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Canagliflozin: Pharmacokinetics, tolerability and glucose/insulin effects of supratherapeutic doses in healthy horses

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ABSTRACT

Sodium-glucose co-transporter 2 inhibitors like canagliflozin (CFZ) have shown promise in preventing hyperinsulinemia-associated laminitis in horses, but data on pharmacokinetics, tolerability, and controlled studies are limited. This randomized, open-label, placebo-controlled, crossover study evaluated these aspects of CFZ treatment in eight healthy Standardbred mares. Each horse received single supratherapeutic oral doses of CFZ (1.8 mg/kg or 3.6 mg/kg) and placebo, with a two-week washout between treatments. A graded glucose infusion (GGI) was administered post-treatment to evaluate glucose and insulin responses. Plasma CFZ, glucose, insulin, urinary glucose, serum biochemistry, and urinalysis samples were collected over 72 h post-treatment. For CFZ 1.8 mg/kg, median C_{max} was 2623 ng/mL, T_{max} 2.2 h, and $T_{1/2Z}$ 21.8 h; for 3.6 mg/kg, C_{max} was 4975 ng/mL, T_{max} 2.8 h, and $T_{1/2Z}$ 23.0 h. The pharmacokinetics of CFZ displayed dose-proportionality across the two tested doses. Insulin and glucose responses to a GGI, measured by the area under the concentration-time curve (AUC), were similar between CFZ doses but significantly reduced compared to placebo ($p < 0.001$). Specifically, mean glucose AUC for CFZ treatments was approximately 14–15 % lower, and mean insulin AUC 22–29 % lower, than for placebo. For CFZ-treated horses, mean urinary glucose concentrations ranged from 277 to 347 mmol/L at 24, 48, and 72 h post-administration, with no significant differences between dose levels. No clinical signs of adverse effects were observed, although a significant increase in GLDH levels compared to placebo ($p < 0.05$) was observed with the CFZ 3.6 mg/kg dose.

Introduction

Laminitis is a painful and potentially life-threatening condition in horses, most commonly caused by hyperinsulinemia, a hallmark of insulin dysregulation (ID) (Durham et al., 2019). While the link between hyperinsulinemia and laminitis is well established (Asplin et al., 2007; de Laat et al., 2010), the exact mechanisms remain unclear (Menzies-Gow and Knowles, 2024). In cases of laminitis due to ID, failure to manage the condition leads to painful laminitis episodes and may result in euthanasia (Sundra et al., 2024). Dietary restriction and exercise remain the cornerstone of managing ID (Durham et al., 2019). Pharmacological therapies such as metformin (Colmer et al., 2023; Durham et al., 2008), levothyroxine (Chameroy et al., 2010), and pioglitazone (Legere et al., 2019; Suagee et al., 2011) have not consistently demonstrated high efficacy in ID horses. In recent years, sodium-glucose co-transporter 2 inhibitors (SGLT2i) have become an off-label alternative for complementary treatment of equine ID (Lindase et al., 2023; Sundra et al., 2024). The primary mechanism of action of SGLT2i is to reduce the glucose load by increasing glucose excretion through the kidneys, achieved by inhibiting the SGLT2 transport protein in the proximal tubule of the nephron (Vallon, 2024).

Although horses with ID are typically normoglycemic, SGLT2 inhibitors like canagliflozin (CFZ), dapagliflozin, ertugliflozin, and velagliflozin have all shown high short-term efficacy in reducing hyperinsulinemia in ID horses, making this class of drugs a promising new tool for managing this condition. (Kellon and Gustafson, 2022,

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2023; Lindase et al., 2023; Meier et al., 2019; Meier et al., 2018; Sundra et al., 2023; Sundra et al., 2025). There is still, however, a lack of long-term studies evaluating their safety and efficacy, along with limited knowledge of their pharmacokinetic properties in horses. Currently, the only published peer-reviewed pharmacokinetic data on SGLT2i in horses is limited to a study investigating the pharmacokinetics after a single dose of 1.8 mg/kg CFZ in eight Icelandic horses (Michanek et al., 2024) along with very limited velagliflozin data from a patent application (Reiche et al., 2015).

This study aims to provide pharmacokinetic data for CFZ in the Standardbred breed of horses, adding to the limited information currently available. The primary aim was to evaluate the dose proportionality of CFZ at elevated plasma concentrations following single supratherapeutic doses (1.8 mg/kg and 3.6 mg/kg). Secondary aims included exploring the effects of CFZ on glucose and insulin responses in healthy horses, along with the tolerability of these supratherapeutic doses and their impact on urinary glucose concentrations, areas minimally explored in prior research.

Material and methods

Horses

Eight Standardbred mares, owned by the Swedish University of Agricultural Sciences and used for teaching and research purposes, were included in this study. All horses were deemed healthy based on a thorough clinical examination, as well as serum biochemistry and urinalysis, with no history or clinical evidence of pituitary pars intermedia dysfunction or ID. The median age of the mares was 15 years (range: 9–23 years), with a median body weight of 601 kg (range: 551–677 kg). The median body condition score (Henneke et al., 1983) (BCS) was 6 (range: 6–7), and the cresty neck score (Carter et al., 2009) (CNS) had a median of 2 (range: 2–3). The horses were housed in individual boxes with daily paddock turnout. Their regular diet consisted of grass haylage provided four times daily, soaked beet-pulp-based mash and a small amount of oat grains mixed with minerals. The horses were not actively exercised.

