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ORIGINAL ARTICLE

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Epidemiological investigation of insulin dysregulation in Shetland and Welsh ponies in Australia

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Abstract

Background: Insulin dysregulation (ID) is central to equine metabolic syndrome. There are limited epidemiological studies investigating dynamic testing of ID in ponies.

Objectives: To evaluate prevalence and risk factors for ID through dynamic testing of hyperinsulinaemia (DHI) and insulin resistance (IR).

Study design: Cross-sectional.

Methods: Sex, age, breed, height, cresty neck score (CNS), body condition score (BCS), laminitis, *HMGA2*:c.83G>A genotype and pituitary pars intermedia dysfunction (PPID) status were documented. Dynamic hyperinsulinaemia was diagnosed with an oral sugar test (OST) and IR with an insulin tolerance test (ITT). Owners completed surveys reporting activity, laminitis history and perception of body condition using a (1–9) visual analogue scale (VASo). Ordinal scores were converted to binary outcomes for CNS (≤2/5 or ≥3/5), BCS and VASo (≤6/9 or ≥7/9). Variables associated with insulin concentrations, glucose reduction after the ITT and laminitis were evaluated with mixed effects regression models accounting for random effects of farms.

Results: Among 167 ponies tested, median (range) age was 9 (4–21) years and BCS was 6 (4–8). Prevalence (95% confidence interval [CI]) of ID was 61 (53–68)%. Factors associated with insulin concentrations (estimate [95% CI]; μIU/mL) 60 min post-OST were: age (1.07 [1.02–1.11]), CNS (≥3/5, 1.52 [1.04–2.23]) and VASo (≥7/9, 1.75 [1.09–2.79]); and 90 min post-OST were: age (1.08 [1.03–1.12]), CNS (≥3/5, 1.80 [1.22–2.64]), VASo (≥7/9, 2.49 [1.52–4.08]) and sex (male, 0.64 [0.45–0.91]). Factors associated with glucose reduction after the ITT (estimate [95% CI]; %) were: age (−1.34 [−2.01 to −0.67]), sex (female, −6.21 [−11.68 to −0.74]) and VASo (≥7/9, −1.74 [−18.89 to −4.78]). Factors associated with laminitis (odds ratio [95% CI]) were DHI (4.60 [1.68–12.58]), IR (3.66 [1.26–10.61]) and PPID (11.75 [1.54–89.40]).

Main limitations: Single time-point sampling, laminitis definition and diet analysis.
Conclusions: Ageing, being female and owner-perceived obesity were associated with ID.

KEYWORDS
equine metabolic syndrome, horse, hyperinsulinaemia, insulin resistance, laminitis

1 | INTRODUCTION

Insulin dysregulation (ID), encompassing both hyperinsulinaemia and insulin resistance (IR), is the central component of equine metabolic syndrome (EMS), which is a collection of phenotypic traits associated with an increased risk for hyperinsulinaemia-associated laminitis (HAL).[1] Ponies are at increased risk for ID, with a complex interplay between genetics and environment influencing disease expression.[2]

Inconsistent epidemiological risk factors are reported, including obesity, regional adiposity, diet, exercise, sex, breed, season, age and pituitary pars intermedia dysfunction (PPID).[3–9] Despite advances in the understanding of ID and EMS, there is a paucity of epidemiological studies investigating ID in horses and ponies through evaluation of both hyperinsulinaemia and IR with dynamic testing methods. Further, there are limited studies exploring epidemiological and genetic factors associated with ID. Recent studies have investigated a genetic variant, *HMGA2*:c.83G>A, that was correlated with basal insulin in ponies, but complete assessment with other epidemiological factors and dynamic assessment of ID is limited.[10,11] Epidemiological investigations in ponies are crucial to evaluate the prevalence and risk factors associated with ID, with the intention to identify at-risk ponies earlier and improve management to limit laminitic episodes.

The objective of this study was to investigate the prevalence and associated risk factors for ID in ponies through dynamic testing of hyperinsulinaemia and IR. We hypothesised that ID prevalence would be higher than previously reported when evaluated with dynamic versus basal tests, and that obesity, regional adiposity and the *HMGA2* variant would be associated with ID.

2 | MATERIALS AND METHODS

2.1 | Animals

Welsh and Shetland ponies, aged ≥4 years, were recruited from two states (Queensland and Victoria) in Australia. Welsh ponies were separated into their four sections (A–D) based on conformation, height and pedigree according to the Welsh Pony and Cob Society of Australia. Recruitment was through direct contact with owners who were primary or secondary contacts of study investigators (approximately 70% of ponies), and advertisements in magazines, social media and at equestrian events. Geldings, stallions and mares were included. Pregnant and lactating mares were excluded. Animals were evaluated between August 2020 and June 2021.

