

## Field Safety and Efficacy of Protamine Zinc Recombinant Human Insulin for Treatment of Diabetes Mellitus in Cats

R.W. Nelson, K. Henley, C. Cole, and the PZIR Clinical Study Group

**Background:** This study describes the efficacy of a new protamine zinc recombinant human insulin (PZIR) preparation for treating diabetic cats.

**Objective:** To evaluate effects of PZIR on control of glycemia in cats with newly diagnosed or poorly controlled diabetes mellitus.

**Animals:** One hundred and thirty-three diabetic cats 120 newly diagnosed and 13 previously treated.

**Methods:** Prospective, uncontrolled clinical trial. Cats were treated with PZIR twice daily for 45 days. Control of glycemia was assessed on days 7, 14, 30, and 45 by evaluation of change in water consumption, frequency of urination, appetite, and body weight, serum fructosamine concentration, and blood glucose concentrations determined 1, 3, 5, 7, and 9 hours after administration of PZIR. Adjustments in dosage of PZIR were made as needed to control glycemia.

**Results:** PZIR administration resulted in a significant decrease in 9-hour mean blood glucose ( $199 \pm 114$  versus  $417 \pm 83$  mg/dL,  $X \pm SD$ ,  $P < .001$ ) and serum fructosamine ( $375 \pm 117$  versus  $505 \pm 96$   $\mu$ mol/L,  $P < .001$ ) concentration and a significant increase in mean body weight ( $5.9 \pm 1.4$  versus  $5.4 \pm 1.5$  kg,  $P = .017$ ) in 133 diabetic cats at day 45 compared with day 0, respectively. By day 45, polyuria and polydipsia had improved in 79% (105 of 133), 89% (118 of 133) had a good body condition, and 9-hour mean blood glucose concentration, serum fructosamine concentration, or both had improved in 84% (112 of 133) of the cats compared with day 0. Hypoglycemia ( $<80$  mg/dL) was identified in 151 of 678, 9-hour serial blood glucose determinations and in 85 of 133 diabetic cats. Hypoglycemia causing clinical signs was confirmed in 2 diabetic cats.

**Conclusions and Clinical Relevance:** PZIR is effective for controlling glycemia in diabetic cats and can be used as an initial treatment or as an alternative treatment in diabetic cats that do not respond to treatment with other insulin preparations.

**Key words:** Endocrinology; Glycosuria; Pancreas.

Treatment of diabetes mellitus in cats often requires daily insulin administration to achieve control of glycemia.<sup>1,2</sup> Commonly used insulin preparations for diabetes in cats include human recombinant NPH, purified pork source lente, beef/pork source PZI, and the human synthetic insulin analog glargine.<sup>1–6</sup> Historically, beef/pork source PZI was the insulin of choice for treating diabetes in cats.<sup>1,2</sup> When the original manufacturer discontinued production and distribution of PZI in 1991, veterinarians used other intermediate (NPH, lente) and long-acting (ultralente) insulin preparations.<sup>3,4</sup> Problems with inconsistent insulin absorption and inadequate duration of effect with these insulin preparations led to resumption of PZI production by an animal health pharmaceutical company<sup>a</sup> in 1998. PZI-VET was subsequently shown to significantly improve control of

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### Abbreviation:

PZIR protamine zinc recombinant human insulin

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glycemia in newly diagnosed diabetic cats and poorly controlled diabetic cats previously treated with other insulin preparations.<sup>7</sup> Currently, PZI-VET is a commonly used insulin for treating diabetes in cats in the United States.

Although PZI-VET has been used with excellent results in diabetic cats, as an animal-derived product use of PZI-VET is problematic. Animal-derived insulins are expensive, primarily because obtaining pancreatic glands is labor intensive and manufacturing and formulating costs are high because of the inherent variability in source material. Although the risk is remote, animal-derived products always carry the concern for transmission of infectious diseases, such as transmissible spongiform encephalopathies.<sup>8</sup> Production of insulin products by recombinant DNA technology has the potential to provide advantages in terms of cost, safety, and supply. Recently recombinant human insulin was formulated as a protamine zinc product (PZIR). Studies conducted in diabetic rats showed similar potency and course of action between PZIR and PZI-VET.<sup>b</sup> A 30-day cross-over-designed pilot study, conducted in 50 diabetic cats further confirmed similarity between PZIR and PZI-VET in diabetic cats.<sup>c</sup> The purpose of this study was to prospectively evaluate the efficacy of PZIR for controlling glycemia in cats with newly diagnosed, untreated diabetes mellitus and cats with previously treated, poorly controlled diabetes under conditions encountered by veterinarians working in general and referral practices.

