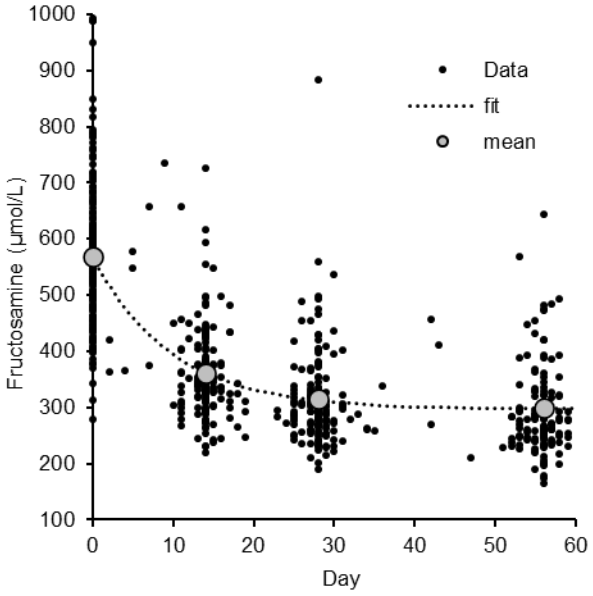
SUPPLEMENTAL INFORMATION

Serum fructosamine half-life in diabetic cats

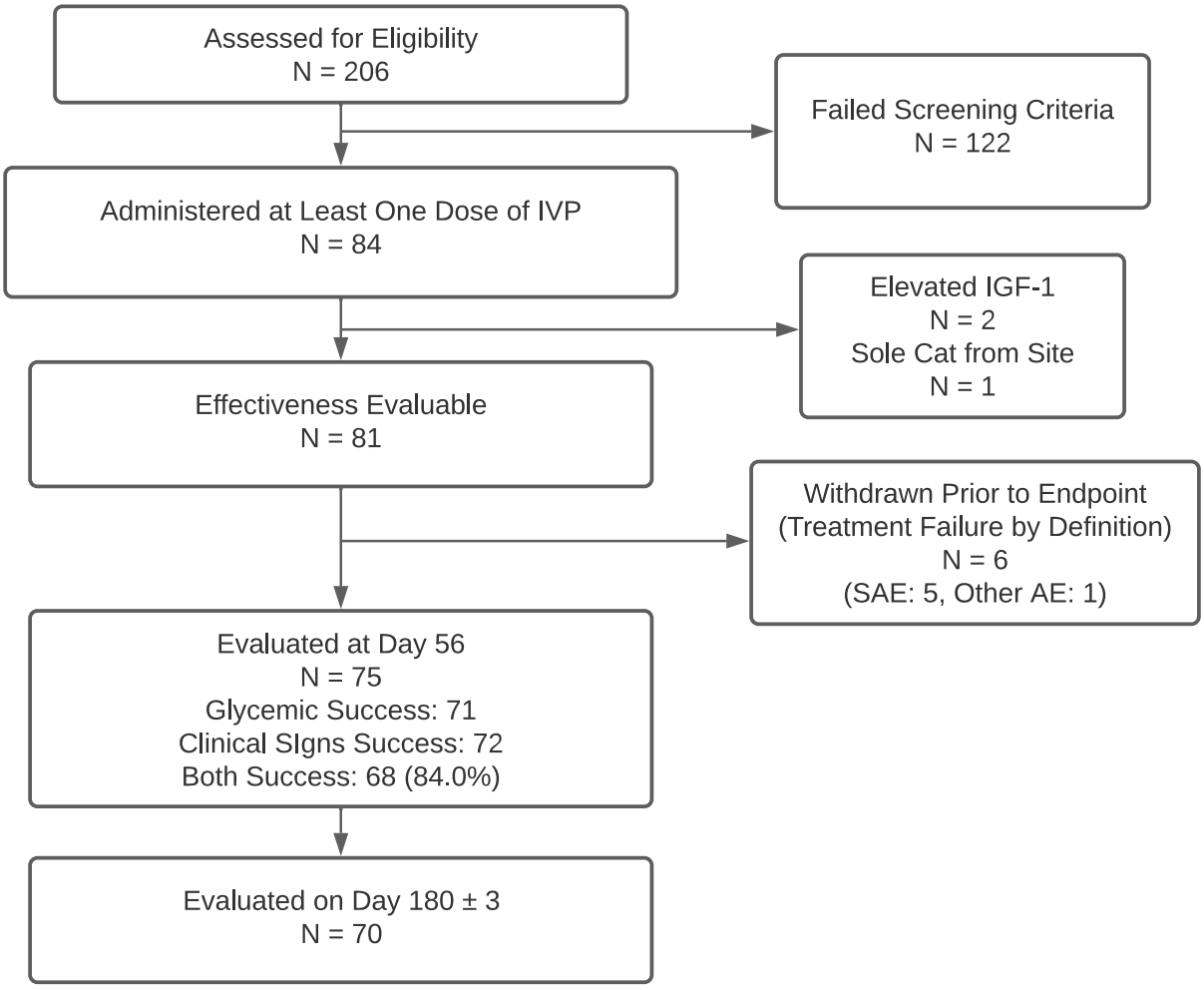
Serum fructosamine concentration measures the mean degree of nonenzymatic glycation of plasma proteins, an indicator of average plasma glucose concentration over the lifetime of the proteins. In humans, the mean half-life of plasma proteins is 2 to 3 weeks, but a similar estimate of protein half-life in cats does not appear to have been reported. The duration of effects of hyperglycemia on fructosamine concentration in healthy cats has been reported in a study conducted by continuous glucose perfusion.[37] The kinetics of the fructosamine response to plasma glucose were markedly asymmetric, with an initial increase over approximately 6 days, and a decrease following cessation of infusion over approximately 20 days.[37] No comparable study has been performed in diabetic cats and there is uncertainty regarding the degree to which changes in fructosamine at day 56 reflect the full therapeutic consequences of administration of bexagliflozin.

