Metformin (1,1-dimethylbiguanide) is an orally administered antihyperglycemic drug used commonly for management of type 2 diabetes mellitus in humans (Figure 1).\(^1,2\) It is used to control blood glucose concentrations by inhibiting hepatic gluconeogenesis and glycogenolysis and by enhancing peripheral tissue sensitivity to insulin.\(^1,2\) Metformin does not appear to affect the production of insulin by pancreatic \(\beta\)-cells and does not cause hypoglycemia when given at therapeutic concentrations.\(^3–5\) A recent study\(^5\) indicates that metformin also increases insulin sensitivity in patients with type 1 diabetes mellitus and helps prevent apoptosis of the pancreatic \(\beta\)-cells. Reported adverse effects of metformin treatment in humans include abdominal discomfort, inappetence, vomiting, and diarrhea.\(^6\) Metformin also causes severe lactic acidosis, which is now recognized to be rare and usually associated with high circulating drug concentrations, often in association with renal dysfunction.\(^5,7\) The drug is excreted unchanged by the kidneys in humans and cats, and normal kidney function is required for adequate clearance of the drug.\(^7,8\) To the authors’ knowledge, adverse effects of metformin use in horses are unknown.

Equine metabolic syndrome is a condition that results in abnormal distribution of adipose tissue, high circulating insulin and glucose concentrations, glucose intolerance, high plasma lipid concentrations, predisposition to laminitis, and infertility in mares.\(^9,10\) Horses with equine metabolic syndrome are commonly glucose intolerant and have insulin resistance.\(^11\) Insulin resistance is also thought to be involved in the pathogenesis of many other equine conditions such as pars intermedia dysfunction, diabetes mellitus, hyperlipemia, laminitis, endotoxemia, and osteochondrosis dissecans.\(^10–16\) In horses, further study is needed to understand the involvement of insulin resistance in these various diseases. Current treatment for horses with equine metabolic syndrome includes limiting them to diets with a low glycemic index and increasing exercise.\(^11,12,16\) In some horses, this approach is not sufficient to reduce blood concentrations of circulating glucose or insulin, especially in individuals that are unable to undertake

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**Pharmacokinetics and bioavailability of metformin in horses**

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**Objective**—To determine pharmacokinetics and oral bioavailability of metformin in healthy horses.

**Animals**—4 adult horses.

**Procedures**—6 g of metformin was administered 3 times IV and PO (fed and unfed) to each horse, by use of a crossover design, with a 1-week washout period between treatments. Plasma metformin concentration was determined via high-pressure liquid chromatography.

**Results**—Mean ± SD distribution half-life of metformin following IV administration was 24.9 ± 0.4 minutes with a volume of distribution of 0.3 ± 0.1 L/kg. Mean area under the curve was 20.9 ± 2.0 h·µg/mL for IV administration; PO administration resulted in area under the curves of 1.6 ± 0.4 h·µg/mL in unfed horses and 0.8 ± 0.2 h·µg/mL in fed horses. Bioavailability was determined to be approximately 7.1 ± 1.5% in unfed horses and 3.9 ± 1.0% in fed horses. The maximal concentration following PO administration in unfed horses was 0.4 ± 0.1 µg/mL with a time at maximal concentration of 0.9 ± 0.1 hours. In fed horses, maximal concentration was reduced to 0.3 ± 0.04 µg/mL with a time at maximal concentration at 1.3 ± 0.3 hours.

**Conclusions and Clinical Relevance**—The low bioavailability of metformin may explain the reported lack of clinical success in improving insulin sensitivity with metformin treatment in horses. Dosages and dose intervals previously used may have been insufficient to achieve plasma concentrations of drug comparable to the therapeutic range achieved in humans. Therefore, a larger and more frequently administered dose may be required to fully evaluate efficacy of metformin in horses. (Am J Vet Res 2009;70:665–668)

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**ABBREVIATIONS**

| **AUC** | Area under the curve |
| **C\(_{\text{max}}\)** | Maximal concentration |
| **HPLC** | High-pressure liquid chromatography |

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Materials and Methods

Horses—Four healthy adult neutered horses (2 geldings and 2 ovariectomized mares) from the Oregon State University research herd aged 4, 10, 15, and 19 years of age and weighing (mean ± SD) 531.8 ± 21.3 kg were available for use in the study. Physical examinations and preliminary blood analyses including a serum biochemical profile and venous blood gas analysis were performed. All horses were considered healthy at the time of the study. The horses were brought into the Oregon State University Large Animal Clinic for acclimation 2 days before the trial and were housed in individual stalls in a climate-controlled barn. Diet included grass hay fed twice daily and ad libitum water for the duration of the study. The horses were housed and cared for in an accredited facility, and the study was approved according to the principles outlined by the Oregon State University Institutional Animal Care and Use Committee.

