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# An ultra-long-acting recombinant insulin for the treatment of diabetes mellitus in cats

Chen Gilor[1,2] | Sean E. Hulsebosch[1] | Jully Pires[1] | Michael J. Bannasch[1] | Thomas Lancaster[3] | Andrea Delpero[3] | Ramya Ragupathy[3] | Sylaja Murikipudi[3] | Todd Zion[3]

[1]Department of Veterinary Medicine and Epidemiology, University of California, Davis, Davis, California

[2]Department of Small Animal Clinical Sciences, University of Florida, College of Veterinary Medicine, Gainesville, Florida

[3]Akston Biosciences, Beverly, Massachusetts

**Correspondence**
Chen Gilor, Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA.
Email: cgilor@ufl.edu

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## Abstract

**Background:** Treatment of diabetes mellitus (DM) in cats typically requires insulin injections q12h-q24h, posing a major compliance barrier for caregivers. Novel treatments enabling decreased injection frequency while maintaining safety are highly desirable. Insulin fused with feline immunoglobulin fragment crystallizable (Fc) has an ultra-long plasma half-life because it recycles through cells where it is protected from proteolysis.

**Hypothesis:** Glycemic control can be achieved in diabetic cats with a recombinant fusion protein of a synthetic insulin and feline Fc (AKS-267c) administered SC weekly.

**Animals:** Five cats with spontaneous DM.

**Methods:** Cats previously controlled using insulin glargine q12h were transitioned to once-weekly injection of AKS-267c. The dose of AKS-267c was titrated weekly for 7 weeks based on continuous glucose monitoring. Clinical signs, body weight, fructosamine concentrations, and mean interstitial glucose concentrations (IG) were compared between baseline (week 0, on insulin glargine) and the last week of treatment. Data were assessed for normality and compared using parametric or nonparametric paired tests (as appropriate).

**Results:** After 7 weeks of once-weekly injections, compared to baseline, there were no significant changes in clinical signs, body weight (median [range] gain, 0.1 kg [−0.1 to +0.7]; $P = .5$), fructosamine (−60 mmol/L [−338 to +206]; $P = .6$), and mean IG concentrations (change = −153 mmol/L [−179 to +29]; $P = .3$), and no adverse reactions were reported.

**Abbreviations:** BG, blood glucose concentration; CGM, continuous glucose monitoring system; CV, coefficient of variation; DM, diabetes mellitus; FcRn, neonatal Fc receptor; FGMS, flash glucose monitoring system; GVP, glucose variability percentage; IG, interstitial glucose concentration; SC, subcutaneous.

**Conclusion:** Successful control of clinical signs and maintenance of glycemia was achieved with this once-weekly novel insulin treatment. The efficacy and safety of this novel formulation should be further assessed in a large clinical trial.

**KEYWORDS**
adherence, compliance, continuous glucose monitoring, FcRn, IgG

## 1 | INTRODUCTION

Diabetes mellitus (DM) is a common endocrinopathy in cats, with an estimated prevalence of 0.5% to 2%.[1] Treatment of DM in cats currently relies upon the SC injection of insulin, usually given twice daily, along with dietary modification. In the majority of cats, insulin treatment is life-long.[2] Even in those cats that achieve remission, insulin treatment is necessary for at least a few weeks after diagnosis. For many cat owners, twice daily injections and the associated feeding schedule have a marked impact on their quality of life and the perceived well-being of their pet; it has been estimated that 30% of affected cats are euthanized within a year of diagnosis.[3] Owners often cite insulin-related issues as a major cause of anxiety, including worrying about hypoglycemic events and inability to have the cat cared for by others.[4]

Insulin is a 51-amino acid peptide that tends to form hexamers, especially in the presence of zinc. After injection into the SC tissue, zinc diffuses out of the SC depot, and the hexamers break down into dimers and monomers that are then free to diffuse into the vasculature.[5] Once in the blood, insulin quickly reaches its target organs. After insulin binds to its receptor on the target cell membrane, the insulin-receptor complex undergoes endocytosis and then insulin is degraded by insulin-degrading enzyme. To extend duration of action, traditional insulin formulations rely on manipulation of the rate of hexamer dissociation in the SC depot to slow down insulin absorption into the blood.[5] Additionally, decreasing the affinity of insulin to its receptor also prolongs duration of action. With manipulation of absorption rate and receptor affinity, currently available insulin formulations have time-action profiles suitable for use as once- or twice-daily SC injections.[6]

