JOURNAL OF
Veterinary Pharmacology and Therapeutics

*J. vet. Pharmacol. Therap.* **35**, 342–350. doi: 10.1111/j.1365-2885.2011.01329.x.

# Pharmacokinetics and pharmacodynamics of protamine zinc recombinant human insulin in healthy dogs

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Clark, M., Thomaseth, K., Heit, M., Hoenig, M. Pharmacokinetics and pharmacodynamics of protamine zinc recombinant human insulin in healthy dogs. *J. vet. Pharmacol. Therap.* **35**, 342–350.

Protamine zinc insulins are generally considered to be long acting, with slow absorption from subcutaneous tissue. Protamine zinc recombinant human insulin (PZIR) may be useful to treat diabetic dogs. The purpose of this study was to describe the pharmacokinetics and pharmacodynamics of PZIR in dogs. PZIR was administered subcutaneously to 10 healthy Beagles using an incomplete crossover design, at doses of 0.3 or 0.5 U/kg (each $n = 5$), 0.8 U/kg ($n = 10$), or 0.8 U/kg at three separate sites ($n = 6$). Insulin and glucose concentrations were measured over 24 h. The shapes of insulin and glucose curves were variable among dogs, and the relationship between insulin dose, concentration, and glucose-lowering effect was nonlinear. For single-site 0.8 U/kg, median (range) onset of action was 3.5 h (0.5–10 h), time to glucose nadir was 14 h (5 to >24 h), and duration of action was >24 h (16 to >24 h). Mathematical model predictions of times to 50% and 90% insulin absorption, and fraction of insulin absorbed in 24 h, were not significantly different among protocols. Results confirm the tendency toward a late onset and long duration of action for PZIR in dogs. This insulin may be an alternative treatment option for diabetic dogs.

(Paper received 4 April 2011; accepted for publication 26 July 2011)

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## INTRODUCTION

Protamine zinc insulin, or PZI, is generally considered to be a long-acting insulin owing to its slow absorption from subcutaneous (s.c.) tissue. Although the insulin molecules in PZI are structurally identical to those in regular insulin, addition of zinc and positively charged protamine in greater than equimolar amounts results in the formation of insulin/zinc/protamine complexes that precipitate at neutral pH (Reiner *et al.*, 1943; Davis, 2006). Administering these complexes as a suspension leads to gradual dissociation and delayed release of insulin monomers or dimers into the systemic circulation (Lawrence & Archer, 1937).

Historically, most PZI products have been composed of a mixture of bovine (90%) and porcine (10%) insulin or of 100% bovine insulin. Bovine insulin differs from feline insulin by one amino acid and from canine insulin by three (Neubauer & Schöne, 1978; Halldén *et al.*, 1986; Hoenig *et al.*, 2006). PZI is no longer commonly used in humans, having been replaced as a basal insulin by 'peakless' insulin analogs such as glargine and detemir (Davis, 2006; Fowler, 2008). However, approved beef or beef-pork PZI products remained on the veterinary market until 2008 in the US (IDEXX, 2009) and until 2010 in the UK (Pfizer, 2010).

Beef or beef-pork PZI products have been used frequently to manage diabetic cats, as their duration of action is perceived to be superior to that of neutral protamin Hagedorn (NPH) insulin in this species (Moise & Reimers, 1983; Wallace *et al.*, 1990; Nelson *et al.*, 2001). Anecdotally, they have not been favored as first-choice insulins in dogs because of variable time-action profiles; however, scientific studies are lacking.

Recently, a recombinant human insulin in a protamine zinc formulation, or PZIR (ProZinc®; Boehringer-Ingelheim Vetmedica, St Joseph, MO, USA), was released onto the veterinary market. This product is licensed for use in cats. The purpose of the study reported here was to characterize the pharmacokinetics and pharmacodynamics of PZIR in healthy dogs, to assess its potential for future use in dogs with diabetes mellitus.

## MATERIALS AND METHODS

### *Animals*

Ten healthy adult male neutered purpose-bred research Beagles (average weight 11 kg; range 9.8–12.7 kg) were used for this study. The dogs were individually housed in a university animal research facility and had been accustomed to their environment and to handling for at least 4 weeks prior to the first study period. All procedures involving them were approved by the University's Institutional Animal Care and Use Committee and were conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Dogs were fed a commercial dry diet (ProPlan Chicken and Rice Adult Shredded Blend; Nestlé Purina, St Louis, MO, USA) twice a day, and food intake was adjusted to maintain a stable body weight.

### *Insulin administration*

Five dogs were randomly selected to receive a s.c. injection of 0.3 U/kg of PZIR (Boehringer-Ingelheim Vetmedica) at a single site on the lateral thorax. The second group of five dogs received the same product at 0.5 U/kg. After at least 4 weeks, the dogs were randomly selected to receive either saline (four dogs) or a s.c. injection of 0.8 U/kg of PZIR (six dogs), at a single site on the lateral thorax. After four subsequent weeks, the dogs that had received saline received 0.8 U/kg of PZIR at a single site, while the dogs that had previously received insulin at a single site received the same dose of insulin, i.e. 0.8 U/kg, at three separate sites (right and left lateral thorax, lateral abdomen).

