Abstract: Metformin may be an effective therapeutic option for insulin-resistant (I-R) horses/ponies because, in humans, it reportedly enhances insulin sensitivity (SI) of peripheral tissues without stimulating insulin secretion. To determine the effect of metformin on insulin and glucose dynamics in I-R ponies, six ponies were studied in a cross-over design by Minimal Model analysis of a frequently-sampled intravenous glucose tolerance test (FSIGT). Metformin was administered at 15 mg/kg bodyweight (BW), orally, twice-daily, for 21 days to the metformin-treated group. The control group received a placebo. A FSIGT was conducted before and after treatment. The Minimal Model of glucose and insulin dynamics rendered indices describing SI, glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and the disposition index (DI). The body condition score (BCS), BW and cresty neck score (CNS) were also assessed. There was no significant change in SI, Sg, AIRg, DI, BW, BCS or CNS in response to metformin, or over time in the control group. There were no measurable benefits of metformin on SI, consistent with recent work showing that the bioavailability of metformin in horses is poor, and chronic dosing may not achieve therapeutic blood concentrations. Alternatively, metformin may only be effective in obese ponies losing weight or with hyperglycaemia.
Original article

The effect of oral metformin on insulin sensitivity in insulin-resistant ponies

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Abstract

Metformin may be an effective therapeutic option for insulin-resistant (I-R) horses/ponies because, in humans, it reportedly enhances insulin sensitivity (SI) of peripheral tissues without stimulating insulin secretion. To determine the effect of metformin on insulin and glucose dynamics in I-R ponies, six ponies were studied in a cross-over design by Minimal Model analysis of a frequently-sampled intravenous glucose tolerance test (FSIGT). Metformin was administered at 15 mg/kg BW, orally, twice-daily, for 21 days to the metformin-treated group. The control group received a placebo. A FSIGT was conducted before and after treatment. The Minimal Model of glucose and insulin dynamics rendered indices describing SI, glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and the disposition index (DI). Bodyweight (BW), body condition score (BCS) and cresty neck score (CNS) were also assessed. There was no significant change in SI, Sg, AIRg, DI, BW, BCS or CNS in response to metformin, or over time in the control group. There were no measurable benefits of metformin on SI, consistent with recent work showing bioavailability of metformin in horses is poor, and chronic dosing may not achieve therapeutic blood concentrations. Alternatively, metformin may only be effective in obese ponies losing weight or with hyperglycaemia.

Keywords: Equine; Hyperinsulinaemia; Laminitis; Obesity; Pharmacology
Introduction

With an increasing appreciation of the pathophysiology of insulin resistance (IR) and hyperinsulinaemia in horses/ponies (Hess et al., 2006; Asplin et al., 2007; Geor, 2008; de Laat et al., 2010), the goal of owners and veterinarians is to prevent IR, or to treat it before the consequences become manifest. While the correct management of energy intake and exercise levels may be effective (Carter et al., 2010; Frank et al., 2010), there are circumstances where pharmacological intervention may be warranted (Geor and Harris, 2009; Frank et al., 2010).

With no licensed medications available for the treatment of IR in horses/ponies, veterinarians increasingly prescribe the off-label use of medications used for IR in humans (eg, metformin, levothyroxine sodium, glibenclamide) (Johnson et al., 2005; Frank et al., 2008; Durham et al., 2009), therefore evaluation of efficacy of these drugs in horses/ponies is necessary. The anti-hyperglycaemic drug metformin (dimethybiguanide) is in widespread human clinical use, where it enhances insulin sensitivity (SI) by increasing peripheral glucose uptake. It also decreases blood glucose concentrations by inhibiting hepatic glucose production and intestinal absorption of glucose (Saenz et al., 2005). Metformin promotes weight loss and reduces lipid levels; adverse effects are rare (Salpeter et al., 2008).

