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Factors associated with insulin responses to oral sugars in a mixed-breed cohort of ponies

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Abstract

Background: Serum insulin concentration at 60 min (InsulinT60) during an oral sugar test (OST) indicates future laminitis risk and insulin dysregulation (ID). Associations between InsulinT60 and physical and owner-reported variables may help clinicians select individuals for ID testing. Associations between InsulinT60 and other metabolic markers may help elucidate ID pathophysiology.

Objectives: To describe associations between (A) season, physically-apparent and owner-reported factors and binary InsulinT60 interpretation (initial models) and (B) variables included in the initial models, other metabolic markers and continuous InsulinT60 (full models).

Study design: Prospective longitudinal.

Methods: Non-laminitic ponies were examined and OSTs (0.3 mL/kg Karo syrup) performed every 6 months (autumn and spring) for ≤4 years. Factors associated with InsulinT60 were determined using mixed-effects models with physical, owner-reported, season and serum/plasma markers as fixed effects and pony and premises identifiers as random effects. Autumn and spring data were analysed separately for full models.

Results: One thousand seven hundred and sixty-three OSTs from 367 ponies were included. High-risk InsulinT60 (>153 μIU/mL) was independently associated with (odds ratio, 95% confidence interval [CI]): age (1.36, 1.16–1.60), body condition score (BCS) (2.38, 1.21–4.69), and bulging supraorbital fatpads (7.25, 2.1–24.98). However, the initial models provided little explanatory power (Nakagawa $R^2 = 0.1–0.27$). LoginsulinT60 was independently associated with (effect estimate, 95% CI): age (0.02, 0.01–0.04), Welsh/Welsh X breed (0.22, 0.05, 0.39), sex (gelding = −0.2, −0.34 to 0.06), BCS (0.16, 0.08–0.23), plasma adiponectin (−0.02, −0.02 to 0.01) and basal insulin (0.01, 0.01–0.01) in spring, and: age (0.03, 0.02–0.04), BCS (0.17, 0.08–0.26), bulging supraorbital fatpads (0.37, 0.2–0.54), turnout score (0.05, 0.02–0.09), plasma adiponectin (−0.01, −0.02 to 0.01), ACTH (per 10 pg/mL) (0.01, 0.00–0.01), triglycerides (0.28, 0.07–0.49) and InsulinT0 (0.01, 0.01–0.01) in autumn.

Main limitations: Only non-laminitic ponies in one region were included.

Conclusions: Owner-reported and physically-apparent data were limited InsulinT60 predictors. InsulinT60 is a complex trait, independently associated with numerous variables, some with seasonal interactions.

KEYWORDS
endocrine, horse, laminitis

1 | INTRODUCTION

Equine insulin dysregulation (ID) manifests in three forms, namely hyperinsulinaemia, an excessive insulin response to oral sugar/starch and peripheral (tissue) insulin resistance.[1] For clinical diagnosis of ID, dynamic tests such as the oral sugar test (OST) are considered to be more sensitive indicators of ID than basal hyperinsulinaemia.[1]

An association between ID and laminitis is well established.[1] Previously we demonstrated that serum insulin concentrations measured in the basal state (InsulinT0) or 60 min after oral administration of 0.3 mL/kg corn syrup (InsulinT60) in a modified OST were important indicators of the risk of future laminitis development in a cohort of non-laminitic ponies in the South-East of England.[2] In this previous study,[2] ponies were categorised as low, medium or high-risk of future laminitis based on InsulinT60. The estimated laminitis incidence was broadly uniform across the lowest six deciles of InsulinT60 (low-risk); with a moderate increase in laminitis incidence associated with the 61st and 90th centiles (medium-risk) and a marked increase associated with the highest decile of InsulinT60 (high-risk).[2] The high-risk group had an estimated 4-year laminitis cumulative incidence of 73%, compared with 3% for ponies with low-risk InsulinT60.[2]

Early detection of ID is likely to be useful for the prevention of laminitis and ideally ponies at higher risk of ID would be selected for further testing based on their signalment, physical attributes or owner-reported information to allow targeted screening. In one other study of British ponies and Cobs, ID was associated with several such easily-identifiable risk-factors that could be used to develop screening strategies including age, breed, sex, levels of exercise, obesity and shorter duration of summer grazing.[3] However, further data from additional cohorts of animals may be useful to improve strategies to select ponies for screening for ID.

While ID is the central feature of equine metabolic syndrome (EMS), affected animals may also have altered plasma concentrations of other metabolic markers such as adiponectin and triglycerides.[1] Additionally, ID is also reported in a subset of cases of pituitary *pars intermedia* dysfunction (PPID), a condition associated with increased immunoreactive plasma adrenocorticotrophic hormone (ACTH).[4] Thus, additional data to describe the relationships between InsulinT60 and: other serum/plasma metabolic markers, season, physical and management variables may help to identify the pathophysiological relationships that underlie ID, EMS and PPID or generate hypotheses for further study. A strong correlation between InsulinT60 and basal metabolic markers (including basal insulin [InsulinT0] perhaps combined with other factors) may also reduce the need to perform an OST.

The current study used an existing dataset from a pony cohort study[2] and aimed to:

A. Describe associations between binary InsulinT60 status and season, physically apparent and owner-reported data to inform strategies to target ID screening.
B. Describe more complex associations between continuous InsulinT60 and other metabolic markers, owner-reported data and physical features to indicate relationships relevant to ID pathophysiology.

2 | MATERIALS AND METHODS

A cohort of ponies was recruited, and each pony was visited every 6 months (spring and autumn) for up to eight visits (2015–2019). Details of the cohort have been reported previously.[2] Briefly, eligible animals were ponies (≤149 cm with shoes), ≥5-years of age with no owner-reported history of veterinary-confirmed laminitis that were kept at a site with at least five eligible ponies. Ponies were excluded if they were pregnant, lactating, receiving pharmaceutical treatment for PPID or if laminitis, significant other lameness or other disease, was evident at the time of data collection. If veterinary-confirmed laminitis developed during the course of the cohort study, the pony was classified as having the event of interest and excluded from further study.

2.1 | Data collection

At each data collection time all eligible ponies (owner consent permitting) at each site were included. Each pony was included for between one and eight data collection visits depending on the duration of their enrolment in the cohort. The study was observational, and owners were asked to treat their ponies as usual between data collection visits. At each visit each pony underwent a standardised veterinary examination and physically apparent variables, blood analyte and owner-reported data were collected. Detailed descriptions of data collection have been reported previously,[2] a brief summary follows.