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*From the Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA (Nelson) and IDEXX Pharmaceuticals Inc, Greensboro, NC (Henley, Cole). The PZIR clinical study group comprised clinicians who investigated protamine zinc recombinant human insulin for the purpose of obtaining FDA approval for treatment of cats with diabetes mellitus. In addition to Drs Nelson, Henley, and Cole, other veterinarian members of the group were Kevin Barcus, Noemi Benitah, Marc Bercovitch, Adrienne Brode, Cheryl Burke, Bonnie Cate, Marcia Chastain, Jeff Dennis, Gary Edlin, Deborah Edwards, Scott Linick, Harrison Monk, Jean Pitcarin, James Prueter, Michelle Purnell, Michael Radcliffe, Roger Sifferman, and Jennifer Usiak.*

*Corresponding author: Richard W. Nelson, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616; email: rwnelson@ucdavis.edu.*

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## Materials and Methods

Nineteen veterinary clinics in the United States participated in the study, including veterinarians in general practice ( $n = 14$ ), referral practice (4), and a university teaching hospital (1). The study was conducted according to Food and Drug Administration guidelines for investigation of new animal drugs<sup>d</sup> and design and conduct of clinical studies of veterinary medical products submitted for approval to the United States.<sup>c</sup> Written owner consent was obtained before entry of each cat into the study.

### Inclusion Criteria

All cats were diagnosed with diabetes mellitus based on the presence of polyuria, polydipsia, polyphagia with or without weight loss, persistent fasting hyperglycemia (blood glucose concentration  $> 250$  mg/dL), and persistent glycosuria. Cats could be newly diagnosed but naïve to treatment or previously diagnosed but considered poorly regulated on their current insulin treatment regimen. Cats could be thin, ideal body weight, or obese. Cats had to complete the study, had to be treated with PZIR throughout the study, and had to be fed the same diet throughout the study to be considered for inclusion. Cats previously treated with PZI-VET<sup>f</sup> insulin were excluded from the study. Cats with suspected concurrent disease such as acromegaly, hyperadrenocorticism, or hyperthyroidism causing insulin resistance or cats with concurrent, potentially life threatening disease such as neoplasia or renal failure that decreased the likelihood of completing the study were not enrolled in the study. Cats treated with glucocorticoids within the past 30 days or megestrol acetate within the past 6 months were also not enrolled.

### Study Design

This was a prospective, uncontrolled clinical trial. The study was conducted over a period of 45 days. On day 0, a history was obtained, a physical examination performed, and blood and serum collected for determination of CBC, serum biochemical analysis, serum thyroxine and fructosamine concentrations, and feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) testing. A urine sample was collected for urinalysis. In untreated diabetic cats, a single morning blood glucose concentration was determined. In insulin-treated diabetic cats, blood glucose concentrations were determined 1, 3, 5, 7, and 9 hours after feeding and administration of the type and dosage of insulin that the cat had been receiving before enrollment in the study. Treatment with PZIR was begun the evening of day 0 and was administered SC every 12 hours. The initial recommended insulin dosage was 0.22–0.66 U/kg/injection (1–3 U/cat/injection). The veterinarian determined the ultimate insulin dose after reviewing the history and results of the physical examination, body weight, and blood glucose concentration(s). Diet at entry into the study was determined by the owner and veterinarian and was kept constant throughout the study. Owners were asked to divide the daily caloric intake in half and feed one half each time PZIR was administered. A dosing diary was provided to each owner to record date, time, and dose of PZIR administered, adverse events, and health-related problems such as lethargy, inappetence, and weakness.

Cats were evaluated on days 7, 14, 30, and 45 after entry into the study. On the morning of each scheduled in-hospital evaluation, owners were asked to feed the cat, administer PZIR, and record their opinion regarding changes (increased, decreased, or no change) in their cat's frequency of urination, water consumption, and appetite, compared with day 0, as well as any adverse events they had observed. At each visit a physical examination was performed, body weight recorded, and blood glucose concentrations determined 1, 3, 5, 7, and 9 hours after administration of PZIR. On

days 14, 30, and 45, serum was collected for determination of fructosamine concentration. The owner's dosing diary was collected and reviewed at each visit and a new dosing diary was provided. Adjustments in the dosage of insulin were made based on the owner's perception of how their cat was doing at home and results of the physical examination, body weight, and blood glucose measurements, with the intent to maintain blood glucose concentrations between 100 and 300 mg/dL and the blood glucose nadir between 80 and 150 mg/dL. Guidelines for adjusting the insulin dose were based on the blood glucose nadir identified during the 9-hour blood glucose curve as follows: blood glucose nadir  $< 80$  mg/dL: decrease insulin dose by 25–100% depending on clinical signs; blood glucose nadir between 80 and 150 mg/dL: no change in the insulin dose depending on clinical signs; and blood glucose nadir  $> 150$  mg/dL: increase insulin dose by 25–100% depending on clinical signs.

