 38.5 C), tachycardia, tachypnea, signs of systemic inflammatory disease,
45 or any other potentially painful condition were excluded. An initial serum biochemistry profile and
46 a CBC were performed on each horse to evaluate health status. Biochemical results were
47 determined using a commercial biochemistry analyzer (Konelab 30 Clinical Chemistry Analyzer,
48 ThermoFisher Scientific, Vantaa, Finland). The CBC was analyzed with an ADVIA 212io
49 hematology analyzer (Siemens, Tarrytown, NY), and plasma fibrinogen concentration was
50 determined using a heat precipitation method²³. Any animal with abnormal biochemical or CBC
51 findings was excluded. Animals with previously diagnosed pituitary pars intermedia dysfunction
52 (PPID) also were excluded.

53 *Questionnaire*

54 A link to a web-based questionnaire was advertised from September to December in 2017 in the
55 University of Helsinki Faculty of Veterinary Medicine webpages seeking study enrollment by
56 owners of purebred Finnhorses ≥ 3 years of age living within approximately 150 km of Helsinki.
57 The Veterinary Medicine webpages provided owner resources and information about the hospital
58 that were regularly accessed by horse owners. The questionnaire requested information about the
59 signalment, history, feeding, exercise and previous and current diseases of each horse.
60 Additionally, owners were asked to estimate their horse's body condition score (BCS, Henneke 1-
61 9 scoring system)²⁴ and cresty neck score (CNS, Carter 0-5 scoring system)²⁵ with the help of
62 illustrative figures. With regard to exercise, owners were asked to report their horse's main use
63 (racing, draft, riding competition, pleasure riding, pet, breeding), estimate how many days per
64 week on average they exercised their horse, how many days per week the horse was sweating
65 during exercise, and how many hours per week the horse was exercised at walk, trot, and canter.
66 Horses were grouped into either intense use (racing, draft, competition) or non-intense use

67 (pleasure riding, pet, breeding). The hours per week spent trotting and cantering (trot + canter)
68 were added together as a single analysis value. Cumulative exercise was calculated by adding
69 walk, trot, and canter hours per week. Finally, the owners were asked to report the amount of
70 roughage (kg) and concentrate (kg) their horse received each day. Concentrate was defined as any
71 feed (commercially prepared or otherwise) given to the horse that was not a vitamin or mineral
72 supplement or both or type of roughage. If owners reported a range, the upper limit value was used
73 for analysis.

74 *Sample size*

75 A convenience sample of recruited horses that were ≥ 3 years old were selected for a stable visit
76 based on their geographical location (within 150 km of the institution). Before the start of testing,
77 sample size was calculated using the online Epitools sample size calculator. Given the population
78 size of approximately 20,000 Finnhorses, an expected incidence of 15-20%, a confidence interval
79 (CI) of 95%, and a power of 80%, the calculated sample size was 150. Operations housing ≥ 5
80 Finnhorses initially were selected but premises with < 5 horses later were included. All available
81 horses at each stable that met inclusion criteria had OST performed.

82 *Physical measurements*

83 One of 2 trained veterinarians (JRB NPK) performed the physical measurements, including
84 phenotypic markers of obesity and hoof wall changes. Phenotypic markers of obesity included
85 BCS, CNS, and supraorbital fat pads. Assessment of macroscopic hoof wall changes that were
86 indicative of laminitis included divergent growth rings, white line separation, and dropped soles.
87 The following physical measurements were obtained using a weight tape designed for horses
88 (Virbac Animal Health): weight, heart-girth, widest part of the abdomen, and neck circumference
89 (midpoint of the neck). Additionally, BCS and CNS were assessed^{24,25}. Horses with BCS of 7 were

90 considered over-conditioned³ and classified as obese if their BCS was ≥ 8 . Horses were considered
91 to have a cresty neck if CNS was ≥ 3 . Supraorbital fat pads were graded on a scale of 0-3; 0 being
92 deep/concave and 3 being rounded/convex.

93 *Basal blood samples*

94 Basal blood glucose concentrations were determined using lithium-heparin blood (Vacuette LH,
95 Greiner Bio-One, Kremsmünster, Austria) immediately after sampling using a handheld veterinary
96 glucometer (AlphaTRAK® II, Zoetis, North Chicago, IL).