2.2 | Blood analytes

Ponies were not fasted prior to sampling. Owners were asked to withhold any additional complementary feed on the day of sampling, but ponies received hay or pasture according to their usual routine. A basal

blood sample was taken by jugular venepuncture into clot activator, EDTA, heparinised and oxalate fluoride vacutainer tubes (Becton Dickinson). Each pony was weighed with a portable weighbridge (Equestrian Weigh Platform, Equestrian Products) and a modified OST was performed. Ponies were syringe dosed 0.3 mL/kg Karo light corn syrup (ACH Food Companies Inc.) orally to the nearest 50 kg. Rarely, ponies were not amenable to syringe dosing and were given the syrup in a small handful of low non-structural carbohydrate feed (typically unmolassed chaff). Blood samples were then taken by jugular venepuncture into clot activator vacutainer tubes 30 and 60 min (±5 min) after corn syrup administration. Basal samples were analysed for serum insulin (InsulinT0) (immunofluorescent assay, IRI Insulin, Tosoh AIA 360, Tosoh Bioscience [Analysis performed and validated at Bell Equine Veterinary Clinic]) and plasma adiponectin (ADPN Adiponectin immunoturbidometric assay, Randox Laboratories [Analysis performed at Bell Equine Veterinary Clinic]), triglycerides (Prestige 24iTG, P.Z. Cormay SA using Biolis 24i premium analyser, Tokyo Boeki [Analysis performed and validated at Bell Equine Veterinary Clinic]), glucose (Colorimetric method performed and validated at the Royal Veterinary College diagnostic laboratory) and immunoreactive ACTH (ACTH Immulite/Immulite 1000, Siemens [Analysis performed and validated at Bell Equine Veterinary Clinic]) concentrations. Samples taken at 30 and 60 min were analysed for insulin (analysis as for basal insulin) only (insulinT30 and InsulinT60).

2.3 | Physical examination, morphometry and management questionnaire

The following physical parameters were recorded: weight, height, body length, neck length, heart girth, belly girth, body condition score (BCS),[5] cresty neck score (CNS),[6] evidence of divergent hoof growth of the forelimbs when viewed from the lateral aspect and the presence or absence of hypertrichosis, bulging supraorbital fat pads, a pot belly (the final three variables were not collected at the autumn 2015 data collection visit). Owners/carers were asked to complete a questionnaire concerning the signalment, diet, management and health (including any PPID signs) for each pony at each visit (returned by post).

2.4 | Data analysis

2.4.1 | Descriptive data

Analysis was performed with statistical software (R version 4.0.3, R Foundation for statistical computing and IBM SPSS Statistics for Windows [Version 28], IBM Corp.). The dataset from the previous cohort study was used in the present analysis.[2] Descriptions of all variables available for inclusion, data distribution and complete rates have been reported.[2] Samples for which either basal insulin (InsulinT0) or InsulinT60 were missing were excluded (105/1868 sampling points) and missing data were not imputed other than for four owner-reported variables described previously.[2] Power calculations for the previous study were based on the expected incidence of laminitis.[2] Separate a priori sample size calculations were not performed for the present analysis. Variables to report were selected based on biological plausibility, data completeness and distribution of data and to avoid reporting of correlated variables. Due to seasonal variation[7] and the presence of autumnal cross-reactants,[8] a binary variable 'ACTH positive' was created to categorise ponies into those with or without plasma ACTH concentrations above the middle of the equivocal range proposed by the Equine Endocrinology Group Guidelines for PPID diagnosis for the relevant assay at the time of data analysis[9] (75 and 40 pg/mL in autumn and spring respectively). To simplify analysis, forelimb hoof divergence scores were collapsed to three categories (none, mild or marked in either hoof) and composite scores (described previously[2]) were created for turnout and exercise to reflect the owner-reported time at pasture and extent of grass cover and the duration and intensity of exercise.

OST results were classified as high-risk if InsulinT60 exceeded 153 μIU/mL and low risk if InsulinT60 <54.2 μIU/mL.[2] The distribution of continuous explanatory variables was assessed by examination of histograms. Descriptive data are presented for each risk group with medians (and IQR) for skewed numerical data and mean (±SD) for data that was approximately normal.

2.4.2 | Mixed-effect models

Data were analysed with statistical software (R version 4.0.3, [primarily 'lme4', 'sjstats' and 'performance' packages], R Foundation for statistical computing). Pre-specified logistic and linear mixed-effects models were created to describe relationships between (A) a binary classification of InsulinT60 and: season, physically-apparent and owner-reported variables (initial models) and (B) continuous (logarithmically transformed) InsulinT60 and: the variables included in the initial models and other blood analytes for spring and autumn separately (full models). For all models, variables were selected based on biological plausibility and to reduce excessive correlation between covariates. There was no iterative model-building. Non-significant variables were not removed from models and model parameters for all tested variables are reported. Sex data were excluded for stallions due to low numbers of stallions and hoof divergence was collapsed to present/absent. For breed comparisons the 'other/unknown' group was arbitrarily assigned as the reference category. Ordinal variables with at least five categories (BCS, CNS, turnout composite and exercise composite) were analysed as continuous variables after assessing the data distribution.[10] For linear mixed-effects models, the distribution of the residuals was assessed for normality using histograms and qq-plots. The explanatory power of each model was calculated as Nakagawa's pseudo R^2 for fixed effects only (marginal r^2) and the full model (conditional r^2).[11]

Season, physical and owner-reported variables and T60 risk group: Initial logistic models

To investigate the potential for physical and owner-reported data to be used to select ponies for screening for high-risk and at least medium-risk InsulinT60, two logistic mixed effect models were created. The first used high-risk InsulinT60 (InsulinT60 >153 μIU/mL) as the outcome, the second model used high or medium-risk InsulinT60 (i.e., InsulinT60 >54.2 μIU/mL) as the outcome.

For both models each individual pony was included as a random (intercept) effect. Each premises could not be included as a random effect in the logistic models as the complexity of the random effects prevented model convergence. However, a linear mixed-effects model using the logarithm of InsulinT60 (LoginsulinT60) as the outcome with pony and premises as random intercepts indicated little effect of premises (Table S1).

Season, physical, blood analyte and owner-reported variables and LoginsulinT60—Full, linear models

A linear mixed-effect model was required for the more complex models that included blood analytes in addition to the variables included in the initial logistic models. InsulinT60 was logarithmically transformed (LoginsulinT60) to improve the distribution of the model residuals. Separate models were created for samples collected in the spring and autumn due to the potential for seasonal interactions. Both pony and premises were included as random intercepts in the models.

3 | RESULTS

3.1 | Animals

In total the study included 1763 OSTs from 367 individual ponies comprising 218 (59%) geldings, 143 (39%) mares, 2 (0.5%) stallions and 4 (1%) unreported. The numbers of examinations, ponies and premises at each stage of the study are illustrated in Figure 1.