Experimental Design

The study was a randomized, open-label, placebo controlled, three-treatment crossover design evaluating the effect of a single dose of oral CFZ (1.8 mg/kg or 3.6 mg/kg) or placebo. To accommodate tablet size, doses were rounded to the nearest 100 mg, resulting in mean administered doses of 1.81 mg/kg (SD ± 0.04) and 3.61 mg/kg (SD ± 0.05) in the respective treatments. The drug was administered in half an apple or with a small amount of concentrates, depending on the horses' preferences. During the placebo phase, only the apple or concentrates were provided. Each treatment period was separated by a minimum two-week washout.

The day before treatment administration, indwelling venous catheters were aseptically placed in both jugular veins to facilitate simultaneous intravenous glucose infusion and blood sampling. Four hours after administering the drug or placebo, a graded glucose infusion (GGI) was initiated to assess the effects of treatment on glucose dynamics. Glucose (Glucos Fresenius Kabi 300 mg/mL, Fresenius Kabi, Uppsala, Sweden) was infused at sequential rates of 0.4, 0.8, 1.2, 1.6, 2.4, and 3.2 mg/kg/min, with each rate sustained for 40 min, leading to a total cumulative glucose infusion of 384 mg/kg over 4 h. The infusion was delivered using an Infusomat Space pump (B. Braun Melsungen AG, Melsungen, Germany). Details of the experimental timeline are presented in Fig. 1.

Horses were fed according to their normal routine, with an additional haylage meal provided at night, approximately 5 h before treatment administration. If any feed remained at the time of CFZ or placebo administration, it was removed, and no additional feed was given until 40 min after completing the GGI. The horses were closely monitored during the first 12 h after drug or placebo administration and subsequently observed several times daily by the stable staff for signs of adverse effects.

The Animal Ethics Committee in Uppsala, Sweden, granted ethical approval for the study (5.8.18–05506/2022).

Sample collection

Blood samples were collected using an intravenous catheter (0–24 h) or by direct venipuncture (32–72 h) into EDTA 2 K tubes for plasma and clot activator tubes for serum. Urine samples were collected using sterile Nelaton catheters (Mediplast AB, Malmö, Sweden) and transferred into sterile additive-free tubes. All samples, except those designated for hematological analysis, were centrifuged immediately after collection at 1950 g for 10 min, and the supernatant was transferred to new tubes. These supernatant samples were immediately stored at −20°C and subsequently moved to −80°C within 24 h, pending analysis. Samples for hematological analysis were collected in EDTA 2 K tubes and analyzed immediately.

Fig. 1 provides an overview of the sampling schedule. Plasma samples for CFZ analysis were collected at 0, 0.33, 0.66, 1, 1.33, 1.66, 2, 2.33, 2.66, 3, 3.5, 4, 5, 6, 8, 12, 24, 32, 48, and 72 h after drug or placebo administration. Plasma samples for glucose analysis was drawn at 0, 1, 2, 3, and 4 h post-treatment administration, followed by sampling every 10 min from the start of the GGI until 40 min after its completion. Additional glucose samples were collected at 12, 24, 32, 48, and 72 h

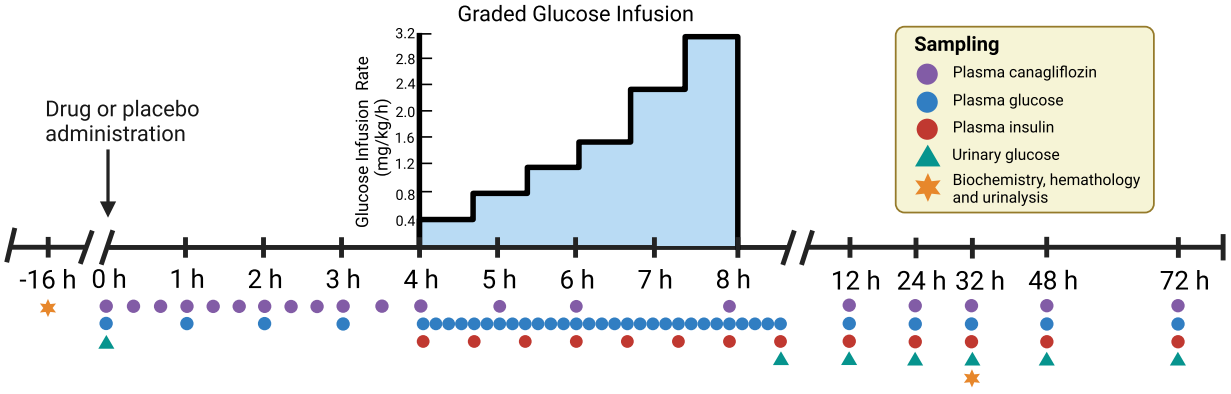

Fig. 1. Overview of the experimental timeline, illustrating treatment administration, graded glucose infusion (GGI), and sample collection time points for canagliflozin (CFZ), glucose, insulin, urinary glucose, biochemistry, hematology, and urinalysis. The GGI was conducted over 4 h, from 4 to 8 h post-administration. Each horse completed this timeline for all three treatments, except that hematology analysis was limited to the 3.6 mg/kg CFZ treatment. Figure created using BioRender (BioRender.com).

post-administration. Plasma samples for insulin analysis were taken at the beginning of the GGI, every 40 min throughout the infusion, until 40 min post-infusion, and at 12, 24, 32, 48, and 72 h post-administration.