97 Blood for adrenocorticotrophic hormone (ACTH) concentration measurement was collected in 6-
98 mL EDTA tubes (Vacuette K2EDTA, Greiner Bio-One, Kremsmünster, Austria) and kept cool
99 until centrifugation (within 8 hr). The separated plasma was frozen and stored at -80 °C until
100 shipment on dry ice to the diagnostic laboratory. Analyses were performed in duplicate using a
101 chemiluminescent immunoassay (Immulite 2000 XPi, The Philip Leverhulme Equine Hospital,
102 Liverpool, UK). All animals with seasonally increased basal plasma ACTH concentrations were
103 excluded from the study. The seasonally adjusted ACTH cut-off concentrations used for the study
104 were 89.4 pg/ml for horses sampled in October and 35.2 pg/ml for horses sampled in November
105 and December (Adams A., Abstract, International Equine Endocrinology Summit, 2017).

106 *Oral sugar test*

107 Oral sugar tests were performed either at the stables (n=139) or in the University of Helsinki (n=5)
108 during a period from the last week of October 2017 through the second week of December 2017.
109 The evening before the test, the horses were stalled and allowed to have a slice of dry hay or
110 haylage (1-2 kg) no later than 22:00. No grain or additional hay was allowed until after the OST
111 was completed. All horses had access to water throughout the entire experiment. The OSTs were
112 performed in the morning between 06:00 and 10:00. Horses were given 0.45 ml/kg body weight

113 (BW) corn syrup PO (Karo Light, ACH Food Companies Inc, Cordova, TN) via 100 ml dosing
114 syringes. Karo Light contains, on average, 158 mg/ml of maltose and 198 mg/ml glucose, so that
115 horses received a combined maltose and glucose dose of 160.3 mg/kg BW¹¹. For insulin
116 concentration measurement, blood was collected into 6 mL serum tubes (Vacuette, Z serum clot
117 activator, Greiner Bio-One, Kremsmünster, Austria) before syrup administration and at 60 (T60)
118 and 90 (T90) minutes thereafter. Blood was allowed to clot at ambient temperature for at least 60
119 minutes. Subsequently, all samples were centrifuged, serum separated within 8 hr, and stored at -
120 80 °C until shipment on dry ice to the laboratory for analysis. All samples were measured in
121 duplicate using a chemiluminescent immunoassay (Immulite 2000 XPi, The Philip Leverhulme
122 Equine Hospital, Liverpool, UK). The reportable range for the Immulite 2000XPi was 2-300
123 μ IU/ml. Our preliminary correlation studies have shown excellent correlation ($r=.996$) between
124 the Immulite 2000XPi and the more commonly used Immulite 2000 (Carslake, H., unpublished
125 data), but the 2000XPi reports consistently higher values than the 2000. Therefore, a higher cutoff
126 concentration of 66 μ IU/ml was used for this study instead of the suggested 40 μ IU/ml. Horses
127 were categorized as having ID based on insulin concentrations ≥ 33 μ IU/ml at T0 or ≥ 66 μ IU/ml
128 at either T60 or T90 or both.

129 **Statistical analysis**

130 The area under the curve (AUC) was calculated for the insulin response (T0-T90) using the
131 trapezoidal method. The normality of each variable distribution was tested using the Shapiro-Wilk
132 test. Correlations among BCS, glucose, and insulin were tested using Spearman rank correlation
133 with Bonferroni correction. Comparison of owner versus investigator BCS and CNS was
134 performed using related samples Friedman's 2-way analysis of variance by ranks.

135 To assess the effect of different covariates on ID, mixed effects logistic regression models,
136 modelling the odds for occurrence of ID, were fitted. First, each covariate was separately modelled
137 with the response, the model including ID status as a response, covariate as a fixed effect and
138 cluster (stable) as a random effect (univariable analysis). Variables with p-value < .2 were taken
139 forward to multivariable analyses. Similar mixed effects logistic regression models as for the
140 individual analyses were fitted. In all models, odds ratios (OR) for comparisons between groups
141 for categorical covariates or increase of 1 unit in continuous or ordinal covariates with 95% CI and
142 p-values were estimated using contrasts from the same model. P-values < .05 were considered
143 statistically significant. Statistical analyses were performed at 4Pharma Ltd using SAS® System
144 for Windows, version 9.4 (SAS Institute Inc., Cary, NC, USA).