Several reports describe the effects of metformin use in horses/ponies with mixed results (Johnson et al., 2005; Vick et al., 2006; Durham et al., 2008; Durham et al., 2009; Firshman et al., 2009; Hustace et al., 2009). A single dose of metformin (1.9 mg/kg bodyweight (BW), orally) administered with an insulin secretagogue to a hyperglycaemic horse reduced plasma glucose concentrations to values within the reference interval (Johnson et al., 2005). Furthermore, metformin (2.8 mg/kg BW, orally, 12-hourly) administered to 14
obese mares for 30 days enhanced SI, measured with the hyperinsulinaemic-euglycaemic clamp (HEC) (Vick et al., 2006). However, in the same study, metformin became ineffective when given for longer or at an increased dose (Vick et al., 2006). In another study, at a dose of 15 mg/kg BW orally, 12-hourly, metformin improved proxy measures of SI and beta-cell function for up to 14 days in 18 insulin resistant (I-R) horses/ponies, compared with pre-treatment values from the same animals (Durham et al., 2008). However, metformin given to a hyperglycaemic mare at the same dose and frequency did not improve blood glucose or serum insulin concentrations (Durham et al., 2009). In six non I-R horses, metformin therapy (15 mg/kg BW, orally, 8-hourly, for 15 days) showed no effect on SI measured with the HEC (Firshman et al., 2009). Pharmacokinetic studies of metformin in horses and I-R ponies show low bioavailability of the drug in this species (Hustace et al., 2009), and that chronic dosing may not achieve therapeutic blood concentrations (Tinworth et al., 2010).

The aim of this study was to determine the effect of oral metformin on insulin and glucose dynamics in I-R ponies using Minimal Model analysis of a frequently-sampled intravenous glucose tolerance test (FSIGT) conducted before and after 21 days of metformin administration (15 mg/kg BW, orally, twice-daily), using six I-R ponies with no signs of laminitis. The study was conducted as part of the aforementioned work that investigated the pharmacokinetics of metformin (Tinworth et al., 2010).

We hypothesised that metformin treatment of IR in I-R ponies would enhance SI, that glucose effectiveness (Sg) would remain unchanged, the acute insulin response to glucose (AIRg) would be lowered, and that the disposition index (DI) would increase. We also hypothesised that if SI was enhanced there would be a concurrent reduction in neck adiposity.
Materials and methods

Animals

Six female Welsh-cross and Shetland-cross ponies were used, with a (mean ± SD) age of 12.00 ± 2.88 years, weighing 206 ± 53.5 kg. The ponies, purchased from local sales, were not obese (BCS: 6 ± 0.89), but did display regional adiposity (Frank et al., 2010) and were deemed to be I-R based on evaluation of neck crest adipose tissue (i.e., cresty neck score (CNS) ≥ 3) (Carter et al., 2009a) and results of a combined glucose-insulin test (CGIT) (Frank et al., 2006). The ponies were otherwise healthy and not suffering from Pituitary Pars Intermedia Dysfunction, based on results of a 19 h dexamethasone suppression test (Dybdal et al., 1994), where testing was conducted during November (Southern Hemisphere late Spring) (Donaldson et al., 2005). The study protocol was approved by the Charles Sturt University Animal Care and Ethics Committee.

Study Design

The study was conducted as a 2 x 2 cross-over trial. The two treatments were control (C) and metformin (M), and the six ponies were randomised to two treatment sequences MC and CM, with three ponies in each group. The two treatment periods were separated by a 21-day wash-out period with ponies kept in a communal paddock. Treatment periods continued for 21 days with a FSIGT conducted on Day 0 and Day 22 on all ponies. At this time, physical characteristics of BW, body condition score (BCS), CNS, neck circumference and the height and thickness of the crest of the neck were also measured. Bodyweight was measured using electronic scales; BCS was scored out of a total of 9 (Henneke et al., 1983). The CNS was assigned according to Carter et al (2009a), with neck circumference, crest height and thickness measured as described by Frank et al (2006). During treatment periods, all ponies were stabled individually, with unrestricted access to water. Stables were cleaned
each morning while the ponies were allowed free exercise in a communal yard. Ponies were fed maintenance rations of early-cut oaten hay at a rate of 1 to 2% of BW twice daily (NRC, 2007). The ponies also received 100 g rice bran pellets (Cool Conditioner, CopRice) twice daily. Ponies were monitored for feeding behaviour at each meal, and received full health checks daily. The C animals received exactly the same management as the M animals.