Study design—One day prior to the start of each treatment, an IV catheter was placed in the jugular vein of each horse to facilitate blood sample collection. Three treatments were administered to each horse in random order in a crossover design with a 1-week washout period between treatments. The 3 treatments were IV administration of metformin (6 g), PO administration of metformin (6 g) after feed withholding for 12 hours, and PO administration of metformin (6 g) with no feed withholding. For IV administration, 6 g of metformin was dissolved in 15 mL of saline (0.9% NaCl) solution and passed through a 0.2-µm filter. In a previous study that used the same technique, the solution for IV use was analyzed by use of HPLC (before and after filtration) and found to contain 98% of the stated dose. Intravenous treatments were administered in 500 mL of saline solution over 5 minutes via the jugular catheter within 1 hour of compounding. The catheter was flushed thoroughly with heparinized saline solution after metformin administration. Oral treatments were administered by crushing metformin tablets and mixing with water and administering by mouth with a syringe within 1 hour after compounding.

Blood samples were collected at times 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, and 24 hours after treatment. For the IV treatment, samples were also taken every 15 minutes for the first 4 hours. To preserve stability, plasma samples were obtained by centrifugation of blood within 1 hour after collection and immediately stored at −80°C for ≤ 3 weeks until shipment to a commercial laboratory for analysis of metformin concentrations via HPLC.

Additional blood samples were collected for measurement of blood lactate concentrations at 0, 4, 8, 12, and 24 hours after IV metformin administration and placed in lithium heparin-containing tubes. Intravenous jugular catheters were removed at the end of each testing period.

Assay—Quantitative analyses for metformin in plasma were performed by a commercial laboratory with an HPLC procedure following derivatization with p-nitrobenzoyl chloride. In summary, an internal standard (buformin, an antidiabetic drug chemically related to metformin) was added to a 0.5-mL aliquot of specimen. Following the addition of sodium hydroxide and approximately 0.6 g of sodium chloride, the sample was mixed well to dissolve the salt. One millilitre of p-nitrobenzoyl chloride reagent (4 g of para-nitrobenzoyl chloride in 100 mL of acetonitrile) was added, the tube was capped, and the solution was mixed for 15 minutes and extracted with a mixed solvent of 30% ethyl acetate and 70% n-butyl chloride. The solvent was removed and back extracted in 1N hydrochloric acid, which was made basic with sodium hydroxide, and again extracted in the mixed solvent. The solvent was evaporated to dryness, and the residue was reconstituted with mobile phase. Extracts were analyzed via HPLC with UV detection at 280 nm. To validate the assay in equine plasma, 6 concentrations of calibrators ranging from 0.05 to 50 µg/mL were included with each analytic batch, and 2 concentrations of control samples were included with each analytic batch. The between-run precision was 13.6% and 8.48% at 1.0 and 2.5 µg/mL, respectively.
The lower limit of quantitation was 0.05 µg/mL, and the upper limit of quantitation was 50 µg/mL. The interassay accuracy was 95% at 1.0 µg/mL and 96% at 2.5 µg/mL. Intra-assay precision was 8.3%, 2.3%, and 5.2% at 0.05, 1.0, and 2.5 µg/mL, respectively. Intra-assay accuracy was 97%, 103%, and 94% at 0.05, 1.0, and 2.5 µg/mL, respectively.

**Data analysis**—Metformin concentration–time data following IV and PO administrations were analyzed by use of noncompartmental analysis and estimated by use of a computer program. The Cₜₐₙ₉₀ and time of maximal concentration were determined via observation. The AUC was calculated by use of the linear trapezoidal method. Volume of distribution, elimination rate constant, mean residence times, and clearance were estimated by use of noncompartmental analysis and estimated by use of a computer program.