Urine samples for glucose measurement were collected from all horses at approximately 0, 8.66, 12, 24, 32, 48, and 72 h post-administration. Additionally, four of the eight horses followed a more frequent urine-sampling schedule for glucose measurement within the first 8 h, detailed in Figure S4 of the supplementary material. For this frequent sampling, indwelling Foley urinary catheters (Medicoplast International GmbH, Illingen, Germany) were used.

Serum for biochemical analysis, urine for urinalysis, and plasma for hematological analysis (only for the 3.6 mg/kg CFZ treatment, not for the placebo or 1.8 mg/kg treatment) were collected twice, once prior to intervention, then again at 32 h post administration.

Sample analyses

We conducted serum biochemical analyses, plasma insulin and glucose measurements, and CFZ quantification as previously described (Michanek et al., 2024). CFZ was quantified by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry with validated precision (0.99 %-1.58 % RSD) and accuracy (98 %-101 %) in plasma samples. The analytical range for CFZ in plasma was 20–10,000 ng/mL. For insulin analysis, using Mercodia Equine Insulin ELISA, duplicate measurements yielded an intra-assay coefficient of variation of 3.8 %, calculated using the root mean square approach. Hematological parameters were analyzed using an IDEXX ProCyte Dx analyzer, with no errors detected in the scatterplots or flagged by the instrument. Urinary samples were batch analyzed for chloride, creatinine, glucose, potassium, sodium and total protein using urinary settings in an automatic biochemistry analyzer, DxC 700 AU with reagents from Beckman Coulter (Beckman Coulter, California, USA).

Data analysis

CFZ pharmacokinetic parameters were determined by non-compartmental analysis using PKanalix™ 2024R1 (Simulations Plus, 2024). Concentration-time profiles were visualized using the same software. The rate constant for the terminal disposition phase (λ_z) was calculated using uniform weighting, with point selection based on adjusted R^2 values. A minimum of the last three time points, covering a period of 40 h, was used. The Area Under the Curve (AUC) was estimated through linear-up log-down method. Concentrations below the lower limit of quantification occurring prior to the time of maximum concentration (T_{max}) were set to zero. Pharmacokinetic parameters estimated included T_{max}, peak plasma concentration (C_{max}), elimination rate constant (k_{el}), terminal half-life ($t_{1/2Z}$), and the apparent clearance (CL/F). The analysis also quantified the AUC from administration to the last observed time point at 72 h ($AUC_{0–72\ h}$) and the total area under the curve ($AUC_{0–inf}$), which included $AUC_{0–72h}$ and its extrapolation to infinity.

The effect of treatment on glucose and insulin concentrations during the GGI was evaluated using the AUC for glucose (AUC_{GLU}) and insulin (AUC_{INS}), incremental AUC ($iAUC_{GLU}$, $iAUC_{INS}$), peak concentrations (Max_{GLU}, Max_{INS}), and beta cell responsiveness indices ($iAUC_{INS}$/$iAUC_{GLU}$ and Δinsulin/Δglucose). Parameters were calculated for the full GGI duration and the 40 min post-infusion, except for Δinsulin/Δglucose, which was derived for the duration of the GGI only. Area Under the Curve values were calculated using the trapezoidal method, with baseline subtraction for iAUCs. The Δinsulin/Δglucose ratio was calculated as the slope of the regression line obtained by least squares regression of insulin vs. glucose concentrations (Lin et al., 2016). The corresponding r^2 value was also determined.

Statistical analyses, excluding the NCA performed in PKanalix, were carried out using R (R Core Team, 2024). Linear mixed-effects models, implemented via the lme4 (Bates et al., 2015) or nlme (Pinheiro et al., 2023) package, were used to evaluate differences between treatments. For biochemical parameters, urinalysis and parameters related to the GGI the models included treatment as fixed effect and individual horses as random effect. Residual vs. fitted plots and Q-Q plots, were performed to evaluate assumptions of homoscedasticity and normality. When necessary, log transformation of the response variable was applied to address violations of assumptions. For glutamate dehydrogenase (GLDH) concentration comparisons between treatments, the non-parametric Friedman test was used because assumptions for the linear mixed-effects model could not be met, even after data transformation. Linear mixed-effects models with an error term following a first-order autoregressive structure (AR(1)) were applied to repeated measures of glucose concentrations during the 0–4 h period post-administration and urinary glucose concentrations at 24, 48, and 72 h post-treatment. For glucose and insulin concentrations during the 12–72 h post-treatment period, a continuous first-order autoregressive structure (CAR(1)) was used to accommodate the non-equidistant time points in this period. The urinary glucose analysis was limited to the 1.8 mg/kg and 3.6 mg/kg CFZ treatments, excluding the placebo to reduce model complexity.

Paired t-tests were used to evaluate differences between pre- and post-treatment samples for hematology and to compare pharmacokinetic parameters between the two dose levels. Exposure parameters ($AUC_{0–72\ h}$, $AUC_{0–inf}$, and C_{max}) were dose-normalized by dividing each parameter by the dose (per kilogram of body weight) before comparison. Hematology parameters were analyzed without transformation, while pharmacokinetic parameters were log-transformed based on the normality of paired differences.

