

Relationships between total adiponectin concentrations and obesity in native-breed ponies in England

Marine A. Barnabé¹  | Jonathan Elliott²  | Patricia A. Harris³  | Nicola J. Menzies-Gow¹ 

¹Department of Clinical Sciences and Services, Royal Veterinary College, Hertfordshire, UK

²Department of Comparative Biomedical Sciences, Royal Veterinary College, Hertfordshire, UK

³Equine Studies Group, Waltham Petcare Science Institute, Leics, UK

Correspondence

Marine A. Barnabé, Department of Clinical Sciences and Services, Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, AL9 7TA, UK.
Email: mbarnabe@rvc.ac.uk

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Abstract

Background: Equine metabolic syndrome is a collection of risk factors associated with an increased risk of endocrinopathic laminitis. All affected animals display insulin dysregulation and some may show adiponectin dysregulation and/or excessive adiposity. However, the relationship between obesity and hypoadiponectinaemia in equids remains unclear.

Objectives: To investigate the relationship between obesity and circulating plasma total adiponectin (TA) concentrations in native-breed ponies in England.

Study design: Secondary data analysis.

Methods: Data collected for three previous studies were retrospectively analysed and cohorts were pooled where possible (maximum sample size: $n = 734$ ponies). Correlations between [TA], age, and morphometric measures were assessed using Spearman's correlation coefficient. [TA] was compared between animals of different body condition score (BCS) classification (ideal-weight, overweight, and obese), breed, and body shape using Kruskal–Wallis with Dunn's post hoc tests, and sex using Mann–Whitney U test. The proportions of obese and ideal-weight ponies with basal hyperinsulinaemia and/or hypoadiponectinaemia were compared using a Chi-square test of homogeneity and post hoc z -test. Logistic regression was used to identify factors that may discriminate ponies with hypoadiponectinaemia.

Results: [TA] was weakly positively correlated with BCS, height, weight, and weight:height ratio (Spearman's $\rho = 0.14$ – 0.29 , $p < 0.05$). There were significant differences in [TA] in ponies with different BCS group classification, body shape, and breed. A greater percentage of obese (54.6%) than ideal-weight ponies (33.1%, $p < 0.001$) had both normal [TA] and [basal insulin], and a greater percentage of ideal-weight (38.6%) than obese ponies (16.5%, $p < 0.001$) showed hypoadiponectinaemia. Weight:height and BCS group were significant variables in a logistic regression of hypoadiponectinaemia but model fit and predictive accuracy were poor.

Main limitations: Retrospective study design, only native-breed ponies included.

Conclusions: Morphometric measures such as BCS do not closely reflect [TA]. Circulating [TA] and [basal insulin] should be determined in all animals with predisposing factors, regardless of obesity status.

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KEYWORDS

adiponectin, body condition score, equine metabolic syndrome, horse, insulin dysregulation, laminitis, pony

1 | INTRODUCTION

Basal hyperinsulinaemia and hypoadiponectinaemia are independent risk factors for the development of endocrinopathic laminitis.^{1,2} Previously laminitic ponies have lower adiponectin concentrations than their never-laminitic counterparts³ and the presence of hypoadiponectinaemia prior to the first development of laminitis suggests that low adiponectin concentrations are not simply a consequence of laminitis.¹ Although adiponectin and insulin concentrations are currently measured in blood, morphometric measurements are a valuable non-invasive alternative to biological sample collection. These measures may allow equine professionals and horse-owners to assess animal health and identify animals at high risk of developing endocrinopathic laminitis, provided these measures are reliable indicators of metabolic status. It is therefore important to investigate and determine the relationships between measures of body size or shape and metabolic parameters, particularly in the context of metabolic abnormalities.

Correlations between various morphometric measures and adiponectin concentrations have been reported in horses and ponies.³⁻⁶ These include subjective measures, such as body condition score (BCS) and cresty neck score (CNS), and objective measures, such as weight, heart girth, or neck circumference. BCS, a subjective measure of generalised adiposity used to assess overall condition, is commonly scored out of 9, with a score of 5/9 regarded as the ideal body condition.⁷ In some previous studies, BCS was positively correlated with total bodyweight (bwt), fat mass, and fat percentage,⁸ inversely associated with high-molecular weight (HMW) adiponectin,⁴ and positively associated with basal insulin concentrations.⁴ However, the accuracy of BCS in estimating body fat content is decreased in obese animals compared with that in their lean counterparts, as the relationship between BCS and fat content in animals with BCS >7 is non-linear.^{9,10} In addition, previous studies have shown that horse-owners do not accurately estimate their animals' BCS.¹¹⁻¹³ Therefore, BCS may not be a reliable marker of metabolic health in certain animals.