Metformin Administration

Metformin was given at 15 mg/kg BW, orally, twice-daily, with meals, at 8 am and 5 pm. For each pony, the dose of metformin was prepared by powdering the appropriate number of metformin tablets (Metforbell 500 mg, CiplaGenpharm Australia, Pty Ltd), using a mortar and pestle, and suspending the powder in 100 mL tap water. This solution was mixed with 100 g rice bran pellets (Cool Conditioner, CopRice) for the ponies to eat. The entire ration was readily consumed within 2 min.

FSIGT

The evening before the FSIGT, a 14G catheter was placed in the jugular vein of each pony, after aseptic preparation of the site and the administration of local anaesthetic. On the morning of the study, the ponies were fed oaten hay at a rate of 1% to 2% of BW. The FSIGT was initiated at 9 am with a bolus of glucose (Dextrose solution 50%, Baxter Healthcare) (300 mg/kg BW, IV) administered within 2 min. Twenty min later, an insulin bolus (Humulin R, Eli Lilly and Co.) (20 mU/kg BW, IV) was administered within 30 s, as described by Yang et al (1987). Basal blood samples (10 mL) were taken 60, 45, and 0 min before the glucose dose. Blood samples were then drawn at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330, and 360 min after the glucose bolus. Blood samples were collected into lithium-heparin vacutainers.
(Vacutainer, Becton-Dickinson), kept on ice and centrifuged at 4 °C (1,000 x g for 10 min) within 20 min of collection.

**Sample Analysis**

Each plasma sample was divided into two aliquots and stored at -20 °C until assayed for glucose and insulin. Plasma glucose concentrations were measured using an enzymatic assay (Glucose Hexokinase, ThermoFisher Scientific Inc.) on a bench-top biochemistry analyzer (Cobas Mira, Roche). Plasma insulin concentrations were determined using a radioimmunoassay (RIA) (Coat-A-Count Insulin RIA, Siemens Medical Solutions Diagnostics), validated for use in equines (Tinworth et al., 2009). Each sample was assayed in duplicate, and intra-assay coefficients of variation < 5 % or < 10 % were required for acceptance of glucose and insulin assay results, respectively.

**Minimal Model Analysis**

Glucose and insulin curves were interpreted according to the Minimal Model of glucose and insulin dynamics (Bergman, 1989; Boston et al., 2003) using MINMOD Millennium software (Boston et al., 2003). Application of this method in horses has been described previously (Hoffman et al., 2003; Treiber et al., 2005a). All results are expressed as mean ± SD.

**Statistical Analyses**

Values for SI, Sg, A1Rg, DI and physical characteristics between C and M groups were compared by calculating the difference in each variable between Day 22 and Day 0 for each pony in each period, then conducting a Wilcoxon Matched Pairs Test comparing ponies
in the two treatment sequences. The null-hypothesis was rejected if \( P \leq 0.05 \). Analyses were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software).

**Results**

Pre-treatment physical characteristics of the six ponies are shown in Table 1. Pre-treatment Minimal Model indices are shown in Table 2. No significant differences were found.

Physical characteristics measured on Day 0 and Day 22, of the C group and the M group at pre-treatment (Day 0) and post-treatment (Day 22) are shown in Figure 1. Results for SI, Sg, AIRg and DI from Minimal Model analysis of the FSIGT of the C Group and the M group at pre-treatment (Day 0) and post-treatment (Day 22) are shown in Figure 2.

Non-fasting basal insulin concentrations of the C Group were 8.67 ± 3.77 (mean ± SD) on Day 0 and 7.80 ± 3.89 on Day 22, and of the M group were 9.43 ± 6.19 on Day 0 and 6.10 ± 0.71 on Day 22. Data from the ponies were compared and no significant treatment effects were observed.