For data analysis, experiments were designated by protocol as follows: A = 0.8 U/kg (one site), B = 0.8 U/kg (three sites), C = saline, D = 0.3 U/kg (one site), and E = 0.5 U/kg (one site). Study design is summarized in Table 1.

### *Sample collection*

In each study period, on day 1, a jugular catheter was placed in each dog. Throughout the study, catheter patency was maintained by flushing with sterile heparinized saline (2.5 U/mL). Food was removed in the evening of day 1 (13–14 h prior to the baseline sampling time point) and was withheld for the duration of the study period. On day 2, two baseline blood samples were obtained, and insulin or saline was administered at time 0.

**Table 1.** Number of dogs studied with each protocol and insulin dose

| | Dose (U/kg) | | | |
|---|---|---|---|---|
| Protocol | 0 | 0.3 | 0.5 | 0.8 |
| A | 0 | 0 | 0 | 10 |
| B | 0 | 0 | 0 | 6 |
| C | 4 | 0 | 0 | 0 |
| D | 0 | 5 | 0 | 0 |
| E | 0 | 0 | 5 | 0 |

Blood samples were taken from the jugular catheter at 30, 60, 90, 120, 180, 240, 300, and 360 min after administration, then at 2-h intervals for a total of 24 h (except for the 0.3 U/kg administration, where 20- and 40-min samples were taken rather than a 30-min sample, and no samples were collected between 14 and 24 h). For each sample, glucose concentrations were measured immediately with a handheld glucometer (WaveSense Presto, AgaMatrix, Salem, NH). If glucometer results were <2.8 mM or if blood glucose appeared to be declining rapidly, the frequency of glucometer measurements was increased, and dogs were monitored for clinical signs of hypoglycemia.

### *Glucose and insulin assays*

Samples were placed in EDTA microcentrifuge tubes and kept on ice until centrifugation (5 min at 5000 ***g***). Plasma was separated for glucose and insulin measurement. Samples for glucose measurement were kept at −4 °C (if assays were performed on the following day) or frozen at −20 °C until assayed. Glucose concentrations were measured with a glucose oxidase assay [Glucose (Trinder) Assay, Genzyme Diagnostics, Charlottetown, PEI]. For insulin measurement, samples were stored at −20 °C, and insulin concentrations were determined using a human insulin radioimmunoassay (Human Insulin-Specific RIA, Millipore, Billerica, MA, USA).

For assay validation, dog plasma containing ProZinc® was diluted serially with charcoal-treated dog plasma. The dilution curves were parallel to the standard curve for human insulin standards. All samples were tested in duplicate. The intra-assay coefficient of variation was 6.3%, and the interassay coefficient of variation was 10.2%. The standard curve for serial dilutions of plasma from clinically normal dogs was observed to be parallel to the standard curve for human insulin standards. Addition of two concentrations of human standard to canine plasma resulted in mean ±SD recovery of 92.0 ± 4.8%. The assay had a working range of 2–200 $\mu$U/L.

### *Statistical analysis of insulin and glucose concentrations*

Tabulation, graphical analysis, and visual inspection were used for data screening and determination of maximum insulin and minimum glucose concentrations for each dog in each protocol. Normality of the data was evaluated using the Kolgorov–Smirnov test, and subsequent calculations, including mixed-effects modeling, were carried out using one of three statistical software packages (Microsoft Office Excel 2007; Microsoft Corporation, URL: http://www.microsoft.com; Graph Pad Prism Version 5.00 for Windows; GraphPad Software, San Diego, CA, USA; R package version 3.1-96; R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org).

Basal insulin ($I_b$) and glucose ($G_b$) concentrations were calculated as the mean of the two pre-injection blood sample measurements for each dog in each protocol. Determination of statistical differences of individual glucose or insulin concentrations from basal was made using the standard deviation of the

baseline glucose or insulin measurements for all dogs. Values of glucose or insulin for an individual dog in a particular protocol were considered to be significantly different from basal if they differed by greater than two standard deviations from $I_b$ or $G_b$ for that dog in that protocol.

Average insulin and glucose concentrations for each dog in each protocol were determined from the area under the corresponding concentration curve (AUC), calculated with the trapezoidal rule between time 0 and 24 h, divided by the interval length (i.e. 24 h). As the adopted factorial experimental design was unbalanced (i.e. all protocols did not include the same number of dogs), determination of statistical differences of average glucose and insulin concentrations among protocols was carried out using linear and nonlinear mixed-effects modeling. Protocol A (0.8 U/kg, single site) was used as the reference protocol in statistical comparisons, as all dogs underwent this protocol. Significance was set at $P < 0.05$.

### *Measures of insulin pharmacokinetics*

For each dog in protocols A (0.8 U/kg, single site), B (0.8 U/kg, three sites), and E (0.5 U/kg), time to maximum insulin concentration was determined by visual inspection of the data. Insulin persistence was calculated as the time from insulin administration to the first time point (after an insulin peak) at which insulin concentrations no longer differed from basal. Data for protocols C (saline) and D (0.3 U/kg) were not analyzed in this manner, as protocol C did not involve insulin administration, and protocol D did not contain time points between 14 and 24 h.