Because bexagliflozin produces a prompt decrease in plasma glucose that is evident on the first day of dosing (Fig. 1), it is possible to estimate with greater precision the kinetics of fructosamine decrease following glycemic normalization in diabetic cats. Supplementary Fig. 2 shows the results of a nonlinear fit to a first-order decay model for all fructosamine data from a single laboratory provided by cats enrolled in a pilot study and the present study. The data analyzed consisted of 661 measurements from 179 cats and the pilot study had a similar design and the same bexagliflozin dosage. The best fit is described by $F = 297 + 271\, e^{-0.102\, t}$, where F is the concentration of fructosamine in μmol/L and t is the time in days. The high quality of the fit is evident from the correspondence with the mean values for binned day 0, 14, 28 and 56

glucose concentrations (grey circles, Supplementary Fig. 2). The inferred fructosamine half-life is 6.78 days and at day 56 the residual contribution of the initial hyperglycemia is predicted to be 0.3%. Hence a measurement at day 56 is anticipated to reflect the full therapeutic effect of bexagliflozin in the study cohort.



Supplemental Figure 1. Change in fructosamine concentration (µmol/L) with time. Data collected prior to treatment are plotted as day 0. Grey circles denote mean values for day 0 and days 14, 28 and 56 ± 3. Values outside the intervals were discarded for the calculation of means. The dotted line represents first-order decay with rate constant 0.1023 days^{-1}.



Supplemental Figure 2. Patient disposition

Investigator Assessment of Cat Condition

At each clinic visit, the investigator gave an assessment of cat condition based on the hair coat quality, muscle mass and neurologic assessment, as shown in the table below. Assessments were made relative to the condition of the cat when healthy.