Alternatively, regional adiposity may be assessed using CNS, a subjective assessment of the height and thickness of the nuchal crest.¹⁴ A high CNS, when complimented with BCS, has previously been associated with an increased risk of metabolic disorders and the development of pasture-associated laminitis¹⁵ and was suggested to be predictive of insulin dysregulation.⁵ CNS has also been associated with low adiponectin concentrations and the authors of that study suggested that circulating adiponectin may also be a measure of insulin dysregulation that is independent of body condition.⁵

In addition to BCS and CNS, other measures such as height,¹⁶ weight:height ratio,⁵ crest height,³ percentage body fat,⁶ fat mass,⁶ and subcutaneous fat thickness¹⁷ have been reportedly correlated with various metabolic parameters including plasma insulin and adiponectin concentrations. However, many of the studies cited were

carried out in relatively small groups of horses and ponies and may have been limited by selection bias and low power. Further studies in large populations are therefore required to fully explore the strength of the associations previously reported.

Although equine obesity is a health issue in its own right,^{18,19} it is understood to be a commonly associated feature rather than a key component of the equine metabolic syndrome.²⁰ However, non-invasive and easy-to-perform measures of body composition, such as BCS, may be used clinically to identify animals with potential metabolic abnormalities. These animals could then be tested to determine circulating insulin and adiponectin concentrations. The aim of this study was therefore to determine the relationships between obesity, morphometric measures, and circulating total adiponectin concentrations in a large population of native-breed ponies in the United Kingdom. Understanding the modifiable factors that are associated with total adiponectin concentrations may help to identify targets for preventive or therapeutic intervention, with the goal of reducing the development of endocrinopathic laminitis in at-risk horses and ponies. A secondary aim was to design a statistical model that could be used to identify at-risk ponies with simple and cost-effective measures available to horse-owners. Considering previously published data, we hypothesised that [total adiponectin] would be consistently negatively associated with measures of obesity (such as BCS, CNS, and weight:height ratio), which may therefore be important factors in a model aiming to identify ponies at risk of developing metabolic abnormalities or endocrinopathic laminitis.

2 | MATERIALS AND METHODS

2.1 | Animals and primary data collection

Data previously collected from three cohorts of ponies for other studies were analysed. Cohort 1 included 446 clinically healthy, client-owned ponies with no known history of laminitis, aged ≥ 7 years, evaluated at a single time-point in August 2010.¹ Cohort 2 included 209 clinically healthy, client-owned ponies evaluated at a single time-point in September or October 2015.² Cohort 3 included 79 native-breed ponies kept either at a sanctuary (Redwings Horse Sanctuary, Norwich, UK) or at the Royal Veterinary College (UK) and evaluated at a single time-point in May 2010. Of these, 38 had a history of laminitis and 41 did not.³ In all cohorts, age, breed, sex, weight, height, and weight:height ratio (W:H) were recorded. Weight was determined using a weighbridge (Equestrian Weigh Platform, Equestrian Products) in cohort 2 and was estimated using a weight tape (Equi Life Ltd) in cohorts 1 and 3. BCS was assessed on a scale of 1-9 according to the method by Henneke et al.⁷ BCS was determined by a single experienced assessor in each cohort (i.e., three assessors in total, all equine veterinarians).

Blood samples were collected from non-fasted animals to determine [basal insulin] and [TA] using commercial assays validated for equine samples. Blood was collected into EDTA-coated tubes for plasma or plain tubes for serum. Plasma tubes were placed on ice until they were returned to the lab for centrifuging to obtain plasma. Blood collected in plain tubes was allowed to clot at room temperature, in a pocket if ambient temperatures were cold, or in a 37°C waterbath (for 30 min) before centrifuging to obtain serum. Serum and plasma samples were stored at –20°C (for up to a week) or –80°C until further analysis. [TA] was determined using a radioimmunoassay (Merck Millipore)^{1,21} and [insulin] was measured using a Coat-a-Count assay (Diagnostic Products Corp) in cohorts 1 and 3, and using a Tosoh AIA360 analyser (Tosoh Bioscience) in cohort 2. The assay used in cohort 2 was extensively validated by the study authors Knowles et al. (2021, 2023) and comparison with the Coat-a-Count assay revealed excellent correlation between the two ($R^2 = 0.97$).^{2,22}

In addition, cresty neck scores (CNS) were recorded in cohort 2 by the same assessor evaluating BCS, on a scale of 0–5 using the method described by Carter et al. (2009)^{2,14} and crest height and thickness were measured in cohorts 1 and 3.