The mean (±SD) age at entry to the study was 13.5 (±6.5) years. Overall, 104 (28%) ponies were reported to be Welsh or Welsh X, 59 (16%) as Cobs or Cob X, 46 (13%) as Shetland or Shetland X and 153 (42%) as other breeds or unknown. Two-hundred and seventy-eight (76%) ponies were reported to be used for ridden or driving exercise and 82 (22%) were reported as unexercised (7 [2%] unreported). Ponies were kept on 24 different properties; 273 (74%) were kept at riding schools or riding schools combined with a livery yard, 63 (17%) were kept at a charity premises and 31 (8%) were kept at a private home or yard.

3.2 | Descriptive OST data

In the low, medium and high-risk groups median (IQR) InsulinT0 concentrations were (μIU/mL): 8.5 (5.5–14.1), 23 (14.2–34.9) and 55.4 (32.9–92.7) while median (IQR) InsulinT60 concentrations were 26.3 (17.4–37), 81.5 (65.6–105.6) and 229.3 (183–311.4), respectively. Descriptive data for selected blood analytes, physical examination variables and owner-reported data are presented in Tables S2–S4 and illustrated in Figures 2 and 3.

3.3 | Initial models-season, owner-reported and physical data and InsulinT60 risk status

Model outputs for the logistic: season, physical and owner-reported variables are shown in Table 1. Increasing age, Welsh and Welsh X ponies, increasing body condition score and the presence of bulging supraorbital fat pads were independently associated with higher odds of a medium to high-risk InsulinT60 compared with low-risk InsulinT60 while geldings and ponies undertaking a greater intensity or duration of exercise were at lower odds. Increasing age, body condition score and bulging supraorbital fat pads were associated with an increased odds of high-risk InsulinT60 compared with low-medium risk groups. The fixed effects explained only 10%–27% of the variation in InsulinT60 risk group depending on the model used; the remaining variation was primarily attributable to uncharacterised within-pony factors. The intra-class correlation coefficients of 0.94 for the high-risk versus low-medium risk and 0.69 for the high-medium risk versus low-risk model indicate relatively high risk-group consistency within each pony.

3.4 | Full models—Factors associated with LoginsulinT60

The linear mixed effect models showing season, owner-reported, physical and other blood analytes variables are presented in Table 2. In both spring and autumn higher LoginsulinT60 was independently associated with increasing age, body condition score, InsulinT0 and plasma triglycerides and inversely associated with plasma adiponectin concentrations. Whereas higher LoginsulinT60 was only independently associated with Welsh/Welsh X ponies and mares in the spring and with increasing immunoreactive ACTH, bulging supraorbital fat pads and the turnout composite score in the autumn. These more complex models explained 46%–49% of the variation in LoginsulinT60.

Univariable linear mixed effect models of the associations between variables included in Table 2 and LoginsulinT60 are shown in Table S5. On a univariable basis, all assessed variables other than hypertrichosis, hoof divergence, exercise and turnout were associated with LoginsulinT60 in autumn and spring. Hypertrichosis was not associated with LoginsulinT60 in either season whereas hoof divergence, exercise and turnout scores were only associated with LoginsulinT60 in the autumn. Most variables had little explanatory power. InsulinT0 had the highest explanatory power and the practical implications of the association between InsulinT0 and InsulinT60 are shown in Table 3 in which categorical (rather than continuous) values are compared. For example, of 1218 samples classified as low-risk based on InsulinT0, 21% had a 'medium-risk' InsulinT60 result and 1% had a high-risk InsulinT60 result.

4 | DISCUSSION

Season, owner-reported and physical features only explained 10%–27% of the differences in InsulinT60 risk status in this population, indicated by the R^2 values for the fixed effects in the logistic mixed-effects models. These values indicate that while physical and owner-reported features can be used to identify ponies with a higher risk of ID that veterinarians should not limit testing for ID to ponies in which