### *Measures of insulin pharmacodynamics*

For each dog in protocols A, B, and E, effects of insulin on blood glucose were described by the following measures:

a) Onset of insulin action, defined as time after insulin administration that glucose concentration first became significantly lower than basal.
b) Time to glucose nadir, defined as time after insulin administration that the lowest glucose concentration was reached.
c) Duration of insulin action, defined as time from insulin administration to the time, after at least two consecutive measurements of glucose concentrations significantly lower than basal that glucose concentration returned to a value not significantly lower than basal.

### *Mathematical modeling of insulin absorption*

As total insulin concentrations in plasma after s.c.injection depend both on absorption and on endogenous secretion, a confounding effect on measured insulin concentrations may be associated with the closed loop operation of glucose-insulin homeostasis, which acts even during fasting conditions. Increased insulin efficacy in reducing blood glucose may be caused either by stronger inhibition of endogenous glucose production or by increased insulin-mediated peripheral glucose uptake. A reduced glucose concentration may also indirectly lower circulating insulin through inhibition of endogenous insulin secretion. To adjust for this confounding effect and to provide an estimate of insulin concentrations as a reflection of absorption alone, a mathematical model was generated as follows:

Insulin kinetics were described by a one-compartment model. Insulin absorption following s.c. injection was modeled as a Weibull distribution characterized by two parameters (scale = $\lambda$; shape = $\kappa$). Endogenous insulin production was assumed related to glucose concentration using a power-law approximation. All quantities except time were normalized with respect to their basal values to obtain dimensionless variables. The model equations were as follows:

$$\frac{\mathrm{d}i(t)}{\mathrm{d}(t)} = \alpha_1\left(g(t)^{\beta_1} - i(t)\right) + \frac{D_1}{\gamma}W(t;\lambda,\kappa); i(0) = 1$$

where time $t$ is expressed in hours (h); $i(t) = I(t)/I_b$ and $g(t) = G(t)/G_b$ represent insulin and glucose concentrations profiles normalized with respect to their basal values; while normalized insulin is fitted to experimental data, the time course of glucose concentration $G(t)$ is viewed as a model input and is obtained by linear interpolation of glucose concentration measurements; $\alpha_I$ is a rate constant (1/h); $\beta_I$ is the sensitivity of fractional insulin variations to fractional glucose variations around basal; $D_I$ is the known administered insulin dose (U/kg); $\gamma_I$ is the insulin pool at basal (U/kg); and $W(t; \lambda; \kappa)$ is the Weibull distribution defined as

$$W(t;\lambda,\kappa) = \begin{cases} \frac{\kappa}{\lambda}\left(\frac{t}{\lambda}\right)^{\kappa-1}\mathrm{e}^{-(t/\lambda)^{\kappa}}, & t \geq 0 \\ 0 & t<0 \end{cases}$$

The estimated model parameters are those indicated with Greek letters, i.e. $\alpha_I$, $\beta_I$, $\gamma_I$, $\lambda$, $\kappa$. Parameter estimates of $\alpha_I$ and $\beta_I$ were obtained after log-transformation to ensure positivity. Estimates of average model parameters and their between-animal variations were obtained by fitting the normalized insulin model output $i(t)$ to normalized insulin concentration measurements using nonlinear mixed-effects modeling of all available datasets simultaneously (Pinheiro & Bates, 2000).

The average time courses of insulin absorption for protocols A, B, and E were described using the measures $T_{50}$ (time to 50% of insulin absorption), $T_{90}$ (time to 90% of insulin absorption), and d24 (fraction of the insulin dose absorbed in 24 h). Estimates for these measures were obtained by nonlinear mixed-effects modeling. The model equations (described previously) and parameter sensitivity equations were implemented using a modeling software tool (Thomaseth, 2003) and solved numerically using a fourth-order, variable-step Runge–Kutta method. Individual values of $T_{50}$, $T_{90}$, and d24 were calculated from individual kinetic parameter estimates provided by the modeling, given the mixed-effects model fitted to all available data simultaneously. Median values for each protocol were compared using the Kruskal–Wallis test for nonparametric data, followed by a Dunn's post-test.

Model predictions, rather than concentration data, were used for comparison of absorption among protocols because the goal of the multiple-site administration was to assess its effects on average absorption of PZIR, not to describe the relative clinical characteristics of three-site administration or to evaluate the fitness of this protocol for clinical use. For the same reason, no statistical comparisons of measures of insulin action among protocols were made, other than evaluation of average glucose and insulin concentrations.

## RESULTS

### *Insulin pharmacokinetics*

No consistent pattern in the shapes of the insulin concentration–time profiles was observed; in each protocol, insulin concentration exhibited multiple peaks in some of the dogs and a single peak in others. Insulin concentration–time curves for each of three dogs receiving 0.8 U/kg of insulin at a single site or at three separate sites are shown in Fig. 1 (in conjunction with glucose concentrations).

Median (range) maximum insulin concentration, time of maximum insulin concentration, and insulin persistence for protocols A (0.8 U/kg, single site), B (0.8 U/kg, three sites), and E (0.5 U/kg) are shown in Table 2. For four of the nine dogs in protocol A for which insulin persistence was determined, insulin concentration differed significantly from baseline at the last measurement of the 24-h study period. Insulin persistence for these dogs was recorded as more than 24 h.