2.2 | Correlations between total adiponectin and continuous variables

[TA], age, and morphometric measurements (BCS, height, weight, and W:H) recorded across all three cohorts were pooled ($n = 734$ ponies). Crest height and thickness recorded in cohorts 1 and 3 were pooled ($n = 525$) and CNS was analysed separately as it was only recorded in cohort 2 ($n = 209$). Data normality was assessed using the Shapiro–Wilk test and correlations between [TA] and all other variables were assessed using Spearman's correlation coefficient. Data were analysed using SPSS Statistics v.27 (IBM) and p values ≤ 0.05 were considered to indicate statistical significance.

2.3 | Association between total adiponectin concentrations and categorical variables

2.3.1 | BCS groups

For BCS subgroup analyses, animals were categorised as (1) ideal-weight ($4 \leq \text{BCS} \leq 5.5$; $n = 149$), (2) overweight ($5.5 < \text{BCS} < 7$; $n = 195$), (3) or obese ($\text{BCS} \geq 7$; $n = 381$).⁴ Animals with $\text{BCS} < 4$ were excluded as their numbers were very small ($n = 12$). To compare [TA] between these BCS groups, data from all three cohorts were pooled.

2.3.2 | Body shape

Using data from cohort 2 only ($n = 209$), body conditions scores for three body sections (forehand, middle, and hindquarter) were calculated as follows: forehand = neck + withers scores; middle = shoulder + rib scores;

and hindquarter = loin + tailhead. The ratio of hindquarter: middle scores was then calculated and the overall shape of the pony was categorised as follows: ratio < 1 , middle-heavy; ratio = 1, even; ratio > 1 , hind-heavy. [TA] was compared between ponies in each of these three groups. This analysis was performed using data from cohort 2 only as scores for individual body areas were not available for the other two cohorts.

2.3.3 | Breed groups

For breed comparisons, animals were separated into six breed groups based on owner-provided information: (1) Shetland/Shetland cross ($n = 117$); (2) Welsh/Welsh cross ($n = 252$); (3) Cob/Cob cross ($n = 68$); (4) other native UK breeds (including Irish, Dartmoor, Exmoor, Fell, New Forest, Dales, and Eriskay; $n = 160$); (5) unspecified crossbreeds/unknown breed ($n = 110$); (6) other breeds (including Haflinger, Fjord, and Camargue; $n = 27$). To compare [TA] between ponies with different BCS groups, body shape, and breed group, a Kruskal–Wallis test followed by Dunn's post hoc test with Bonferroni multiple testing correction was used.

2.3.4 | Sex

A Mann–Whitney U test was used to compare [TA] between geldings ($n = 378$) and mares ($n = 352$).

2.4 | Proportions of obese and ideal-weight ponies with basal hyperinsulinaemia and/or hypoadiponectinaemia

Obese ($\text{BCS} \geq 7$, $n = 381$) and ideal-weight ponies ($4 \leq \text{BCS} \leq 5.5$, $n = 149$) were grouped according to their [TA] and [basal insulin] into the following four groups:

1. normal: normal [basal insulin] and normal [TA]
2. abnormal: high [basal insulin] and low [TA]
3. hyperinsulinaemia only: high [basal insulin] and normal [TA]
4. hypoadiponectinaemia only: normal [basal insulin] and low [TA]

Threshold values used for [basal insulin] and [TA] were assay-specific, as determined in previous studies.^{1,2,15} The proportions of obese and ideal-weight ponies within these four categories were compared using a Chi-square test of homogeneity followed by post hoc z -test of two proportions with Bonferroni correction.

2.5 | Binary logistic model for the prediction of hypoadiponectinaemia

Binary logistic regression was used to explore the possibility of identifying ponies with hypoadiponectinaemia using explanatory variables.