**Discussion**

The results of this study were disappointing but not surprising, in light of pharmacokinetic results obtained concurrently. The lack of apparent long-term efficacy of metformin is attributable to its low bioavailability in the horse/pony. The bioavailability of metformin in horses after oral administration was reportedly 3.9% to 7.1%, depending on feeding status (Hustace et al., 2009). In contrast, bioavailability in humans is 40% to 60% (Scheen, 1996). This bioavailability factor may be clinically relevant to metformin.
concentrations at steady state (C_{ss}). From our study of the pharmacokinetics of metformin in these I-R ponies, the C_{ss} of metformin administration (15 mg/kg BW orally, twice-daily) was 122 ng/mL (Tinworth et al., 2010), approximately 5 times lower than the C_{ss} of 500 to 1,000 ng/mL considered therapeutic in humans (Scheen, 1996). However, work by Stepensky et al (1998) and Sambol et al (1996) in rodents and humans, have shown a lack of direct correlation between the magnitude of glucose-lowering effect and blood metformin concentrations, with a significant “first-pass” pharmacodynamic effect, in the liver and the gastrointestinal wall, that contributes to the efficacy of metformin (Stepensky et al., 2002). It is unknown if these pharmacodynamics are reflected in the horse/pony, but due to the equivocal results obtained by other researchers using metformin to treat I-R horses/ponies, the findings of the current study need to be presented.

The appeal of metformin as a therapeutic option for I-R horses/ponies is that it reportedly enhances the SI of peripheral tissues (Salpeter et al., 2008), without stimulating insulin secretion by pancreatic beta-cells (Klip and Leiter, 1990). In horses/ponies with a history of endocrinopathic laminitis, such as the horses/ponies studied by Durham et al (2008), their IR is characterised by basal hyperinsulinaemia (Treiber et al., 2005c; Walsh et al., 2009). There seems to be another subgroup of horses/ponies, such as the ponies studied here, with the pre-laminitic metabolic syndrome (Treiber et al., 2005c) where the animals may not present with basal hyperinsulinaemia and do not show signs of laminitis, but are IR, displaying hyperinsulinaemia in response to a glucose challenge. Prolonged hyperinsulinaemia increases the risk for laminitis and other diseases (Hess et al., 2006; Asplin et al., 2007; Geor, 2008; de Laat et al., 2010). Hence the aim to decrease insulin secretion is paramount.
In humans, therapeutic concentrations of metformin decrease fasting plasma glucose concentrations within 3 to 5 days of commencing therapy, stabilising within 1 to 2 weeks (Sambol et al., 1996). Based on this, the 21 days allowed in the present study to assess the efficacy of the drug should have been adequate.

Reports of metformin use in horses/ponies (Johnson et al., 2005; Vick et al., 2006; Durham et al., 2008; Durham et al., 2009; Firshman et al., 2009; Hustace et al., 2009) indicated some degree of effectiveness in treating IR and were valuable in suggesting an optimal design for this study. Although the case study described by Johnson et al (2005) showed metformin may be a therapeutic option for I-R horses/ponies, a randomised, controlled study provides stronger clinical evidence. Our study furthered that of Firshman et al (2009), by examining the response to metformin of ponies with IR. Studies by Durham et al (2008; 2009) were important in assuring us that chronic administration of metformin to horses/ponies was safe, using a dosing regimen comparable to that used in humans. Earlier studies described were limited by the use of proxy measures of SI (Durham et al., 2008).

Proxy measures of SI, derived from basal insulin and glucose concentrations, have been determined to improve the predictive ability of IR and the risk for subsequent disease (Treiber et al., 2005b). Predicting IR by using these proxies in an inbred population, where a major gene may be responsible for this tendency, gave a moderate predictive power of 78%. The power of this method in the wider outbred population may be limited by greater individual variability and may be inappropriate for assessing the general population of horses/ponies. The use of basal blood samples to measure blood glucose concentrations (Durham et al., 2009) in type 2 diabetes mellitus was appropriate to monitor hyperglycaemia in the cases described. However, non-specific indicators of IR derived from basal blood
samples are limited in usefulness because they fail to provide values for SI, Sg or beta cell function. Basal measurements of glucose and insulin are also subject to seasonal, diurnal, stress, pain and feeding history influences. A dynamic challenge test, such as the HEC or the FSIGT (Bailey et al., 2007), is a more discriminating and controlled method of determining IR in horses/ponies (Frank et al., 2010). Currently, the gold-standard method of determining IR, used in this study, is by Minimal Model analysis of the FSIGT (Monzillo and Hamdy, 2003). Minimal Model analysis has been used primarily to elucidate etiologies of diabetes in humans and other species (Bergman, 1989), but has effectively estimated SI and Sg in horses (Hoffman et al., 2003).