### Analytical Methods

Blood glucose concentrations were determined by use of a hand-held portable blood glucose monitor.<sup>g</sup> Detectable glucose concentrations ranged from 20 to 600 mg/dL. Blood glucose concentrations  $< 20$  mg/dL registered as "LO" on the portable blood glucose monitor and were arbitrarily assigned a value of 19. Blood glucose concentrations  $> 600$  mg/dL registered as "HI" and were assigned a value of 601 mg/dL. Intra-assay repeatability was assessed with 3 pooled plasma samples containing low (52 mg/dL), reference range (103 mg/dL), and high (379 mg/dL) blood glucose concentrations. The respective intra-assay coefficients of variation for 10 measurements on each plasma sample were 3.0, 4.6, and 5.4% for low, normal, and high blood glucose concentrations. Quality control procedures recommended by the manufacturer were followed to assure accurate functioning of the monitor. Serum fructosamine concentration was determined by means of the nitroblue tetrazolium reduction method.<sup>9</sup> Glycosuria was confirmed with urine reagent test strips.<sup>h</sup> Other analyses carried out on blood, urine, and serum samples were performed at a central reference laboratory.<sup>i</sup>

### Assessment of Efficacy

Blood parameters used to assess control of glycemia included the mean of the 5 blood glucose concentrations measured over a 9-hour period after administration of PZIR (9-hour mean blood glucose concentration), blood glucose nadir, and serum fructosamine concentration. Control of glycemia was classified as good, moderate, or poor according to the following criteria: 9-hour mean blood glucose concentrations  $< 200$  mg/dL were considered good, 200–300 mg/dL moderate, and  $> 300$  mg/dL poor; blood glucose nadir concentrations  $< 150$  mg/dL were considered good, 150–200 mg/dL moderate, and  $> 200$  mg/dL poor; and serum fructosamine concentrations  $< 450$   $\mu$ mol/L were considered good, 450–500  $\mu$ mol/L moderate, and  $> 500$   $\mu$ mol/L poor. Clinical parameters used to assess efficacy of treatment included owner's subjective assessment of polyuria and polydipsia, findings on physical examination, and stability of body weight. For purposes of this study, treatment with PZIR was considered successful if the owner observed improvement in polyuria and polydipsia, the veterinarian believed the cat was in good body condition on physical examination, body weight was stable or increasing, and there was improvement in the 9-hour mean blood glucose concentration, serum fructosamine concentration, or both when day 0 and day 45 results were compared. Improvement was defined as an increase by at least 1 control of glycemia classification category, that is "poor" to "moderate," "moderate" to "good," or "poor" to "good" from day 0 to day 45.

### Data Analysis

Summary statistics were calculated for all continuous endpoints showing the number of observations, mean, standard deviation, minimum, and maximum. For categorical data the number of observations, median, minimum, and maximum were calculated and presented. All analyses were performed by a statistical software program.<sup>1</sup>

For analyses conducted between different days, continuous endpoints (insulin dosage, body weight, 9-hour mean blood glucose concentration, and serum fructosamine concentration) were analyzed by analysis of variance (ANOVA) techniques with day as the classification variable. Post ANOVA contrasts were performed among all days. The experimental unit for this analysis was the animal and all hypotheses were conducted at an  $\alpha$  of 0.05. For data that were not normally distributed (blood glucose nadir) a Friedman (nonparametric) ANOVA was used to assess differences across time, with Wilcoxon' signed-rank tests for paired data to perform posthoc comparison of days 14, 30, and 45 values with day 7 values. The categorical endpoint "time of blood glucose nadir" was analyzed by generalized estimation techniques (a nonparametric analog to ANOVA) with day as the classification variable. Post-ANOVA contrasts were performed among all days. The experimental unit for this analysis was the animal and all hypotheses were conducted at an  $\alpha$  of 0.05.

For analyses conducted between newly diagnosed and previously treated diabetic cats, continuous endpoints (insulin dosage, body weight, 9-hour mean blood glucose concentration, blood glucose nadir, and serum fructosamine concentration) were analyzed by ANOVA techniques with treatment (prior, naïve) as the classification variable. Post-ANOVA contrasts were performed between treatments by day (0, 7, 14, 30, and 45). The experimental unit for this analysis was the animal and all hypotheses were conducted at an  $\alpha$  of 0.05. The categorical endpoint "time of blood glucose nadir" was analyzed by generalized estimation techniques (a nonparametric analog to ANOVA) with treatment (prior, naïve) as the classification variable. Post-ANOVA contrasts were performed between treatments by day. The experimental unit for this analysis was the animal and all hypotheses were conducted at an  $\alpha$  of 0.05.

## Results

### Cats

One hundred seventy-five diabetic cats met the inclusion criteria for entry into the study. Forty-two of the 175 cats were removed from the study because the investigator did not enroll the minimum number (4) of diabetic cats required to be considered a study site (17 cats), owners or investigators did not adhere to the study protocol (12), cats were euthanized or died before study day 45 (8), cats reverted to a non-insulin-requiring diabetic state (2), cats became too fractious to handle (2), or were referred by the investigator to a university for treatment (1). Reasons for euthanasia or death before study day 45 included renal failure ( $n = 3$ ), owner unwillingness to continue treatment (2), pancreatitis (1), heart failure (1), and diabetic ketoacidosis (1).