Most ponies were evaluated during on-farm visits, with a small subset presenting to UQ VETS Equine Specialist Hospital. After clinical assessment to assess general health, height at the withers, weight (estimated using a pony-standardised weight tape),[12] cresty neck score (CNS; 0–5 scale)[13] and body condition score (BCS; 1–9 scale)[14] were determined. Current laminitis was assessed utilising the modified-Obel scoring system (0–12 scale) involving a three-stage examination, including examining the pony standing, lifting the forelimbs and walking a straight line and circle.[15] Assessment of BCS, CNS and modified-Obel score were all performed by a single veterinarian. Hair roots were collected from the mane.

2.2 | Endocrine testing

Dynamic assessment of IR and hyperinsulinaemia were performed on sequential days. On the first day with no feed restriction, IR was evaluated with an insulin tolerance test (ITT).[16] A temporary catheter (18G; Surflo, Terumo) was placed in a jugular vein and blood was collected at baseline and 30 min following intravenous injection of 0.1 IU/kg BW of regular insulin (Actrapid, Novo Nordisk). Blood glucose was measured using a glucometer (AlphaTrak 2, Zoetis).[17] Ponies were monitored closely during and up to 90 min after the test for clinical signs of hypoglycaemia, such as sweating or weakness. If any pony showed clinical signs of hypoglycaemia or if blood glucose was <2 mmol/L, 50 mL/300 kg of 50% dextrose solution (Baxter) was given intravenously. After the 30-min blood sample, all ponies were given 60 mL of molasses orally and offered a small grain meal. Blood glucose was subsequently measured until concentrations returned to baseline values.

The following day, dynamic hyperinsulinaemia (DHI) was assessed with an oral sugar test (OST), with 0.45 mL/kg of corn syrup (Karo Light™) administered orally and blood collected 60 and 90 min later.[18,19] No grain or concentrates were given for 8 h, hay for 4 h and ponies were taken off pasture at least 1 h prior to testing.[20] Blood was collected into silica-containing clot tubes (serum vacutainer, BD) and allowed to clot at ambient temperature, then placed on ice. Samples were centrifuged within 6 h and serum aliquots were stored at −80°C until analysis. Insulin concentrations were measured using a chemiluminescent assay (Immulite 1000, Siemens) with the lower and upper limits of detection being 2 and 300 μIU/mL, respectively, with samples >300 μIU/mL not diluted.[21]

For ponies older than 10 years of age, PPID was evaluated by collecting blood into EDTA tubes (EDTA vacutainer, BD) for

measurement of basal adrenocorticotropic hormone (ACTH) concentration using a chemiluminescent assay (Immulite 1000, Siemens).[22] This age was selected as PPID has been rarely reported in horses and ponies <10 years of age.[23]

2.3 | Surveys

Owners completed a survey, which included closed-ended questions on breed, sex, activity, diet components and amounts (pasture, hay and complementary feeds), exercise level and laminitis history (Supplementary Item S1). Owner assessment of body condition was performed using a visual analogue scale (VASo) with a linear score of 1–9; no formal training was provided by the investigators.

2.4 | Case classification

IR was diagnosed if blood glucose concentration failed to decrease by ≥50% from baseline after insulin administration.[16] Basal hyperinsulinaemia (BHI) and DHI were defined as a serum insulin concentration >20 μIU/mL at baseline and >65 μIU/mL at 60 or 90 min after corn syrup administration.[20] ID was defined as the presence of one or more of BHI, DHI or IR. Laminitis was defined as modified-Obel score >1[15] or if owners reported a previous episode of laminitis in the absence of systemic illness. A diagnosis of PPID was based on seasonally adjusted basal ACTH concentration cut-offs and clinical signs.[24]

2.5 | Genotyping

From hair roots, DNA was isolated as per the manufacturer's instructions (Gentra Puregene Tissue Kit, Qiagen). A TaqMan SNP genotyping assay for the *HMGA2*:c.83G>A variant was performed, as previously described.[11] All samples were run in duplicate with positive controls. BioRad's CFX Manager Software (version 3.1) was used to analyse the results.

2.6 | Data analysis

Prior to study commencement, a power calculation was performed to detect a significant difference between ID and non-ID ponies using BHI cut-off of >20 μIU/mL,[20] with $\alpha = 0.05$ and 80% power. A total of 72 ponies per group was calculated as being required.