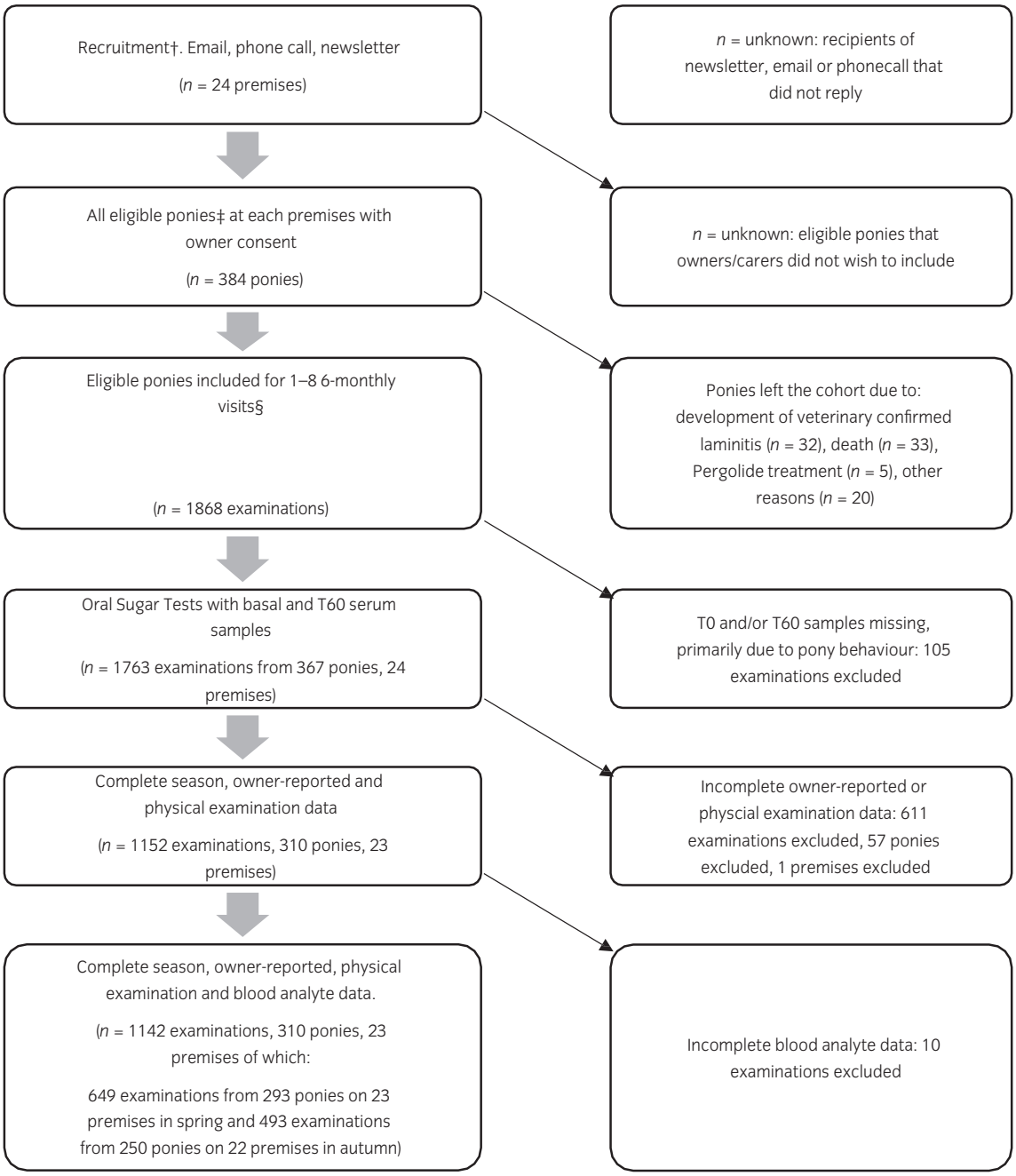


FIGURE 1 Flowchart of pony and sample numbers. †Recruitment: All eligible premises for which contact details were available retrieved from: Pony Club, Riding for the Disabled and British Horse Society online databases. The first 30 Google™ results for 'Kent pony sanctuary' and 'Kent equine charity'. The first 40 Google™ results for 'Kent riding school'. All members of South Eastern Welsh Pony and Cob Society via newsletter. ‡ Inclusion criteria (premises): within Kent or 25 mile radius of Bell Equine Veterinary Clinic with 1 additional premises included at 36 miles) with ≥5 eligible ponies at initial recruitment. ‡Inclusion criteria (ponies): (≤149 cm at the withers with shoes or ≤148 cm without), ≥5 years old. Exclusion criteria (ponies): owner-reported history of likely or veterinary confirmed laminitis, pregnant or lactating, receiving medical treatment for PPID, systemically unwell at examination, laminitis or significant other lameness at examination. §Further details of the subjects included at each time point are included in reference 2.

these risk factors are present. Doing so would miss identifying ponies at moderate to high risk of laminitis because the variables included in the present study were not sensitive indicators of high-risk InsulinT60. It is notable that highrisk InsulinT60 concentrations were recorded in ponies of all values of BCS (3–9/9), turnout score, exercise and CNS studied. For example, a healthy BCS did not rule out ID and 8% of OSTs from ponies with BCS ≤6/9 were in the high-risk InsulinT60 group (compared with 17% of ponies with BCS ≥7/9; Table S3). This may, in part reflect the limitations of body condition scoring as an indicator of total fat mass[12] and/or that the metabolic

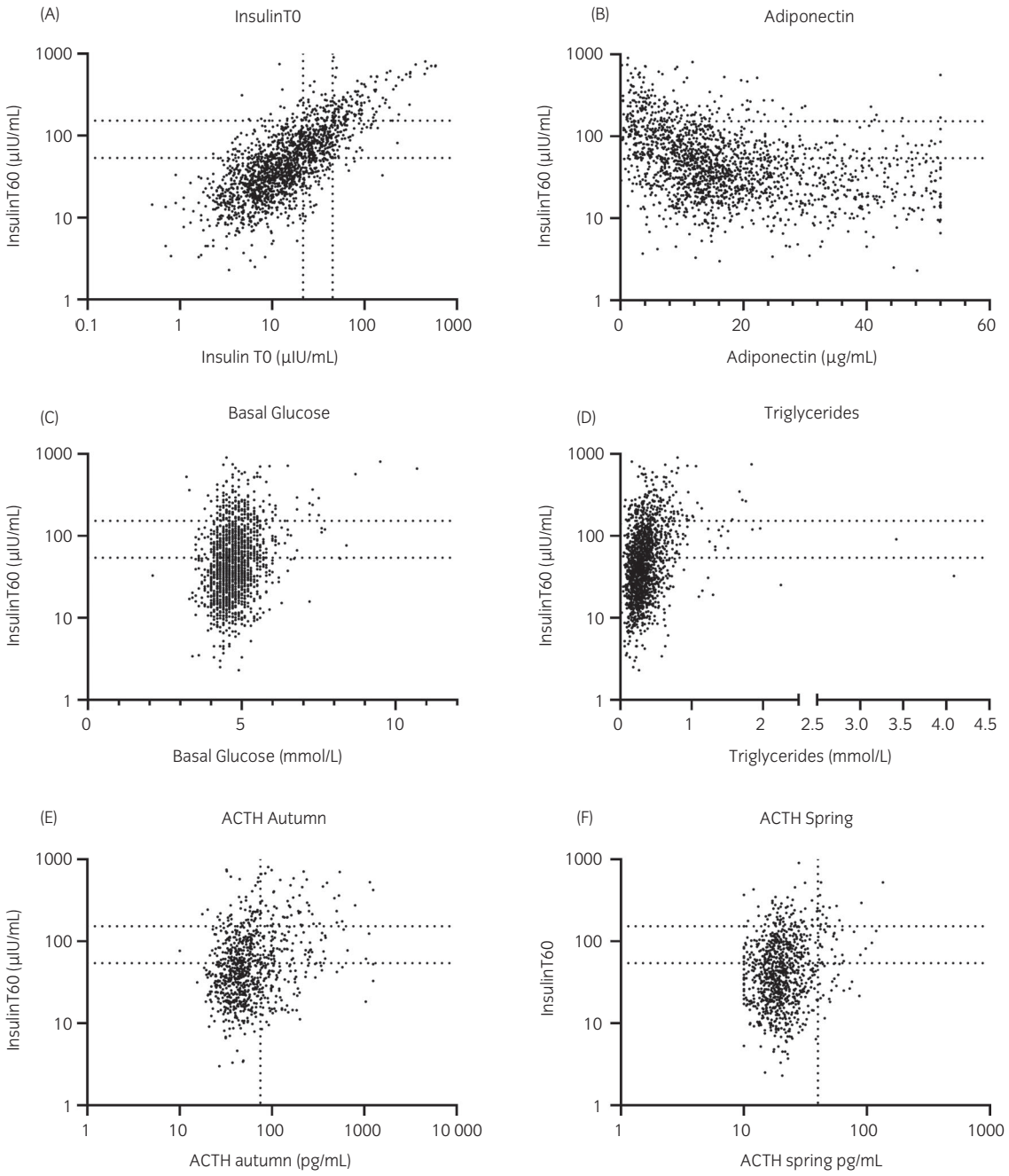
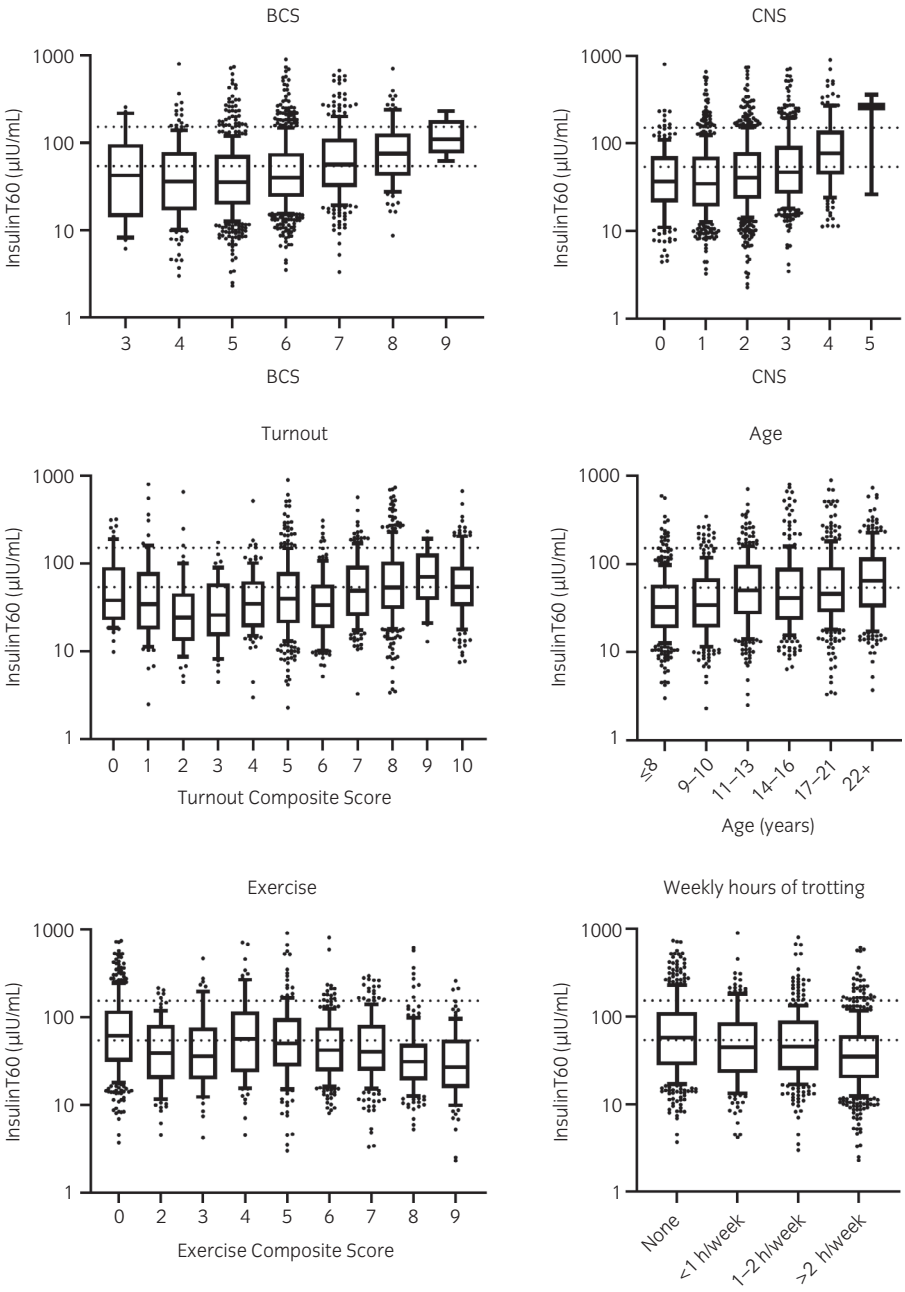