Here we present data on a novel ultra-long-acting insulin formulation (AKS-267c) intended for once-weekly administration in cats. The active molecule in this formulation is a fusion protein of synthetic insulin and the feline fragment crystallizable (Fc) region of immunoglobulins. This nonimmunogenic fusion protein is a ligand to the insulin receptor but also binds to the host neonatal Fc receptor (FcRn). Binding to the FcRn leads to recycling of the insulin fusion molecule intracellularly, which greatly extends its half-life in comparison to native insulin.[7] Preliminary data from healthy laboratory cats suggest that the half-life of this molecule would allow for once-weekly administration with prolonged glucose-lowering effect. We hypothesized that in cats with naturally occurring DM, this Fc-insulin fusion protein would control clinical signs, weight, and blood glucose concentrations (BG) with once-weekly injections. Ours was a dose-escalation pilot study, with the aim of assessing the ability of once-weekly AKS-267c injections to maintain or improve glycemic control in diabetic cats previously treated using conventional twice-daily insulin administration.

## 2 | MATERIALS AND METHODS

Cats were recruited from the University of California, Davis Veterinary Medical Teaching Hospital and local veterinary clinics. Cats were included if they were previously diagnosed with naturally occurring DM (in accordance with the Project Agreeing Language in Veterinary Endocrinology [ALIVE] definition for DM diagnosis)[8] and had been treated with any insulin formulation (≤4 U per injection) for at least 2 months before enrollment. Cats were required to have moderate to good glycemic control as defined by minimal clinical signs and stable serum fructosamine concentrations (2 serum fructosamine concentrations measured at least 3 weeks apart that were within 100 mmol/L of each other). After inclusion, degree of glycemic control also was assessed by measuring interstitial glucose concentration (IG) continuously during the 1st week (Figures 1 and 2). Body weight was required to be between 3 and 8 kg with no recent change in body weight (defined as <5% in the past 3 months). Finally, cats must have been fed a consistent diet at study enrollment with the expectation that for each cat, consistency of diet type and feeding regimen would be maintained throughout the study period. There was no requirement for feeding a specific diet or specific carbohydrate content upon entry.

Exclusion criteria included uncontrolled hyperthyroidism, previous insulin dose >4 U per injection, increased insulin-like growth factor-1 (IGF-1) concentration above the reference interval (12-92 nmol/L), clinically relevant concurrent illness (that might preclude following the patient >3 months and that might affect insulin requirement), positive urine culture, inability to tolerate the flash glucose monitor (see below), and history of diabetic ketoacidosis within the past 2 months.

Upon inclusion in the study, informed consent was obtained from all owners. The study protocol was approved by University of California, Davis Institutional Animal Care and Use Committee (protocol #19844). On visit 1, a CBC, serum biochemistry panel, serum thyroxine concentration, IGF-1 concentration, urinalysis by cystocentesis, aerobic bacterial urine culture, and urine protein : creatinine ratio were performed in the University of California, Davis clinical pathology laboratory. A flash glucose monitoring system (FGMS; FreeStyle Libre, Abbott), validated for use in cats, was used throughout the study.[9] A FGMS sensor was applied to the skin on the dorsolateral neck as previously described.[9] This FGMS measures IG once per

