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Case Report
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Hypertriglyceridemia in equines with refractory hyperinsulinemia treated with SGLT2 inhibitors

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Abstract

Background: Sodium-Glucose CoTransporter-2 (SGLT2) inhibitors, the -flozin group of drugs, which block glucose reuptake in the renal proximal tubule, are being increasingly used off-label to treat horses with refractory hyperinsulinemia. After 2 years of use by animals in our group, a horse on canagliflozin was incidentally noted to be hyperlipemic.

Case Description: We have been following a cohort of equines (n = 20) treated with SGLT2 inhibitors due to refractory hyperinsulinemia. The animals are owned by members of the Equine Cushing's and Insulin Resistance Group and treated by their attending veterinarians. The index case was a 23 years old gelding with a 2 years history of recurring laminitis that began canagliflozin therapy to control hyperinsulinemia which was no longer responsive to metformin. Between 6 and 10 weeks post start of therapy, significant weight loss was noticed. Two days later he was hospitalized with colic symptoms and hyperlipemia but was bright, alert, and eating well throughout. Canagliflozin was discontinued and triglycerides returned to normal reference values within 10 days. A subsequent study of 19 other horses on SGLT2 inhibitors revealed varying degrees of hypertriglyceridemia, all asymptomatic.

Conclusion: While this class of drugs holds great promise for cases of refractory hyperinsulinemia and laminitis that do not respond to diet or metformin therapy, hypertriglyceridemia is a potential side effect. In our experience, animals remained asymptomatic and eating well. Further study of hypertriglyceridemia in horses on SGLT2 inhibitors and the possible mitigating effect of diet is indicated. To our knowledge, this is the first report of hypertriglyceridemia with canagliflozin treatment in equines.

Keywords: Equine, Hyperinsulinemia, Hypertriglyceridemia, Canagliflozin, Ertugliflozin.

Introduction

Hyperinsulinemia in horses is associated with pituitary pars intermedia dysfunction (PPID) or equine metabolic syndrome (EMS) (Vaughn *et al.*, 2022). There is some evidence to suggest that, at least in ponies, hyperinsulinemia may result from enhanced intestinal glucose absorption and incretin excretion in the absence of insulin resistance (de Laat *et al.*, 2016) but this finding has not been duplicated either in ponies (Fitzgerald *et al.*, 2019a) or horses (Chameroy *et al.*, 2016).

Exercise is an effective method for controlling hyperinsulinemia (Karikoski *et al.*, 2022) but not an option with laminitic horses. Horses with PPID require dopaminergic medication but that alone often does not control circulating insulin levels (Donaldson *et al.*, 2002; Kienzle and Bockhorni, 2018).

The recognition of the role of insulin in laminitis (Knowles *et al.*, 2023) has led to dietary modifications (Johnson *et al.*, 2004) which have greatly improved the prognosis for horses with endocrinopathic laminitis, whether due to PPID or EMS, by reducing the total dietary percentage of simple sugars and starches. However, animals that fail to respond to pergolide, if indicated, and/or dietary restriction of simple carbohydrates have few alternatives to effectively reduce insulin levels.

Metformin, at 30 mg/kg BW twice daily, was a promising therapeutic initially (Rendle *et al.*, 2013). Early trials showed it effectively lowered glucose and insulin in naturally occurring and experimentally induced hyperinsulinemia. However, some horses fail to respond adequately, and the drug's effectiveness has been shown to diminish with time (Durham *et al.*, 2008).

Velagliflozin, belonging to a class of drugs known as sodium-glucose cotransporter 2 (SGLT2) inhibitors, has also been reported to reduce glucose and insulin effectively and rapidly in ponies. A controlled trial using velagliflozin in 75 ponies fed a high carbohydrate challenge diet resulted in a significant reduction in glucose and insulin levels 2 days after drug administration and reduced the incidence of laminitis (Meier *et al.*, 2018, 2019). These promising reports have

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prompted considerable interest in this class of drugs around the globe because refractory hyperinsulinemia and laminitis are significant human problems.

We recently published the case reports of equines with refractory hyperinsulinemia treated with SGLT2 inhibitors belonging to members of the international Equine Cushing's and Insulin Resistance Group (ECIR) (Kellon and Gustafson, 2022). The ECIR group is a non-profit organization that has maintained an outreach group for over 20 years. The group has over 10,000 members caring for horses with PPID and/or EMS (https://ecir.groups.io/g/main). Individual case histories are stored in a central database. Private owners and their attending veterinarians made the clinical decision to trial SGLT2 inhibitors (canagliflozin; 0.3–0.6 mg/kg, ertugliflozin; 0.05 mg/kg) in cases of refractory hyperinsulinemia and laminitis that did not respond to reduced dietary carbohydrate, exercise, and metformin. All cases responded with a marked reduction in insulin concentration and laminitis. There were no observed side effects except for the expected occasional weight loss and mild diuresis in some horses during the first 2 years horses were followed. However, an incidental finding of hyperlipemia in one horse led us to question the effect of SGLT2 inhibitors on triglycerides.

SGLT2 inhibitors in humans and experimental animals have not been linked to worsening of the lipid profile. In fact, improvement has been reported in fatty liver parameters as well as blood levels and liver enzymes (Euh *et al.*, 2021; Li *et al.*, 2021; Luo *et al.*, 2021; Zhao *et al.*, 2021; Chen *et al.*, 2022; Lee *et al.*, 2022). When details of the index case were reported, we notified veterinarians overseeing horses on SGLT2 inhibitors and suggested adding triglycerides to pretreatment screening and posttreatment monitoring.