One hundred thirty-three diabetic cats completed the study. Breeds of the 133 cats included Domestic Short Hair ( $n = 93$ ), Long Hair (14), and Medium Hair (8), Siamese (5), Maine Coon (3), Manx (3), Siamese mix (2), Scottish Fold (2), Himalayan (1), Maine Coon mix (1), and Ragamuffin (1). Eighty-eight cats were castrated males and 45 were spayed females. Age ranged from 3 to

19 years (median, 10.6 years) and body weight ranged from 2.1 to 9.5 kg (mean, 5.5 kg). One hundred and twenty cats were naïve to treatment and 13 had been previously treated with insulin other than PZI, including NPH ( $n = 8$ ), ultralente (2), glargine (2), and lente (1). Diabetes in previously treated cats was considered poorly controlled based on persistence of clinical signs, marked hyperglycemia (blood glucose nadir  $> 250$  mg/dL during a 9-hour serial blood glucose analysis), and glycosuria that persisted despite treatment. Cats were fed a high-protein/low-carbohydrate ( $n = 62$ ), high-fiber (16), commercial dry (31), commercial canned (11), or a mixture of commercial dry and canned (13) diets.

Concurrent diseases identified in the 133 cats after review of their histories and results of physical examinations, CBC, serum biochemistry analyses, and urinalyses on day 0 included urinary tract infection ( $n = 26$ ), periodontal disease (24), diabetic ketosis (2), and otitis externa (1). Cats with urinary tract infections were treated with antimicrobials. Serum thyroxine concentrations were within the reference range for all cats. FeLV was negative in all cats; 1 cat was positive for FIV. Diabetic ketosis resolved after initiation of PZIR treatment. Results of the CBC, serum biochemical analyses (except for serum glucose concentration), and urinalyses (except for glycosuria) were similar on day 0 and day 45.

There was a significant ( $P < .05$ ) increase in the median dosage of PZIR over the course of the study (Table 1). In 1 cat, the initial PZIR dose (0.5 U/injection) was less than study recommendations. There was a corresponding significant ( $P < .001$ ) decrease in all blood parameters used to assess control of glycemia (Table 1; Figs 1–3). The percentage of diabetic cats with poor glycemic control based on results of the 9-hour mean blood glucose (insulin-treated cats) or serum glucose (naïve cats) concentration and serum fructosamine concentration was 92 and 50% on day 0 and 26 and 15% on day 45, respectively. Similarly, the percentage of diabetic cats with results consistent with good glycemic control was 0 and 23% on day 0 and 54 and 74% on day 45, respectively.

A significant change over time for glucose nadir was observed ( $P < .0001$ ). Compared with day 7 values, days 14, 30, and 45 were significantly different ( $P < .0001$  for all 3 contrasts; Table 1). The percentage of diabetic cats with good and poor glycemic control based on results of the blood glucose nadir were 19 and 72% on day 7 and 60 and 29% on day 45, respectively. The blood glucose nadir was  $< 60$  mg/dL in 46 cats and between 60 and 80 mg/dL in 16 cats on day 45. The median insulin dosage was 0.5 U/kg/injection (range, 0.1–1.3 U/kg/injection) in the 46 cats with a blood glucose nadir  $< 60$  mg/dL. The time of the blood glucose nadir varied between cats, ranging from 1 to 9 hours throughout the study, and the time interval from PZIR administration to blood glucose nadir was significantly ( $P < .05$ ) shorter at day 45, compared with days 7, 14, and 30 (Table 1). The lowest blood glucose concentration was at the last blood sampling time (ie, 9 hours) in 32 cats and presumably the blood glucose nadir occurred later than 9 hours after PZIR administration in some of these cats.

**Table 1.** Variables (mean  $\pm$  SD [range] or median [range]) used to assess control of glycemia in 133 cats with naturally acquired diabetes mellitus treated with protamine zinc insulin twice daily for 45 days.