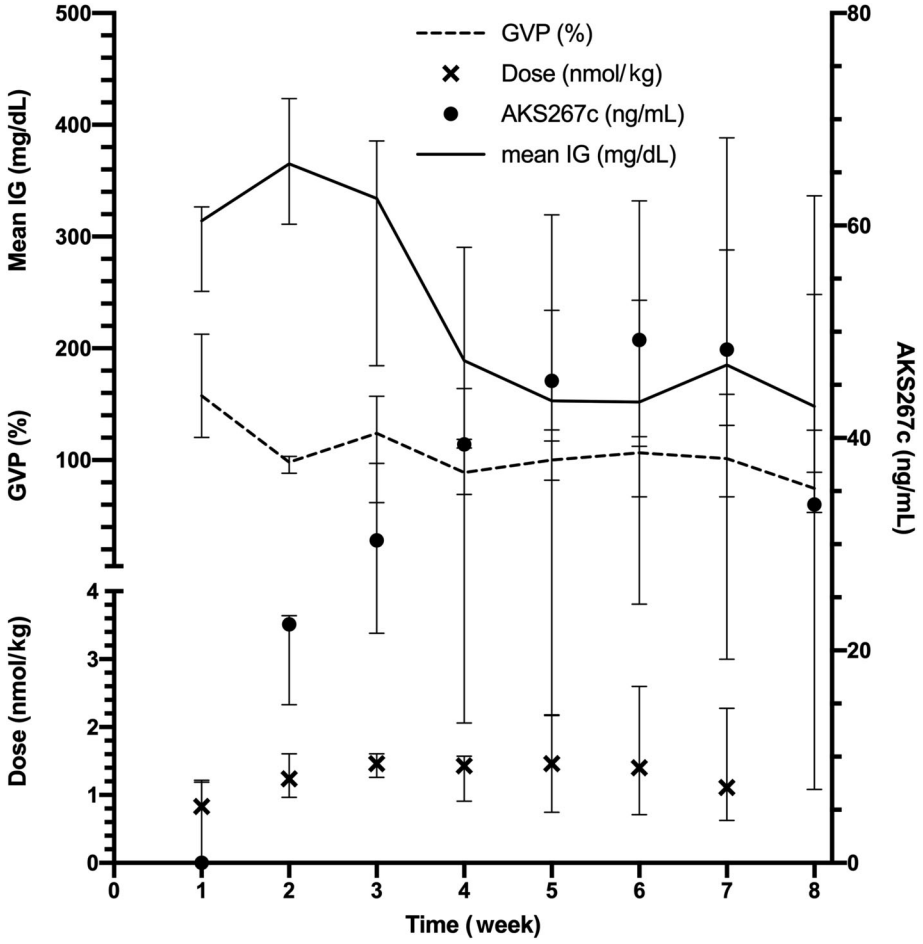


**FIGURE 1** Median and interquartile ranges of AKS-267c dose, serum AKS-267c concentrations, mean interstitial glucose (IG; over the preceding week), and glucose variability percentage (GVP; over the preceding week) in 5 cats treated with AKS-267c once-weekly. All cats were treated with insulin glargine on week 0 and received the 1st dose of study insulin (AKS-267c) on week 1

minute and records 15-minute IG averages for up to 14 days. Minute-by-minute measurements can be obtained by scanning the sensor frequently. Cats were discharged after this visit for 10 to 14 days to continue receiving their prestudy insulin treatment twice daily and to ensure patient compliance with the FGMS.

On visit 2, serum fructosamine concentration was measured and thoracic radiography and abdominal ultrasound examination were performed. If no exclusion criteria were met, a new FGMS sensor was applied and the cat received the 1st injection of AKS-267c at approximately 1 nmol/kg SC. This dose was chosen based on preliminary data from healthy purpose-bred cats with the intention of approximating a 0.3 U/kg/d dose of conventional insulin. It was anticipated that hypoglycemia would be unlikely at this dose and that insulin dose escalation over the succeeding weeks would be required to maintain glycemic control. Also based on preliminary data from healthy purpose-bred cats, AKS-267c effects were expected to peak within the first 24 to 36 hours after injection. Because the potency of AKS-267c was not fully elucidated in cats with naturally occurring DM, and in order to minimize the risk of hypoglycemia, cats were hospitalized for monitoring for 48 hours after the 1st injection. If no hypoglycemia was observed, cats were discharged from the hospital after 48 hours. No insulin was administered by the owner at home unless instructed otherwise by the study investigators.

After visit 2, cats were evaluated weekly by a full physical examination, collection of a serum sample for drug concentrations, replacement of the FGMS sensor, and administration of AKS-267c. The dose of AKS-267c was adjusted weekly as necessary based on FGMS data, body weight, and clinical response (drug concentration data was not available to the investigators during the study). In total, cats were treated with AKS-267c 7 times (ie, for 49 days). At the final visit, which was 1 week after the 7th injection, samples were collected again for CBC, serum biochemistry panel, serum fructosamine concentration, urinalysis and urine culture via cystocentesis, and a urine protein : creatinine ratio to assess for potential adverse drug effects.

The concentrations of AKS-267c were measured with a sandwich ELISA specifically developed by Akston Biosciences for the purpose of this study. In brief, microtiter plates were coated with purified anti-insulin antibodies to capture the AKS-267c therapeutic molecules in the samples and the captured AKS-267c then was quantitated using a goat anti-cat IgG-Fc-horseradish peroxidase (HRP) detection antibody followed by a tetramethylbenzidine substrate system. The tetramethylbenzidine substrate changes color when it reacts with the HRP that is conjugated to the detection antibody. The enzyme substrate reaction was stopped by the addition of a stop reagent (1% $H_2SO_4$) and the color intensity was measured in a microplate reader at 450 nm.

