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Effect of pergolide treatment on insulin dysregulation in horses and ponies with pituitary pars intermedia dysfunction

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Abstract

Background: Due to the high frequency of laminitis reported for both conditions, the relationship between pituitary pars intermedia dysfunction (PPID) and insulin dysregulation (ID), and the potential role of dopamine in modifying insulin secretion, requires further investigation.

Objectives: To evaluate the effect of pergolide mesylate on insulin sensitivity and postprandial insulin and glucose responses in horses and ponies with ID, both with or without concurrent PPID.

Study design: Randomised crossover study.

Methods: Sixteen horses and ponies, comprising eight matched pairs (PPID+ID or ID-only), were given pergolide mesylate at a dose of 2 μg/kg bwt orally once daily for 4 weeks (plus a 4-week non-treatment control period, with a 4-week washout between phases). A combined glucose and insulin tolerance test (CGIT) and a standard meal test (SMT; containing 1.1 g/kg bwt of starch and 0.1 g/kg bwt of free sugars), were performed before and after each treatment period to determine insulin sensitivity and postprandial insulin and glucose responses, respectively. Variables derived from the CGIT and SMT were analysed using linear mixed models.

Results: Pergolide treatment did not alter any of the variables derived from the CGIT in either the PPID+ID or ID-only groups (all $p > 0.05$). For the SMT, insulin responses were reduced by pergolide treatment for the PPID+ID group, with Δ change values for the total area under the curve for insulin over 300 mins (estimated marginal mean [95% confidence interval]) being −25.4 (−39.9 to −7.3) min·mIU/mL ($p = 0.03$) and Δ change values for peak insulin concentration being −100 (−167 to −29) μIU/mL ($p = 0.04$). No effect of pergolide treatment was detected for the ID-only group.

Main limitations: Number of animals and heterogeneity among groups.

Conclusions: Pergolide had no effect on tissue insulin sensitivity. However, the results suggest that postprandial hyperinsulinaemia may be limited by this dopamine receptor agonist in animals with PPID plus ID.

KEYWORDS
dopamine, endocrine, horse, hyperinsulinaemia, insulin resistance, laminitis

1 | INTRODUCTION

Pituitary pars intermedia dysfunction (PPID) is a common endocrine disorder of horses and ponies older than 15 years.[1] Laminitis is a serious potential sequela in clinical cases of PPID,[2] and is a common reason for euthanasia.[3] Insulin dysregulation (ID), characterised by hyperinsulinaemia and/or tissue insulin resistance (IR), is the underlying cause of endocrinopathic laminitis and is frequently identified in cases of PPID with laminitis.[4–6] High insulin concentrations have been correlated with laminitis severity and are predictive of non-survival among horses and ponies with PPID.[7,8] The relationship between ID and PPID remains unclear, as not all horses and ponies with PPID have ID,[9] although the overproduction of pro-opiomelanocortin-derived peptides has been speculated to exacerbate ID in cases of PPID.[10]

The most common treatment for PPID is pergolide mesylate, a dopamine receptor agonist with demonstrated efficacy for reducing adrenocorticotropic hormone (ACTH) concentrations and improving clinical signs.[11,12] Since PPID might exacerbate ID, there is interest in whether dopaminergic agonists can reduce hyperinsulinaemia and improve tissue insulin sensitivity. Dopamine has been demonstrated to attenuate glucose-stimulated insulin secretion in other species by acting through dopamine receptors on pancreatic β-cells.[13–16] Two previous studies in horses found that pergolide and cabergoline (both dopamine receptor agonists) did not improve tissue insulin sensitivity when evaluated using intravenous testing methods.[17,18] However, bromocriptine (another dopamine receptor agonist) was shown to reduce postprandial insulin secretion after a high starch meal.[19]

The objective of the current project was to evaluate the effect of pergolide treatment on insulin and glucose dynamics in horses and ponies with ID, with and without concurrent PPID, using both intravenous and oral dynamic testing protocols. We hypothesised that, in horses and ponies with ID, pergolide treatment would reduce postprandial insulin responses only in animals with concurrent PPID, without affecting tissue insulin sensitivity.