Scoring Guide for Assessment of Cat Condition

	Excellent = 0	Good = 1	Fair = 2	Poor = 3
Hair coat quality	Hair coat is soft and shiny with no or minimal shedding. Looks excellent.	Hair coat is smooth with no mats, bare spots or excessive shedding. Looks normal.	Hair coat is slightly dull or dry. May have mild increase in shedding. No mats or bare spots.	Hair coat is dry and dull. May have mats, excess shedding, bare spots, or flakes.
Muscle mass	Normal muscle mass. Back, ribs, and limbs are well muscled with normal muscle tone.	Slightly decreased muscle mass. Spinal processes are not evident. Minor loss of muscle over ribs, lower back and limbs. Muscles have good tone.	Markedly decreased muscle mass. Spinal processes are evident, loss of some muscle over ribs and lower back. Limb musculature is reduced but normal muscle tone.	Emaciated appearance. Spinal processes are prominent and ribs easily palpated. Limbs are thin with bones prominent, Appendicular muscles are atrophic and flaccid.
Neurologic assessment	Normal neurologic examination. No evidence of diabetic neuropathy.	Neurologic examination reveals mild plantigrade stance. Neurologic examination otherwise normal.	Neurologic examination reveals mild hindlimb weakness and plantigrade stance.	Neurologic examination reveals marked hind limb weakness and marked plantigrade stance.

Quality of Life Questionnaire

The rating system as described[22] was followed with the exception that the descriptions of the lowest ranked of the frequency attributes were "Rarely," whereas in the previous questionnaire it was "Never." The score is the arithmetic average of individual scores constructed by multiplying the importance of a response by the frequency with which the response occurred. In this scheme, the more negative the overall score is, the greater the adverse impact of the disease on owner quality of life. Frequency was assessed as "All the time" (-3), "Often" (-2), "Occasionally" (-1), or "Rarely" (0). Importance was assessed as "Very important" (4), "Important" (3), "Moderately important" (2), "Low importance" (1), or "Not at all important" (0). For questions 8, 25 and 28, the frequency numerical scores are positive.

The original questionnaire was predicated on the management of diabetes by insulin. For this study, questions that refer to insulin or injection were reformulated in terms of general provision of care. For example, question 3 of previous instrument was "Do you feel your life is restricted because of the daily insulin injections?" whereas in this study question 3 was "Do you feel your life is restricted because of the treatment requirements?" Similarly, question 4, "Does your pet ever react annoyed or in pain when injected?" became "Does your pet ever act annoyed when treated?"

Questions in this study:

1. Do you worry about your pet's diabetes? ($p < 0.0001$)
2. Do you ever feel you want to give your pet treats but you don't because of the diabetes? ($p = 0.0003$)
3. Do you feel your life is restricted because of the treatment requirements? ($p = 0.0080$)
4. Does your pet ever act annoyed when treated? ($p = 0.12$)
5. Do you ever worry about whether you have given the treatment correctly? ($p < 0.0001$)

6. Do you resent having to treat your pet? ($p = 0.11$)
7. Do you ever find the diabetes of your pet restricts or limits what you are doing or what you want to do, like going on holidays, away for weekends, away for the day/night, working? ($p = 0.53$)
8. Do you ever give your pet extra things, like snacks, treats, extra attention or extra walks because of the diabetes? ($p = 0.075$)
9. Do you ever feel you want to take more control of the diabetes on your own, without the help from vets and other people? ($p = 0.091$)
10. Do you think the diabetes affects your pet's moods? ($p = 0.095$)
11. Does your pet ever feel unwell, tired or in any other way negatively affected since treatment was started? ($p = 0.076$)
12. Do you ever choose not to put your pet into boarding kennels because of the diabetes? ($p = 0.84$)
13. Do you ever choose not to leave your pet to stay with friends or family because of the diabetes? ($p = 0.92$)
14. Does your pet ever show signs of a low blood sugar (*e.g.*, wobbliness, collapse)? ($p = 0.0066$)
15. Do you ever choose not to take your pet with you on an active day (*e.g.*, walking longer distances, going to the beach, etc.) because of the diabetes? ($p = 0.70$)
16. Does your pet still drink more than before the diagnosis? ($p < 0.0001$)
17. Is your pet still hungrier than before the diagnosis? ($p = 0.062$)
18. Does your pet still urinate more than before the diagnosis? ($p < 0.0001$)
19. Is your pet still losing weight since treatment has begun? ($p = 0.0034$)
20. Do you ever feel worried you will not be able to take care of your pet in the future because of the diabetes? ($p = 0.028$)
21. Do you ever feel worried about your pet suffering from an episode of low blood glucose? ($p = 0.0023$)
22. Do you ever feel worried about your pet suffering from an episode of ketoacidosis? ($p < 0.0001$)
23. Do you ever worry about your pet getting vision problems because of cataracts or did you worry about this before your pet suffering from such problems? ($p = 0.015$)
24. Are you less inclined to play with your pet now that he/she has diabetes? ($p = 0.38$)
25. Are you more inclined to play with your pet now that he/she has diabetes? ($p = 0.30$)
26. Do you ever find you need to fit your pet's diabetes into your social life (*e.g.*, carrying treatment, food, providing food on time)? ($p = 0.15$)
27. Do you ever find you need to fit your pet's diabetes into your working life (*e.g.*, having to make special arrangements when you need to work late or need to start working earlier)? ($p = 0.074$)