145 **Results**

146 Two-hundred and thirty-three owners completed the online questionnaire, representing 291 horses.
147 One-hundred forty-four horses from 30 premises were selected for detailed examination and
148 testing. Of the 144 horses sampled, 1 horse was excluded because of increased body temperature
149 and 15 were excluded because of seasonally increased basal plasma ACTH concentrations. The
150 remaining 128 horses consisted of 63 geldings (49%), 58 mares (45%), and 7 stallions (5%).
151 Owners categorized the use of their horses in the following manner: 106 as “pleasure riding”
152 horses, 7 as “riding competition” horses, 2 as “breeding” horses, 10 as “racehorses”, 2 as a “pet”,
153 and 1 as a “draft horse”. Therefore, 18 horses were considered as experiencing intense use and 110
154 non-intense use.

155 The phenotypic marker results of the 128 horses are presented in Table 1. There were 77/128 (60%;
156 95% CI, 51-68%) over-conditioned or obese horses, of which 35/128 (27%; 95% CI, 20-36%)
157 were over-conditioned and 42/128 (33%; 95% CI, 25-41%) were obese. The BCS given by the

158 owners were significantly lower than the BCS given by the investigator ($p < .001$). The CNS given
159 by the owners, however, were significantly higher than those given by the investigator ($p < .001$).
160 Forty-two horses (33%; 95% CI, 25-41%) were considered to have a cresty neck. Seventy-three
161 (57%; 95% CI, 48-65%) horses had visible growth rings on ≥ 1 hooves, but none of the horses had
162 divergent hoof rings, white line separation, or dropped soles indicative of a history of laminitis.
163 Six horses had a history of laminitis diagnosed by a veterinarian and 2 additional horses had
164 historical owner-suspected but not veterinarian-confirmed laminitis.

165 *Oral Sugar Test*

166 No adverse events were noticed by the investigators during the OST or reported by the owners
167 after the study. All but 1 of the horses readily accepted the PO dosing of the syrup. The 1 horse
168 that refused the dosing syringe consumed all of the syrup (within 1-2 minutes) after the entire
169 volume was ejected into the horse's empty food bucket. In total, 20/128 (16 %; 95% CI, 10-23%)
170 horses met the criteria for ID (Table 2). Of these, only 1 animal had increased insulin concentration
171 at T0, 9 at T60 and 19 at T90. Eight horses had increased insulin concentrations at both T60 and
172 T90. Seven of the 8 horses with owner-reported history of laminitis were categorized in the ID
173 group.

174 Body condition scores correlated significantly with insulin T0 ($\rho = .253$, $p=.036$), T60 ($\rho = .309$,
175 $p < .01$), T90 ($\rho=.270$, $p= .018$), and AUC ($\rho = .305$, $p < .01$). Basal glucose concentration
176 correlated significantly with insulin T60 ($\rho = .267$, $p=.027$) and AUC ($\rho = .261$, $p = .027$). No
177 other significant correlations were detected among BCS, glucose, and insulin.

178 Of the 42 obese horses, only 11 (26%; 95% CI, 15-41%) met ID criteria, and of the 35 over-
179 conditioned horses, only 4 (11%; 95% CI, 5-26%) met ID criteria. Five (4%; 95% CI, 2-9%) non-
180 over-conditioned or obese horses were categorized as having ID.

181 Several variables were found to be significant in univariable analysis (Table 3) and these were
182 moved forward into a multivariable model. However, because several significant obesity-related
183 variables were found in univariable analysis that correlated with each other, the variables that were
184 not affected by the horse's height (BCS, CNS and obesity vs. heart-girth, weight, widest part of
185 abdomen) were selected for multivariable analysis. Each of the obesity variables was analyzed
186 separately in a multivariate model with the other 4 (age, sex, glucose, combined trot and canter
187 hours/week) variables (3 separate models). Finally, only obesity ($BCS \geq 8$) was shown to be
188 associated with ID in multivariable models (OR, 3.29; 95% CI, 1.04-10.37; $p=.043$).

189 **Discussion**

190 In this population of Finnhorses in southern Finland tested between October and December in
191 2017, obesity was the only variable associated with ID in multivariable analysis. The risk for ID
192 was 3.29 times higher in horses with $BCS \geq 8$ than in horses with lower BCS. In addition, several
193 phenotypic markers related to obesity (BCS, CNS, BW, heart-girth, widest part of the abdomen)
194 were found to be significant on univariable analysis. However, variables associated with feeding
195 or exercise were not significant risk factors in this Finnhorse population. Small sample size could
196 be a reason for the lack of association with these variables, with the majority of horses being over
197 conditioned or obese and not undergoing intense exercise.