2 | MATERIALS AND METHODS

2.1 | Animals

Thirty-five horses and ponies aged ≥15 years, kept at an equine rescue sanctuary and a nearby private farm in Victoria, Australia, were considered for inclusion in this study. From the 35 horses, two groups of 8 animals were selected: (1) animals with PPID and ID (PPID+ID group) and (2) animals with ID but without PPID (ID-only group). The number of animals to be enrolled in each group was based on previous studies where insulin sensitivity was compared between groups,[20,21] where $n = 8$ had a power $(1 - \beta)$ of 0.8 to detect a 23% change in insulin sensitivity and 13% change in basal insulin concentration when $\alpha = 0.05$. Screening for PPID and ID was performed using a combined thyrotropin-releasing hormone (TRH) stimulation test and insulin tolerance test (ITT) protocol, as previously described.[22] Briefly, a temporary catheter (18G; Surflo, Terumo) was placed in a jugular vein and a baseline blood sample was collected, followed by administration of recombinant TRH (1 mg for animals ≥250 kg bwt and 0.5 mg for animals <250 kg bwt; Sigma-Aldrich) and insulin (0.1 IU/kg bwt; Actrapid, Novo Nordisk), intravenously. Blood samples were collected 10 and 30 min after TRH and insulin administration and placed into tubes containing EDTA or lithium heparin anticoagulant (Vacutainer, BD). Blood glucose was evaluated using a hand-held glucometer (Accu-Chek, Roche) and plasma ACTH concentrations were determined by chemiluminescent immunoassay (Immulite 1000, Siemens).[23]

For logistical reasons, so that the main study could be performed during winter and spring, screening was performed at the end of May (late autumn in Southern hemisphere). Animals were diagnosed with PPID if they demonstrated at least two consistent clinical signs (for example, hypertrichosis and epaxial muscle atrophy) and had basal ACTH concentrations >100 pg/mL and/or ACTH concentrations >430 pg/mL at 10 min after TRH administration.[24] Animals were classified as non-PPID if they did not demonstrate any consistent clinical signs and had basal ACTH concentrations <100 pg/mL. Animals were diagnosed with ID if blood glucose concentration failed to decrease by at least 50% from baseline concentrations.[25] Following screening, 8 PPID+ID animals were paired with 8 control animals (ID-only); matched as far as possible for age and breed. Signalment details and results of screening tests of individual animals are provided in Table S1.

2.2 | Study design

A randomised crossover study (2 × 2 design) with two groups (PPID+ID or ID-only) and two treatments (pergolide or no treatment) was performed. Treatments were administered to four animals from each group for 4 weeks in each phase, with a 4-week washout period between phases. Animals received the alternative treatment (pergolide or no treatment) in the second phase. During treatment, pergolide mesylate (Prascend, Boehringer Ingelheim Vetmedica) was administered at 2 μg/kg bwt (rounded to the nearest 0.5 mg increment) orally, once daily. Two separate tests were performed to evaluate insulin and glucose dynamics at the start and finish of each treatment phase, 5 days apart: firstly, a combined glucose-insulin tolerance test (CGIT) on day −5 and day 29, and secondly, a standard meal test (SMT) on

day 0 and day 34. Bodyweight was measured using calibrated horse scales at the start and finish of each phase. Before each test (CGIT and SMT), animals were allowed access to hay and water overnight. On the morning of each test, animals were moved into individual 3 × 3 m pens without feed ~2 h before the start of the test and a temporary catheter (18G; Surflo, Terumo) was placed into a jugular vein. The study was performed from July to November (winter and spring in Southern hemisphere). Throughout the study period no changes were made to regular husbandry conditions, and paired animals were managed identically.

2.3 | Combined glucose-insulin test

Tissue insulin sensitivity was evaluated using the CGIT, as previously described.[26] Following collection of a baseline blood sample, a bolus of 150 mg/kg bwt glucose solution (50% w/v solution; Baxter) was administered intravenously in <1 min, immediately followed by a dose of 0.1 IU/kg bwt insulin (Actrapid, Novo Nordisk) and flushed with 10 mL sterile saline. Serial blood samples were taken 1, 5, 15, 30, 45, 60, 75, 90, 120, 150 and 180 min after glucose and insulin administration, with blood glucose concentration determined at each time point using a glucometer (Accu-Chek, Roche),[27] and the remaining blood (10 mL) placed into tubes containing lithium heparin anticoagulant (Vacutainer, BD).

2.4 | Standard meal test

Postprandial insulin and glucose dynamics were evaluated through the SMT. After collection of a baseline blood sample, the horses and ponies were provided with a meal of commercial pellets (475 g/100 kg bwt, as fed; Allrounder, Hygain), providing 1.1 g of starch and 0.1 g of free sugars per kg bwt, as fed (determined by near-infrared spectroscopic analysis of pellets; Agrifood Technology, Australia). This was comparable to the amount of cereal starch used in other studies where a robust glycaemic and insulinaemic response was observed,[28] and would be considered a 'high starch' meal for ID animals. Further blood samples were collected 15, 30, 60, 120, 150, 180, 240 and 300 min after the meal was provided, with blood glucose concentration determined at each time point using a glucometer (Accu-Chek, Roche), and remaining blood (10 mL) placed into tubes containing lithium heparin anticoagulant (Vacutainer, BD). The time taken to finish the meal was recorded.