Note that for the purposes of this analysis, hypoadiponectinaemia was defined as circulating [total adiponectin] $<2.5 \mu\text{g/mL}$, as ponies with such concentrations have been shown to be at greater risk of developing endocrinopathic laminitis within 3 years.¹ Binary outcomes were having low [TA] ($<2.5 \mu\text{g/mL}$) versus having normal [total adiponectin] ($\geq 2.5 \mu\text{g/mL}$) and data from all cohorts were pooled ($n = 734$). Explanatory factors included were only those that could be easily measured without the need for laboratory assays, as the aim was to design a model that could be used to identify at-risk ponies with simple and cost-effective measures available to horse-owners. Continuous variables entered into the initial model thus were age and W:H, and categorical variables included were BCS group, breed group, sex, and laminitis history. No interaction terms were included. Linearity was assessed using the Box-Tidwell procedure with Bonferroni correction and all continuous variables were linearly related to the logit of the dependent variable. Multicollinearity between explanatory factors was assessed using the variance inflation factor (VIF). Height and weight were not included in this model as they violated the multicollinearity assumption ($\text{VIF} > 5$). VIF values for all remaining variables were <1.6 . Variables were entered into the initial model and those with $p > 0.05$ were sequentially removed via backward elimination

until all variables left contributed significantly to correctly predicting the outcome ($p \leq 0.05$). Model fit was estimated using the Hosmer and Lemeshow test (where a non-significant result [$p > 0.05$] indicates a good fit of the model to the data) and Nagelkerke R^2 .

3 | RESULTS

3.1 | Animals and cohort characteristics

The characteristics of animals in each cohort are presented in Table 1.

3.2 | Correlations between total adiponectin concentrations and morphometric measurements

BCS, height, weight, and W:H were significantly positively correlated with [TA] ($p < 0.001$ for all), although correlations were weak (Spearman's $\rho = 0.14$ – 0.29 ; Table 2; Figures S1–S4). There were no significant correlations between [TA] and crest height, crest thickness, or CNS.

TABLE 1 Characteristics of animals included in each cohort.

Characteristic	Cohort 1, $n = 446$	Cohort 2, $n = 209$	Cohort 3, $n = 79$	Total, $n = 734$
Age (years)	15.8 \pm 6.6	13.8 \pm 6.5	15.0 \pm 5.4	15.2 \pm 6.5
Sex				
Gelding	220 (49.3)	120 (57.4)	38 (48.1)	378 (51.5)
Mare	226 (50.7)	85 (40.7)	41 (51.9)	352 (48.0)
Missing data	0 (0.0)	4 (1.9)	0 (0.0)	4 (0.5)
Height (cm)	126.4 \pm 23.0	128.4 \pm 16.5	120.3 \pm 16.9	126.3 \pm 20.8
Weight (kg)	368.2 \pm 130.8	337.9 \pm 106.4	322.3 \pm 103.6	354.7 \pm 122.7
BCS	7.4 \pm 1.3	6.0 \pm 1.0	5.2 \pm 1.3	6.7 \pm 1.5
BCS group				
Underweight (BCS < 4)	2 (0.4)	1 (0.5)	9 (11.4)	12 (1.6)
Ideal weight ($4 \leq \text{BCS} \leq 5.5$)	24 (5.4)	81 (38.8)	41 (51.9)	149 (20.3)
Overweight ($5.5 < \text{BCS} < 7$)	98 (22.0)	83 (39.7)	14 (17.7)	195 (26.6)
Obese (BCS ≥ 7)	322 (72.2)	44 (21.1)	15 (19.0)	381 (51.9)
CNS	ND	2.1 \pm 1.0	ND	
Breed				
Shetland/Shetland cross	77 (17.3)	30 (14.4)	10 (12.7)	117 (15.9)
Welsh/Welsh cross	158 (35.4)	62 (29.7)	32 (40.5)	252 (34.3)
Cob/Cob cross	43 (9.6)	23 (11.0)	2 (2.5)	68 (9.3)
Other native UK breed ^a	90 (20.2)	40 (19.1)	30 (38.0)	160 (21.8)
Crossbreed/unknown	59 (13.2)	47 (22.5)	4 (5.1)	110 (14.9)
Other breed ^b	19 (4.3)	7 (3.3)	1 (1.3)	27 (3.7)
Previously laminitic	0 (0.0)	0 (0.0)	38 (48.1)	38 (5.2)

Note: Data are presented as n (%) or mean \pm SD.

Abbreviations: BCS, body condition score; CNS, cresty neck score; ND, not determined.

^aOther native UK breeds include Irish, Dartmoor, Exmoor, Fell, New Forest, Dales, and Eriskay.

^bOther breeds include Haflinger, Camargue, and Fjord.

TABLE 2 Correlations between total adiponectin concentrations and morphometric measurements.