Median maximum insulin concentration and time to maximum insulin concentration were similar among protocols. Range was wide for all measures calculated.

### *Insulin pharmacodynamics*

The shapes of the glucose concentration–time profiles among dogs were variable. Blood glucose curves for each of three dogs receiving insulin at 0.8 U/kg at a single site and at three separate sites are shown in Fig. 1 (in conjunction with insulin concentrations). Blood glucose curves for all ten dogs receiving insulin at 0.8 U/kg at a single site are shown in Fig. 2.

Mean ± standard deviation of glucose nadir, and median (range) onset of insulin action, time to glucose nadir, and duration of insulin action for protocols A (0.8 U/kg, single site), B (0.8 U/kg, three sites), and E (0.5 U/kg) are shown in Table 3. For five of the nine dogs in protocol A for which duration of insulin action was determined, and for one of the six dogs in protocol B, glucose concentration was significantly lower than basal at the last measurement of the 24-h study period. Duration of action for these dogs was recorded as more than 24 h.

Median onset of insulin action, time to glucose nadir, and duration of insulin action were similar among protocols.

One of 10 dogs in protocol A and one of six dogs in protocol B had an initial brief increase in blood glucose of slightly greater than two standard deviations above basal, despite being fasted. In the same dog in protocols A and B, glucose concentrations slightly exceeded basal values for all time points after the one at which blood glucose was no longer significantly decreased (i.e. after return to baseline). In one dog in protocol A and one dog in

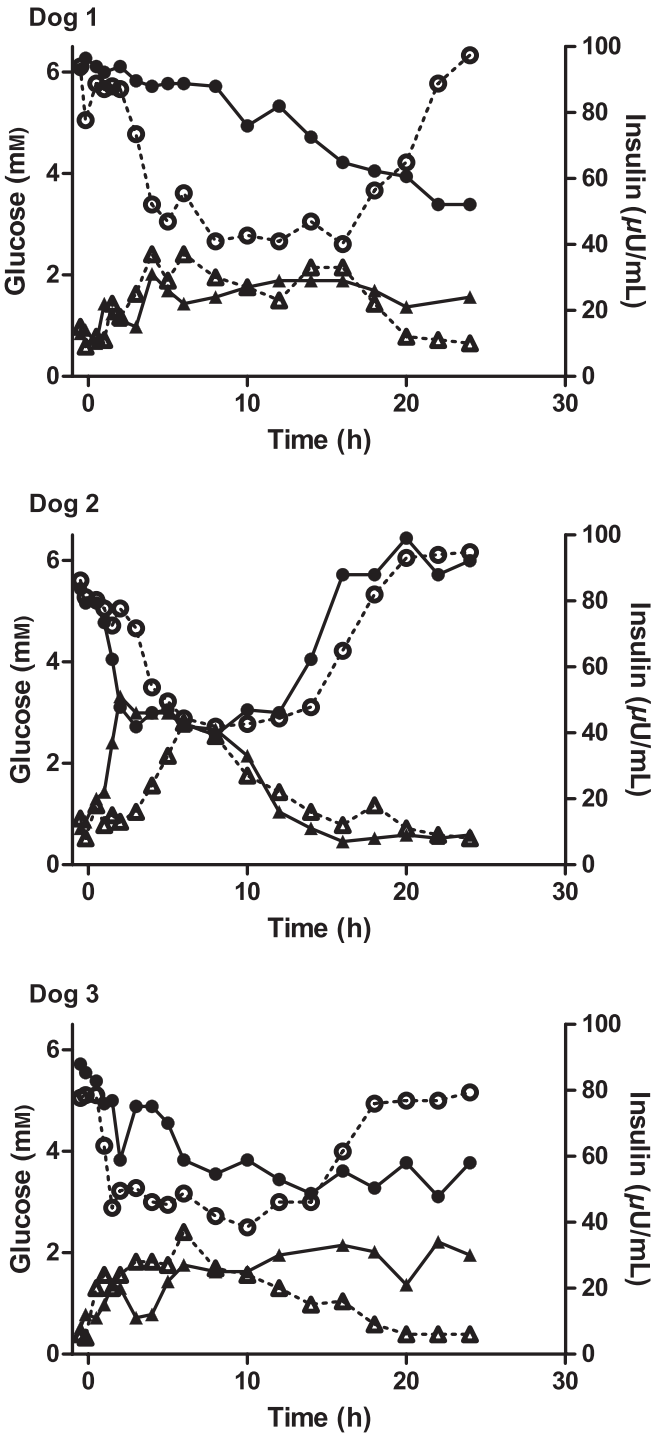


**Fig. 1.** Blood glucose and insulin concentrations for each of three dogs receiving 0.8 U/kg protamine zinc recombinant human insulin at a single site or at three separate sites. –●– Glucose, single site; –▲–insulin, single site; -⊙-glucose, multiple sites; -△-insulin, multiple sites.