Statistical analyses were performed using R software (version 4.2.2).[25] Descriptive statistics, followed by regression analyses (lme4 package)[26] applying univariable and multivariable mixed effects models were performed. Insulin concentration at baseline and 60 and 90 min after OST, and percent reduction in baseline glucose concentration after the ITT were analysed as continuous outcomes and reported as estimates, whereas ID and laminitis status were analysed as dichotomous outcomes and reported as odds ratios. Normality was evaluated for each dependent variable using the Shapiro–Wilk test. Insulin concentrations (basal and dynamic) were not normally distributed, so were log-transformed for analyses and back-transformed for reporting.

Variables evaluated were breed, sex, age, season, diet, pony activity, PPID status, BCS, CNS, VASo and genotype (*HMGA2*:c.83G>A variant). A small number of ponies had missing data within each variable; therefore, different denominators are reported for certain variables. Welsh Sections A and B, and Sections C and D, were grouped together due to similarities with height and low numbers in some sections. Pony activity was grouped into breeding and paddock, showing or other (low-intensity riding, including trail riding and pony club). Season groupings included summer (December–February), autumn (March–May), winter (June–August) and spring (September–November). Diet-related information in the owner surveys was inconsistent. Attempts were made to categorise the type and amount of feed provided; however, none of the diet variables were found to be significant during initial model screening. Therefore, diet was not evaluated further.

Regional obesity was categorised as CNS ≤ 2/5 or ≥ 3/5.[13] Adiposity was categorised as BCS ≤ 6/9 or ≥7/9.[13] Genotypes were categorised as homozygous for the variant allele (AA), heterozygous (GA) or homozygous for the wild-type allele (GG). Initial analysis for insulin concentration and percent reduction after the ITT found no difference between mares, stallions and geldings and they were subsequently categorised as female or male. Univariable mixed models were performed on each predictor variable using the clients (farms) as random effects. A bidirectional stepwise model selection approach was then used, with the initial set of predictors having $p < 0.2$ from univariable models considered for inclusion. Predictors that remained with $p < 0.05$ after stepwise elimination of the least significant variables were retained in the final multivariable regression analyses. Additionally, potential confounding effects, interactions and multicollinearity were explored in the multivariable mixed model analyses by identifying significantly associated covariates and interaction terms. Although significant interactions were observed (e.g. season, breed and genotype), due to the substantial disparities in sample sizes across various categories, analyses involving interactions were omitted. The inclusion of interactions in this context was avoided to prevent potential confounding effects and ensure the reliability of findings. Marginal and conditional *R*-squared values were computed to examine the impact of fixed effects, both excluding and including the random effect of farm, on the mixed effects models. Agreement between body condition assessment by the veterinarian and owners was evaluated using the intraclass correlation coefficient (ICC). A two-way model was implemented to assess absolute agreement between the single score of BCS and VASo for each horse. Additionally, Cohen's Kappa (*K*) was employed to assess agreement after transforming the 1 to 9 scale scores into a dichotomous grouping (≤6 or ≥7). Both ICC and K were computed using the irr package.[27] For ICC interpretation, < 50% is poor, 50–75% is moderate, 75%–90% is good and > 90% is excellent reproducibility.[28]

TABLE 1 Final multivariable mixed effect linear regression models with 'farm' as a random effect for insulin concentrations (μIU/mL) at baseline, 60 and 90 min after the oral sugar test (OST) and percent glucose reduction for the insulin tolerance test (ITT). All outcome variables were log transformed for analyses and the model outputs were subsequently back-transformed for reporting of estimates with 95% confidence intervals (CI).

	Estimates	95% CI	*p* value	Marginal r^2	Conditional r^2
Outcome: basal insulin ($n = 166$)				0.09	0.45
Genotype					
G/G	Reference				
G/A	1.89	1.24–2.89	0.004		
A/A	1.31	0.77–2.23	0.31		
VASo					
≤6/9	Reference				
≥7/9	2.30	1.47–3.59	<0.001		
Outcome: insulin 60 min post-OST ($n = 161$)				0.03	0.26
Age	1.07	1.02–1.11	0.004		
VASo					
≤6/9	Reference				
≥7/9	1.75	1.09–2.79	0.021		
CNS					
≤2/5	Reference				
≥3/5	1.52	1.04–2.23	0.031		
Outcome: insulin 90 min post-OST ($n = 148$)				0.21	0.41
Age	1.08	1.03–1.12	0.001		
VASo					
≤6/9	Reference				
≥7/9	2.49	1.52–4.08	<0.001		
CNS					
≤2/5	Reference				
≥3/5	1.80	1.22–2.64	0.003		
Sex					
Female	Reference				
Male	0.64	0.45–0.91	0.013		
Outcome: % glucose reduction post-ITT ($n = 164$)				0.15	0.27
Age	−1.34	−2.01 to −0.67	<0.001		
VASo					
≤6/9	Reference				
≥7/9	−11.74	−18.89 to −4.78	0.001		
Sex					
Male	Reference				
Female	−6.21	−11.68 to −0.74	0.026		

Note: All outcomes were log-transformed for analyses and back-transformed for reporting.
Abbreviations: CNS, cresty neck score; VASo, owner-assessed body condition using visual analogue scale.