FIGURE 2 Scatter plots of [Insulin]T60 against other blood analytes. InsulinT60, InsulinT0 and ACTH are shown on a logarithmic scale. Dotted lines on Y axes (54.2 and 153 µIU/mL) indicate thresholds for low, medium and high laminitis risk categories from previous analysis of the present dataset. Dotted lines on X axes indicate thresholds for low, medium and high laminitis risk for InsulinT0 (21.6 and 45.2 µIU/mL) and the middle of the ACTH equivocal ranges for PPID diagnosis (40 and 75 pg/mL).

consequences of obesity are diet dependent.[13,14] Regardless of cause, it is of important clinical relevance.

Although the variables studied were neither highly sensitive nor specific indicators of exaggerated OST responses, the associations shown are useful to increase the clinical suspicion of ID and are consistent with findings in other similar populations.[3,15,16] In particular, Carslake et al.[3] investigated factors associated with EMS in a population of UK ponies. Consistent with the present study, Carslake et al.[3] identified associations between EMS and age, breed, sex, obesity, turnout, reduced levels of activity, bulging supra-orbital fat pads and divergent hoof growth.[3] However, in the present study some of these associations varied with season. These factors cannot be used to rule in or rule out an exaggerated insulin response with any degree of confidence but can be used to identify populations in which ID is likely to be particularly prevalent.

The association between InsulinT60 and bulging supraorbital fat pads warrants further investigation, particularly as the presence of bulging supraorbital fat pads was associated with a seven-fold



increase in the odds of being in the high-risk InsulinT60 group in the initial logistic models. The separate autumn and spring analysis indicated an interaction with season. On further analysis there was no association between season and the presence of supraorbital fat pads and the within-pony correlation coefficient for bulging fat pads was high (data not shown) that is, there was no indication that the presence or absence of supraorbital pads varied by season. To the authors' knowledge, the pathology of supraorbital fat accumulation has not been investigated but regional adiposity is a well-described feature of ID[1] and supraorbital deposits may be a particularly important, or readily identifiable local adipose deposit.

In all models the fixed effects had relatively low explanatory power. However, when the random effects were included, there was a marked increase in the explanatory power of the models. Analysis of the variance in InsulinT60 attributable to the random effects indicated that the between-pony variance was much higher than between-premises variance. The within-pony intra-class correlation coefficient (ICC) was also much higher than the within-premises ICC. Taken together, these findings indicate that a high proportion of the variation in InsulinT60 was due to within-pony factors incompletely characterised in the present models (rather than to factors relating to the management on particular premises). Given the high heritability of serum insulin concentrations in Welsh ponies,[17] genetic factors are likely contributors to this uncharacterised variance and require further investigation. The amount of unexplained variation in InsulinT60 is also consistent with the complexity of InsulinT60 as a trait, particularly when the high number of independent explanatory variables and the seasonal differences in the effects of explanatory variables are

TABLE 1 Multivariable logistic mixed effect models of season, physical and owner-reported factors associated with high-risk InsulinT60 (>153 μIU/mL) or medium and high risk InsulinT60 (>54.2 μIU/mL) (initial models).