**Table 2.** Median and range of maximum insulin concentration, time to maximum insulin concentration, and median insulin persistence for dogs in protocols A (0.8 U/kg PZIR, single site), B (0.8 U/kg PZIR, three sites), and E (0.5 U/kg PZIR)

| Protocol | Dosage | Median (range) maximum insulin concentration, μU/mL | Median (range) time to maximum insulin concentration, h | Median (range) insulin persistence, h |
|---|---|---|---|---|
| A ($n$ = 10) | 0.8 U/kg, single site | 34 (25–69) | 7 (1–22) | 22 (12 to >24)* |
| B ($n$ = 6) | 0.8 U/kg, three sites | 37 (30–49) | 6 (5–24) | 17 (10 to >24) |
| E ($n$ = 5) | 0.5 U/kg | 29 (24–50) | 6 (0.5–18) | 20 (6–24) |

*$n$ = 9. PZIR, protamine zinc recombinant human insulin.

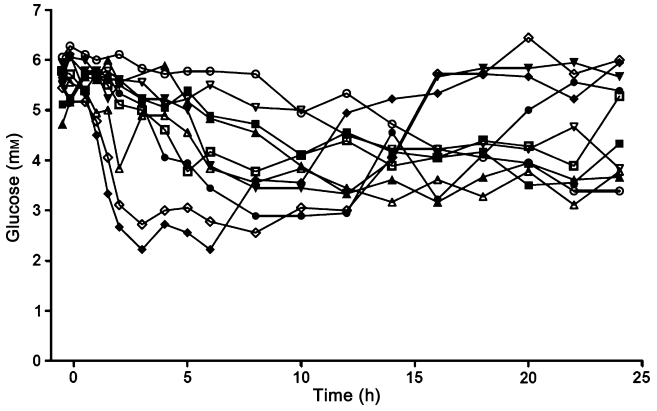


**Fig. 2.** Blood glucose curves for ten dogs receiving 0.8 U/kg protamine zinc recombinant human insulin at a single site.

protocol E, insulin concentrations differed significantly from basal at only one time point. However, blood glucose concentrations for both dogs were significantly lower than basal for a period of at least 20 h postadministration.

Clinical signs of hypoglycemia (weakness, mental dullness, and ataxia) occurred in one dog in protocol A, 6.5 h after insulin administration. Blood glucose measured via glucometer at that time was 1.7 mM. Signs resolved rapidly when the dog was fed. In this dog, blood glucose concentration (via glucose oxidase assay) had already become significantly lower than basal at the first postadministration time point and was between 2.2 and 2.8 mM from 2 to 6 h postadministration. An insulin peak of 69 μU/mL had occurred at the 4-h time point. Insulin persistence and duration of action were not calculated for this dog.

*Average insulin and glucose concentrations and comparison among protocols*

Box plots of the average insulin and glucose concentrations over 24 h for the dogs in each protocol are shown in Figs 3 & 4, respectively. The corresponding mean values, differences with respect to protocol A, and statistical significance levels are reported in Table 4 for insulin and in Table 5 for glucose, respectively. Average insulin concentrations between protocols A (0.8 U/kg, single site) and E (0.5 U/kg) were not significantly different ($P$ = 0.202), despite the difference in dose. However,

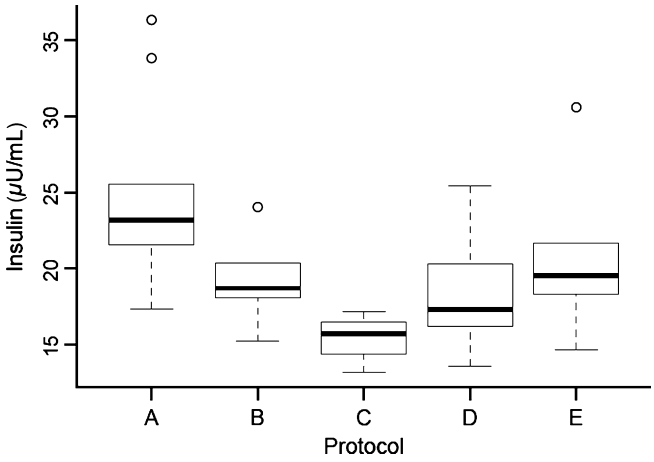


**Fig. 3.** Box plots of average insulin concentrations over 24 h for dogs in each protocol. Circles represent outliers. A = 0.8 U/kg (one site), B = 0.8 U/kg (three sites), C = saline, D = 0.3 U/kg (one site), and E = 0.5 U/kg (one site).

**Table 3.** Mean and standard deviation of glucose nadir, and median and range of onset of insulin action, time to glucose nadir, and duration of insulin action for dogs in protocols A (0.8 U/kg PZIR, single site), B (0.8 U/kg PZIR, three sites), and E (0.5 U/kg PZIR)

| Protocol | Dosage | Mean ± SD of glucose nadir (mM) | Median (range) onset of insulin action (h) | Median (range) time to glucose nadir (h) | Median (range) duration of insulin action (h) |
|---|---|---|---|---|---|
| A ($n$ = 10) | 0.8 U/kg, single site | 3.2 ± 0.5 | 3.5 (0.5–10) | 14 (5 to >24) | >24 (16 to >24)* |
| B ($n$ = 6) | 0.8 U/kg, three sites | 2.9 ± 0.4 | 4 (1–10) | 13 (8–20) | 22 (18 to >24) |
| E ($n$ = 5) | 0.5 U/kg | 3.6 ± 0.2 | 3 (1–14) | 16 (6–16) | 20 (16 to >24) |

*$n$ = 9. PZIR, protamine zinc recombinant human insulin.