Variables	Day 0	Day 7	Day 14	Day 30	Day 45
Median insulin dosage (U/kg injection)	0.37 [0.09–0.56]	0.45 <sup>c</sup> [0.18–0.9]	0.53 <sup>c</sup> [0.18–1.25]	0.52 <sup>c</sup> [0.18–1.51]	0.59 <sup>a,b,c,e</sup> [0.10–1.40]
Body weight (kg)	5.4 $\pm$ 1.5 [2.1–9.5]	5.5 $\pm$ 1.4 <sup>c</sup> [2.2–9.1]	5.6 $\pm$ 1.4 <sup>c</sup> [2.5–9.2]	5.7 $\pm$ 1.4 <sup>c</sup> [2.6–9.3]	5.9 $\pm$ 1.4 <sup>a,b,e</sup> [2.7–9.2]
9-hour mean blood glucose concentration (mg/dL) <sup>f</sup>	417 $\pm$ 83 <sup>g</sup> [259–601]	338 $\pm$ 117 <sup>c</sup> [86–597]	287 $\pm$ 124 <sup>c</sup> [33.8–601]	229 $\pm$ 129 <sup>c</sup> [37–532]	199 $\pm$ 114 <sup>a,b,c,e</sup> [29–462]
Median blood glucose nadir (mg/dL)	ND	286 <sup>c</sup> [19–590]	237.5 <sup>c</sup> [19–601]	113.5 <sup>c</sup> [19–504]	94 <sup>b,c,e</sup> [19–424]
Time of blood glucose nadir (h)	ND	5.4 $\pm$ 2.5 <sup>c</sup> [1.0–9.0]	5.4 $\pm$ 2.5 <sup>c</sup> [1.0–9.0]	5.5 $\pm$ 2.5 <sup>c</sup> [1.0–9.0]	4.6 $\pm$ 2.2 <sup>b,c,d,e</sup> [1.0–9.0]
Serum fructosamine ( $\mu$ mol/L)	505 $\pm$ 96 <sup>c</sup> [182–923]	ND	454 $\pm$ 104 <sup>c</sup> [201–826]	407 $\pm$ 110 <sup>c</sup> [190–648]	375 $\pm$ 117 <sup>a,b,h</sup> [185–641]

<sup>a</sup> $P < .05$  significantly different from value obtained on day 0.

<sup>b</sup> $P < .05$  significantly different from value obtained on day 7.

<sup>c</sup> $P < .05$  significantly different from value obtained on day 14.

<sup>d</sup> $P < .05$  significantly different from value obtained on day 30.

<sup>e</sup> $n = 133$  cats.

<sup>f</sup>Values were calculated from blood glucose concentrations determined 1, 3, 5, 7, and 9 hours after administration of insulin.

<sup>g</sup>Values were obtained from a single blood glucose concentration in 120 cats with newly diagnosed diabetes and from a 9-hour serial glucose curve determined after administration of insulin in 13 cats with previously treated but poorly controlled diabetes before entry into the study.

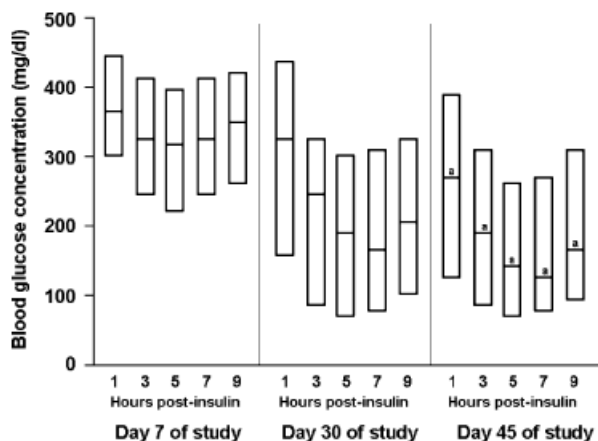
<sup>h</sup> $n = 131$  cats.

Treatment was initiated during the evening of day 0.

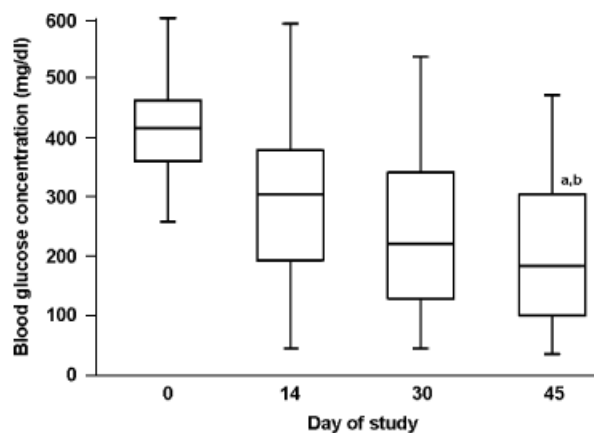
ND = not done.

At the end of the study, 100 (75%) and 105 (79%) of the 133 owners reported improvement in their cats' polydipsia and polyuria, respectively, and 118 (89%) of the cats were determined to be in good body condition based on the veterinarian's clinical assessment. There

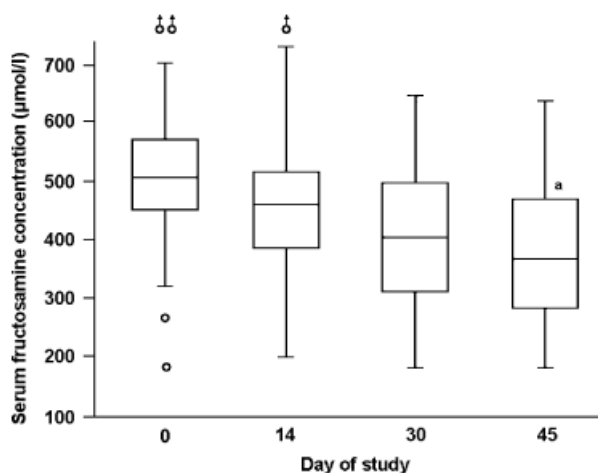
was a significant ( $P < .05$ ) increase in mean body weight from day 0 to day 45 (Table 1) and 108 of 133 cats gained or maintained their body weight. The 9-hour mean blood glucose concentration and serum fructosamine concentration had improved in 55 of 133 diabetic cats, the