3 | RESULTS

3.1 | Animals

A total of 167 ponies were recruited from 27 farms, with the median number of ponies at each farm being 3 (range 1–37; interquartile range [IQR] 1–9). There were 94 mares (57%), 50 geldings (30%) and 22 stallions (13%) among 61 Welsh Section A/B (37%), 34 Welsh Section C/D (20%) and 72 Shetland ponies (43%). Median age was 9 (range, 4–21; IQR, 6–12) years; median BCS was 6 (range, 4–8; IQR, 6–7) and median CNS was 3 (range, 0–5; IQR, 2–3). Eleven ponies (7%) were diagnosed with PPID based on clinical signs and ACTH

concentrations. Twenty-nine (17%) ponies were evaluated in summer, 88 in autumn (53%), 14 in winter (8%) and 36 in spring (22%).

Ninety ponies (54%) had activity assigned as breeding/paddock, 62 (37%) as showing and 15 (9%) as other riding activities. Median VASo was 5 (range, 3–8; IQR, 5–8), with 114/164 (70%) owners considering their pony to be in ideal condition. Owners underestimated their pony's body condition by an average of 1 score (range, −2 to 4; IQR, 0–2) using the VASo scale compared with BCS performed by the veterinarian. The agreement between body condition scoring by owners and the veterinarian was found to be in the poor to moderate range (ICC = 0.34; $p = 0.005$) (Figure S1). Cohen's Kappa for the dichotomous groupings also indicated poor to fair agreement (K = 11.7%, 95% CI: 3.7–30%).

HMGA2:c.83G>A A allele frequency among 160 ponies was 55 (95% confidence interval [CI], 47%–63% (Table S1).

3.2 | Basal insulin concentration

Median basal insulin concentration was 4.3 (IQR, 2–11.1) μIU/mL with 25 ponies (15%) classified as having BHI. Univariable analysis found genotype, age and VASo to be significantly associated with basal insulin concentration (Table S2). In the final multivariable model, basal insulin was associated with genotype (G/A compared with reference G/G, estimate 1.89; 95% CI 124–2.89) and VASo ≥ 7/9 (estimate 2.30; 95% CI 1.47–3.59) (Table 1).

3.3 | Insulin concentration after OST

Median insulin concentration 60 min after corn syrup administration was 34.5 (IQR, 13.5–91.9) μIU/mL with 52 ponies (32%) classified as having DHI at 60 min. Univariable analysis found age, CNS, VASo and PPID status to be significantly associated with insulin concentration 60 min after the OST (Table S2). In the final multivariable model, insulin concentration after the OST at 60 min was associated with increasing age (estimate 1.07; 95% CI 1.02–1.11), CNS ≥ 3/5 (estimate 1.52; 95% CI 1.04–2.23) and VASo ≥ 7/9 (estimate 1.75; 95% CI 1.09–2.79) (Table 1).

Median insulin concentration 90 min after OST was 32.2 (IQR, 14.2–127.5) μIU/mL with 54 ponies (36%) classified as having DHI at