2.5 | Sample handling and laboratory analysis

For both the CGIT and SMT, blood samples were placed on ice immediately after collection until centrifugation, with 1-mL plasma aliquots stored at −80°C until analysis. Plasma insulin concentrations were measured in all samples by radioimmunoassay using purified anti-human insulin antiserum raised in a guinea pig (Antibodies Australia, Melbourne, Australia), purified human insulin for iodination (Sigma-Aldrich, Sydney, Australia) and porcine insulin for the standards (Sigma-Aldrich, Sydney, Australia). The assay followed the same procedure as a commercial human-specific insulin radioimmunoassay that uses guinea pig anti-human antibodies that has been validated for equine plasma.[29] All samples were analysed at the conclusion of the study, with assays performed across several days due to large number of samples. The sensitivity of the assay was 0.94 μIU/mL, the intra-assay coefficient of variation (CV) was <10% between 6.6 and 162.8 μIU/mL and the inter-assay CV was 9.3% at 116 μIU/mL, 6.9% at 220.4 μIU/mL and 3.1% at 414.7 μIU/mL.

2.6 | Data analysis

Descriptive statistics and simple group comparisons were performed using GraphPad Prism software (version 9.1; GraphPad Inc, La Jolla, CA). Data were evaluated for normality using the Shapiro–Wilk test, and where data did not conform to a normal distribution, non-parametric tests were used. The ages of animals enrolled in the study were compared using a Mann–Whitney *U* test. Basal ACTH concentrations and bodyweight were compared before and after each treatment phase using a Wilcoxon signed-rank test. Statistical significance was set at $p < 0.05$.

Glucose and insulin curves obtained from the CGIT were evaluated as previously described.[30] Briefly, the glucose 'positive phase' was defined as the time that glucose concentrations remained above baseline or until the nadir was reached (whichever occurred first). The total glucose area under the curve (AUCg) for the positive phase was calculated using the trapezoidal method (GraphPad Prism). Glucose positive phase clearance rate was calculated by taking the difference between peak glucose concentration and lowest glucose concentration during the positive phase and divided by the time between these points. A value of delta (Δ) 45 glucose was calculated as the difference between glucose concentration at 45 min and baseline. The insulin area under the curve (AUCi) was calculated using the trapezoidal method (GraphPad Prism) and the insulin clearance rate was calculated using the same method as for glucose clearance. Insulin resistance was defined as glucose-positive phase >45 min and/or insulin concentration >20 μIU/mL at 75 min.

For the SMT, peak glucose and insulin concentrations were determined, and AUCg and AUCi were calculated using the trapezoidal method (GraphPad Prism). Peak insulin and AUCi values were calculated and analysed two ways: total (where baseline was defined as a value of $y = 0$) and incremental (where baseline was defined as the value of y at $t = 0$). Peak glucose and AUCg were calculated as incremental values only. Animals that took >90 min to consume <75% of the meal were excluded from statistical analysis. The time to finish the meal was compared pre- and post-treatment using a two-way analysis of variance for repeated measures, following log transformation of the data.

Statistical analysis of CGIT data was performed using a linear mixed model in the R package (version 4.2, R Core Team) after log

transformation. The only variable not subject to log transformation was 'Δ45 glucose' as it was normally distributed. For each outcome, the dependent variable was the post-treatment value, with the pre-treatment value included as a covariate. Statistical analysis of SMT data was performed similarly, using a linear mixed model in the R package, except that due to heterogeneity and heteroscedasticity of data among SMT variables, delta (Δ) change values (calculated as the difference between post-treatment and pre-treatment values) were used, with log transformations applied before analysis.

For both CGIT and SMT models, the factors of treatment, disease, and phase, together with their interactions, were included as fixed effects, with the individual animal included as a random effect. The models used the methodology of Kenward–Roger for degrees of freedom. When fixed effects were significant, least square means were compared using the estimated marginal means (EMM; emmeans) package in R. Results obtained from the mixed model analyses were back-transformed from the log scale and reported as EMM with 95% confidence interval (CI), unless otherwise indicated.

3 | RESULTS

3.1 | Animals

Among the 16 selected animals, breeds included Andalusian or Andalusian-cross ($n = 5$), Stock horse ($n = 1$), Standardbred ($n = 3$), miniature pony ($n = 2$) and other pony breeds ($n = 5$), with 9 geldings and 7 mares (Table S1). The median age was 19.5 (range, 16–24) years for the PPID+ID group and 18 (15–32) years for the ID-only group ($p = 0.8$). One horse from the PPID+ID group that received pergolide during the first treatment phase was excluded from the second phase of the study after developing self-limiting diarrhoea during the washout period. There were no adverse effects associated with any of the CGIT or SMT procedures. Baseline comparisons of insulin response variables derived from CGIT and SMT analyses for PPID+ID and ID-only groups are presented in Table S2. No changes in bodyweight were observed during the study period for any groups (all $p > 0.1$; Figure S1).