	Cohorts 1 + 2 + 3 (n = 734)				Cohorts 1 + 3 (n = 525)		Cohort 2 (n = 209)
	BCS	Height	Weight	W:H	Crest height	Crest thickness	CNS
<i>p</i>	0.293	0.144	0.201	0.213	0.031	−0.010	−0.062
<i>p</i> value	<0.001	<0.001	<0.001	<0.001	0.483	0.826	0.389

Note: Values in bold are those with $p < 0.05$.

Abbreviations: BCS, body condition score; CNS, cresty neck score; W:H, weight:height ratio.

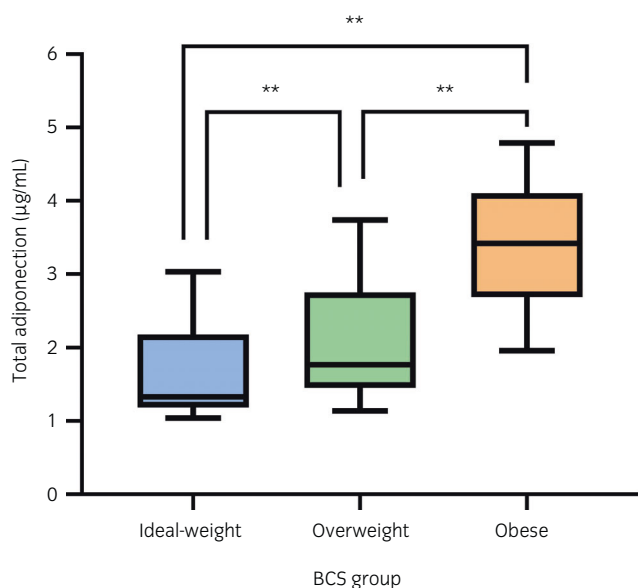


FIGURE 1 Box-and-Whisker plot comparing total adiponectin concentrations across body condition score (BCS) groups. Data are shown as medians \pm interquartile range. Ideal-weight ($4 \leq \text{BCS} \leq 5.5$; $n = 149$), overweight ($5.5 < \text{BCS} < 7$; $n = 195$), or obese ($\text{BCS} \geq 7$; $n = 381$). $**p < 0.01$.

3.3 | Categorical variables associated with total adiponectin concentrations

Significant differences were observed in [TA] between the three BCS groups ($H(2) = 94.48$, $p < 0.001$; Figure 1) and all pair-wise comparisons were significant ($p < 0.01$). Obese ponies had the highest (median [interquartile range]: 3.42 [1.96–4.79] $\mu\text{g/mL}$) total adiponectin concentrations, followed by overweight (1.77 [1.14–3.74] $\mu\text{g/mL}$) and ideal-weight ponies (1.33 [1.04–3.03] $\mu\text{g/mL}$).

There were significant differences in [TA] in animals with different body shapes ($H(2) = 7.07$, $p = 0.03$; Figure 2). Hind-heavy animals had lower [TA] than both even ($p = 0.02$) and middle-heavy ponies ($p = 0.03$).

There were also differences in [TA] between different breed groups ($H(5) = 16.29$, $p = 0.01$; Figure 3). [TA] was significantly lower in the Shetland/Shetland cross group (2.09 [1.27–3.51] $\mu\text{g/mL}$) than in the Welsh/Welsh cross group (3.11 [1.60–4.60] $\mu\text{g/mL}$; $p = 0.01$).

There was no significant association between age and [TA] ($p = 0.07$) or between sex and [TA] ($p = 0.06$).

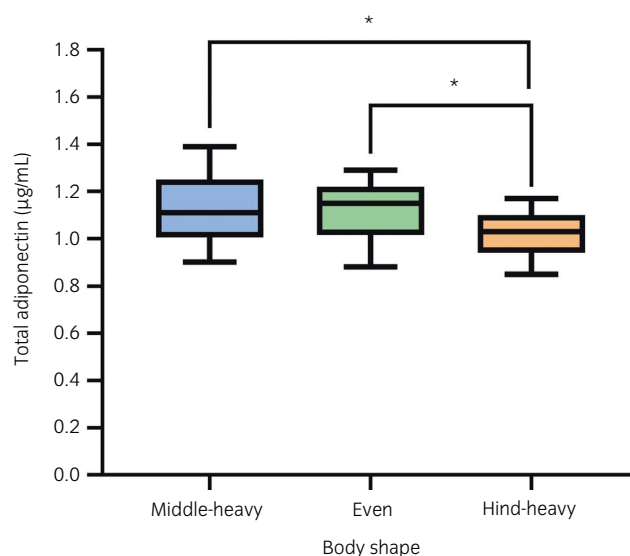


FIGURE 2 Box-and-Whisker plot comparing total adiponectin concentrations in ponies with different body shapes (cohort 2 only). Data are shown as medians \pm interquartile range. Middle-heavy, $n = 58$; even, $n = 70$; hind-heavy, $n = 81$. $*p < 0.05$.