28. Do you feel you have a more special bond with your pet now that you are managing his/her diabetes? ($p = 0.76$)
29. Do you ever worry about how much money your pet's diabetes costs you and your family? ($p = 0.0083$)

A summary of the results by question and visit is provided in Supplementary Table 1 below. Scores on questions 1, 2, 3, 5, 14, 16, 18, 19, 20, 21, 22, 23 and 29 increased (improved) from Visit 2 to Visit 5 ($p < 0.05$). These questions mostly addressed clinical signs of hyperglycemia (14, 16, 18, 19), and possible side effects or disease complications (21 – 23). Owner overall anxiety was addressed by question 1, and anxiety about management effectiveness and financial impact were addressed by questions 5, 20 and 29. Questions 2 and 3 related to a change in owner behavior due to the disease.

Supplemental Table 1. Quality of life responses by question

Day	Question	N	Mean	SD	95% Confidence Intervals		p-value [a]
					Lower Bound	Upper Bound	
0	1	81	-8.47	3.08	-9.15	-7.79	< 0.0001
56	1	74	-6.55	3.69	-7.41	-5.70	
0	2	80	-3.69	4.06	-4.59	-2.78	0.0003
56	2	74	-2.07	3.12	-2.79	-1.34	
0	3	80	-1.99	2.68	-2.58	-1.39	0.0080
56	3	74	-1.05	1.52	-1.41	-0.70	
0	4	73	-1.96	2.90	-2.64	-1.28	0.1202
56	4	74	-1.34	2.23	-1.86	-0.82	
0	5	72	-3.07	3.37	-3.86	-2.28	< 0.0001
56	5	74	-0.62	1.51	-0.97	-0.27	
0	6	76	-0.49	1.67	-0.87	-0.11	0.1135
56	6	74	-0.16	0.60	-0.30	-0.02	
0	7	77	-1.83	3.00	-2.51	-1.15	0.5322
56	7	74	-1.61	2.60	-2.21	-1.01	
0	8	80	-3.01	3.65	-3.82	-2.20	0.0746
56	8	73	-2.23	3.13	-2.96	-1.50	
0	9	81	-1.49	3.37	-2.24	-0.75	0.0913
56	9	74	-0.97	2.49	-1.55	-0.40	
0	10	81	-3.47	3.67	-4.28	-2.66	0.0947
56	10	74	-2.65	3.57	-3.48	-1.82	