3.2 | Effect of pergolide on ACTH

Basal plasma ACTH concentrations were decreased in the PPID+ID group after 4 weeks of pergolide treatment, with 7 out of 8 animals demonstrating >50% reduction in basal ACTH concentration and 1 animal remaining unchanged. Pre-treatment (median [IQR]) values were 94 (50–404) pg/mL compared with post-treatment values of 57 (24–72) pg/mL ($p = 0.02$). No changes in plasma ACTH concentrations were observed for the ID-only group after pergolide treatment, or in either the PPID+ID or ID-only groups after receiving no treatment (all $p > 0.1$; Figure S2).

3.3 | Effect of pergolide on CGIT results

Given that the selection criteria included pre-existing IR based on ITT results, all animals were confirmed as being IR from the results of the pre-treatment CGIT. Parameters derived from the CGIT analysis are presented in Tables 1 and S3. Pergolide treatment did not affect AUCg ($p = 0.5$) or AUCi ($p = 0.1$). There was a significant interaction between treatment and phase for glucose time to nadir ($p = 0.03$), where during

TABLE 1 Effect of pergolide treatment on combined glucose-insulin tolerance test (CGIT) results in horses and ponies with insulin dysregulation (ID) only (ID-only group; $n = 8$) or with pituitary pars intermedia dysfunction (PPID) plus ID (PPID+ID group; $n = 8$).

	PPID+ID		ID only		*p* values			
	Pergolide	No treatment	Pergolide	No treatment	Phase	Treatment	Disease	Treat. × dis.
Basal glucose (mmol/L)	5.6 (5.0 to 6.2)	5.6 (5.0 to 6.2)	5.8 (5.2–6.5)	5.3 (4.8 to 5.8)	**0.01**	0.3	>0.9	0.3
Δ 45 glucose (mmol/L)	0.8 (−0.2 to 1.8)	0.0 (−1.2 to 1.2)	−0.1 (−1.1 to 0.9)	0.4 (−0.7 to 1.4)	0.4	0.7	0.6	0.2
Glucose pos. phase clear. (mmol/L/min)	0.1 (0.1 to 0.2)	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.3)	**0.04**	0.2	0.1	0.3
Glucose pos. phase AUC (mmol/L·min)	184 (135 to 251)	132 (94 to 186)	137 (98 to 192)	153 (110 to 213)	0.7	0.5	0.7	0.2
Glucose pos. phase duration (min)	87 (60 to 127)	55 (36 to 83)	56 (37 to 86)	57 (38 to 86)	0.08	0.2	0.4	0.2
Glucose time to nadir (min)	141 (111 to 178)	107 (83 to 138)	96 (76 to 122)	102 (78 to 125)	0.1	0.4	0.1	0.2
Basal insulin (μIU/mL)	73 (40 to 100)	80 (53 to 123)	70 (45 to 107)	82 (53 to 127)	0.6	0.3	0.8	0.9
Insulin 75 min (μIU/mL)	110 (66 to 183)	119 (70 to 202)	106 (63 to 179)	108 (64 to 183)	0.5	0.8	0.8	0.9
Insulin pos. phase clear. (μIU/mL/min)	16.1 (10.5 to 24.8)	22.8 (14.4 to 36.1)	18.5 (12.5 to 28.0)	26.6 (17.6 to 40.2)	0.3	0.06	0.5	>0.9
Insulin AUC (mIU/mL·min)	26.2 (20.8 to 32.8)	31.7 (24.9 to 40.3)	26.3 (20.7 to 33.4)	31.9 (25.3 to 40.4)	0.5	0.1	>0.9	>0.9

Data presented as back-transformed estimated marginal means (95% confidence intervals) derived from linear mixed model analysis. Delta (Δ) 45 glucose was calculated as the difference between glucose concentration at 45 min and baseline. Significant *p* values are indicated in bold.
Abbreviation: AUC, area under the curve.