Degrees of freedom for the mixed-effects models were estimated using the Kenward-Roger method and Tukey's post-hoc method was applied for multiple comparisons for the mixed effects models using the emmeans (Lenth, 2024) package. For the Friedman test, Nemenyi post-hoc analysis, from the DescTools (Signorell, 2024) package, was used to identify differences between treatment pairs. All tests were two-sided, and statistical significance was set at an alpha level of 0.05. Plots were created using the package ggplot2 (Wickham, 2016). Arithmetic means ± standard deviation are presented for most data. For linear mixed-effects models utilizing log transformation and covariate matrices, back-transformed estimated marginal means (emmeans) with or without 95 % confidence intervals are reported. Standard error of the mean is used only in figures.

Results

All horses successfully underwent all treatments and no clinical signs of adverse effects were observed. Data limitations included one missing hematology sample, one missing CFZ sample, and one missing urinary glucose sample. Additionally, one urinary sodium outlier was excluded post-analysis (placebo treatment). All other available data from these affected cases were utilized (intention to treat).

Plasma concentration-time course of canagliflozin

CFZ plasma concentrations were below the limit of quantification at 0 h but detectable within the quantifiable range (20–10,000 ng/mL) in all horses by 20 min post-administration. For both the 1.8 mg/kg and 3.6 mg/kg doses, concentrations remained quantifiable throughout the entire 72-hour sampling period. The concentration-time profiles for each dose are illustrated in Fig. 2, and the corresponding pharmacokinetic parameters are summarized in Table 1. Mean adjusted r^2 for estimation of λ_z was 0.99. No significant differences were observed in pharmacokinetic parameters between the two dose levels, with exposure parameters ($AUC_{0–72h}$, $AUC_{0\text{-}inf}$ and C_{max}) being dose-adjusted (all comparisons: $p > 0.17$). Boxplots illustrating these comparisons are presented in Figure S1 of the supplementary material.

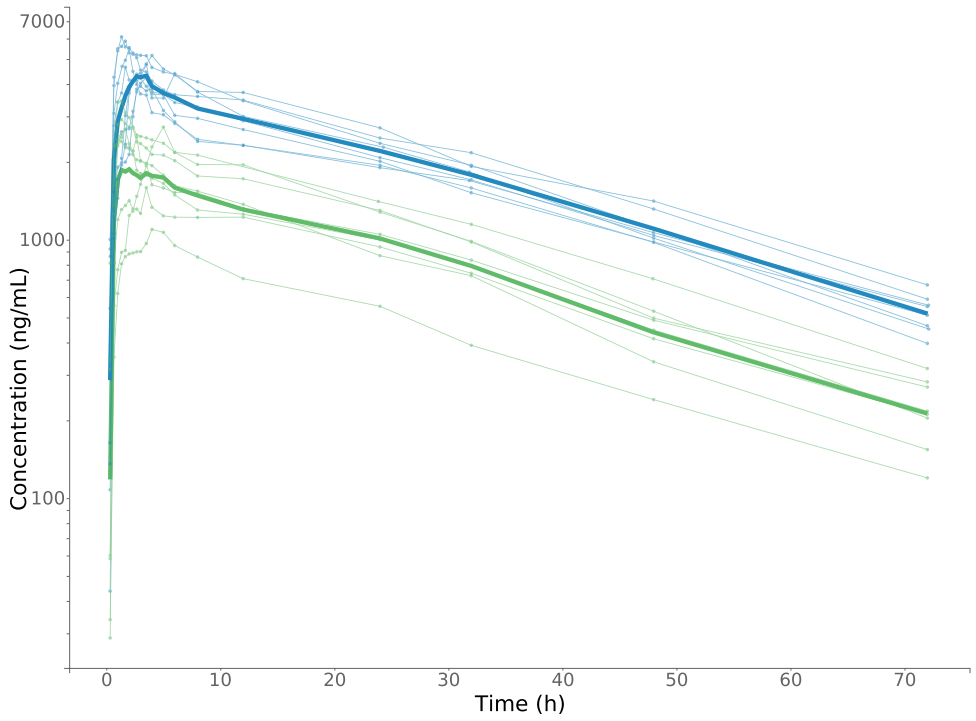

Fig. 2. Concentration-time profile of canagliflozin in eight Standardbred horses. Green lines represent the 1.8 mg/kg dose, while blue lines represent the 3.6 mg/kg dose. Thin lines depict individual horse data, and thick lines indicate the geometric mean concentrations for each CFZ treatment. Points represent observed data points.

Table 1
Pharmacokinetic parameters derived from non-compartmental analysis in 8 healthy Standardbred horses following administration of canagliflozin at 1.8 mg/kg and 3.6 mg/kg, with median, geometric means, and variability (Geometric CV%).