	High risk versus low and medium risk			High and medium risk versus low risk		
Predictors	**Odds ratios**	**CI**	***p***	**Odds ratios**	**CI**	***p***
Season (spring)	0.75	0.35–1.61	0.5	0.91	0.59–1.39	0.7
Age	1.36***	1.16–1.60	**<0.001**	1.21***	1.13–1.30	**<0.001**
Cob/Cob X	0.76	0.04–15.69	0.9	0.6	0.17–2.05	0.4
Shetland/Shetland X	2.22	0.13–36.56	0.6	0.55	0.15–2.05	0.4
Welsh/Welsh X	5.05	0.59–42.97	0.1	4.95**	1.82–13.42	**0.002**
Sex (gelding)	0.41	0.07–2.38	0.3	0.35*	0.16–0.8	**0.01**
Body condition score	2.38**	1.21–4.69	**0.01**	2.43***	1.67–3.54	**<0.001**
Cresty neck score	1.39	0.83–2.35	0.2	>0.9	0.74–1.33	>0.9
Bulging supraorbital fat pads Y/N	7.25**	2.1–24.98	**0.002**	3.39***	1.68–6.82	**0.001**
Hypertrichosis Y/N	0.38	0.05–2.87	0.3	0.87	0.16–4.61	0.9
Pot belly Y/N	1.46	0.55–3.88	0.4	1.54	0.84–2.81	0.2
Hoof divergence Y/N	0.82	0.36–1.87	0.6	1.08	0.68–1.72	0.8
Exercise composite	0.84	0.67–1.06	0.1	0.81***	0.72–0.91	**<0.001**
Turnout composite	1.04	0.84–1.29	0.7	1.11	0.99–1.24	0.07
Random effects	τ_{00} pony = 49.1, σ^2 = 3.29, ICC = 0.94			τ_{00} pony = 7.37, σ^2 = 3.29, ICC = 0.69		
Model fit	Marginal R^2 = 0.1/conditional R^2 = 0.94			Marginal R^2 = 0.27/conditional R^2 = 0.77		

Note: One thousand one hundred and fifty-two observations were included from 310 individual ponies were included in each model. τ_{00} = mean between-subject variance for each random effect; σ^2 = residual variance; ICC = intra-class coefficient.
*p < 0.05; **p < 0.01; ***p < 0.001. p values <0.05 are also shown in bold.

considered. It should also be remembered that the associations reported do not inherently imply causation.

Although owner-reported and physical findings had relatively weak explanatory power, they should not be discounted as owners and veterinarians can only seek to alter factors that are actually modifiable. In the present study, exercise and turnout were the only directly modifiable variables. Body condition score is indirectly modifiable though tailored diet and exercise[18] and was independently associated with InsulinT60 in all models. The exercise composite score was associated with LoginsulinT60 in the autumn on univariable analysis but in the multivariable models was only independently associated with InsulinT60 in one logistic model. This suggests that metabolic changes associated with higher duration or intensity of exercise are correlated with other explanatory variables (such as body condition score) in the multivariable model. It must be acknowledged that the exercise composite score used in the present study is based on subjective owner-reported information that may be difficult to extrapolate to other populations. However, the relationship between the reported weekly hours of trotting and InsulinT60 in Figure 3 and Table S4 suggests that relatively small increments in exercise routines may be beneficial. For example, 18% of samples from ponies that were reported to do no trotting exercise were in the high-risk InsulinT60 category compared with only 9% of samples from those reported to undertake 1–2 h of trotting per week. The benefits of low intensity exercise were previously reported in a more controlled experimental study.[19] The reason for a lack of association between LoginsulinT60 and exercise in spring is unclear although we speculate that greater between-pony variation in exercise levels during summer may have enabled detection of an effect in autumn whereas in winter levels of exercise were more consistently low.

The turnout composite score was associated with LoginsulinT60 in the autumn on a univariable and multivariable basis but did not reach significance in the logistic models. This may also reflect a greater variation in turnout conditions over the summer when fresh grass was likely to be a greater constituent of the pony's diet than in the winter. Ponies may also show heterogenous metabolic adaptations to summer grazing that are not revealed by winter diets.[20] Overall, the present data therefore suggest that strategies to alter exercise, turnout and body condition score may help to reduce ID but the effects may be small given the contribution of non-modifiable factors. Further interventional studies are required to demonstrate the magnitude of any benefit.

The univariable linear mixed-effect models (Table S5) describe associations between LoginsulinT60 and all included blood analytes in both autumn and spring. Plasma concentrations of adiponectin and triglycerides and InsulinT0 all also showed consistent independent effects in the autumn and spring multivariable models. The lack of multivariable associations for basal glucose is likely to relate to collinearity with InsulinT0. An independent association between ACTH and LoginsulinT60 was shown in the autumn but not the spring. The reason for this difference is unclear but could reflect the effects of endogenous peptides with ACTH-like immunoreactivity that are present in pony plasma in the autumn.[9] However, any effects of such

TABLE 2 Multivariable linear mixed effects model to describe associations between LoginsulinT60 and physical, owner-reported and other blood variables in spring and autumn (full models).

	Spring			Autumn		
Predictors	**Estimates**	**CI**	***p***	**Estimates**	**CI**	***p***
Age	0.02***	0.01–0.04	**<0.001**	0.03***	0.02–0.04	**<0.001**
Breed = Cob/Cob X	−0.12	−0.34 to 0.09	0.3	−0.22	−0.45 to 0.01	0.06
Breed = Shetland/Shetland X	−0.21	−0.44 to 0.02	0.08	−0.12	−0.37 to 0.12	0.3
Breed = Welsh/Welsh X	0.22*	0.05–0.39	**0.01**	0.12	−0.06 to 0.29	0.2
Sex = gelding	−0.20**	−0.34 to −0.06	**0.005**	−0.08	−0.22 to 0.07	0.3
Body condition score	0.16***	0.08–0.23	**<0.001**	0.17***	0.08–0.26	**<0.001**
Cresty neck score	0.01	−0.05 to 0.07	0.8	−0.01	−0.08 to 0.06	0.8
Bulging supraorbital fat pads Y/N	0.11	−0.05 to 0.26	0.2	0.37***	0.20–0.54	**<0.001**
Hypertrichosis Y/N	0.07	−0.23 to 0.38	0.6	0.14	−0.34 to 0.62	0.6
Pot belly Y/N	−0.02	−0.16 to 0.12	0.8	0.03	−0.12 to 0.19	0.7
Hoof divergence Y/N	−0.03	−0.13 to 0.07	0.6	−0.02	−0.14 to 0.10	0.7
Exercise composite	−0.01	−0.04 to 0.01	0.3	−0.03	−0.05 to 0.00	0.06
Turnout composite	0.01	−0.02 to 0.03	0.6	0.05**	0.02–0.09	**0.001**
Adiponectin	−0.02***	−0.02 to −0.01	**<0.001**	−0.01***	−0.02 to −0.01	**<0.001**
ACTH/10[a]	0.03	−0.02 to 0.07	0.2	0.01*	0.00–0.01	**0.01**
Glucose	0.06	−0.04 to 0.16	0.3	0.05	−0.07 to 0.16	0.4
Triglycerides	0.42***	0.20–0.64	**<0.001**	0.28**	0.07–0.49	**0.009**
InsulinT0 (basal)	0.01***	0.01–0.01	**<0.001**	0.01***	0.01–0.01	**<0.001**
Random effects						
τ_{00}	0.19 pony, 0.03 premises			0.15 pony, 0.01 premises		
σ^2	0.25			0.27		
ICC	0.46			0.39		
Marginal R^2/conditional R^2	0.459/0.705			0.494/0.689		