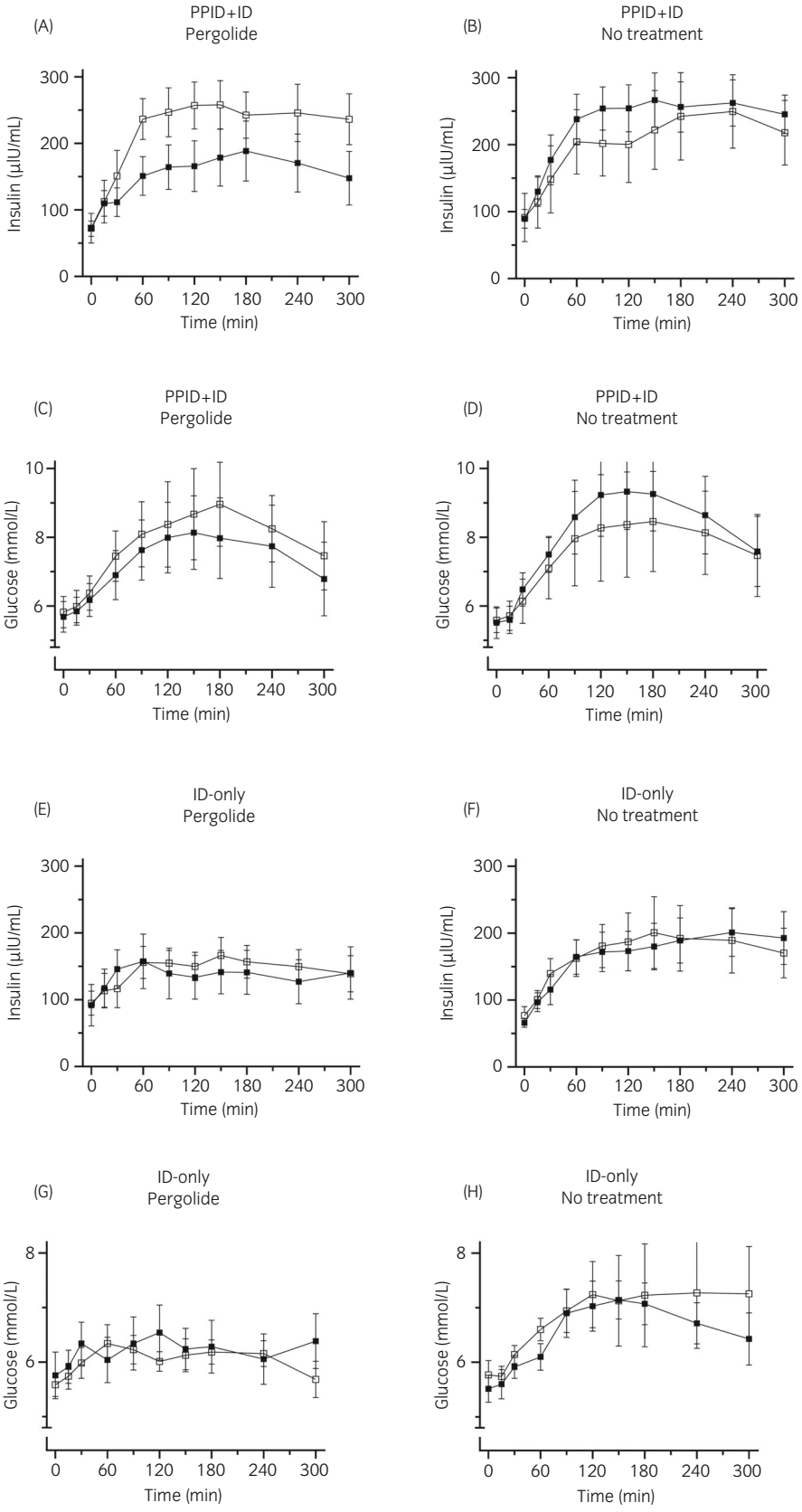

FIGURE 1 Insulin and glucose curves derived from standard meal tests (SMT) that provided 1.1 g/kg bwt of starch and 0.1 g/kg bwt of free sugars to horses and ponies with insulin dysregulation (ID) only (ID-only group; $n = 7$) or pituitary pars intermedia dysfunction (PPID) plus ID (PPID+ID group; $n = 7$). Individual panels show results (mean ± SEM) from SMT performed before (open squares) and after (closed circles) 4 weeks of treatment with pergolide or a non-treatment period. (A) Insulin and (C) glucose curves for the PPID+ID group from the pergolide treatment period. (B) Insulin and (D) glucose curves for the PPID+ID group from the non-treatment period. (E) Insulin and (G) glucose curves for the ID-only group from the pergolide treatment period. (F) Insulin and (H) glucose curves for the ID-only group from the non-treatment period.

TABLE 2 Effect of pergolide treatment on standard meal test (SMT) results in horses and ponies with insulin dysregulation (ID) only (ID-only group; $n = 7$) or with pituitary pars intermedia dysfunction (PPID) plus ID (PPID+ID group; $n = 7$).

	PPID+ID		ID only		*p* values			
	Pergolide	**No treatment**	**Pergolide**	**No treatment**	**Phase**	**Treatment**	**Disease**	**Treat. × dis.**
Glucose								
Δ Basal (mmol/L)	0.0 (−0.8 to 1.0)	−0.2 (−1.0 to 1.0)	0.1 (−0.7 to 1.0)	−0.2 (−1.0 to 1.0)	**0.01**	0.3	0.9	0.7
Δ Peak (mmol/L)	−0.9 (−2.4 to 0.0)	1.0 (−0.5 to 3.0)	0.2 (−1.3 to 2.0)	−0.4 (−1.9 to 1.0)	0.8	0.4	0.9	0.1
Δ AUC (min·mmol/L)	−230 (−511 to 214)	41 (−357 to 687)	−7 (−384 to 601)	−243 (−531 to 220)	0.5	0.9	0.9	0.2
Insulin								
Δ Basal (μIU/mL)	−30 (−62 to 10)	−6 (−45 to 42)	10 (−62 to 10)	−19 (−55 to 25)	**0.004**	0.9	0.5	0.2
Δ Peak total (μIU/mL)	−100 (−167 to −29)	45 (−36 to 134)	15 (−63 to 100)	−15 (−91 to 68)	**0.03**	0.1	0.4	**0.04**
Δ Peak inc. (μIU/mL)	−86 (−138 to −31)	33 (−29 to 99)	17 (−44 to 82)	−1 (−60 to 63)	0.3	0.1	0.2	**0.04**
Δ AUC total (min·mIU/mL)	−25.4 (−39.9 to −7.3)	13.0 (−10.1 to 42.2)	1.1 (−19.5 to 26.9)	−12.3 (−30.2 to 42.2)	**0.02**	0.2	0.8	**0.03**
Δ AUC inc. (min·mIU/mL)	−19.8 (−31.2 to −6.5)	11.8 (−5.0 to 31.6)	2.2 (−31.0 to 20.2)	0.2 (−14.8 to 17.8)	0.5	0.07	0.4	**0.04**