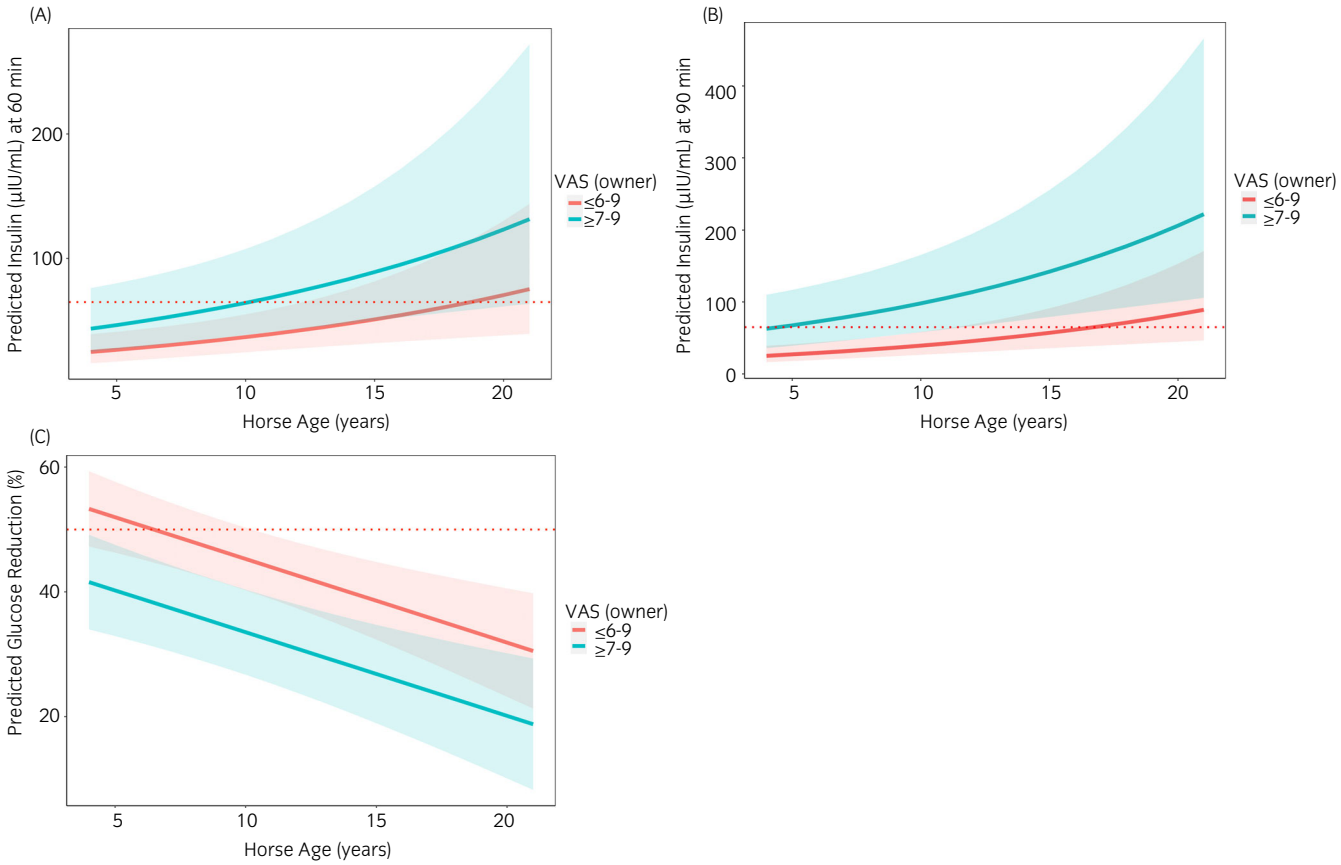

FIGURE 1 Predicted insulin concentration (A) 60 min and (B) 90 min after an oral sugar test, and (C) percent glucose reduction after the insulin tolerance test with the multivariable generalised linear regression mixed effects model for increasing age and visual analogue score as assessment of body condition by the owner (VASo) ≤6/9 and ≥7/9, with client (farm) as random effect. Data of dependent variables were log-transformed for analysis and subsequently back-transformed for reporting. Red line represents body condition score ≤6/9 and blue line represents ≥7/9. Shading represents 95% confidence intervals. Red dashed line represents accepted cut-offs for dynamic hyperinsulinaemia (A and B; insulin concentration >65 μIU/mL 60 and 90 min after administration of corn syrup) and insulin resistance (C; glucose reduction <50% from baseline 30 min after administration of insulin IV).

TABLE 2 Final multivariable mixed effects logistic regression models with 'farm' as a random effect for insulin dysregulation (ID), defined as the presence of one or more of basal hyperinsulinaemia, dynamic hyperinsulinaemia (DHI) or insulin resistance (IR) and laminitis. Model outputs transformed to odds ratio (OR) with 95% confidence intervals (CI).

	OR	95% CI	*p* value	Marginal r^2	Conditional r^2
Outcome: ID ($n = 167$)				0.28	0.43
Age	1.27	1.14–1.41	<0.001		
VASo					
≤6/9	Reference				
≥7/9	9.64	2.44–38.16	0.001		
Outcome: laminitis ($n = 167$)				0.26	0.53
DHI	4.60	1.68–12.58	0.02		
IR	3.66	1.26–10.61	0.02		
PPID	11.75	1.54–89.40	0.003		

Abbreviations: PPID, pituitary pars intermedia dysfunction; VASo, owner-assessed body condition using visual analogue scale.