3.4 | Proportions of obese and ideal-weight ponies with basal hyperinsulinaemia and/or hypoadiponectinaemia

There were significant differences in the distributions of the four metabolic categories between obese and ideal-weight ponies ($\chi^2(3) = 37.41$, $p < 0.001$; Figure 4). A significantly greater proportion (54.6%) of obese ponies had normal [basal insulin] and [TA] than their ideal-weight counterparts (33.1%; $p < 0.001$). In addition, a greater proportion of ideal-weight ponies (38.6%) had low [TA] and normal [basal insulin] than obese ponies (16.5%; $p < 0.001$). There were no statistically significant differences in the proportions of obese and ideal-weight ponies with hyperinsulinaemia only or those with abnormal concentrations of both hormones.

3.5 | Binary logistic regression predicting hypoadiponectinaemia

Logistic regression was used to predict the binary outcome low [TA] versus normal [TA]. The null model predicted the outcome correctly in 61% of cases but did not correctly identify any ponies with

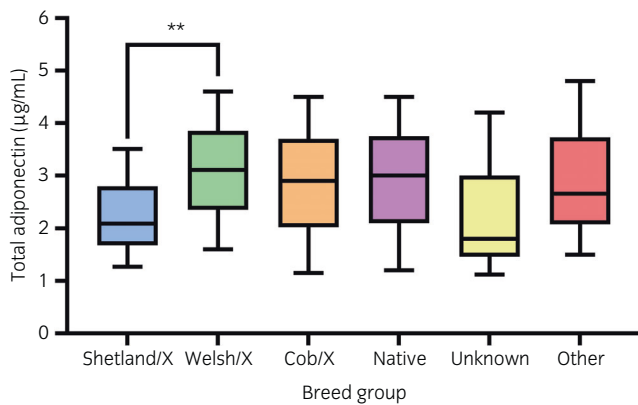


FIGURE 3 Box-and-Whisker plot comparing total adiponectin concentrations across breed groups. Data are shown as medians \pm interquartile range. Shetland/X: $n = 110$; Welsh/X: $n = 252$; Cob/X: 68; Native UK breeds: 160; Unknown/X: 110; other: $n = 38$. Other UK native breeds include Irish, Dartmoor, Exmoor, Fell, New Forest, Dales, and Eriskay. Other breeds include Haflinger, Camargue, and Fjord. $**p = 0.006$.

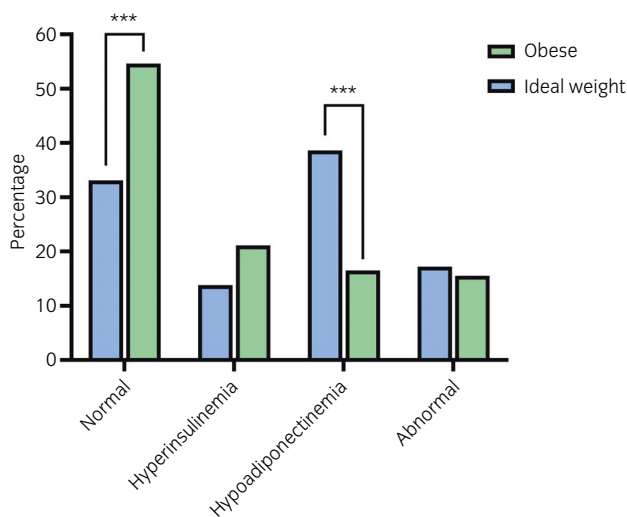


FIGURE 4 Percentage of obese and ideal-weight ponies with normal and abnormal concentrations of total adiponectin and/or basal insulin. Ideal-weight ($4 \leq$ body condition score [BCS] ≤ 5.5 ; $n = 149$), obese (BCS ≥ 7 ; $n = 381$). $***p < 0.001$.

hypoadiponectinaemia only. After backward elimination, two significant factors remained in the full model: W:H and BCS group. The logistic regression model was statistically significant ($\chi^2(4) = 46.49$, $p < 0.001$) and accounted for 8.5% of the variation in the data (Nagelkerke R^2). However, the Hosmer and Lemeshow goodness of fit test indicated the model was not a good fit of the data ($p < 0.05$). The overall accuracy of the full model was 62.%, specificity was 79.5%, and sensitivity was 35.5%. Increasing W:H ratio was associated with decreased odds of hypoadiponectinaemia, and obese ponies had lower odds of having hypoadiponectinaemia than ideal-weight ponies (Table 3).