**Results**

Results of the pharmacokinetic analysis (Table 1) revealed that, following IV administration, metformin was rapidly eliminated from the plasma (Figure 2). The concentration half-life was quantifiable for > 5 half-lives. The mean distribution half-life of metformin following IV administration was 24.9 ± 0.4 minutes with a mean residence time of 27.5 ± 2.1 minutes. The mean AUC was 20.9 ± 0.2 h·µg/mL for IV administration, whereas PO administration resulted in AUCs of 1.6 ± 0.4 h·µg/mL in unfed horses and 0.8 ± 0.2 h·µg/mL in fed horses. The AUCs determined for each horse were used to estimate the bioavailability, and the mean bioavailability of metformin was approximately 7.1 ± 1.5% in unfed horses and 3.9 ± 1.0% without feed withholding. This estimate was based on the plasma data collected from fed and unfed horses, compared with IV infusion for those samples with quantifiable amounts of metformin. Quantitative data were obtained for samples collected up to 8 hours following administration. The maximal concentration of metformin achieved with the 6-g dose following PO administration was 0.4 ± 0.1 µg/mL in unfed horses. The Cₜₐₙ₉₀ was reduced to 0.3 ± 0.04 µg/mL in fed horses; however, this was not significantly different from values in unfed horses.

**Discussion**

Results of the present study revealed marked differences in the pharmacokinetics of metformin in horses, compared with humans. In humans, metformin has a half-life of 6.2 hours as judged on the basis of a 3-compartment open model with initial elimination half-life estimated to be 1.7 to 3.0 hours and a terminal elimination of 9 to 17 hours. In
the present study, metformin was rapidly eliminated and no longer detectable in plasma after 4 hours. Following IV administration in humans, the drug is completely excreted unchanged in urine with a renal clearance of (mean ± SD) 454 ± 47 mL/min.6 Our estimates suggested that the clearance of metformin in horses is >10 times that in humans and may, in part, explain the low plasma concentrations following PO administration.

Results of a human study10 indicate an absolute oral bioavailability of 40% to 60%, with gastrointestinal absorption complete within 6 hours of ingestion. In rats and humans, the rate of absorption is slower than the rate of renal elimination, which results in what has been described as a flip-flop pharmacokinetic model.2,3 Furthermore, an inverse relationship between the dose ingested and the relative absorption has been observed as doses are increased,2,3 suggesting the involvement of an active, saturable absorption process that we hypothesize may be lacking in horses. On the basis of our results, it is possible that the rate of absorption and clearance of metformin may be higher in horses than in humans, which may in part account for the drug’s low bioavailability. Low fractional bioavailability, as measured in the study reported here, can be caused by either low absorption or high excretion. Additional studies, including analysis of urinary excretion data, are needed to determine which of these is responsible for the low bioavailability of metformin in horses. Despite the rapid clearance from plasma, the maximal concentrations reached were close to the reported therapeutic range for humans of 0.5 to 1 µg/mL.20 Our data suggested that dosages and administration intervals used in earlier equine studies were insufficient to achieve plasma concentrations of drug comparable to the therapeutic range achieved in humans. More information regarding metformin pharmacodynamics and pharmacokinetics in horses may be obtained by use of a larger sample size.

Low bioavailability only partially explains the lack of success with administration of metformin in horses. Metformin is reported to have a substantial first-pass pharmacodynamic effect that occurs in the liver and gastrointestinal wall in rats, and PO administration of metformin provides greater clinical response than IV administration in humans.22,23 These pharmacodynamic and pharmacokinetic effects are hypothesized to be linked to the therapeutic effects of treatment because stronger, sustained glucose-lowering responses are observed when metformin is administered orally, compared with the equivalent dose administered IV.21 On the basis of the results of the study reported here, we hypothesize that the lack of clinical success of treatment with metformin in horses may be attributable to a combination of low bioavailability and increased rate of elimination. Although the horses used in this study had a wide age range, we did not detect a significant difference in bioavailability in young versus old horses. Therefore, it is apparent that a study designed to measure efficacy of metformin in horses may require larger doses and more frequent administration or perhaps a sustained-release formulation.

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