	Canagliflozin 1.8 mg/kg			Canagliflozin 3.6 mg/kg		
Parameter	Median	Geometric mean	Geo CV%	Median	Geometric mean	Geo CV%
C_{max} (ng·mL^{-1})	2623	2314	38.7	4975	4941	16.0
$AUC_{0\text{-}72h}$ (h·ng·mL^{-1})	58364	57165	31.3	124643	128030	13.8
$AUC_{0\text{-}inf}$ (h·ng·mL^{-1})	66794	63963	30.9	141171	145250	13.5
$t_{1/2z}$ (h)	21.8	21.5	12.4	23.0	22.5	12.0
k_{el} (h^{-1})	0.032	0.032	12.4	0.030	0.031	12.0
CL/F (mL·h^{-1}·kg^{-1})	27.3	28.4	29.4	25.4	24.9	13.2
T_{max} (h)	2.17	2.28	59.2	2.83	2.72	52.3

Abbreviations: C_{max}, peak plasma concentration; $AUC_{0–72\ h}$, area under the curve from the time of administration to the last observation at 72 h post administration; $AUC_{0–inf}$, the sum of $AUC_{0–72\ h}$ and its extrapolation to infinity; $t_{1/2Z}$, terminal half-life; k_{el}, elimination rate constant; CL/F, apparent clearance; T_{max}, time of maximum concentration.

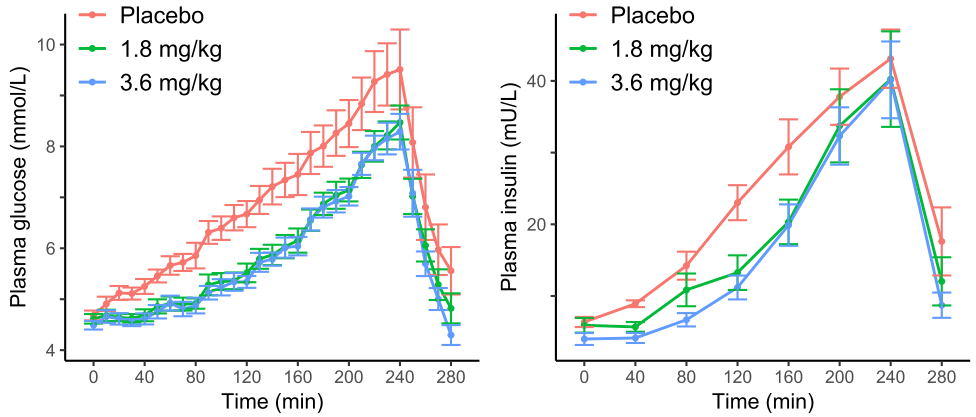

Fig. 3. Mean concentration-time profiles for plasma glucose (left) and plasma insulin (right) during and after a graded glucose infusion in eight Standardbred horses, starting at time 0 and completed at 240 min. Data for an additional 40 min post-infusion are also shown. The red line represents the placebo treatment, the green line represents the 1.8 mg/kg canagliflozin dose, and the blue line represents the 3.6 mg/kg dose. Error bars represent ± SEM.

Plasma glucose and insulin

The concentration-time profiles for glucose and insulin during the GGI, and 40 min post-infusion are presented in Fig. 3. There were no significant differences in glucose or insulin AUC or iAUC parameters between the 1.8 mg/kg and 3.6 mg/kg doses of CFZ (all comparisons: $p > 0.28$), but both doses resulted in significantly lower values compared to placebo (all comparisons: $p < 0.01$). Both doses of CFZ significantly reduced MAX_{GLU} compared to placebo ($p < 0.05$), whereas no significant differences were found between treatments for MAX_{INS} ($p = 0.63$), $iAUC_{INS}/iAUC_{GLU}$ ($p = 0.31$), or Δinsulin/Δglucose ($p = 0.78$). Mean ± SD of the GGI-related parameters and treatment comparisons are presented in Table 2.

Plasma glucose levels from 0 to 4 h post-administration, and plasma glucose and insulin levels from 12 to 72 h post-administration, are presented in Figures S2 and S3 in the supplementary material. Additionally, back-transformed emmeans with 95 % confidence intervals (CI) for all time points, along with statistical comparisons between treatments are presented in Table S1 of the supplementary material.

At treatment administration, back-transformed emmeans of fasting glucose concentrations were comparable across treatments (ranging 4.6–4.7 mmol/L; all comparisons $p > 0.57$). At 1 h post-administration, glucose concentrations were significantly lower in CFZ-treated animals (back-transformed emmean: 4.5 mmol/L for both CFZ doses) compared to placebo (4.9 mmol/L; both comparisons $p < 0.01$). At 2 h post-administration, the back-transformed emmean glucose concentration was significantly lower for the 3.6 mg/kg treatment (4.5 mmol/L) compared to the placebo treatment (4.8 mmol/L, $p < 0.05$). No statistically significant differences between treatments were detected at 3 and 4 h post-administration (all comparisons: $p > 0.14$). Plasma glucose concentrations 12–72 h post-administration did not differ significantly between treatments at any sampling point (all comparisons: $p > 0.21$). Insulin concentrations were significantly lower in 3.6 mg/kg CFZ-treated horses compared to placebo at 48 h (10.7 mU/L for 3.6 mg/kg dose vs. 17.2 mU/L for placebo, $p < 0.05$). No significant differences in insulin concentrations were observed at other time points between 12 and 72 h (all comparisons: $p > 0.17$).