Data presented as delta (Δ) change (calculated as the difference between post-treatment and pre-treatment values) with back-transformed estimated marginal means (95% confidence intervals) derived from linear mixed model analysis. Peak and AUC values for insulin are reported as both total values (calculated from baseline of $y = 0$) and incremental values (calculated from baseline of y at $t = 0$). Significant p values are indicated in bold.

Abbreviation: AUC, area under the curve.

the second phase there was a decrease in time for the treatment group and an increase in time for the non-treatment group. No other parameters derived from the CGIT showed significant differences by treatment or any interactions between treatment and other predictors.

3.4 | Effect of pergolide on SMT results

One horse from the ID-only group was excluded from the SMT statistical analysis, as this horse did not consume >75% of the meal within 90 min during any phase of the study, which seemed to be an appetite or palatability issue given that it was otherwise clinically healthy. There was no effect of pergolide treatment on meal consumption, with median (IQR) time to finish the meal being 60 (26–95) min before treatment and 53 (32–120) min after treatment ($p = 0.5$). Median time to consume the meal was 49 (31–97) min before and 39 (27–60) min after the non-treatment phase ($p = 0.3$).

Glucose and insulin curves obtained from the SMT are reported in Figure 1, with parameters derived from curve analysis reported in Table 2 and Table S4. Peak insulin concentration (total and incremental) and AUCi (total and incremental) were reduced in the PPID+ID group following treatment with pergolide (treatment × disease effects all $p < 0.05$). Glucose variables were not different between groups or treatments (all $p > 0.05$).

A significant main effect of phase was detected, with Δ insulin values (magnitude of effect [95% CI]) being higher during the first phase of the study for basal insulin (1.4 [1.2–1.7]), peak insulin (total) (1.1 [1.0–1.2]) and AUCi (total) (1.4 [1.1–1.7]).

4 | DISCUSSION

Pergolide mesylate is the most common treatment for PPID and, as well as the potential to reduce plasma ACTH concentrations and improve clinical signs, studies have suggested that it may reduce the incidence of laminitis among treated horses.[11] As most cases of laminitis in animals with PPID are associated with ID,[2,5] and based on evidence in other species regarding the role of dopamine (both central and peripheral) in insulin regulation,[16] further investigation of the role of dopamine receptor agonist drugs to treat PPID and ID are warranted. Our study included animals with PPID plus ID, and animals with ID only, to allow for the potential confounding effect of PPID to be separated from ID within the analysis, although the PPID+ID group demonstrated higher pre-treatment insulin response variables compared with the ID-only group. Overall, pergolide treatment did not affect tissue insulin sensitivity; however, pergolide treatment reduced postprandial insulin responses following consumption of a high starch meal in animals with PPID plus ID.

Four weeks of treatment with pergolide at the recommended starting dose was effective in reducing plasma ACTH concentrations for horses and ponies with PPID, confirming a pharmacological effect of the drug. Given the relatively short treatment period, improvements in clinical signs were not anticipated, especially as clinical signs such as hair coat changes and epaxial muscle mass can take time to demonstrate a noticeable improvement. Basal and post-TRH stimulation ACTH concentrations, along with clinical signs, were evaluated for the diagnosis of PPID, and basal ACTH concentrations were monitored at the start and end of each treatment period during the study. Currently, there are no clinical guidelines to indicate a particular level of reduction in basal ACTH concentration that represents an appropriate response to treatment. However, seven out of eight animals with PPID demonstrated a reduction in basal ACTH concentrations by more than 50%, and the one animal in which basal ACTH was unchanged was ranked fourth in terms of the magnitude of the reduction in postprandial insulin responses after treatment.