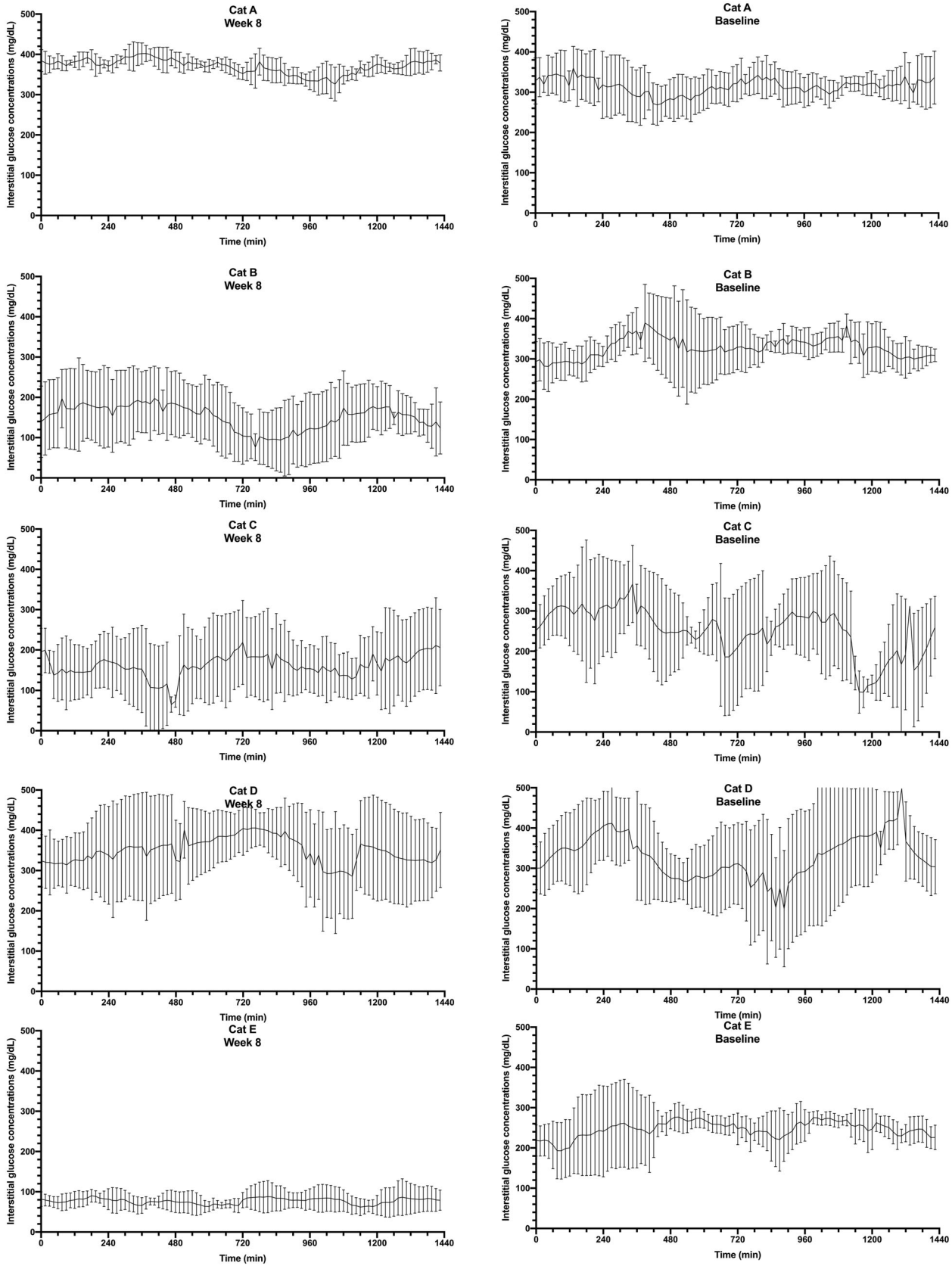


**FIGURE 2** Interstitial glucose concentrations in 5 individual cats treated with twice daily insulin glargine (left panels: baseline) and after 7 doses of once-weekly AKS-267c insulin (right panels: week 8). Each time point represents the mean ± SD over 6 days of measurement, with the x-axis representing 24 hours