198 Not all obese or over-conditioned horses had ID. In fact, most of the obese or over-conditioned
199 horses (81%) did not have ID. Supraorbital fat pad and neck circumference were not significant
200 risk factors in univariable analysis. In 2 previous studies, ponies with ID (basal hyperinsulinemia)
201 did not have significantly higher BCS or CNS than did ponies with normal insulin concentrations,
202 and therefore the authors suggested that assessment of physical obesity parameters might not be
203 an accurate predictor for ID in native pony breeds^{8,12}. However, the majority of ponies in these

204 studies were over-conditioned or obese, which may have affected the results. In another more
205 heterogenous group of ponies, CNS was positively associated with postprandial insulin
206 concentration (oral glucose test), and ponies with a cresty neck had 5 times higher risk of having
207 ID than did ponies with a normal neck⁷. In addition, in a mixed horse population in the US, over-
208 conditioned and obese horses had significantly higher basal plasma insulin concentrations
209 (indicative of ID) compared to optimally conditioned horses¹³. Additionally, that study found
210 breed differences in IS and insulin concentrations. Therefore, the association between ID and
211 obesity indeed may be breed-related, and this possibility should be taken into consideration when
212 evaluating the status of and risks for ID in an individual horse.

213 Exercise was not shown to be a protective factor for ID in our study. However, none of the animals
214 registered as trotting racehorses (n=10) met the criteria of ID or had a history of laminitis, nor were
215 any of them obese. Previously published studies indicate that moderate-intensity, short- (45
216 minutes per day for 7 days)²¹ and long-term (60 minutes per day for 1 month)²⁷ training have been
217 shown to improve ID in horses. Additionally, a recent study showed that ID was significantly
218 improved by diet modification and low-intensity exercise when compared with diet modification
219 alone²⁸. The study also found that low-intensity exercise without diet change was insufficient to
220 improve IS despite decreases in total body fat mass. Another study demonstrated that even long-
221 term low-intensity exercise, such as walking 2 hours twice daily for 3 months, did not improve IS
222 although the animals lost weight during the research period²⁹. Therefore, light exercise alone, even
223 if done regularly several hours per week, may not be sufficient to protect horses from ID. Accuracy
224 in owner reporting may have been a factor in the non-significance of exercise data in our study.
225 The questionnaire was designed to be as straightforward as possible, but some owners' perceptions

226 regarding health, nutrition and exercise intensity may not have been realistic, as has been shown
227 in some previous studies.^{30,31}

228 Owners underestimated their horses' BCS by 1 grade compared to the investigators, in agreement
229 with previous study reports,^{2,32,33} which is relevant when assessing weight management of horses.
230 Because body weight, heart-girth and widest part of the abdomen were taken with a standard,
231 commercially available weight and measuring tape designed for horses, owners can record and
232 track measurements of their horses without the need of a veterinarian or expensive equipment. Use
233 of this tool allows owners of horses with ID to identify horses at risk, or monitor treatment success,
234 such as diet changes. Weight tapes have been shown to overestimate weight of horses^{34,35} and
235 therefore, the animals in our study may have been marginally lighter, on average, than what is
236 presented in Table 1. However, change in BW is what often dictates owner management, not actual
237 weight. Therefore, especially where weighbridge scales are unavailable, use of a weight tape, as
238 employed in our study, represents a practical monitoring tool compatible with typical field
239 conditions.

240 The frequency of ID in this population of Finnhorses was 16%, which is close to previously
241 published reports of 18-27 % in other breeds^{12,13}. Despite phenotypic markers of obesity being
242 significantly higher in the ID group, the frequency of ID was low compared to the percentage of
243 over-conditioned or obese Finnhorses (60%). This observation supports previous findings of a
244 lower breed representation of laminitis in this breed¹⁴. Arbitrarily decreasing the cutoff to 50
245 μ IU/ml increased the number of horses with ID to 24 (19%; 95% CI, 13-26%). This possibly could
246 be a more sensitive cutoff for samples analyzed using the Immulite 2000XPi.

247 Fasting or resting blood glucose measurement is not a useful diagnostic test to determine ID.
248 Instead, it should be used as part of a comprehensive diagnostic plan⁶. Although fasting blood

249 glucose concentration was a risk factor for ID in univariable analysis, the concentrations of all
250 horses were within the normal reference range.