There was no observable effect of pergolide treatment on tissue insulin sensitivity for either the PPID+ID or ID-only groups after 4 weeks of treatment, as evaluated using the CGIT. This agrees with previous work using another dopamine receptor agonist, which showed that short- or long-term administration of cabergoline did not affect insulin sensitivity in insulin-resistant and non-insulin resistant mares.[18] More recently, administration of bromocriptine for 2 weeks was found to *decrease* insulin sensitivity in horses, as evaluated using the CGIT, which is in contrast to the findings in humans and rodents.[19] Other previous work regarding pergolide administration in horses with PPID did not find any difference in tissue insulin sensitivity between pergolide-treated and non-treated horses, although pre-treatment assessments were not performed to evaluate the response to treatment over time.[17] The reasons that these findings are different in horses compared with humans and rodents are not readily apparent and warrant further investigation. For practical reasons, the CGIT was used to evaluate tissue insulin sensitivity in the present study, as it is a simplified version of the 'gold standard' frequently-sampled intravenous glucose tolerance test (FSIGT).[31] While the CGIT might have reduced sensitivity and specificity to diagnose IR as a binary outcome compared with the FSIGT,[32] this test has acceptable repeatability, although insulin curves demonstrate superior repeatability compared with glucose curves.[33] The CGIT has been used successfully in research settings to evaluate changes in insulin sensitivity over time in the same animals.[34]

As IR and postprandial hyperinsulinaemia represent different aspects of ID (and the relationship between the two remains unclear), it was important to evaluate both tissue IR and postprandial insulin responses, as they can coexist or occur one without the other within the same animal.[35] For example, in insulin-sensitive breeds, an improvement in tissue insulin sensitivity was observed when horses were fed starch and sugar-rich diets while no improvement was seen in postprandial insulin responses.[36]

Treatment with pergolide reduced the postprandial insulin response to a high starch meal in the PPID+ID group, while there was no apparent effect on the ID-only group. Insulin variables derived from analysis of the SMT were higher for peak insulin and AUCi values among the PPID+ID animals compared with ID-only animals at the outset of the study. This agrees with the findings of previous studies, in which horses with concurrent PPID and ID tended to exhibit more severe ID than horses with either disease alone.[5,37] In light of

this observation, treatment with pergolide seemed to mitigate the additional postprandial insulin response associated with PPID to a value that was broadly similar to that seen in animals with ID alone. Heterogeneity among groups and the small sample size reduced the power of statistical analyses, however, the effect of pergolide to reduce postprandial insulin responses in the PPID+ID group was significant across all variables evaluated in the statistical model, including peak insulin (total and incremental) and AUCi (total and incremental).

Clear evidence that horses with PPID have a systemic loss of dopamine is currently lacking, although there is one report of mildly decreased dopamine concentrations in a small number of horses with PPID, as measured by radioimmunoassay.[38] Recently, it has been reported that there are dopamine D2 receptors on pancreatic β cells of horses,[39] while another recent study demonstrated that pharmacological reduction of dopamine (central and circulating) by a tyrosine hydroxylase inhibitor increases the postprandial insulin response in healthy horses.[40] Therefore, if there is a reduction in peripheral dopamine in horses with PPID (leading to increased insulin secretion) then pergolide could directly reduce insulin secretion, and these potential effects warrant further investigation. Since peptides derived from pro-opiomelanocortin (which are overproduced by the pars intermedia in PPID) have the potential to stimulate insulin secretion, there could also be a direct effect of pergolide to modify the pituitary secretome that could explain the observed effects, and that too warrants further investigation.

There was a significant effect of study phase on insulin variables derived from the SMT, with the magnitude of responses being lower during the second phase. The effect of phase was observed for basal insulin, as well as peak insulin (total) and AUCi (total), both of which are calculated using a baseline of $y = 0$ and are therefore affected by changes in basal values. However, the effect of phase was absent for peak insulin (incremental) and AUCi (incremental), indicating that the incremental increase in postprandial insulin concentrations above basal values was not affected by study phase. There is not a clear explanation for these observations, as husbandry and management practices were consistent throughout the study period. Possibilities include a seasonal effect of moving from winter to spring, pasture composition or consumption (which were not quantified), weather conditions, or due to other unknown factors. While it would have been preferable to not have an effect of phase on study results, this finding supports the randomised cross-over study design to ensure the equal assessment of the disease by treatment interaction within each period as effects within the statistical model, as a longitudinal study design would not have accounted for this potential effect. Further work is necessary to explore the effect of phase and the potential for season or other factors to influence insulin responses.

A reduction in AUCi was previously reported following 2 weeks of bromocriptine administration in horses both with and without ID, in which the SMT comprised a similar amount of starch (1 g/kg bwt) as whole oats.[19] However, bromocriptine is not as selective as other dopamine agonist drugs,[41] and its effects on the pancreas have been shown to be mediated by α_2 adrenoceptors in other species.[42] Furthermore, horses in the bromocriptine study did not have PPID. The interesting findings of the bromocriptine study formed part of the rationale for using a SMT in the present study over other tests, such as the oral sugar test, that are used more commonly in clinical practice, as it was thought important to distend the stomach with a large meal to generate the strongest possible postprandial physiological response. Another reason is the prior publication of studies that evaluated changes in the oral sugar test in response to pergolide treatment that did not identify any significant effects.[24,43] Dopamine and dopamine receptor agonists have been shown to reduce glucose-mediated insulin secretion in human and mouse pancreatic islets.[14,16,44,45] In other species, it has been shown that dopamine may also lead to a reduction in postprandial insulin due to an 'anti-incretin' effect.[46,47] In vitro, insulin secretion by pancreatic islets in response to incretin hormones could be reduced when exposed to increasing concentrations of dopamine.[47] Whether dopamine has any effect on the insulin response to incretin hormones in horses is not yet known. Further studies to understand the effects of dopamine agonists on postprandial insulin secretion in horses are warranted.