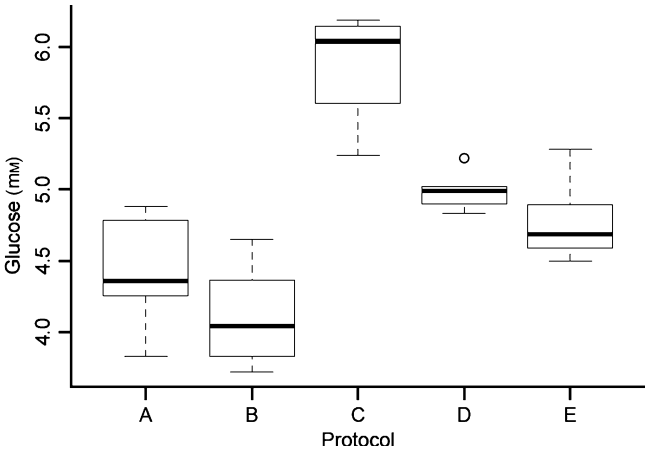


**Fig. 4.** Box plots of average glucose concentrations over 24 h for dogs in each protocol. Circles represent outliers. A = 0.8 U/kg (one site), B = 0.8 U/kg (three sites), C = saline, D = 0.3 U/kg (one site), and E = 0.5 U/kg (one site).

**Table 4.** Mean average insulin concentrations for different protocols, differences with respect to protocol A, and statistical significance levels. A = 0.8 U/kg (one site), B = 0.8 U/kg (three sites), C = saline, D = 0.3 U/kg (one site), and E = 0.5 U/kg (one site)

| Protocol | Mean | Different vs. A | SE | *P*-value |
|---|---|---|---|---|
| A | 24.55 | – | 1.56 | – |
| B | 19.18 | −5.36 | 2.55 | 0.052 |
| C | 15.43 | −9.11 | 2.92 | 0.007 |
| D | 18.57 | −5.98 | 2.71 | 0.042 |
| E | 20.94 | −3.60 | 2.71 | 0.202 |

**Table 5.** Mean average glucose concentrations for different protocols, differences with respect to protocol A, and statistical significance levels. A = 0.8 U/kg (one site), B = 0.8 U/kg (three sites), C = saline, D = 0.3 U/kg (one site), and E = 0.5 U/kg (one site)

| Protocol | Mean | Different vs. A | SE | *P*-value |
|---|---|---|---|---|
| A | 79.58 | – | 1.86 | – |
| B | 74.56 | −5.01 | 2.70 | 0.082 |
| C | 104.8 | 25.25 | 3.14 | <0.001 |
| D | 89.74 | 10.16 | 2.89 | 0.003 |
| E | 86.35 | 6.78 | 2.89 | 0.032 |

average glucose concentration for protocol A was significantly lower than for protocol E ($P = 0.032$).

### *Modeling of insulin absorption*

Best-fit model predictions compared with measured insulin concentrations collected in all experiments are shown in Fig. 5, and within each different protocol in Fig. 6. Table 6 summarizes the estimated model parameters that characterize the average behavior of the proposed insulin kinetics model (fixed effects) and the standard deviation of the between-animal variations (random effects). Parameters $\beta_I$ and $\gamma_I$ were assumed constant among all dogs and experiments to ensure practical identifiability and plausibility of results.

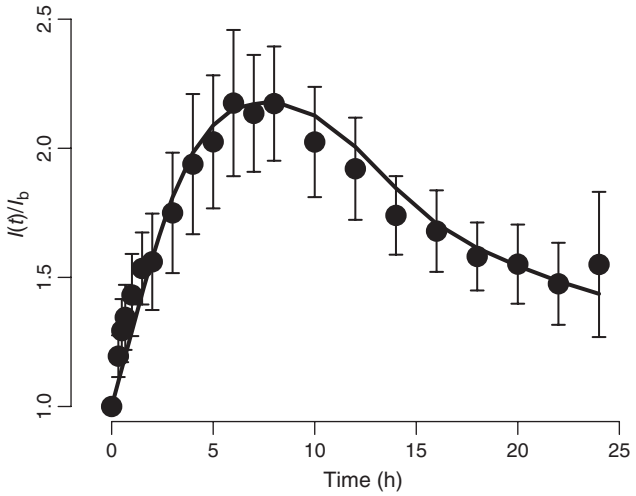


**Fig. 5.** Mean ± SE of insulin increment over basal (filled circles = measured concentrations; solid line = average model predictions) for all experiments.

For each protocol, median and range for model-derived times to 50% and 90% of insulin absorption ($T_{50}$, $T_{90}$) and fraction of the dose absorbed in 24 h (d24) are shown in Table 7. Statistical significance levels (*P*-values) for differences among protocols are also reported in Table 7. No significant differences among protocols A, B, and E were found in model-derived values for any of these descriptors of insulin absorption.