### 2.1 | Statistical analysis

Descriptive statistics of all variables were calculated as median (range) and all comparisons were performed using nonparametric tests because of the small number of cats in the study. Wilcoxon matched-pairs signed-rank tests were used for comparisons between median values of the group at baseline (week 0, immediately before the 1st AKS-267c injection) and week 7 (1 week after the last AKS-267c injection). All statistical tests were performed as 2-tailed tests and a $P$ value ≤.05 was considered significant. Raw IG data extracted from the Libre CSV files were compiled for each week of the study starting at baseline and for each week that followed, corresponding to study insulin injections 1 to 7. For each week, IG data were included from midnight on the night after sensor placement until midnight of the night before AKS-267c injection (for a total of 6 full days, excluding the 24 hours surrounding the weekly hospital visit to minimize the effect of stress-induced hyperglycemia). These data were used to calculate mean IG for the week as well as SD, coefficient of variation (CV) between days of the week, and glucose variability percentage (GVP), which was calculated as previously described.[10] In brief, the GVP method calculates the length of line from continuous data by using a trigonometric analysis of the data and is used as a superior indicator of intraday BG variability.[10] It is a more comprehensive measure of variability compared to CV and SD because it captures fluctuations in both amplitude and frequency. Data were graphed to represent measurements as obtained on the day of each visit. As such, mean IG and GVP correspond to the week before the visit (eg, week 1 mean IG represents treatment with standard insulin during the week before the 1st injection of AKS-267c [baseline] and week 8 mean IG represents the mean of the week after the injection on week 7).

## 3 | RESULTS

Eight cats were screened for enrollment and 3 were excluded for the following reasons: 1 cat had uncontrolled hyperthyroidism; 1 cat had both uncontrolled hyperthyroidism and a liver mass; and 1 cat was going into diabetic remission.

Of the 5 cats enrolled in the study, 2 were spayed females and 3 were neutered males. There were 3 domestic short hairs and 2 Abyssinian cats. The median (range) age was 10 (7-16) years. Median weight was 6.6 (4.9-7.9) kg. Median body condition score was 6 of 9 (5-8 of 9). One of the cats was fed a prescription low-carbohydrate diet (Purina Feline DM) whereas the other 4 cats were fed a variety of commercial cat foods at study entry; no diet change was made throughout the study period. Cats were diagnosed with spontaneous DM a median of 11 (2-28) months before enrollment and all had normal IGF-1 concentrations upon enrollment. No clinically relevant comorbidities were detected by CBC, serum biochemistry panel, serum thyroxine concentration, urinalysis, urine culture, and thoracic radiographs. One cat was FIV positive. This cat also had mild mesenteric lymphadenomegaly on abdominal ultrasonography, but at the time these were not considered reasons for exclusion because the cat had no associated gastrointestinal signs. Before enrollment, all cats were treated with insulin glargine at a median dosage of 0.5 (0.4-0.6) U/kg and were considered controlled based on minimal-to-no clinical signs at home, stable body weight, and stable serum fructosamine concentration. At baseline, median serum fructosamine concentrations were 556 (318-628) μmol/L and the week mean IG at baseline was 314 (247-327) mg/dL.

The starting AKS-267c dosage was 0.83 (0.72-1.53) nmol/kg. Week mean IG tended to increase on week 2 but then decreased throughout the study as the dose of the study insulin was adjusted (Figure 1). Insulin dose was increased in 4 of the 5 cats on week 2 (by 27%-55%) and in 3 of these 4 cats on week 3 (by 17%-21%). After that, the AKS-267c dose was decreased or unchanged weekly in these 4 cats until the end of the study. In the 5th cat, the dose was unchanged on week 2, but gradually increased after that throughout week 6. At week 7, the median final dosage of AKS-267c in the 5 cats was 1.11 (0.46-2.92) nmol/kg.

At the final visit (week 8), the median (range) of week mean IG was not different from baseline (148 [76-355] mg/dL; $P = .3$), serum fructosamine concentration (454 [290-641] μmol/L) was not different from baseline ($P = .6$; Figure 3), and owners reported good control of clinical signs and body weight was nearly identical to baseline (6.5 [5.0-7.8] kg; $P = .5$; Figure 4). Glucose variability tended to decrease from baseline at week 1 and that tendency continue throughout week 7 (GVP = 158% [107-268] vs 75% [50-159]; $P = .09$; Figures 1 and 2). No significant change was observed in CV or SD between baseline and the last week of treatment (data not shown).