TABLE 3 Odds ratio of significant variables included in the prediction of hypoadiponectinaemia.

Predictive factor	Odds ratio	95% confidence interval	p value
Weight:height ratio	0.713	0.547–0.930	0.012
BCS group			<0.001
Overweight vs. ideal	0.786	0.505–1.222	0.285
Obese vs. ideal	0.400	0.259–0.617	<0.001

Note: For the term body condition score (BCS) group, the reference category was “ideal weight.” There was a total number of 721 ponies included in this model ($n = 439$ with normal total adiponectin concentrations and $n = 282$ with hypoadiponectinaemia).

4 | DISCUSSION

In some previous studies, EMS and endocrinopathic laminitis were associated with generalised and/or regional adiposity.^{5,15,23} In addition, HMW adiponectin concentrations were reported to be inversely associated with BCS and insulin concentrations in equids.^{4,5} However, obesity is no longer seen as a cause of EMS, but rather a commonly associated feature that is present in some (but not all) animals, in addition to insulin dysregulation as the key feature.²⁰ Morphometric measures remain of interest as they represent a non-invasive alternative for the assessment of EMS risk that does not require specialist equipment or training. They may be of value, but only if they provide an accurate reflection of metabolic health.

In the present study, the relationships between various morphometric measurements and circulating [TA] concentrations were investigated in three cohorts of ponies in England, encompassing obese and ideal-weight ponies, with and without a history of laminitis. The hypothesis that [TA] would be negatively associated with morphometric measures is rejected, as [TA] was weakly positively correlated with BCS (as a continuous measure). It was also higher in obese (BCS ≥ 7) than overweight ($5.5 < \text{BCS} < 7$) and ideal-weight ($4 \leq \text{BCS} \leq 5.5$) ponies when animals were grouped by BCS. The hypothesis was based on previous literature, including Wooldridge et al.⁴ who previously reported a negative correlation between [HMW adiponectin] and BCS, and higher [HMW adiponectin] in lean ($4 \leq \text{BCS} \leq 6$) compared with obese horses (BCS ≥ 7). Present results therefore directly contradict previous reports, although such discrepancies may be partially explained by differences in study population (ponies vs. horses, as well as the inclusion of different breeds) and methodology (e.g., measuring total vs. HMW adiponectin). Although HMW adiponectin is thought to be the most biologically active form, total adiponectin (which includes HMW isoforms) is the only form of adiponectin currently measured in clinical practice in the United Kingdom.

In contrast to BCS, CNS is used to determine regional rather than generalised adiposity. A study by Fitzgerald et al.⁵ showed that CNS was a strong predictor of insulin dysregulation in ponies and performed better than BCS. However, CNS was not significantly correlated with [TA] in the present study. In contrast to the subjective BCS

and CNS, height and weight are two objective measures that are relatively easy to determine by horse-owners using simple equipment (e.g., measuring tape). Height and [TA] were weakly positively correlated, in contrast with a previous study reporting a significant negative correlation.¹⁶ W:H was significantly positively correlated with [TA], as reported previously.⁵ Overall, the present study highlights the high inherent variability present within the equine population with regard to various parameters and suggests that observations made in one study may not be applicable to other populations. Ultimately, few conclusions can be drawn using simple statistical methods in such a heterogeneous and variable population, despite its size. Several factors not considered here including feeding and husbandry practices, medical history, exercise habits, and environment may also affect the relationships between morphometric measurements and biochemical parameters in horses and ponies and contribute to the heterogeneity of the findings.

In addition to correlations and group comparisons, logistic regression was used to potentially identify morphometric measures that may be used to discriminate ponies with hypoadiponectinaemia. W:H and BCS group (categorical) were significant predictive factors, although the model fit was poor and prediction accuracy was relatively low. Finally, there were significant differences in the percentage of obese and ideal-weight ponies with basal hyperinsulinaemia and/or hypoadiponectinaemia. Over half of obese ponies had normal concentrations of both hormones compared with only a third of ideal-weight ponies. Furthermore, a greater proportion of ideal-weight ponies than obese ponies showed hypoadiponectinaemia. Taken together, these results indicate that body condition scoring should not be used to evaluate an animal's metabolic risk for laminitis. Instead, circulating insulin and adiponectin concentrations should be measured, especially in any animal with a genetic predisposition (such as breed) or environmental risk factors (e.g., access to rich pasture, lack of exercise, previous episodes of laminitis) and thus deemed to be at risk of developing EMS. This may allow early identification of non-obese animals with basal hyperinsulinaemia and/or hypoadiponectinaemia that are not showing any clinical signs, and which may otherwise have been overlooked if focusing only on BCS or obesity.