**Fig 1.** Median blood glucose concentrations in 133 cats with naturally acquired diabetes mellitus treated by administration of various dosages of protamine zinc recombinant human insulin twice daily for 45 days. Administration of protamine zinc recombinant human insulin and consumption of half of daily caloric intake occurred 1 hour before 1st blood glucose measurement on each day of evaluation. The box represents the interquartile range (ie, 25–75% range). The horizontal bar in the box represents the median value. Median (range) daily insulin dosage was 0.45 (0.18–1.25) U/kg on day 7, 0.52 (0.18–1.57) U/kg on day 30, and 0.59 (0.38–1.69) U/kg on day 45. (a) ( $P < .0001$ ), compared with corresponding glucose concentrations obtained on day 7.



**Fig 2.** Box plots of 9-hour mean blood glucose concentrations in 133 cats with naturally acquired diabetes mellitus treated by administration of various dosages of protamine zinc recombinant human insulin twice daily for 45 days. The 9-hour mean blood glucose concentration is the mean of 5 blood glucose concentrations measured over a 9-hour period after administration of protamine zinc recombinant human insulin. The box represents the interquartile range (ie, 25–75% range). The horizontal bar in the box represents the median value. For each box plot, the T-bars represent the main body of the data. (a)  $P < .001$ , compared with results on day 0. (b)  $P < .05$ , compared with results on day 14.



**Fig 3.** Box plots of serum fructosamine concentrations in 133 cats with naturally acquired diabetes mellitus treated by administration of various dosages of protamine zinc recombinant human insulin twice daily for 45 days. The box represents the interquartile range (ie, 25–75% range). The horizontal bar in the box represents the median value. Circles represent outlying data points. For each box plot, the T-bars represent the main body of the data. (a)  $P < .001$ , compared with results on day 0.

9-hour mean blood glucose concentration improved in an additional 40 cats, and the serum fructosamine concentration improved in an additional 17 cats. There was no improvement in the 9-hour mean blood glucose concentration or the serum fructosamine concentration in 21 diabetic cats when day 0 and day 45 results were compared. Based on the criteria used to judge success of PZIR treatment, 113 (85%) of 133 diabetic cats, including 9 previously treated, poorly controlled diabetic cats, attained good diabetic control by day 45 of the study.

Hypoglycemia (blood glucose nadir  $< 80$  mg/dL) was the only consistent problem with PZIR, identified in 151 (22%) of 678 9-hour serial blood glucose determinations and in 85 (64%) of 133 diabetic cats. Symptomatic hypoglycemia was confirmed in 2 diabetic cats that presented to the veterinarian with clinical signs (lateral recumbency, paddling, stupor) consistent with hypoglycemia. Blood glucose concentration was  $< 20$  mg/dL in both cats and clinical signs resolved with IV administration of

dextrose-containing fluids. There were an additional 26 episodes of owners or veterinarians reporting clinical signs (lethargy, weakness, unsteady gait) that could have been caused by hypoglycemia but hypoglycemia was not confirmed by blood glucose determinations in any of the cats.

Possible reactions at the site of PZIR administration were reported in 3 cats. In 1 cat 4 punctate red lesions were observed on study day 6 in the shaved area where PZIR injections were given and resolved by day 13 despite continued administration of PZIR. In 1 cat a palpable subcutaneous thickening was noted at the injection site on study day 29 but resolved by day 34 despite continued administration of PZIR. In 1 cat painful lesions involving the skin near the area of PZIR injections were noted on study day 12. The lesions were near pre-existing lesions that were being treated with a topical antibiotic cream. The new lesions improved after treatment with the topical antibiotic cream but did not resolve over the course of the study.

On study day 45, the 9-hour mean blood glucose concentration was significantly ( $P < .05$ ) lower for newly diagnosed, untreated diabetic cats, compared with previously treated, poorly controlled diabetic cats ( $192 \pm 114$  versus  $267 \pm 122$  mg/dL, respectively). There was no significant difference in PZIR dose, mean serum fructosamine concentration, blood glucose nadir concentration, time from PZIR administration to the blood glucose nadir, or body weight between newly diagnosed and previously treated diabetic cats, respectively. One hundred and four (87%) of 120 newly diagnosed diabetic cats and 9 (69%) of 13 previously treated diabetic cats were considered treatment successes by day 45 of the study.