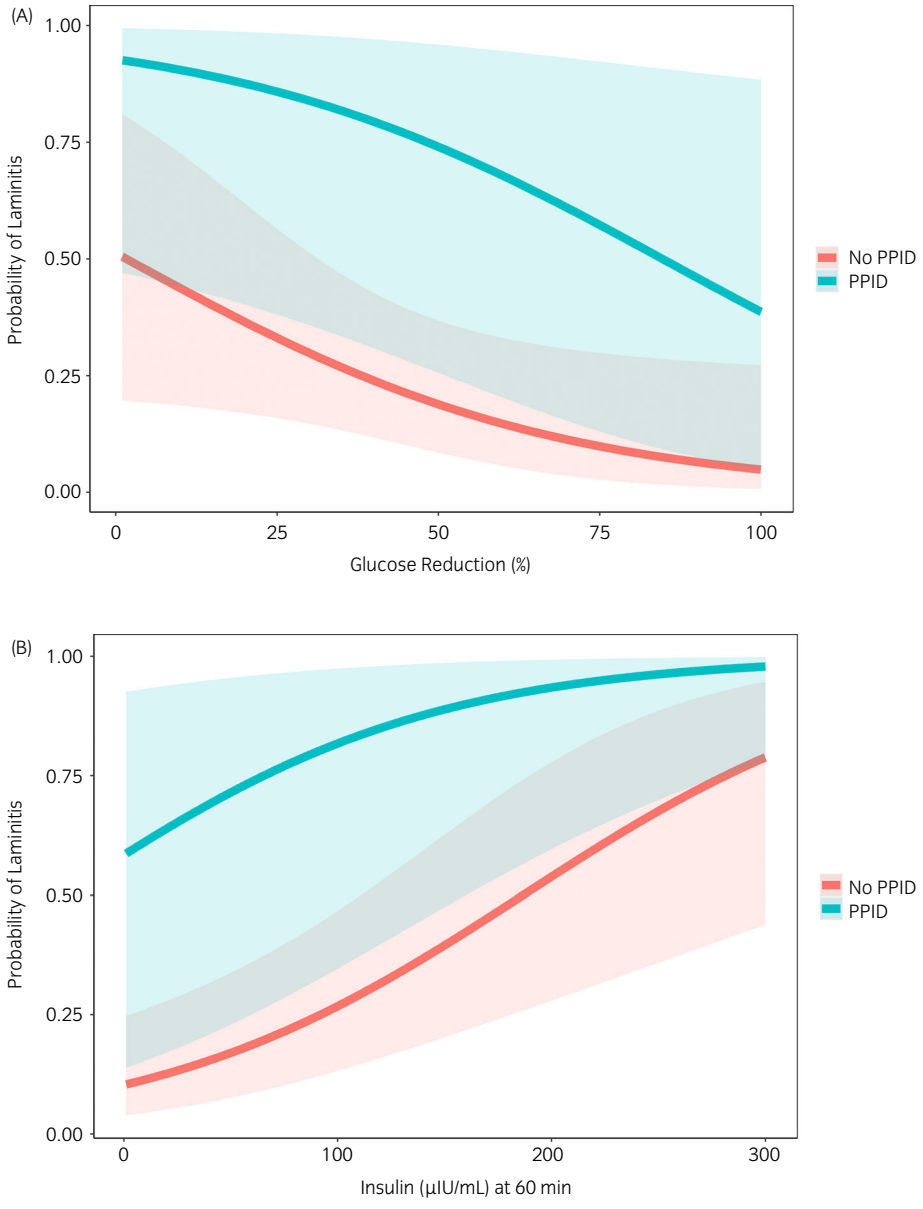

FIGURE 2 Probability of laminitis predicted with the multivariable generalised logistic regression mixed effects model for (A) percent glucose reduction after the insulin tolerance test, and (B) insulin concentration 60 min after the oral sugar test, with client (farm) as random effect. Red line represents pituitary pars intermedia dysfunction (PPID) negative and blue line represents PPID positive. Shading represents 95% confidence intervals.

90 min. Univariable analysis found age, sex, BCS, CNS and VASo to be significantly associated with insulin concentration 90 min after the OST (Table S2). In the final multivariable model, insulin concentration after the OST at 90 min was associated with increasing age (estimate 1.08; 95% CI 1.03–1.12), sex (male estimate 0.64; 95% CI 0.45–0.91), CNS ≥ 3/5 (estimate 1.80; 95% CI 1.22–2.64) and VASo ≥ 7/9 (estimate 2.49; 95% CI 1.52–4.08) (Table 1, Figure 1).