Finally, an interesting finding in the present study was the relationship between [TA] and body shape. Previous research in humans has shown that certain morphologies (increased thigh fat or "pear" shape) are protective against insulin resistance (compared with increased visceral fat or "apple" shape),²⁴⁻²⁶ leading to the hypothesis that similar findings may be observed in equids. In the present study, there were significant differences in total adiponectin concentrations between ponies of different body shapes. The middle-heavy body shape was associated with higher total adiponectin concentrations than both other shapes, suggesting this fat accumulation pattern may be protective in ponies. Alternatively, increased adiponectin concentrations in these animals may form part of a compensatory mechanism secondary to an inflammatory state, as different fat depots are thought to perform

slightly different functions and have different effects on systemic inflammation.^{27,28} This approach requires further research and validation with a more diverse cohort including both ponies and horses. In addition, results indicated breed differences in adiponectin concentrations, which were significantly lower in Shetlands/Shetland cross ponies than in Welsh/Welsh cross ponies. However, breed classification was based on owner information and this finding would need to be confirmed in a different cohort or using genetic data.

Some of the strengths of this study include the type of recorded data and the sample size, as this is the largest retrospective study to investigate the relationship between adiponectin and obesity. By pooling data from three different cohorts, a sample size of up to 734 animals was obtained. The diversity of the population group was increased by pooling data to include ponies with and without a history of laminitis, under different types of ownership (privately owned or kept at a sanctuary), stabled at different locations (although all in England), and representing different breeds and body shapes/sizes.

There are however several limitations to this study as several important factors (e.g., body composition indicators such as body fat percentage and fat mass, exercise, dietary, and seasonal factors) were not investigated. Furthermore, BCS, which is a subjective measure, was assessed by three different assessors (one for each cohort) and this may have introduced additional variability in the data. Similarly, although weight is an objective measure, it was estimated using a weight tape in cohorts 1 and 3, which is likely to have been less accurate than the use of a weighbridge as in cohort 2. This will have been partially mitigated by the fact that weight assessment using the weight tape was done by the same assessor and in the same consistent manner in each of cohorts 1 and 3. Finally, data were analysed at a single time-point for each subject, thus limiting the complexity of the analysis.

In conclusion, body condition scoring and other morphometric measures of obesity do not reliably reflect circulating total adiponectin concentrations and should not be used to assess metabolic risk factors for EMS or endocrinopathic laminitis in ponies. Basal hyperinsulinaemia and hypoadiponectinaemia may be highly prevalent in lean native-breed ponies and circulating concentrations of both these hormones should be measured in animals with predisposing factors, regardless of BCS. Further analyses of relationships between morphometric measures and other metabolic parameters should be carried out in different sample populations representing a broader range of breeds, geographical locations, husbandry practices, and exercise habits in order to draw conclusions applicable to a wider range of animals.

AUTHOR CONTRIBUTIONS

Marine Barnabé contributed to study design, study execution, data analysis and interpretation, preparation of the manuscript, final approval of the manuscript and takes responsibility for the integrity of the data and the accuracy of data analysis. Jonathan Elliott, Patricia A. Harris and Nicola J. Menzies-Gow contributed to study design, data

interpretation, preparation of the manuscript, and final approval of the manuscript.

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CONFLICT OF INTEREST STATEMENT

P. Harris is an employee of Waltham Petcare Science Institute.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/evj.14013>.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ETHICAL ANIMAL RESEARCH

This study involved retrospective analysis of data collected during three studies that were approved by the Animal Ethics and Welfare Committee of the Royal Veterinary College (London, UK).

INFORMED CONSENT

Not applicable.

ORCID

Marine A. Barnabé  <https://orcid.org/0000-0002-8844-0179>

Jonathan Elliott  <https://orcid.org/0000-0002-4517-6590>

Patricia A. Harris  <https://orcid.org/0000-0003-4068-1624>

Nicola J. Menzies-Gow  <https://orcid.org/0000-0002-3803-8069>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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