Urinary Glucose

Urinary glucose concentrations severely increased in all horses following CFZ administration, remaining markedly elevated across the 72-hour sampling period, independent of dose, as depicted in Fig. 4. No significant differences were observed in urinary glucose concentrations between the 1.8 mg/kg and 3.6 mg/kg doses at 24, 48, and 72 h (p-values: 0.93, 0.15, and 0.65, respectively). Across both the 1.8 and 3.6 mg/kg CFZ doses, mean urinary glucose concentrations ranged from 277 to 347 mmol/L at 24, 48, and 72 h post-administration.

Four of the eight horses had additional urinary samples collected frequently during the first 8 h post-treatment, not shown in Fig. 4. Figure S4 of the supplementary material presents urinary and plasma glucose concentrations for these horses, showing increased urinary glucose concentrations already in the first sample 1 h post-CFZ administration. At time 0, urinary glucose concentrations in both CFZ treatments ranged from 0.1 to 0.4 mmol/L, increasing to 9.8–225.2 mmol/L by 1 h post-administration.

Biochemistry, hematology, and urinalysis

The hematological evaluation showed no significant differences between pre- and post-treatment samples for horses administered 3.6 mg/kg of CFZ (all comparisons: $p > 0.08$). There were a few significant differences between treatments for serum biochemical variables and urinalysis and they are presented in Tables S2 and S3 of the supplementary material. Mean urinary creatinine increased by 22.0 % in the placebo treatment, while it decreased by 38.0 % and 38.2 % in the

Table 2
Summary of glucose and insulin parameters related to the graded glucose infusion in eight Standardbred horses following placebo, canagliflozin (CFZ) 1.8 mg/kg, and CFZ 3.6 mg/kg treatments. Pairwise comparisons are reported with Tukey-adjusted p-values where significant ($p < 0.05$), indicated by superscript letters. When the overall ANOVA from the mixed effects model is non-significant ($p \geq 0.05$), only the ANOVA p-value is shown.

Parameter	Treatment	Mean ± SD	P-value (Pairwise Tukey-adjusted or overall ANOVA)
AUC_{GLU} (min·mmol·L^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	1936 ± 260^{a} 1666 ± 140^{b} 1640 ± 116^{b}	$^{a\text{-}b} < 0.001$
$iAUC_{GLU}$ (min·mmol·L^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	626 ± 227^{a} 374 ± 104^{b} 382 ± 94^{b}	$^{a\text{-}b} < 0.01$
AUC_{INS} (min·mU·L^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	6794 ± 1901^{a} 5324 ± 2144^{b} 4829 ± 1736^{b}	$^{a\text{-}b} < 0.001$
$iAUC_{INS}$ (min·mU·L^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	5009 ± 1600^{a} 3668 ± 1897^{b} 3709 ± 1179^{b}	$^{a\text{-}b} < 0.01$
Max_{GLU} (mmol·L^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	9.7 ± 1.9^{a} 8.5 ± 0.9^{b} 8.4 ± 1.0^{b}	$^{a\text{-}b} < 0.05$
Max_{INS} (mU·L^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	43 ± 11 41 ± 19 40 ± 15	Overall ANOVA: 0.63
Δinsulin/Δglucose (mU·mmol^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	8.9 ± 4.3 9.6 ± 5.3 10.3 ± 4.5	Overall ANOVA: 0.31
$iAUC_{INS}/iAUC_{GLU}$ (mU·mmol^{-1})	Placebo CFZ 1.8 mg/kg CFZ 3.6 mg/kg	9.2 ± 4.6 9.8 ± 4.5 10.0 ± 3.1	Overall ANOVA: 0.78

Abbreviations: AUC_{GLU}, area under the curve for glucose; $iAUC_{GLU}$, incremental area under the curve for glucose; AUC_{INS}, area under the curve for insulin; $iAUC_{INS}$, incremental area under the curve for insulin; Max_{GLU}, peak glucose concentration; Max_{INS}, peak insulin concentration; Δinsulin/Δglucose, change in insulin relative to the change in glucose

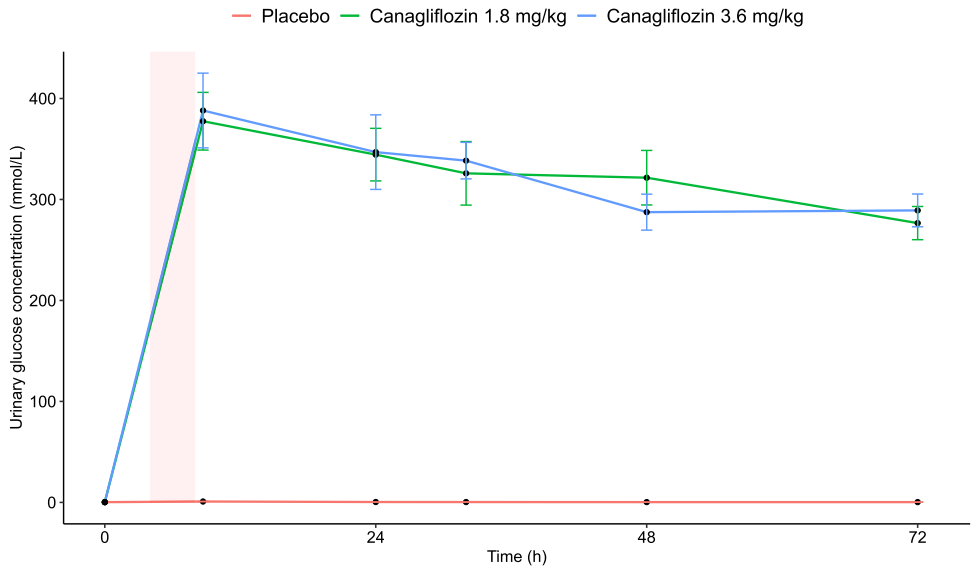

Fig. 4. Mean urinary glucose concentrations over time in eight Standardbred horses. Treatment was administered at time 0. The red line represents the placebo treatment, the green line represents the 1.8 mg/kg canagliflozin dose, and the blue line represents the 3.6 mg/kg dose. Error bars represent ± SEM. The red shaded area represents the time of the graded glucose infusion.