Note: Six hundred and forty-nine observations from 293 ponies on 23 premises were included in the spring and 493 observations from 250 ponies on 22 premises were included in the autumn. τ_{00} = mean between-subject variance for each random effect, σ^2 = residual variance, ICC = intra-class coefficient.
[a]ACTH concentrations were divided by 10 to illustrate the effect of a 10 pg/mL change in ACTH.
*p < 0.05; **p < 0.01; ***p < 0.001. p values <0.05 are also shown in bold.

TABLE 3 Contingency table (within-row percentages) of categorical interpretation of InsulinT0 (basal) and InsulinT60 for low, medium and high laminitis risk categories.

		T60 insulin (μIU/mL)			
		Low (<53.4)	**Medium (53.4–153)**	**High (>153)**	**Total**
InsulinT0 (basal) (μIU/mL)	Low (<21.6)	951 (78%)	250 (21%)	17 (1%)	1218 (100%)
	Medium (21.6–45.2)	88 (24%)	223 (61%)	52 (14%)	363 (100%)
	High (>45.2)	5 (3%)	64 (35%)	113 (62%)	182 (100%)
	Total	1044	537	182	1763

Note: InsulinT0 (μIU/mL): low risk <21.6, medium-risk 21.6–45.2, high risk >45.2 InsulinT60 (μIU/mL): low risk <54.2, medium-risk 54.2–153, high risk >153.[2]

peptides are likely to be minor given the very small R^2 value associated with ACTH.

A negative association between insulin responses and plasma adiponectin in horses is well described.[21] The shape of the adiponectin/InsulinT60 scatter plot shown in Figure 2 is interesting with little association between adiponectin and InsulinT60 throughout much of the range of adiponectin. High levels of InsulinT60 were only associated with the very lowest adiponectin concentrations consistent with a threshold effect for adiponectin on insulin regulation.

InsulinT0 (basal) explained more variation in LoginsulinT60 than any other variable (Table S5). There was good correlation between InsulinT0 and InsulinT60 when results were interpreted on a

categorical basis according to the previously reported laminitis risk groups[2] (Table 3). Although dynamic tests are considered to be more sensitive for detection of ID than basal tests,[1] the present data indicate that from a practical perspective, basal (InsulinT0) and OST results are likely to have equivalent interpretation in a significant majority of cases, but not all. The other independent variables associated with InsulinT60 shown in Table 2 should not be forgotten. In a pony with a normal InsulinT0 for example, a high InsulinT60 would be more likely if the pony were older, had low plasma adiponectin and had a higher body condition score (in both autumn and spring).

The approach to creating explanatory models for InsulinT60 warrants discussion. The underlying distribution of InsulinT60 values was right skewed, with a small proportion of outliers with very high values (maximum value 902 μIU/mL). Applying a logarithmic transformation to InsulinT60 improved the fit of the models (with much reduced kurtosis of residuals when compared with untransformed values). Although the logarithmic transformation improved model fit, the approach has disadvantages from a clinical perspective. Primarily, the distribution of laminitis risk shows the inverse distribution (i.e., exponential) in the present dataset.[2] For example, the difference in laminitis risk between InsulinT60 values of 1 and 10 μIU/mL differs considerably from the risk associated with an increase from 10 to 100 μIU/mL or 100 to 1000 μIU/mL, but these are single unit increments on a logarithmic scale. Second, interpretation of logarithmically transformed parameter estimates is not intuitive nor familiar to many clinicians. The logistic models may provide more clinically intuitive results but also have disadvantages. The dichotomisation of continuous variables results in a loss of information and statistical power,[22] in particular for the relatively small high-risk group. The more equal group sizes created by categorising the outcome as low-risk compared with high/medium-risk increased the statistical power of the model. Logistic model convergence was also problematic when greater numbers of continuous covariates were included. The selection of variables for each model was subjective and different approaches may have been justified. A large number of variables were included in each model with the intention of including the most important independent variables. However, models were not refined iteratively. As indicated by the univariable models a 'non-significant' p value in the multivariable model should not be assumed to indicate that no association exists. Correlations between variables are inevitable in such studies and may mask associations between correlated covariates and the outcome.

The present study had several additional limitations. The quality of owner-reported data was not verified and in some cases was missing, although this may be akin to clinical practice. Data collection was only conducted in spring and autumn and so seasonal effects or interactions outside of these periods could not be tested. By the nature of the study and data-collection techniques additional sources of variation may have affected the data for example there was some variation in the time of day that data collection was undertaken. The study selected ponies without current or a known history of laminitis and so the study findings are most readily extrapolated to pony populations in which the prevention of laminitis development is sought. Different findings are likely to apply in currently or recently laminitic equids when presented for assessment of ID in clinical practice. There was also a selection bias towards premises with larger numbers of ponies and therefore potentially more experienced owners. Selection bias may also have affected the ponies that were recruited. Ponies receiving pharmaceutical treatment for PPID were excluded from the study and the vast majority (79%) of ponies had some level of exercise such that few very aged, retired ponies were included. Therefore, although around 26% of samples were classified as 'ACTH positive' during the autumn, the prevalence of hypertrichosis was very low (3%) and it is unlikely that many advanced PPID cases were included in the cohort, limiting the analysis of variables such as hypertrichosis. Conversely given that the sensitivity of basal ACTH is more limited than its specificity,[23] early PPID may have been prevalent in this population. Finally, it should be noted that the insulin concentrations apply to the assay used in the present analysis and concentrations reported using other assays will not be identical. Equivalent diagnostic thresholds for the commonly used Immulite 2000XPI analyser have been reported.[24]

In conclusion, the likelihood of an exaggerated OST response (InsulinT60) could not be confidently ruled in or out based on season, physical or owner-reported data. Veterinarians must be aware that high InsulinT60 may occur in ponies across a wide range of body conditions, ages and levels of exercise. InsulinT60 is a complex trait, independently associated with many variables, some with seasonal interactions. Modifiable factors were associated with a healthier metabolic phenotype but non-modifiable factors such as age and breed were also important explanatory variables.