Serum AKS-267c concentrations of approximately 40 ng/mL corresponded to good glycemic control (as represented by mean IG < 200 mg/dL). These concentrations were achieved in 4 cats after

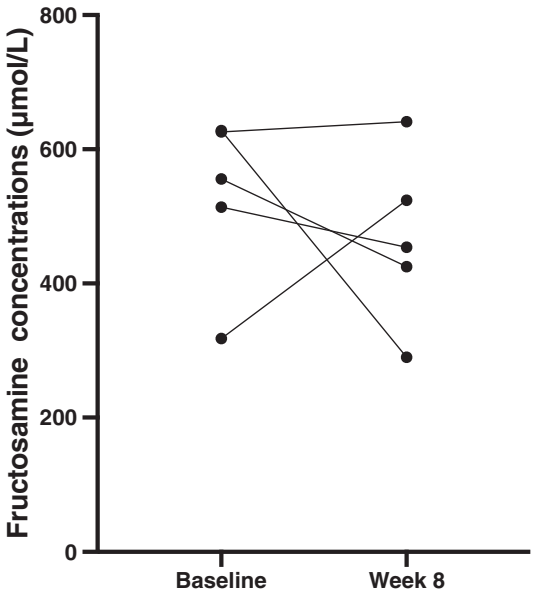


**FIGURE 3** Serum fructosamine concentrations of 5 diabetic cats at baseline (treated with daily insulin glargine) and at week 8 (treated with AKS-267c once-weekly for 7 weeks)

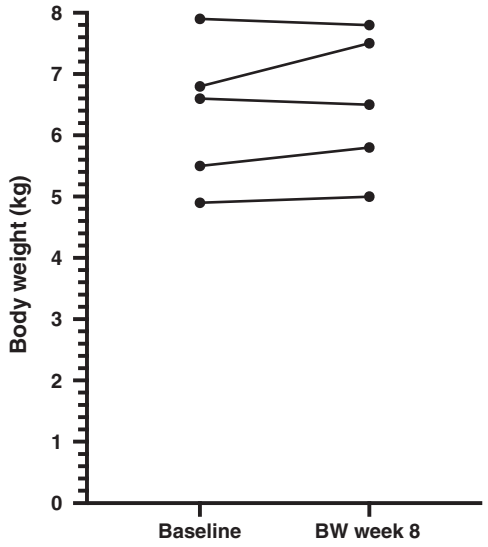


**FIGURE 4** Body weight of 5 diabetic cats at baseline (treated with insulin glargine daily) and at week 8 (treated with AKS-267c once-weekly for 7 weeks)

the 3rd injection. Serum insulin concentrations continued to increase during the next 2 weeks despite a stable dose, suggesting drug accumulation. Drug concentrations on week 8 (a week after the last dose) suggest that AKS-267c has a half-life of approximately 2 weeks in diabetic cats.

Two cats had no IG results <70 mg/dL throughout the study. In the other 3 cats, low IG results were more frequent on the last week of treatment compared to baseline. In these 3 cats, the total time spent with IG < 70 mg/dL was 0%, 4%, and 5% at baseline and 12%, 9%, and 52% (respectively) at week 7. The total time spent with IG < 40 mg/dL was 0%, 0%, and 1% (respectively) at baseline and 4% in all 3 cats at week 7. Clinical hypoglycemia was not reported in any of the cats. No adverse events were reported throughout the study either locally at the injection site or systemically, as assessed by weekly physical examination and blood and urine tests at the end of the study.

In 1 cat, based on lack of clinical signs of DM, a normal serum fructosamine concentration, and mean IG = 76 mg/dL during week 7, insulin treatment was discontinued after study completion and the cat is currently in remission (12 months later). This cat was diagnosed with DM 4 months before enrollment. It was the last to be enrolled in the study and received the highest dose of AKS-267c on week 1 (1.53 nmol/kg) based experience with the previous 4 cats. This cat's week mean IG decreased from 247 mg/dL at baseline to 121 mg/dL at week 3 and the dose was gradually decreased from week 3 to week 7. The cat's GVP was 157% at baseline (equal to the group median) but decreased to 56% on week 3 and was consistently the lowest in the group from week 3 to week 7 (ranging from 37% to 76%).