From the physiologic perspective, SI is the capacity of insulin to stimulate glucose uptake into tissue (Bergman, 1989). We hypothesized that SI would be enhanced after 21 days of metformin administration. In humans, metformin enhances hepatic and muscle SI (Saenz et al., 2005; Salpeter et al., 2008) through direct (Stith et al., 1996) and indirect effects (Cusi et al., 1996). Our results failed to show a significant change in response to metformin in this small population of ponies.

The fractional disposal rate of glucose (Sg) is the capacity of the cells to take up glucose and suppress hepatic glucose production without insulin mediation (Bergman, 1989). Metformin is reported to enhance SI of peripheral tissues (Saenz et al., 2005; Salpeter et al., 2008), by pathways independent of insulin-mediated phosphoinositide-3 kinase and that may involve 5'-AMP-activated kinase (Zhou et al., 2001), thus enhancing Sg. This action is important in patients suffering from hyperglycaemia. In this study, as hypothesised, Sg remained unchanged. It is likely hyperglycaemia is a pre-requisite to this pathway becoming effective, and our ponies were not hyperglycaemic. Because Sg remained unaffected by
metformin treatment, and the ponies were normoglycaemic at baseline, it appears glucose uptake into tissues via insulin-independent pathways is intact, and/or that elevated hepatic gluconeogenesis is not a feature of the condition in these ponies. This is in contrast to the horses studied by Durham et al (2009).

Responsiveness of beta-cells to the glucose load is described by the AIRg, the increase in plasma insulin above basal concentration integrated from 0 to 10 min after the glucose dose (Bergman, 1989). By enhancing SI (Saenz et al., 2005; Salpeter et al., 2008), metformin was expected to decrease the insulin concentration required to metabolise the glucose load and AIRg to be lowered. In this study, AIRg did not change significantly. Therefore, our results suggest that insulin secretion was unaffected by metformin treatment and the control management; corroborating our finding that SI was not significantly affected by the metformin treatment.

The product of AIRg and SI determines the DI, the appropriateness of the beta-cell response relative to the degree of IR in tissues (Bergman, 1989). In this study, DI did not change. This suggests an adequate capacity of AIRg to compensate for limited SI. Our ponies were able to compensate for their IR as they maintained euglycaemia, but secreted more insulin than normal, to do so.

In I-R humans, metformin reduces BW (Salpeter et al., 2008). However, metformin (2.8 mg/kg BW, orally, 12-hourly) in 14 obese mares for 30 days did not affect BW (Vick et al., 2006). Similarly, in our study, the ponies’ BW was unchanged in response to metformin administration.
Some adipocytes secrete endocrine factors that directly cause IR (Lyon et al., 2003). These endocrinologically-active adipocytes are especially plentiful in visceral fat in humans. Although there is a clear correlation between obesity and IR (Hoffman et al., 2003; Frank et al., 2006; Vick et al., 2007), some I-R animals, such as our I-R ponies, are not obese on the basis of BCS (ie, BCS < 7), but had enlarged fat deposits on the neck (a cresty neck) (Carter et al., 2009b). Numerous endocrine signals produced by adipocytes in visceral fat are collectively known as ‘adipokines’ (Dandona et al., 2004) and increased production of adipokines by individuals with visceral fat is strongly associated with IR. Since it has been speculated that visceral fat in humans may be equivalent to cresty necks in ponies (Carter et al., 2009b), we expected any enhancement of SI may be accompanied by a reduction in neck ‘crestiness’. However, no significant change in apparent neck adiposity was evident.

Conclusions

In this study with non-obese, non-laminitic I-R ponies, our results do not support the hypothesis that metformin is effective, but should be interpreted with caution due to the limited sample size and large individual variation. However, in light of the pharmacokinetics of metformin studied in these same I-R ponies (Tinworth et al., 2010), and the results reported here, we speculate that metformin was not therapeutic, because the drug did not circulate at concentrations required to enhance insulin-dependent glucose uptake; nor did it affect insulin-independent glucose uptake pathways associated with hyperglycaemia.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.
Animal Care and Ethics statement

The protocol described was reviewed on Wednesday 5 March 2008 by the Charles Sturt University Animal Care and Ethics Committee, and was approved with the approval number 08/030. The ponies were owned by Charles Sturt University.