The results of this PZIR study are similar to those of a comparable study<sup>7</sup> performed in 67 diabetic cats treated with PZI insulin. As shown in Table 2 both insulin formulations resulted in comparable levels of glycemic control.

## Discussion

Administration of PZIR was effective in decreasing blood glucose concentrations and improving clinical signs within 45 days of initiating treatment in approxi-

**Table 2.** Variables (mean  $\pm$  SD) used to assess control of glycemia in 133 diabetic cats treated with protamine zinc recombinant human insulin (PZIR) and 67 diabetic cats treated with beef/pork-source protamine zinc insulin (PZI-VET) twice daily for 45 days.

Study Visit Day	Fructosamine ( $\mu$ mol/L)		Glucose Nadir (mg/dL)		Glucose Mean (mg/dL)	
	PZIR	PZI-VET	PZIR	PZI-VET	PZIR	PZI-VET
0	505 $\pm$ 96	598 $\pm$ 110	ND	ND	417 $\pm$ 83	420 $\pm$ 61
7	ND	ND	271 $\pm$ 124	318 $\pm$ 104	338 $\pm$ 117	380 $\pm$ 89
14	454 $\pm$ 104	546 $\pm$ 130	221 $\pm$ 124	261 $\pm$ 117	287 $\pm$ 124	326 $\pm$ 118
30	407 $\pm$ 110	481 $\pm$ 141	162 $\pm$ 128	198 $\pm$ 104	229 $\pm$ 129	261 $\pm$ 102
45	375 $\pm$ 117	419 $\pm$ 128	138 $\pm$ 108	163 $\pm$ 93	199 $\pm$ 114	218 $\pm$ 99

Results of the 67 cats are reported elsewhere.<sup>7</sup>

ND = not done.

mately 85% of diabetic cats evaluated in this study. Cats with newly diagnosed diabetes were more likely to respond favorably to PZIR treatment than previously treated, poorly controlled diabetic cats. The difference in response between groups of cats could be due, in part, to a selection bias for problems with unrecognized disorders, such as chronic pancreatitis, that can interfere with insulin sensitivity, problems with insulin dosage, duration of effect or absorption, or a combination of these problems in previously treated, poorly regulated diabetic cats; problems which might have persisted during the PZIR study.<sup>3-5,10,11</sup> Cats with identifiable concurrent disease or medications known to cause insulin resistance were excluded from the study. Obesity was not an exclusion criterion in this study because of the causal relationship between obesity and diabetes in cats and the common need to treat obese diabetic cats with insulin. It is noteworthy that 9 of the 13 previously treated, poorly controlled diabetic cats responded to PZIR and were classified as treatment successes by day 45 of the study. These findings suggest that poor control in the 9 cats could have been caused by problems with dosage, absorption, or duration of effect of the previously administered insulin preparation, problems that were corrected with use of PZIR administered twice a day. Based on results of this study, PZIR is a viable alternative for diabetic cats that have not responded to other insulin preparations.

The only important adverse event associated with PZIR was hypoglycemia, which is commonly associated with administration of any insulin preparation. Hypoglycemia was confirmed in 2 cats presenting with clinical signs of hypoglycemia and was suspected but not confirmed in 26 additional instances. Hypoglycemia presumably resulted from an overdose of PZIR. Considerable overlap in the PZIR dosage range that caused hypoglycemia, established control of glycemia, and had not established control of glycemia by day 45 was identified in this study. For example, hypoglycemia developed at dosages of PZIR as low as 0.1 U/kg per injection and as high as 1.3 U/kg per injection. Control of glycemia was established over a similar insulin dosage range. Predicting an effective dosage of PZIR that does not cause hypoglycemia is difficult, in part, because of variability between cats in their response to insulin. Experience with PZI-VET insulin and results of this study support using low dosages of insulin (0.22 U/kg) initially in newly diagnosed diabetic cats and adjusting the dosage of PZIR based on the cat's response to treatment and results of blood glucose and serum fructosamine concentrations.

Asymptomatic hypoglycemia was a common finding during the 9-hour serial blood glucose determinations. Possible reasons for the common identification of asymptomatic hypoglycemia in this study include differences in the approach to treating diabetic cats between veterinarians participating in the study, the bias of the portable blood glucose monitoring device, and potential reversion of some cats to a subclinical diabetic state. Although guidelines for the initial insulin dosage and for subsequent dosage adjustments were provided, insulin dosage decisions were ultimately at the discretion of the

attending veterinarian. Several veterinarians were aggressive in attempting to obtain and maintain tight control of glycemia. As such, the initial dosage of PZIR ranged from 0.22 to 1.1 U/kg and by the end of the study, approximately 34% of the cats had a low blood glucose nadir, 9-hour mean blood glucose concentration, and/or serum fructosamine concentration. In our experience, the bias of the portable blood glucose-monitoring device used in this study is consistently low, compared with reference hexokinase results measured from the same blood sample. The prevalence and severity of hypoglycemia was presumably increased because of this bias. Although all cats were still receiving PZIR at the end of the study, some of the diabetic cats, especially those with low 9-hour mean blood glucose and serum fructosamine concentrations, were probably in the process of reverting to a subclinical diabetic state.<sup>12</sup> Decreasing the insulin dosage in response to the low blood glucose and serum fructosamine concentrations would undoubtedly have resulted in discontinuation of PZIR in some of these cats.