3.4 | Insulin sensitivity with insulin tolerance test

Mean percent glucose reduction after the ITT was 45% from baseline (IQR, 30%–63%), with 90 (55%) ponies classified as IR. Univariable analysis found age, sex, CNS, VASo and activity to be significantly associated with percent glucose reduction after the ITT (Table S2). In the final multivariable model, percent reduction after the ITT was associated with age (estimate −1.24; 95% CI −2.01 to −0.67), sex (female estimate −6.21; 95% CI −11.68 to −0.74) and VASo ≥ 7/9 (estimate −11.74; 95% CI −18.89 to −4.78) (Table 1, Figure 1).

3.5 | Insulin dysregulation

Overall, 102 (61%) ponies were classified as ID (95% CI, 53%–68%). Univariable analysis found breed, age, CNS, VASo and activity to be significantly associated with ID (Table S2). In the final multivariable model, ID was associated with increasing age (OR 1.27; 95% CI 1.14–1.41) and VASo ≥ 7/9 (OR 9.64; 95% CI 2.44–38.16) (Table 2).

3.6 | Laminitis

Forty seven ponies (28%) were classified as laminitic, 10% (16/167) had current laminitis at the time of examination (median modified-Obel score of 4 [range, 2–8; IQR, 3–5]). Univariable analysis found age, PPID status, IR status, BHI status and DHI status at 60 and 90 min after the OST to be significantly associated with laminitis (Table S2). In the final multivariable model, laminitis was associated with DHI (OR 4.60; 95% CI 1.68–12.58), IR (OR 3.66; 95% CI 1.26–10.61) and PPID (Table 2, Figure 2).

Of those with laminitis, 15% (7/47) had PPID (OR 11.75; 95% CI 1.54–89.40), of which six of seven had IR and only three of six had concurrent DHI.

4 | DISCUSSION

This epidemiological study found that: (1) ID was highly prevalent, affecting 61% of Shetland and Welsh ponies; (2) increasing age, being female and owner-perceived obesity were associated with hyperinsulinaemia and IR; (3) having DHI, IR or PPID were associated with an increased risk for laminitis; and (4) owners underestimated body condition relative to appraisal by a veterinarian.

This study found a higher prevalence of ID than previously reported, with 61% of ponies diagnosed with ID; comprising of 55% with IR, 15% with BHI and 31% and 32% with DHI at 60 and 90 min, respectively. Previous studies identified a higher prevalence of BHI than our study, with 18%–27% of horses and ponies being diagnosed with BHI.[9,29] In contrast, a higher prevalence of DHI was identified in our study compared with 16% reported in a recent study of light-breed horses.[7] Breed differences might explain the higher prevalence among our population of ponies, as ponies exhibit a higher incidence of ID than light-breed horses.[6] Dynamic tests for hyperinsulinaemia, such as the OST are considered superior to basal insulin concentration due to increased sensitivity, as they stimulate the enteroinsular axis.[30,31]

Obesity was associated with IR, BHI and DHI, with ponies scoring ≥7/9 by their owners using a VAS, almost 10 times more likely to have ID. Interestingly, it was owner assessment of obesity using the VASo, rather than scoring of BCS by the study investigators, that was associated with ID. Owners underestimated body condition by an average of 1 grade using the VASo scale compared to a veterinarian using the BCS scale, which is consistent with previous studies that found owners to have difficulties in identifying obesity, especially in their own horses and ponies.[32,33] The propensity for owners to underscore their ponies suggests that marked obesity (BCS ≥ 8) might be associated with ID. We further tested this theory by reanalysing data with adiposity categorised as BCS ≤ 7/9 or ≥8/9 but due to the relatively small numbers of ponies with BCS and VASo ≥ 8/9 statistical significance was not quite reached. Studies in horses have found varying associations between adiposity and inflammatory markers that might contribute to the development of ID, with regional adiposity (such as cresty neck) appearing to play a more important role.[9,34–36] It is unknown why regional adiposity was associated with DHI, but not with IR in our cohort. We had a large proportion of Shetland ponies and stallions, that have a broader neck, potentially creating a falsely increased CNS.

Ponies with PPID were almost 12 times more likely to have current or previous laminitis, although wide odds ratios were obtained due to the relatively small number of ponies with PPID. This is an interesting finding, as there have been conflicting results in previous studies, depending on whether ID was included in the clinical evaluation. Studies have found that horses with PPID (but without hyperinsulinaemia) did not have histological evidence of laminitis,[37] but a combined diagnosis of EMS and PPID increased hyperinsulinaemia and laminitis risk,[38] with the degree of BHI correlated with laminitis severity at the time of PPID diagnosis.[39] No direct association between PPID and tests for ID were identified. This is consistent with a prior study in which horses with PPID did not have reduced insulin sensitivity compared with age-matched controls, and not all PPID horses were hyperinsulinaemic.[40] PPID and ID are two separate entities, and it is unlikely that PPID directly causes ID, but it could exacerbate concurrent ID via the action of pro-opiomelanocortin-derived peptides.[41] Another plausible explanation is that with increased age, there is increased chance of PPID and ID occurring in the same animal.