Supplemental Table 1. Quality of life responses by question

Day	Question	N	Mean	SD	95% Confidence Intervals		p-value [a]
					Lower Bound	Upper Bound	
0	11	68	-1.40	2.79	-2.07	-0.72	0.0756
56	11	74	-0.69	1.74	-1.09	-0.29	
0	12	78	-0.97	2.74	-1.59	-0.36	0.8366
56	12	73	-0.88	2.83	-1.54	-0.22	
0	13	78	-1.71	3.16	-2.42	-0.99	0.9246
56	13	73	-1.77	3.54	-2.59	-0.94	
0	14	78	-0.72	1.86	-1.14	-0.30	0.0066
56	14	74	-0.08	0.49	-0.19	0.03	
0	15	77	-0.40	1.73	-0.80	-0.01	0.7004
56	15	73	-0.37	1.81	-0.79	0.05	
0	16	80	-5.56	4.70	-6.61	-4.52	<.0001
56	16	74	-2.14	3.04	-2.84	-1.43	
0	17	78	-4.37	4.73	-5.44	-3.31	0.0623
56	17	73	-3.19	3.56	-4.02	-2.36	
0	18	79	-5.57	4.48	-6.57	-4.57	< 0.0001
56	18	74	-2.82	3.60	-3.66	-1.99	
0	19	72	-2.39	3.78	-3.28	-1.50	0.0034
56	19	74	-0.96	2.25	-1.48	-0.44	
0	20	81	-3.63	4.12	-4.54	-2.72	0.0282
56	20	74	-2.58	3.24	-3.33	-1.83	
0	21	81	-5.05	4.32	-6.00	-4.09	0.0023
56	21	74	-3.47	3.54	-4.29	-2.65	
0	22	79	-5.03	4.43	-6.02	-4.03	< 0.0001
56	22	74	-2.81	3.58	-3.64	-1.98	
0	23	80	-3.40	4.11	-4.31	-2.49	0.0148
56	23	74	-2.39	3.42	-3.18	-1.60	
0	24	81	-0.32	1.19	-0.58	-0.06	0.3810
56	24	74	-0.18	0.90	-0.38	0.03	
0	25	81	-3.72	3.74	-4.54	-2.89	0.2987
56	25	74	-3.20	3.24	-3.95	-2.45	
0	26	78	-2.35	3.53	-3.14	-1.55	0.1493
56	26	74	-1.82	3.25	-2.58	-1.07	
0	27	80	-2.14	3.51	-2.92	-1.36	0.0738
56	27	74	-1.34	2.83	-1.99	-0.68	
0	28	79	-4.75	4.17	-5.68	-3.81	0.7592
56	28	74	-4.96	3.85	-5.85	-4.07	
0	29	81	-4.25	4.05	-5.14	-3.35	0.0083
56	29	74	-2.97	3.70	-3.83	-2.11	

Brief Histories for Cats with SAEs

The 8 cats that experienced SAEs in this study and their outcomes are presented in the following section

Case 1 (Dehydration and weight loss, transition to insulin)

Case 1 was a 13-year-old male neutered domestic shorthair cat enrolled on 2019-09-09. His initial laboratory findings at screening were notable for poorly-controlled diabetes mellitus. In repeated visits over the course of the study his clinical status continued to reflect poor glycemic control. At a scheduled visit on 2019-11-01 (day 53), he was found to be dehydrated and losing weight. He was seen by a non-study veterinarian for an unscheduled visit on 2019-11-06 (day 58) for continued dehydration and weight loss. He exited the study at this visit and was managed with insulin thereafter.

Case 2 (Fatal weight loss with anemia)

Case 2 was a 13-year-old male castrated domestic medium-hair cat enrolled on 2020-02-21. His initial laboratory findings at screening were notable for poorly-controlled diabetes mellitus, hyperglobulinemia, mild hematuria, mildly increased symmetrical dimethylarginine and mildly increased pancreatic lipase. At visit 4 on 2020-03-20 (day 28), anemia developed, with an elevation in liver enzymes and a further increase of pancreatic lipase. These abnormalities persisted, and he began losing weight. He was seen for an unscheduled visit on 2020-04-27 (day 66) for follow up on the weight loss and anemia. Despite supportive care and additional diagnostic tests to try to elucidate the underlying cause, he died at home on 2020-05-08 (day 77).

Case 3 (Death of unknown cause)

Case 3 was an 8-year-old male neutered domestic longhair cat enrolled on 2019-09-24. His laboratory findings at screening were notable for poorly-controlled diabetes mellitus. He was seen for an unscheduled visit on 2019-10-01 (day 7) after the owner noticed anorexia and lethargy commencing 2019-09-29 (day 5). The owner had stopped providing the investigational product upon observing these clinical signs, with the last dose administered on 2019-09-28 (day 4), for a total of 5 doses. During the visit the owner declined medical management and opted for euthanasia. Approval for necropsy was not granted.