In the other 4 cats, AKS-267c was continued after the study ended at the owners' request. Two months after study conclusion, the cat that was FIV positive was diagnosed with gastrointestinal large cell lymphoma and was euthanized. The other 3 cats continued to be well controlled on AKS-267c for 5, 5, and 18 months with no reported adverse events.

## 4 | DISCUSSION

In our study, 5 cats with naturally-occurring DM were transitioned from twice daily insulin injections to a novel formulation of ultra-long-acting fusion insulin. After 7 weeks of treatment, the once-weekly formulation was as effective at controlling clinical signs, body weight, and glycemia. These positive outcomes were achieved with no evidence of clinical hypoglycemia and without adverse events. Glycemic control was no different between the once-weekly and twice daily protocols, and the former offers considerable benefits to the cat and pet owner.

In people, increasing dosing frequency of any drug is associated with decreased adherence to treatment protocol.[11] In diabetic people, poor adherence is compounded by the pain of insulin injection.[12,13] Human patients consider insulin injections to be a serious burden and they have a negative impact on quality of life.[12] Although adherence has not been studied in people giving insulin injections to cats, it is anticipated that the problems noted in human patients will be compounded by the physical and emotional challenges of performing repeated injections in cats. It is also unknown how the act of injection itself affects compliance and the owners' short- and long-term decisions to treat. Although not exclusively associated with these problems, it is reasonable to assume that the high euthanasia rate (30%) soon after DM diagnosis is associated with compliance issues.[3] Once-weekly injections likely would minimize these barriers to greater treatment success, improve quality of life for the pet and owner, and increase patient survival. In this context, it is also important to consider not just the treatment choice per se, but also how it affects monitoring intensity and cost. An insulin that leads to lesser intraday and between-day variability likely would require simpler monitoring protocols compared to formulations that are associated with more variability. One of the major factors contributing to between-day variability of insulin dosage is erratic absorption of insulin crystals that vary in size and shape.[14,15] In contrast, AKS-267c does not precipitate in the SC tissue, but instead is recycled in SC cells, thus prolonging duration of effect. That, together with the fact that AKS-267c likely would achieve steady state within a few weeks of treatment, likely would be beneficial in decreasing between-day variability, making AKS-267c easier for the veterinarian and client to monitor long term.

Throughout the study, glucose variability was lower on the study insulin compared to baseline insulin. This difference in variability was observed in the 1st week after the study insulin was administered, even while mean IG was increasing. Decreased glucose variability might be important, considering recent evidence that decreased glucose variability in cats is associated with increased frequency of diabetic remission.[16] Whether decreased glucose variability was the cause or effect of normalizing glycemia was difficult to discern in that study, but the sequence of events in the cats presented here suggests

that normalizing glycemia was not the cause of decreasing GVP. The 1 cat that achieved diabetic remission at the end of the study had started with an unremarkable glucose variability (ie, GVP equal to the group median) but the cat's glucose variability quickly decreased on the study insulin and was consistently the lowest of the group from week 3 onward. This lowest GVP corresponded to the lowest mean IG observed in this cat weekly compared to the other 4 cats, starting at week 3.

Glucose variability is affected not just by fluctuations in insulin concentrations but also by other factors such as stress and dietary carbohydrates. Here, dietary carbohydrate content was not standardized across cats, but each cat was fed the same diet and on the same schedule during baseline measurements and while receiving the study insulin. As such, it is unlikely that diet contributed to differences in GVP between baseline and week 8. It is likely, however, that absorption of carbohydrates from food contributed to intraday fluctuation in IG overall.

Because ours was the 1st study to assess the efficacy of this novel ultra-long-acting insulin formulation in diabetic cats, we only recruited patients that were well controlled, with the intention of comparing the novel to the standard insulin formulation. We did not include cats that were newly diagnosed because of the initial concern that glycemic control might take longer than usual to achieve, putting newly diagnosed cats at risk for ketoacidosis. Because we were observing the glycemic response to this novel insulin formulation, we tended to increase the dose of insulin more rapidly and eventually start treatment at a higher dose with subsequent enrollees. However, considering drug accumulation over time (resulting from a prolonged half-life of >1 week), a conservative starting dose and low rate of dose increase still would be advised in the future. This approach would be especially critical in newly diagnosed patients in which resolution of glucolipotoxicity could lead to increased insulin sensitivity, improved beta cell function, and a rapidly decreasing requirement for exogenous insulin. Although no different from treatment with shorter acting formulations, more caution would be advised in this respect when using ultra-long-acting formulations, especially when nearing euglycemia and imminent diabetic remission.