Age was associated with IR and hyperinsulinaemia in our study. This finding is not novel, with increasing age associated with reduced insulin sensitivity and increased insulin secretion.[9,42–44] This emphasises the importance of monitoring ponies as they age; for every year older, ponies were almost 30% more likely to have ID. Studies have highlighted the effects of inflammation and age, coined 'inflammaging'.[45] Investigation of the pathophysiological features was beyond the scope of our study; nonetheless, the importance of increasing age on ID likelihood in ponies was confirmed.

Consistent with previous reports, mares had an increased risk of DHI and IR.[42] No associations were identified with ID and laminitis between breed or season, as found previously,[4,8,9] although both Shetlands and Welsh ponies are anecdotally prone to both conditions. Further, we did find significant interactions between season and breed in initial model analysis, but there were significant disparities in sample sizes and thus reliable interpretation was not possible. Larger sample sizes and equal sample sizes would have alleviated this issue and improved the final model. There were also no associations found for pony activity, as observed in a recent study.[42] However, sampling was performed during COVID-19 restrictions with many ponies not in usual levels of work.

Consistent with previous reports, *HMGA2* genotype was associated with basal insulin concentration,[10,11] although not with other measures of ID. Since EMS is a complex genetic trait,[2] further studies are required to unravel the effects of contributing genes, including associations with other EMS-associated biochemical parameters such as plasma lipid profiles.

Limitations of this study include the potential for centripetal bias in the recruitment of ponies, laminitis classification and quality of owner-reported information. Ponies were not followed prospectively to monitor for development of laminitis and there is no gold-standard definition of laminitis.[46] Our laminitis definition was deemed acceptable, as recent studies have identified that owner-reported laminitis has good agreement with veterinary-confirmed laminitis,[47] and not including these cases would underestimate the incidence of laminitis. Compared with veterinarian-diagnosed laminitis, owner-reported has good specificity, though lower sensitivity.[47]

Convenience sampling might have introduced the potential for centripetal bias and a reduction in extrinsic validity with variation in owners. Our study included many stud farms, and breeders might be more educated than the average owner of a single pony. We attempted to sample from a range of owners, including those involved in breeding, showing and pleasure activities, and owners with small numbers of ponies, to reflect the general population of ponies. Selection bias was also possible, with owners volunteering their ponies for inclusion, potentially falsely increasing the prevalence of ID. We attempted to mitigate bias through direct contact of owners and breeders and sampling all available ponies on each farm. Owner-perceived body condition was performed using a 1–9 linear VASo without providing owners with detailed scoring instructions or training; however, some owners might already have been familiar with the 9-point Henneke system.[14]

5 | CONCLUSIONS

This study identified a high prevalence of ID in Shetland and Welsh ponies. Ageing, being female and owner-perceived obesity were associated with an increasing risk for ID, likely increasing the risk of laminitis. Periodic endocrine testing as ponies age is warranted.

AUTHOR CONTRIBUTIONS

Brianna L. Clark: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; writing – original draft; writing – review and editing. **Elaine M. Norton:** Conceptualization; funding acquisition; methodology; resources; supervision; validation; writing – review and editing. **Nicholas J. Bamford:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing – review and editing. **Imtiaz A. S. Randhawa:** Formal analysis; resources; software; validation; writing – review and editing. **Kate L. Kemp:** Data curation; investigation; project administration; writing – review and editing. **Molly E. McCue:** Conceptualization; funding acquisition; resources; writing – review and editing. **François-René Bertin:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – review and editing. **Allison J. Stewart:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing – review and editing.

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[Correction added on 9 January 2024, after first online publication: CAUL funding statement has been added.]

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest.

DATA INTEGRITY STATEMENT

BL Clark had full access to all data and took responsibility for the integrity of the data and accuracy of the data analysis.

ETHICAL ANIMAL RESEARCH

Approved by the University of Queensland Animal Ethics Committee, approval number SVS/210/20 and the University of Queensland Institutional Human Research Ethics, approval number 2020001208.

INFORMED CONSENT

Signed owner consent was obtained for animals' inclusion and survey data collection.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/evj.14044.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Data sharing exemption granted by the editor.

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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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