Historically, PZI was often administered only once per day to diabetic cats; a frequency of administration that was based more on clinical perceptions of response to treatment than on results of absorption kinetic studies. Studies have identified substantial variability in absorption kinetics of PZI among cats. Time to peak blood insulin concentration ranged from 4 to 12 hours, time of the blood glucose nadir ranged from 1 to 12 hours, and time for blood insulin concentration to return to baseline ranged from 8 to 24 hours after SC administration of PZI to healthy and diabetic cats.<sup>1,13,14</sup> PZIR was administered twice daily in our study because of prior clinical experiences of 1 investigator (RWN) and results of a study involving PZI-VET insulin.<sup>7</sup> Although absorption kinetics were not performed and efficacy of once daily treatment was not evaluated in the study, mean time of the blood glucose nadir was between 5 and 7 hours and subsequent blood glucose concentrations were increasing in most cats by 9 hours after administration of PZIR. These results suggest that PZIR should be administered twice a day in most diabetic cats to maintain control of glycemia. However, the lowest blood glucose concentration occurred 9 hours after administration of PZIR and the glucose nadir had not been definitively identified in approximately 25% of the cats on day 45. Once-daily administration of PZIR may be effective in maintaining control of glycemia in some diabetic cats and should be considered in those cats where the blood glucose nadir occurs 10 hours or longer after administration of PZIR, especially if hypoglycemia or the Somogyi response become recurring problems.<sup>15</sup>

Although type of diet can affect control of glycemia in diabetic cats<sup>16,17</sup> feeding the same diet to all cats was not attempted in this study primarily because in our experience many owners are reluctant to switch diets and not all diabetic cats will eat diets recommended for diabetic cats. A goal of this study was to evaluate the efficacy of PZIR in situations commonly encountered in general practice, including diabetic cats that are not

fed an ideal diet. The majority of cats consumed a high-protein/low-carbohydrate diet and each cat was fed the same diet during the study, thereby minimizing diet as a possible factor for improved control of glycemia in any given cat. Because a variety of diets were fed to the cats, the impact of any specific diet on response of cats to treatment with PZIR could not be determined in this study, other than to state that PZIR was effective in treating diabetes in approximately 85% of the cats regardless of the diet fed.

The protocol used to evaluate the efficacy of PZIR in this study was similar to a prior study evaluating beef/pork source PZI insulin (PZI-VET) in 67 newly diagnosed or previously treated, poorly controlled diabetic cats.<sup>7</sup> Results of both studies were comparable, including the percentage of cats that had a good response to PZI-VET insulin and PZIR (90 versus 85%), median insulin dosage (0.80 versus 0.59 U/kg/injection), mean 9-hour mean blood glucose concentration (218 versus 199 mg/dL), and mean serum fructosamine concentration (419 versus 376  $\mu\text{mol/L}$ ), respectively, at day 45 of each study (Table 2). These findings suggest that there is minimal to no difference in efficacy of PZI preparations that utilize beef/pork source or recombinant human insulin. Administration of PZI-VET insulin or PZIR twice a day was effective in establishing control of glycemia in cats with newly diagnosed diabetes and significantly improved control of glycemia in most cats with poorly controlled diabetes that were being treated with insulin at the time treatment with PZI-VET insulin or PZIR was initiated.

### Footnotes

<sup>a</sup> PZI-VET, Blue Ridge Pharmaceuticals Inc, Greensboro, NC

<sup>b</sup> Data on file IDEXX Pharmaceuticals Inc, Greensboro, NC

<sup>c</sup> Norsworthy DG, Lynn RC. Clinical study to evaluate a new formulation of protamine zinc insulin for treatment of diabetes mellitus in cats. *J Vet Int Med* 2008; 22:729 (abstract)

<sup>d</sup> Code of Federal Regulations, Title 21, Chapter 1, Part 511: New animal drugs for investigational use, US Department of Health and Human Services, Food and Drug Administration, April 1, 2003

<sup>e</sup> Document VICH GL9, Guidance for Industry, Good Clinic Practice, US Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine, May 9, 2001

<sup>f</sup> PZI-VET, IDEXX Pharmaceuticals Inc

<sup>g</sup> Elite XL glucometer, Bayer, Mishawaka, IN

<sup>h</sup> Diastix, Bayer, Elkhart, IN

<sup>i</sup> IDEXX Preclinical Research Services, West Sacramento, CA

<sup>j</sup> SAS Statistical Analysis System version 9.1, SAS Institute, Cary, NC

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