1.8 mg/kg and 3.6 mg/kg CFZ treatments, respectively. Both decreases in urinary creatinine were significantly different from placebo (both comparisons: $p < 0.01$). When urinary variables were normalized to urinary creatinine, no significant differences were observed between treatments (all comparisons: $p > 0.19$) (data not shown). Serum GLDH levels significantly increased in the CFZ 3.6 mg/kg treatment compared to placebo ($p < 0.05$), with a median change of + 185 nkat/L. In contrast, the CFZ 1.8 mg/kg treatment did not demonstrate a significant difference from placebo ($p = 0.56$), showing a median change of + 41 nkat/L. The placebo group had a median change of + 5 nkat/L. Triglycerides increased slightly by 0.09 mmol/L in the CFZ 3.6 mg/kg treatment. This increase was statistically significant compared to the placebo treatment (decrease of −0.09 mmol/L, $p < 0.01$) and the CFZ 1.8 mg/kg treatment (decrease of −0.04 mmol/L, $p < 0.05$).

Discussion

This placebo-controlled crossover study demonstrates that CFZ significantly lowers plasma glucose and insulin concentrations in response to a GGI in healthy Standardbreds. This finding is consistent with results from a similar study in healthy Icelandic horses (Michanek et al., 2024). In both studies, beta-cell responsiveness was not significantly altered by CFZ treatment, suggesting that the reduction in insulin levels is mainly driven by decreased glucose exposure. However, beta-cell responsiveness may require ID and/or a longer treatment duration to change, as a significant reduction was observed after four weeks of treatment in ID horses. (Lindase et al., 2023)

The pharmacokinetics of CFZ were characterized following two supratherapeutic dose levels (1.8 mg/kg and 3.6 mg/kg), revealing rapid absorption and sustained plasma exposure over 72 h. The variation in exposure-related parameters was notably higher at the 1.8 mg/kg dose compared to the 3.6 mg/kg dose. However, this difference largely disappeared when one individual with substantially lower drug exposure was excluded from the analysis. It is suspected that this horse may not have received the full dose due to tablet loss during administration. The pharmacokinetic parameters observed in this study are consistent with those previously reported in Icelandic horses (Michanek et al., 2024), though some differences were noted. The Standardbred horses in this study exhibited a faster drug elimination, as indicated by a shorter terminal half-life with a median of 21.8 h compared to 28.5 h reported in Icelandic horses, both following a 1.8 mg/kg dose. There was also, naturally, a decreased $AUC_{0\text{-inf}}$ in this study. Additionally, T_{max} was notably shorter in this study (median 2.2 h vs. 7 h in Icelandic horses). These pharmacokinetic differences may be linked to breed-related factors, though other explanations should also be considered. The shorter T_{max} observed in this study could be due to differences in feeding practices, as the Icelandic horses were fed closer in time (1 h vs 5 h in the present study) in relation to drug administration, potentially slowing gastric emptying and delaying drug absorption. Variability related to the small sample sizes (8 horses in each study) may have also contributed to the observed differences. Further pharmacokinetic analysis with a larger sample size is warranted to clarify these findings.

Non-linearity is sometimes observed at high plasma concentrations due to the saturation of metabolic or elimination pathways, which can reduce clearance and increase the risk of toxicity. No evidence of non-linearity was observed in the present study, following single supratherapeutic doses of 1.8 mg/kg and 3.6 mg/kg. These doses are substantially higher than the clinically effective daily doses of 0.3–0.6 mg/kg reported in previous studies (Kellon and Gustafson, 2022, 2023; Lindase et al., 2023). The observed linearity, coupled with the fact that the latter portion of the concentration-time profiles likely reflects concentrations similar to those achieved with lower, clinically effective daily doses, underscores the broader relevance of these findings beyond an overdose safety assessment. A key area for future research is to determine whether ID horses show similar or altered pharmacokinetics for CFZ, as they are the primary candidates for treatment with this drug. However, CFZ may also help prevent glucocorticoid-associated laminitis in predisposed horses, even those without diagnosed ID (Menzies-Gow and Knowles, 2024).

Glucose and insulin responses to the GGI were similar between the 1.8 mg/kg and 3.6 mg/kg CFZ treatments, indicating comparable pharmacodynamic effects early on. Additionally, urinary glucose concentrations remained highly elevated for both dose levels, with no significant differences between treatments at any time point during the 72-hour sampling period. Together, these findings suggest that both treatments induced similar pharmacological effects, with renal glucose excretion remaining near maximal for both doses even after 72 h. No pharmacokinetic/pharmacodynamic model for CFZ-induced glucose excretion exists for horses, but a half-maximal effective concentration (EC_{50}) of 21 ng/mL has been reported in healthy humans (Devineni

et al., 2015). Plasma concentrations in the present study far exceeded these human EC_{50} values, with the lowest observed concentration being 120 ng/mL, measured 72 h after a 1.8 mg/kg dose, thus supporting high and sustained pharmacological activity at both dose levels.