251 We used a higher corn syrup dose (0.45 ml/kg), which has been suggested to have higher sensitivity
252 for ID than the previously used lower doses¹¹. However, the data originates from a study in which
253 the purpose was to differentiate previously laminitic and non-laminitic ponies from each other, not
254 to find ID animals in a random population¹¹. Therefore, this higher dose may be suboptimal, and
255 more research is warranted in this area.

256 **Conclusions**

257 In this sample population of Finnhorses, obesity was shown to be associated with ID. Several
258 phenotypic indicators of obesity were found to be significantly higher in horses with ID in
259 univariable analysis, suggesting that generalized obesity is associated with ID in cold-blooded type
260 horses. The frequency of ID in this population when tested with .45 ml/kg OST was 16 %. Because
261 owners were found to underestimate the BCS of their horses, they should be encouraged to
262 regularly measure and record BCS and weight estimates to track changes over time.

263 **References**

- 264 1. Frank N. Equine Metabolic Syndrome. *Veterinary Clinics of North America: Equine Practice*.
265 2011;27(1):73-92.
- 266 2. Wyse CA, McNie KA, Tannahil VJ, et al. Prevalence of obesity in riding horses in Scotland. *Vet*
267 *Rec*. 2008;162(18):590-1.
- 268 3. Thatcher CD, Pleasant RS, Geor RJ, et al. Prevalence of Overconditioning in Mature Horses in
269 Southwest Virginia during the Summer. *J Vet Intern Med*. 2012;26(6):1413-8.
- 270 4. Frank N, Tadros EM. Insulin dysregulation. *Equine Vet J*. 2014;46(1):103-12.

- 271 5. Asplin KE, Sillence MN, Pollitt CC, et al. Induction of laminitis by prolonged hyperinsulinaemia
272 in clinically normal ponies. *The Veterinary Journal*. 2007;174(3):530-5.
- 273 6. Durham AE, Frank N, McGowan CM, et al. ECEIM consensus statement on equine metabolic
274 syndrome. *J Vet Intern Med*. 2019;1-15. <https://doi.org/10.1111/jvim.15423>.
- 275 7. Fitzgerald DM, Anderson ST, Sillence MN, et al. The cresty neck score is an independent
276 predictor of insulin dysregulation in ponies. *Plos One* 2019; 14(7):e0220203.
- 277 8. Menzies-Gow N, Harris PA, Elliott J. Prospective cohort study evaluating risk factors for the
278 development of pasture-associated laminitis in the United Kingdom. *Equine Vet J*;49(3):300-6.
- 279 9. Schuver A, Frank N, Chameroy KA, et al. Assessment of Insulin and Glucose Dynamics by
280 Using an Oral Sugar Test in Horses. *Journal of Equine Veterinary Science*. 2014;34(4):465-70.
- 281 10. Knowles EJ, Harris PA, Elliott J, et al. Use of the oral sugar test in ponies when performed with
282 or without prior fasting. *Equine Vet J*. 2016;49(4):519-24.
- 283 11. Jocelyn NA, Harris PA, Menzies-Gow N. Effect of varying the dose of corn syrup on the insulin
284 and glucose response to the oral sugar test. *Equine Vet J*. 2018;50(6):836-41.
- 285 12. Morgan RA, McGowan TW, McGowan CM. Prevalence and risk factors for hyperinsulinaemia in
286 ponies in Queensland, Australia. *Aust Vet J*. 2014;92(4):101-6.
- 287 13. Pleasant RS, Suagee JK, Thatcher CD, et al. Adiposity, Plasma Insulin, Leptin, Lipids, and
288 Oxidative Stress in Mature Light Breed Horses. *J Vet Intern Med*. 2013; 2018/11;27(3):576-82.
- 289 14. Bamford NJ, Potter SJ, Harris PA, et al. Breed differences in insulin sensitivity and insulinemic
290 responses to oral glucose in horses and ponies of moderate body condition score. *Domestic
291 Animal Endocrinology*. 2014;47:101-7.
- 292 15. Karikoski NP, Horn I, McGowan TW, et al. The prevalence of endocrinopathic laminitis among
293 horses presented for laminitis at a first-opinion/referral equine hospital. *Domestic Animal
294 Endocrinology*. 2011;41(3):111-7.
- 295 16. Luthersson N, Mannfalk M, Parkin TDH, et al. Laminitis: Risk Factors and Outcome in a Group
296 of Danish Horses. *J Equine Vet Sci*. 2017;53:68-73.