In contrast to the situation in newly diagnosed patients, based on the previous response to treatment with insulin glargine in these patients, we also had an estimation of what insulin dose to target. As a precautionary measure to minimize the risk of hypoglycemia, our initial starting dose was intended to approximate 0.3 U/kg/d (or 2.1 U/kg/wk), contrasting with 1 U/kg/d (or 7 U/kg/wk), the insulin glargine dose at which cats already were controlled. Each unit of insulin glargine contains 6 nmol of insulin. Unexpectedly, at the end of the study, the AKS-267c dose that was controlling glycemia and clinical signs was only 1.1 nmol/kg/wk, contrasting with 42 nmol/kg of total weekly dose of insulin glargine. The relatively high potency of AKS-267c compared to insulin glargine might be explained by the increased residence time of insulin glargine in the SC depot, exposing it to degradation by tissue proteases, and effectively lowering the dose of intact insulin that ultimately diffuses into the blood.[17] In contrast to other SC formulation, the prolonged duration of action of AKS-267c does not rely on slowing absorption of insulin from the SC tissue but rather on intracellular circulation. The AKS-267c is free to diffuse from the SC tissue into blood where it is distributed throughout the body. The FcRn is ubiquitously expressed in epithelia, endothelia, cells of hematopoietic origin, and other cells.[18] Upon binding to the FcRn, AKS-267c undergoes pinocytosis and eventually exocytosis. While inside the cell, AKS-267c is protected from proteolysis similar to other ligands of the FcRn.[18]

In most insulin formulations, retardation of absorption from the SC depot relies on the formation of insulin hexamers, a process that is a function of insulin concentration and the precise ratio of insulin to other molecules (such as zinc, protamine, or m-cresol) and local tissue pH.[5] This markedly limits the ability to vary the concentrations of insulin formulations and is the reason why dilute formulations have failed to maintain prolonged duration of action in the past.[19] Because the prolonged duration of action of AKS-267c does not rely on slowing absorption of insulin from the SC tissue, it can be formulated in any concentration, making it exceedingly convenient for use in small animals.

Interstitial glucose concentrations were measured continuously throughout the study, but these data were used primarily to aid in dose adjustments and only secondarily as outcome measures. Although the optimal target IG in cats is unknown, optimal BG targets have not been established in cats either.[20,21] In addition, although more data are available regarding BG targets, an argument can be made that IG would be safer to rely upon when adjusting insulin doses because IG better reflects tissue physiologic requirements for insulin and because they provide a broader perspective on trends and not just single time points.[22] Use of a continuous glucose monitoring system (CGM) is especially important in the context of identifying subclinical hypoglycemic events. The CGM used in our study recently was validated for use in cats, showing good correlation between BG and IG.[9] However, like other glucometers intended for use in people, this CGM underestimates glucose concentrations in cats by 23.3 ± 18.1 mg/dL.[9] Although the performance of this CGM was not well studied in the hypoglycemic range, it is likely that this bias extends to some extent into that range as well. This possibility means that the frequency of low IG events in our study may overestimate the frequency of true hypoglycemic events, potentially explaining why we did not observe clinical hypoglycemia. In the future, it will be important to develop feline-specific CGM systems, IG targets, and monitoring protocols, in order to optimize the use of once-weekly insulin formulations.

In summary, AKS-267c is a promising formulation for clinical control of DM in cats with only 1 injection per week. Despite lack of any previous clinical experience with this formulation, glycemic control using CGM data was achieved within a few weeks with no incidents of clinical hypoglycemia. Once-weekly administration of AKS-267c was sufficient for maintaining body weight and serum fructosamine concentrations. Future clinical trials should examine the ability to control DM in cats with even lower frequency of AKS-267c administration and develop protocols for its safe introduction in newly

diagnosed diabetic cats as well as how to transition to it from standard insulin formulations.

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## CONFLICT OF INTEREST DECLARATION

Authors declare no conflicts of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Study design and protocol was approved by the UC Davis IACUC, protocol #19844.

## HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

## ORCID

*Chen Gilor* https://orcid.org/0000-0003-0393-4135

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