## DISCUSSION

Previous reports of the pharmacokinetics and pharmacodynamics of PZI in dogs are limited. One study using beef-pork origin PZI at 0.5 U/kg in eight diabetic dogs reported a peak activity time of 12 ± 4 h and a duration of action of more than 24 h (Church, 1981). Stenner *et al.*, (2004) reported, in abstract form, an onset and duration of action of 3.1 ± 0.7 and 19.0 ± 1.6 h, respectively, for a 0.5 U/kg dose of beef-pork PZI in nine healthy dogs. These data are largely consistent with the time course of insulin action observed here for the recombinant formulation of PZI, although median rather than mean was reported in this study because of non-normality of the data.

Substantial between-dog variability in the time course of insulin action was evident in this study (see Figs 1 & 2, and Tables 2 & 3). This was also noted in the study by Church *et al.*, in which significant dog-to-dog variation was found in blood glucose response not only to PZI, but also to NPH insulin and to a porcine lente insulin. Porcine lente insulin had a more predictable time to peak activity (6 ± 1 h, compared with 9 ± 4 h for NPH and 12 ± 4 h for PZI).

In other studies, administration of 0.5 U/kg of porcine NPH insulin to seven healthy dogs resulted in a range of times to maximum insulin concentrations of 0.5–2 h, times of insulin persistence of 4–12 h, and peak activity times of 0.5–4 h (Goeders *et al.*, 1987). For recombinant human NPH in ten diabetic dogs, Palm *et al.*, (2009) reported ranges for the same

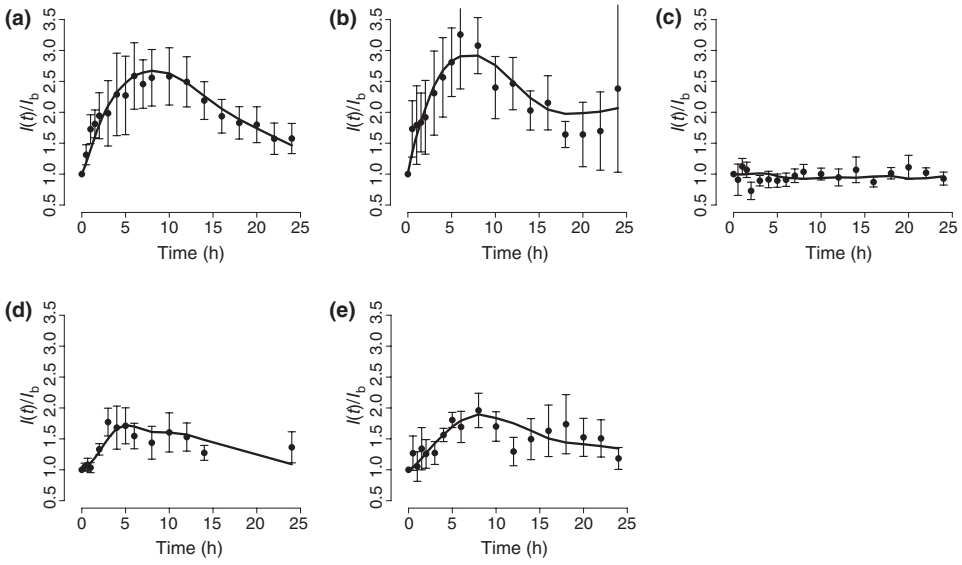


**Fig. 6.** Mean ± SE of insulin increment over basal (filled circles = measured concentrations; solid line = average model predictions) for each protocol. A = 0.8 U/kg (one site), B = 0.8 U/kg (three sites), C = saline, D = 0.3 U/kg (one site), and E = 0.5 U/kg (one site).

**Table 6.** Parameter estimates of the insulin kinetics model

| Parameter | Value | SE | 95% CI | SE* |
|---|---|---|---|---|
| $\alpha_I$ | 0.338 | 0.28† | 0.197, 0.579 | 0.50† |
| $ß_I$ | 2.43 | 0.31† | 1.33, 4.46 | – |
| $\gamma_I$ | 0.059 | 0.013 | 0.034, 0.085 | – |
| $\lambda$ | 12.51 | 1.20 | 10.2, 14.9 | 4.7 |
| $\kappa$ | 1.88 | 0.16 | 1.56, 2.20 | 0.69 |

*Estimated between-animal variability (random effects). †Refers to log-transformed parameter.

parameters of 0.5–4 h (median, 1.5 h), 3.1 to >10 h (median, 8.5 h), and 1 to >10 h (median, 4 h), respectively.

Variable pharmacokinetics and pharmacodynamics have also been observed for porcine lente insulin administered to diabetic dogs at different doses. Insulin persistence ranged from 14 to >24 h (mean 17.4 ± 3.65 h) in ten diabetic dogs receiving this insulin (Graham *et al.*, 1997). In eight diabetic dogs with no detectable endogenous insulin secretion, ranges for times to the two peak concentrations of porcine lente insulin were 1–6 h (mean 3.1 ± 2.2 h) and 2–14 h (mean 8.9 ± 4 h), respectively. Insulin persistence had a range of 8–22 h (mean 15.5 ± 4.5 h), and ranges for time to glucose nadir and duration of insulin action were 4–22 h and 10 to >24 h, respectively (Fleeman *et al.*, 2009). Although it might be argued that some of the variation in the diabetic dogs was caused by dose differences, Graham *et al.*, (1997) found no correlation between dose and insulin persistence or insulin AUC. In addition to between-dog variation, within-dog variation in serial blood glucose curves has been observed in diabetic dogs given porcine lente insulin (Fleeman & Rand, 2003).