Urinary glucose concentrations were elevated in all horses post CFZ administration, consistent with the expected mechanism of SGLT2 inhibition. In the days following CFZ administration, mean urinary glucose concentrations ranged from 277 to 347 mmol/L, compared to 0.3–0.4 mmol/L in the placebo treatment. Assuming a normal daily urinary output of approximately 15–30 mL/kg/day (McKenzie, 2007) and an average urinary glucose concentration of 300 mmol/L in CFZ-treated horses, a 500 kg horse is expected to excrete roughly 0.4–0.8 kg of glucose per day. This loss is substantial, considering that a 500 kg horse fed 2 % of its body weight in dry-matter roughage containing 10 % non-structural carbohydrates (NSC) would have an NSC intake of 1 kg per day. Given this, it is not surprising that the drug is highly effective in ID horses, as it likely facilitates the excretion of a significant proportion of the sugar intake. This estimate of glucose excretion in healthy horses does not account for the drug's potential diuretic effects or the elevated plasma glucose concentrations sometimes observed in ID horses (Frank et al., 2006), both of which could further increase glucose losses. However, these calculations rely on several assumptions, and to obtain a more precise estimate, both urinary volume and glucose concentration would need to be measured, which could be explored in future studies.

No clinical signs of adverse effects were observed, and hematological variables remained unchanged after CFZ 3.6 mg/kg treatment. The reduction in all measured urinary variables except glucose following CFZ treatment is likely due to osmotic diuresis, given the absence of significant differences between treatments upon normalization to urinary creatinine. Serum biochemistry variables were generally within normal ranges and showed no treatment-related differences, though some changes were noted. The most notable finding was an increase in the liver enzyme GLDH in the CFZ 3.6 mg/kg treatment, potentially linked to the high dose administered. However, elevated GLDH levels have also been reported with a commonly used dose of ertugliflozin over seven days (Sundra et al., 2025). The clinical significance of these hepatic enzyme elevations remains uncertain.

Hypertriglyceridemia has been reported as an adverse effect in studies on ID horses treated with SGLT2i (Kellon and Gustafson, 2023; Lindase et al., 2023; Sundra et al., 2025). In this study, a slight increase in triglycerides (0.09 mmol/L) was observed following CFZ treatment at 3.6 mg/kg. Although statistically significant, this increase is unlikely to be clinically relevant. However, it should be noted that in the current study included only eight horses, and hypertriglyceridemia appears to develop in only a subset of SGLT2i-treated individuals (Sundra et al., 2025), and these metabolic changes may also take longer to develop and vary depending on breed and ID status.

Conclusion

Single supratherapeutic oral doses of CFZ (1.8 mg/kg and 3.6 mg/kg) decreased insulin and glucose concentrations in response to a GGI. No evidence of non-linearity in pharmacokinetic parameters was observed at these doses. Urinary glucose concentrations markedly increased, consistent with the expected mechanism of SGLT2 inhibition. No clinical signs of adverse effects were observed, but an increase in GLDH was noted, which may warrant clinical consideration.

CRediT authorship contribution statement

Mikael Hedeland: Writing – review & editing, Validation, Resources. **Minerva Löwgren:** Writing – review & editing, Investigation. **Malin Erkas:** Writing – review & editing, Investigation. **Cathrine Fjordbakk:** Writing – review & editing, Methodology, Funding acquisition. **Jonas Bergquist:** Writing – review & editing, Validation, Resources. **Carl Ekstrand:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Inger Lilliehöök:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition. **Johan Bröjer:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Peter Michanek:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis.

Declaration of Generative AI and AI-assisted technologies in the writing process

The principal author acknowledges the use of the large language model, GPT-4, for language editing and grammar checks. The authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2025.106412.

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Glossary (Field-specific terms)

$AUC_{0–72\ h}$: area under the curve from the time of administration to the last observation at 72 h post administration
$AUC_{0–inf}$: the sum of $AUC_{0–72\ h}$ and its extrapolation to infinity
AUC_{GLU}: area under the curve for glucose
AUC_{INS}: area under the curve for insulin
BCS: Body condition score
CFZ: Canagliflozin
CL/F: the ratio between total clearance and bioavailability
CNS: Cresty Neck Score
C_{max}: peak plasma concentration
EC_{50}: half-maximal effective concentration
GGI: Graded glucose infusion
$iAUC_{GLU}$: incremental area under the curve for glucose
$iAUC_{INS}$: incremental area under the curve for insulin
ID: Insulin dysregulation
kel: elimination rate constant
Max_{GLU}: peak glucose concentration
Max_{INS}: peak insulin concentration
NSC: non-structural carbohydrates
SGLT2i: sodium-glucose co-transporter 2 inhibitor
$t_{1/2z}$: terminal half-life
T_{max}: time of maximum concentration
Δinsulin/Δglucose: change in insulin relative to the change in glucose