- 297 17. Lewis SL, Holl HM, Streeter C, et al. Genomewide association study reveals a risk locus for
298 equine metabolic syndrome in the Arabian horse. *Journal of Animal Science*. 2017;95(3):1071-
299 1079.
- 300 18. McCue ME, Geor RJ, Schultz N. Equine Metabolic Syndrome: A Complex Disease Influenced by
301 Genetics and the Environment. *Journal of Equine Veterinary Science*. 2015;35(5):367-75.
- 302 19. Bird SR, Hawley JA. Update on the effects of physical activity on insulin sensitivity in humans.
303 *BMJ Open Sport Exerc Med*. 2017;2(1):e000143. doi:10.1136/bmjsem-2016-000143
- 304 20. Menzies-Gow N, Wray H, Bailey SR, et al. The effect of exercise on plasma concentrations of
305 inflammatory markers in normal and previously laminitic ponies. *Equine Vet J*. 2014;46(3):317-
306 21.
- 307 21. Stewart-Hunt L, Geor RJ, McCutcheon LJ. Effects of short-term training on insulin sensitivity
308 and skeletal muscle glucose metabolism in Standardbred horses. *Equine Vet J*. 2006;38:226-32.
- 309 22. McCutcheon LJ, Geor RJ, Hinchcliff KW. Changes in skeletal muscle GLUT4 content and
310 muscle membrane glucose transport following 6 weeks of exercise training. *Equine Vet J*.
311 2002;34:199-204.
- 312 23. Millar HR. An evaluation of the heat precipitation method for plasma fibrinogen estimation. *J*
313 *Clin Pathol*. 1971;24(9):827.
- 314 24. Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical
315 measurements and body fat percentage in mares. *Equine Vet J*. 1983;15(4):371-2.
- 316 25. Carter RA, Geor RJ, Burton Staniar W, et al. Apparent adiposity assessed by standardised scoring
317 systems and morphometric measurements in horses and ponies. *The Veterinary Journal*.
318 2009;179(2):204-10.
- 319 26. Sergeant, ESG, 2019. Epitools epidemiological calculators. Ausvet Pty Ltd. Available at:
320 <http://epitools.ausvet.com.au>.
- 321 27. Bonelli F, Sgorbini M, Meucci V, et al. How swimming affects plasma insulin and glucose
322 concentration in Thoroughbreds: A pilot study. *The Veterinary Journal*. 2017;226:1-3.

- 323 28. Bamford NJ, Potter SJ, Baskerville CL, et al. Influence of dietary restriction and low-intensity
324 exercise on weight loss and insulin sensitivity in obese equids. *J Vet Intern Med.*
325 2019;01;33(1):280-6.
- 326 29. de Laat MA, Hampson BA, Sillence MN, et al. Sustained, Low-Intensity Exercise Achieved by a
327 Dynamic Feeding System Decreases Body Fat in Ponies. *J Vet Intern Med.* 2016;30(5):1732-8.
- 328 30. Ireland JL, Clegg PD, McGowan CM, et al. Comparison of owner-reported health problems with
329 veterinary assessment of geriatric horses in the United Kingdom. *Equine Vet J.* 2012;44(1):94-
330 100.
- 331 31. Murray JMD, Bloxham C, Kulifay J, et al. Equine Nutrition: A Survey of Perceptions and
332 Practices of Horse Owners Undertaking a Massive Open Online Course in Equine Nutrition.
333 *Journal of Equine Veterinary Science.* 2015;35(6):510-7.
- 334 32. Jensen RB, Danielsen SH, Tauson A. Body condition score, morphometric measurements and
335 estimation of body weight in mature Icelandic horses in Denmark. *Acta Vet Scand.*
336 2016;58(1):59.
- 337 33. Stephenson HM, Green MJ, Freeman SL. Prevalence of obesity in a population of horses in the
338 UK. *Vet Rec.* 2011;168(5):131-.
- 339 34. Wagner EL, Tyler PJ. A Comparison of Weight Estimation Methods in Adult Horses. *Journal of*
340 *Equine Veterinary Science.* 2011 December 2011;31(12):706-10.
- 341 35. Hoffmann G, Bentke A, Rose-Meierhöfer S, et al. Estimation of the Body Weight of Icelandic
342 Horses. *Journal of Equine Veterinary Science.* 2013;33(11):893-5.