Marked interpatient and intrapatient differences in the time-action profile of insulin have been recognized in human medicine for decades and are ascribed partially to the inconsistency of insulin absorption even from dose to dose (Lauritzen *et al.*, 1979). Absorption of depot insulins such as NPH, lente, and PZI is an inherently irregular process, involving noncontrolled dissociation of insulin molecules from a heterogeneous aggregate. Rates of dissociation, diffusion from the injection site, and entry into the vasculature are affected by temperature, local blood flow, and depth of injection (Binder *et al.*, 1984). For nonmonomeric insulins, insulin concentration and injection volume also affect the rate of absorption. Higher concentrations favor persistence of insulin hexamers rather than monomers and retard dissociation (Soeborg *et al.*, 2009). Higher injection volumes lead to slower diffusion (Binder *et al.*, 1984).

Variability in absorption tends to increase as physicochemical complexity of the insulin preparation increases (i.e. as more

| Protocol | Dosage | $T_{50}$ (h) | $T_{90}$ (h) | d24 |
|---|---|---|---|---|
| A | 0.8 U/kg, single site | 13.1 (3.4–15.0) | 24.6 (8.3–41) | 0.88 (0.74–1.0) |
| B | 0.8 U/kg, three sites | 8.5 (3.0–19.6) | 18.0 (11.0–33.0) | 0.98 (0.67–1.0) |
| E | 0.5 U/kg | 11.1 (6.6–13.3) | 21.2 (10.6–31.1) | 0.95 (0.81–1.0) |
| *P*-value | – | 0.37 | 0.78 | 0.83 |

Statistical significance levels for differences among protocols are reported as *P*-values; significance was set at $P < 0.05$. PZIR, protamine zinc recombinant human insulin.

**Table 7.** Median and range for model-derived times to 50% of insulin absorption ($T_{50}$) and 90% of insulin absorption ($T_{90}$), and fraction of insulin dose absorbed in 24 h (d24) for dogs in protocols A (0.8 U/kg PZIR, single site), B (0.8 U/kg PZIR, multiple sites), and E (0.5 U/kg PZIR)

retarding substances are added), although even regular insulin injected subcutaneously does not produce a consistent absorption profile (Goeders *et al.*, 1987). However, effects of concentration, injection volume, and injection site location have been reported to be less pronounced for long-acting insulins such as PZI (Binder *et al.*, 1984). In the current study, a mathematical model was used to generate average insulin absorption profiles for PZIR. The lack of a significant difference in model-predicted absorption parameters among protocols A (0.8 U/kg, single site) and B (0.8 U/kg, three sites) supports a relatively minimal effect of injection volume or injection site location on the rate or extent of absorption of PZIR, compared with intraanimal and interanimal variability. Lack of a difference in these parameters between protocols A and E suggests that small dose increases likewise exert less of an effect on the time course of absorption of this long-acting insulin than does individual animal variation.

In humans, dose increases in an intermediate-acting insulin were shown to cause nonproportional changes in plasma insulin concentration and to prolong duration of action without changing time to maximal insulin concentration (Binder *et al.*, 1984). In two instances in the dogs studied here, insulin concentration was increased above basal at only one time point, yet blood glucose remained significantly lower than basal for up to 20 h. As noted from these data and from the average insulin concentrations over 24 h in protocols A (0.8 U/kg, single site) and E (0.5 U/kg), the relationship between insulin dose, plasma concentration, and glucose-lowering effect is complex and is nonlinear in dogs with the capacity for endogenous insulin secretion. In conjunction with absorption differences, the nonlinearity of this dose-concentration-response relationship may contribute to observed variation in insulin action.

A consequence of the high between-dog variability observed for most insulin formulations is that no one insulin is ideal for all dogs, and an increase in the number of insulin options available for diabetic dogs is desirable. This study was performed in healthy dogs, which were fasted on the day of insulin administration. Characteristics of insulin absorption would not be expected to be consistently different in diabetic dogs; however, the pharmacodynamics of insulin, as measured by blood glucose response, would certainly be affected by lack of endogenous insulin secretion and by feeding. Thus, pharmacodynamic data from dogs lacking endogenous insulin secretion would be necessary to predict the effects of repeated dosing of this insulin on blood glucose concentration. As well, studies using this insulin in a clinical setting are needed before conclusions can be drawn regarding its suitability in diabetic canine patients. Nonetheless, data from this study do verify that absorption of PZIR is adequate to lower blood glucose in normal dogs.

In conclusion, PZIR administered to dogs exhibits the variability in absorption and action that is typical of a complex hexameric insulin formulation. The trend toward a late onset and long duration of action for PZIR is similar to what has been reported for animal source PZI in dogs. This insulin preparation may be a useful treatment option for diabetic dogs.

## ACKNOWLEDGMENTS

Funding for this study was provided in part by Boehringer-Ingelheim Vetmedica, St Joseph, MO, USA.

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