The time taken to consume the SMT meal was not different following pergolide treatment. Reduced appetite is a reported potential consequence of pergolide treatment in horses,[48,49] so it was important to quantify meal consumption to ensure that any observed differences could not be attributed to slow or incomplete meal consumption. One horse in the ID-only group was excluded from the statistical analysis of SMT parameters, as it did not eat the complete SMT meal at any time, regardless of pergolide treatment.

The results obtained in this study may be specific to the dose and duration of pergolide treatment, and the methods used to assess ID. A longer treatment period, the use of a higher pergolide dose or evaluation across different seasons might yield different results. This is worthy of note, given that horses diagnosed with PPID are often treated with pergolide for the remainder of their lives, with dose adjustments occurring based on clinical and laboratory evaluations. For example, a longitudinal treatment study detected weight loss among horses with PPID that received higher doses of pergolide for a longer period, which might have implications for insulin sensitivity.[43] While efforts were made to ensure accurate classification of PPID, it cannot be guaranteed that animals in the ID-only group were not in the early stages of PPID; however, they did not exhibit any apparent clinical signs (and have been confirmed to remain free from clinical signs 1 year after study completion), and basal ACTH concentrations were <50 pg/mL throughout the study period, with the exception of a one-off result of 56 pg/mL for one horse. The use of different tests to evaluate glucose and insulin dynamics, such as the oral sugar test or 2-step insulin tolerance test, could be worth investigating, as these tests are more commonly performed by practitioners,[35] but for the reasons discussed above, these tests were not performed. The ability to directly compare absolute insulin concentrations between this study and others is limited by the choice of assay, whereby the radioimmunoassay used in this study has not been directly compared with other commercially available assays. Therefore, we have focussed the presentation of results and discussion on the relative changes that occurred within the present study. Finally, due to heterogeneity

among groups across variables evaluated for the CGIT and SMT, it cannot be discounted that a larger number of animals, or animals that were more closely matched for their degree of ID, might have revealed more subtle effects of pergolide on insulin sensitivity, although an a priori power calculation, based on published evidence, was considered in the study design.

5 | CONCLUSIONS

Pergolide treatment did not alter tissue insulin sensitivity, as measured by the CGIT. However, postprandial insulin responses to a high starch meal were reduced following pergolide treatment in horses and ponies with PPID plus ID. The potential for pergolide to reduce the postprandial insulin response in horses and ponies with PPID is encouraging and may provide a mechanism by which pergolide could help to reduce the risk of laminitis in equids with PPID; although dietary management and other measures are likely to still be important. Further work to explore the effect of dopamine and dopamine agonist drugs on insulin secretion is warranted.

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CONFLICT OF INTEREST STATEMENT

Tobias Warnken is an employee of Boehringer Ingelheim Vetmedica GmbH, which manufactures a registered formulation of pergolide mesylate. Patricia Harris is an employee of Waltham Petcare Science Institute. No other authors have a conflict of interest.

AUTHOR CONTRIBUTIONS

Nicolas C. Galinelli: Investigation; conceptualization; writing – original draft; methodology; validation; software; formal analysis; data curation. **Nicholas J. Bamford:** Conceptualization; investigation; writing – original draft; methodology; validation; writing – review and editing; formal analysis; project administration; supervision. **Madison L. Erdody:** Investigation; writing – review and editing; methodology. **Skye A. Mackenzie:** Investigation; methodology; writing – review and editing. **Tobias Warnken:** Conceptualization; funding acquisition; writing – review and editing; methodology. **Patricia A. Harris:** Conceptualization; funding acquisition; methodology; writing – review and editing; supervision. **Martin N. Sillence:** Conceptualization; funding acquisition; writing – review and editing; methodology; supervision. **Simon R. Bailey:** Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; writing – review and editing; software; formal analysis; project administration; supervision; resources.

DATA INTEGRITY STATEMENT

Nicolas Galinelli had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

ETHICAL ANIMAL RESEARCH

The study was approved by the University of Melbourne Animal Ethics Committee (ID 23234).

INFORMED CONSENT

Signed owner consent was obtained for all animals enrolled.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at http://doi.org/10.26188/25721883.

ANTIMICROBIAL STEWARDSHIP POLICY

Not applicable.

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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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