Acknowledgments

The authors would like to acknowledge WALTHAM Centre for Pet Nutrition, Waltham-on-the-Wolds, U.K. and Rural Industries Research and Development Corporation, Australia for their continuing support of Kellie Tinworth’s PhD candidature. We would also like to express our gratitude to Naomie Tidd for her expert technical assistance and intellectual support, and to the staff and students of the School of Animal and Veterinary Sciences, Charles Sturt University, for their assistance with the pony studies, especially Dr Chris Petzel for his clever advice on blood sampling.

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dexamethasone suppression test results with season, age, and sex in healthy ponies and horses. Journal of Veterinary Internal Medicine 19, 217-222.


Table 1. The baseline physical characteristics of the ponies at the frequently-sampled intravenous glucose tolerance test, before the treatment period. Each group comprised a sample size of six. Data are presented as mean ± SD.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Metformin-Treated Ponies</th>
<th>Control Ponies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodyweight (kg)</td>
<td>209.50 ± 49.95</td>
<td>209.8 ± 54.05</td>
</tr>
<tr>
<td>Body Condition Score (scale: 1 to 9)</td>
<td>6.00 ± 0.89</td>
<td>6.00 ± 0.89</td>
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<tr>
<td>Cresty Neck Score (scale: 1 to 5)</td>
<td>3.17 ± 0.41</td>
<td>3.00 ± 0.00</td>
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<tr>
<td>Neck Circumference (cm)</td>
<td>84.50 ± 6.03</td>
<td>85.67 ± 5.50</td>
</tr>
<tr>
<td>Crest Height (cm)</td>
<td>10.53 ± 1.86</td>
<td>10.38 ± 1.29</td>
</tr>
<tr>
<td>Crest Thickness (cm)</td>
<td>7.37 ± 1.31</td>
<td>7.73 ± 1.69</td>
</tr>
</tbody>
</table>
Table 2. The baseline values for insulin sensitivity (SI), glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and disposition index (DI) from Minimal Model analysis of the frequently-sampled intravenous glucose tolerance test of the ponies before treatment (Day 0). Each group comprised a sample size of six. Data are presented as mean ± SD.

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Metformin-Treated Ponies</th>
<th>Control Ponies</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI ((mU/L)⁻¹•min⁻¹)</td>
<td>0.36 ± 0.31</td>
<td>0.32 ± 0.31</td>
</tr>
<tr>
<td>Sg (min⁻¹•10²)</td>
<td>0.98 ± 0.60</td>
<td>0.93 ± 0.58</td>
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<tr>
<td>AIRg (mU•L⁻¹•min⁻¹)</td>
<td>1079 ± 905.8</td>
<td>840.6 ± 557.2</td>
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<tr>
<td>DI</td>
<td>430.9 ± 406.9</td>
<td>355.3 ± 334.2</td>
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</table>
Figure legends

Figure 1: Physical characteristics of the six ponies, illustrating the change in these variables over time, with or without metformin treatment. Day 0 (○) and Day 22 (○) illustrate the change when no metformin treatment was applied. Day 0 (●) and Day 22 (●) illustrate the change when the ponies also received metformin (15 mg/kg BW orally, twice-daily for 21 days). Horizontal bars represent the mean for each set of observations. There was no significant effect of time or treatment on any of the variables measured. Significance was set at $P \leq 0.05$.

Figure 2: Insulin sensitivity (SI, A), glucose effectiveness (Sg, B), acute insulin response to glucose (AIRg, C) and disposition index (DI, D) from Minimal Model analysis of the frequently-sampled intravenous glucose tolerance test of the of the six ponies, illustrating the change in these variables over time, with or without metformin treatment. Day 0 (○) and Day 22 (○) illustrate the change when no metformin treatment was applied. Day 0 (●) and Day 22 (●) illustrate the change when the ponies also received metformin (15 mg/kg BW orally, twice-daily for 21 days). Horizontal bars represent the mean for each set of observations. There was no significant effect of time or treatment on any of the variables measured. Significance was set at $P \leq 0.05$. 