AUTHOR CONTRIBUTIONS

Edward J. Knowles, Nicola J. Menzies-Gow, Patricia A. Harris and Jonathan Elliott contributed to the study design. Edward J. Knowles was responsible for data collection, study execution and data integrity and had full access to all data. Edward J. Knowles analysed the data with assistance from Yu-Mei Chang. All authors contributed to data interpretation, manuscript preparation and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

Edward J. Knowles is employed by CVS Ltd and provides diagnostic laboratory services through Axiom Veterinary Laboratories. He has received fees for speaking/CPD material from Boehringer Ingelheim. Patricia A. Harris is employed by the 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.13983.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request: Open sharing exemption granted by editor due to lack of provision in owner informed consent form.

ETHICAL ANIMAL RESEARCH

The study was approved by the Royal Veterinary College Animal Welfare and Ethical Review Board (2015-5128) and was conducted under a UK Home Office Licence (PPL 70/8195/PED 1AA054).

INFORMED CONSENT

Owners gave consent for their animals' inclusion in the study.

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REFERENCES

1. Durham AE, Frank N, McGowan CM, Menzies-Gow NJ, Roelfsema E, Vervuert I, et al. ECEIM consensus statement on equine metabolic syndrome. J Vet Intern Med. 2019;33:335–49.
2. Knowles EJ, Elliott J, Harris PA, Chang Y, Menzies-Gow NJ. Predictors of laminitis development in a cohort of nonlaminitic ponies. Equine Vet J. 2022;55(1):12–23.
3. Carslake HB, Pinchbeck GL, McGowan CM. Equine metabolic syndrome in UK native ponies and cobs is highly prevalent with modifiable risk factors. Equine Vet J. 2021;53:923–34.
4. McGowan TW, Pinchbeck GP, McGowan CM. Prevalence, risk factors and clinical signs predictive for equine pituitary pars intermedia dysfunction in aged horses. Equine Vet J. 2013;45:74–9.
5. 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.
6. Carter RA, Geor RJ, Burton Staniar W, Cubitt TA, Harris PA. Apparent adiposity assessed by standardised scoring systems and morphometric measurements in horses and ponies. Vet J. 2009;179:204–10.
7. Beech J, Boston RC, McFarlane D, Lindborg S. Evaluation of plasma ACTH, alpha melanocyte stimulating hormone, and insulin concentrations during various photoperiods in clinically normal horses and ponies and those with pituitary pars intermedia dysfunction. J Am Vet Med Assoc. 2009;235:715–22.
8. Knowles EJ, Shaw S, Harris PA, Elliott J. Plasma adrenocorticotropic hormone (ACTH) concentrations in ponies measured by two different assays suggests seasonal cross-reactivity or interference. Equine Vet J. 2018;50:672–7.
9. The Equine Endocrinology Group. Recommendations for the diagnosis and treatment of pituitary pars intermedia dysfunction (PPID); 2019. https://sites.tufts.edu/equineendogroup/files/2019/12/2019-PPID_EEGbooklet.pdf. Accessed Dec 2021.
10. Johnson DR, Creech JC. Ordinal measures in multiple indicator models: a simulation study of categorization error. Am Sociol Rev. 1983;48:398–407.
11. Nakagawa S, Schielzeth H. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol Evol. 2013;2:133–42.
12. Dugdale AHA, Grove-White D, Curtis GC, Harris PA, McG AC. Body condition scoring as a predictor of body fat in horses and ponies. Vet J. 2012;194:173–8.
13. Bamford NJ, Potter SJ, Harris PA, Bailey SR. Effect of increased adiposity on insulin sensitivity and adipokine concentrations in horses and ponies fed a high fat diet, with or without a once daily high glycaemic meal. Equine Vet J. 2016;48:368–73.
14. Bamford NJ, Potter SJ, Baskerville CL, Harris PA, Bailey SR. Effect of increased adiposity on insulin sensitivity and adipokine concentrations in different equine breeds adapted to cereal-rich or fat-rich meals. Vet J. 2016;214:14–20.
15. Box JR, McGowan CM, Raekallio MR, Mykkänen AK, Carslake H, Karikoski NP. Insulin dysregulation in a population of Finnhorses and associated phenotypic markers of obesity. J Vet Intern Med. 2020;34:1599–605.
16. Morgan RA, McGowan TW, McGowan CM. Prevalence and risk factors for hyperinsulinaemia in ponies in Queensland, Australia. Aust Vet J. 2014;92:101–6.
17. Norton EM, Schultz NE, Rendahl AK, Mcfarlane D, Geor RJ, Mickelson JR, et al. Heritability of metabolic traits associated with equine metabolic syndrome in Welsh ponies and Morgan horses. Equine Vet J. 2019;51:475–80.
18. Morgan RA, Keen JA, McGowan CM. Treatment of equine metabolic syndrome: a clinical case series. Equine Vet J. 2016;48:422–6.
19. Bamford NJ, Potter SJ, Baskerville CL, Harris PA, Bailey SR. Influence of dietary restriction and low-intensity exercise on weight loss and insulin sensitivity in obese equids. J Vet Intern Med. 2019;33:280–6.
20. Bailey SR, Habershon-Butcher JL, Ransom KJ, Elliott J, Menzies-Gow NJ. Hypertension and insulin resistance in a mixed-breed population of ponies predisposed to laminitis. Am J Vet Res. 2008;69:122–9.
21. Ungru J, Blüher M, Coenen M, Raila J, Boston R, Vervuert I. Effects of body weight reduction on blood adipokines and subcutaneous adipose tissue adipokine mRNA expression profiles in obese ponies. Vet Rec. 2012;171:528.
22. Altman DG, Royston P. The cost of dichotomising continuous variables. Br Med J. 2006;332:1080.
23. Tatum RC, McGowan CM, Ireland JL. Evaluation of the sensitivity and specificity of basal plasma adrenocorticotrophic hormone concentration for diagnosing pituitary pars intermedia dysfunction in horses: a systematic review. Vet J. 2021;275:105695.
24. Knowles EJ, Elliott J, Harris PA, Menzies-Gow NJ. Laminitis risk categories for serum insulin concentrations differ between two analysers. Equine Vet J. 2022;54(S57):24–5.

SUPPORTING INFORMATION

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

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