Case 4 (Constipation and pancreatitis)

Case 4 was a 10-year-old female spayed domestic shorthair cat enrolled on 2019-09-27. Her initial laboratory findings at screening were notable for poorly-controlled diabetes mellitus. She was seen by a non-study veterinarian for non-study visits on 2019-10-31 (day 34) and 2019-11-01 (day 35) for anorexia, vomiting and constipation. She was found to be severely constipated and pancreatitis was suspected. She was seen for an unscheduled visit on 2019-11-02 (day 36) for continued constipation and anorexia. She was referred to an emergency hospital for continued treatment of the constipation and pancreatitis. Bexagliflozin was administered once daily with a missed dose on 2019-10-31 (day 34).

Case 5 (DKA, transition to insulin)

Case 5 was a 9-year-old male neutered domestic shorthair cat enrolled on 2020-02-24. His initial laboratory findings at screening were notable for poorly-controlled diabetes mellitus. His initial dose was administered on 2020-02-24 (day 0). He was seen for an unscheduled visit on 2020-02-26 (day 2) after the owner noticed anorexia and lethargy. During the visit, he was diagnosed with euglycemic DKA and hospitalized for treatment. He exited the study on 2020-02-29 (day 5) and

was started on glargine insulin. The last dose of investigational product was administered on 2020-02-25 (day 1), for a total of 2 doses.

Case 6 (DKA, transition to insulin)

Case 6 was a 12-year-old female spayed domestic short-hair cat enrolled on 2020-05-22. Her initial laboratory findings at screening were notable for poorly-controlled diabetes mellitus and a resolving previous urinary tract infection. Between the screening visit on 2020-05-15 (day -7) and the scheduled visit on 2020-06-05 (day 14), her serum β-hydroxybutyrate concentration increased from 5.4 mg/dL to 14.0 mg/dL, but she was clinically doing well. On 2020-06-19 (day 28), she had lost 1.4 kg from the previous visit, her liver values were elevated, her β-hydroxybutyrate had increased to 96.9 mg/dL and ketonuria was present. She was seen for an unscheduled visit on 2020-06-22 (day 31) for anorexia of 48-hour duration and lethargy. She was diagnosed with DKA and hospitalized for treatment. She exited the study on 2020-06-23 (day 32) and her diabetes was managed with insulin thereafter. Investigational product was administered once daily without missed doses, with the last dose being administered on 2020-06-19 (day 28).

Case 7 (fatal hepatic lipidosis with DKA)

Case 7 was a 13-year-old female spayed domestic short-hair cat enrolled on 2020-07-09. Her initial laboratory findings at screening were notable for poorly-controlled diabetes mellitus, mildly increased anion gap, mild hypochloremia and mild hypokalemia. She was seen for an unscheduled visit on 2020-07-13 (day 4) for anorexia, weakness and lethargy. During the visit, she was diagnosed with euglycemic DKA, electrolyte abnormalities and suspected hepatic lipidosis based on elevated liver enzymes. She exited the study on 2020-07-13 (day 4), with the

last dose of investigational veterinary product administered on 2020-07-11 (day 2), for a total of 3 doses delivered. Despite supportive care during hospitalization, she showed minimal improvement with a progression of liver enzyme elevation and emergence of a mild anemia. After being sent home for the weekend, she did poorly and was brought to an emergency clinic, where she was found to have progressive hypokalemia and anemia. The owner declined further treatment and opted for euthanasia. Approval for a necropsy was granted.

Case 8 (presumed DKA, transition to insulin)

Case 8 was a 9-year-old female spayed Russian Blue cat enrolled on 2020-06-01. Her initial laboratory findings at screening were notable for poorly-controlled diabetes mellitus and a urinary tract infection. Her initial dose of investigational product was administered on 2020-06-01 (day 0). She was seen in an unscheduled visit on 2020-06-04 (day 3), diagnosed with presumptive pyelonephritis and DKA and hospitalized for treatment, which included insulin. She exited the study on 2020-06-11 (day 10), with the last dose of investigational product being administered on 2020-06-03 (day 2), for a total of 3 doses administered. As of 2020-07-29, she was reported to be doing well on insulin.