Due to the promising effects of SGLT2 inhibitors on hyperinsulinemia, we anticipate increased use in horses with refractory hyperinsulinemia and laminitis. The purpose of this report is to inform the veterinary medical community about the possible complication of hypertriglyceridemia associated with drugs in this class as observed from a cross-section of horses in the home setting.

Case Details

This is a retrospective, observational study of the case records of animals owned by members of the international ECIR Group Inc. All animals were attended to by their private veterinarians. Animals were included in this analysis if they had a history of refractory hyperinsulinemia unresponsive to dietary carbohydrate restriction, were treated with SGLT2 inhibitors and had triglycerides determined at any test period after initiation of SGLT2 inhibitor.

Animals

There were 15 horses, 4 ponies, and 1 miniature mule; 8 mares and 12 geldings. Breeds of ponies were 1 Welsh, 1 Shetland, 1 grade, 1 New Forest pony. Breeds of horses were 2 Tennessee Walking horse, 1 Paint, 2 Quarter horse cross, 2 Arabian, 2 Arabian cross, 3 Missouri Fox Trotter, 1 Westphalian, 1 Icelandic, 1 Kentucky Mountain Horse. Ages were 6 to 31 years. There was 1 horse under 10 years, 11 animals were 11 to 19 years, and 5 were over 20 years of age. Fourteen of 20 animals had a history of PPID and were regularly monitored with ACTH testing, with pergolide dosage adjustments as needed. Nineteen of 20 had a history of laminitis in addition to refractory hyperinsulinemia. Eighteen of 20 were actively laminitic at the time they began SGLT2 inhibitor therapy. Follow-up time varied from 1 to 21 months, with a mean of 7 months.

Animals included in this report were maintained by their owners on a forage-based diet of controlled simple carbohydrates with the summed percentage of sugar (ethanol soluble carbohydrate; ESC) and starch at or below 10%. Maintenance intake was calculated as 2% of ideal body weight or 1.5% of current body weight when overweight, whichever was higher.

Attending veterinarians prescribed either ertugliflozin at 0.05 mg/kg daily (Merck & Co, Inc., Rahway, NJ) or canagliflozin at 0.3–0.6 mg/kg daily (Janssen Pharmaceuticals Inc., Titusville, NJ). Both drugs are approved for human use and were purchased from commercial pharmacies.

Laboratory testing

Laboratory testing was done by a variety of university and licensed commercial laboratories in the United States and abroad. All results were converted to SI units for reporting. A conversion factor of 6 was used for insulin originally reported in uIU/ml; 0.0556 for glucose reported in mg/dl; 0.0113 for triglycerides reported in mg/dl.

Statistical analysis

Descriptive statistics were generated for glucose, insulin, and triglycerides at baseline (pre-therapy) and each monitoring period. Triglyceride values did not conform to a normal distribution and were log-transformed. To test whether the reported increase in triglycerides was an effect of treatment we used a paired *t*-test to compare baseline (PRE) triglycerides to the first retest values (R1) in animals that had results at both time points. Non-transformed means were used to calculate the percent increase or decrease between the 3 test periods.

Cases

Index case (Case #11)

This was a 23-year-old Arabian gelding with a history of laminitis for 2 years. Metformin therapy was trialed but while initially helpful, over time it was less effective and could no longer control hyperinsulinemia. Glucose and insulin levels prior to starting canagliflozin (0.6 mg/kg) were 6.3 mmol/l and 1,190.2 pmol/l, respectively. Four weeks post therapy, both glucose and insulin levels decreased. (Glucose, 5.4 mmol/l; Insulin, 246.6 pmol/l.) Triglycerides were not tested at either of these time points.

During canagliflozin therapy, a high-fiber, mature, and unpalatable hay was introduced resulting in decreased consumption. Weight loss was noted approximately 6 weeks into therapy. Hoof comfort had been variable, but the horse had other pathologies including advanced pastern arthritis. Due to suspicion of PPID, pergolide was prescribed, although the diagnosis of PPID was not confirmed by laboratory testing. This led to a further decrease in food intake. At 10 weeks, glucose was 5.3 mmol/l, and insulin normal at 181.4 pmol/l. At the blood draw, the attending veterinarian noted an opaque appearance of the blood sample and subsequently tested triglycerides. The triglyceride concentration was 12.8 mmol/l. Two days later, the horse presented with possible colic symptoms.

The horse was hospitalized, pergolide was reduced from 1 mg to 0.5 mg/day and canagliflozin was discontinued. A physical examination revealed no evidence of colic. He was treated with intravenous amino acids, low molecular weight heparin, and glucose. Elevated lactate was noted initially but quickly resolved. His attitude remained bright and appetite was good throughout. Triglycerides decreased rapidly to 6.47 mmol/l over 3 days, then to 1.37 mmol/l at 5 days, and within normal reference values at 10 days.

After discharge, the horse became laminitic again and canagliflozin therapy was restarted using various very low dosages with no improvement. He was subsequently titrated to a dose of 0.47 mg/kg. When retested, glucose was 5.7 mmol/l, insulin 185.4 pmol/l, and triglycerides 6.42 mmol/l. His weight was stable on a diet liberalized to approximately two times maintenance digestible energy. At the last report, the horse remains sound, active, and alert.

Clinical notes, signalment, and laboratory results for the remaining 19 animals are in Table 1.

Seventeen animals were treated with canagliflozin and three (cases #1, 4, 9) were treated with ertugliflozin (0.05 mg/kg). Case #1 was administered a compounded paste and subsequently had rebound insulin to >1,200 pmol/l at 6 weeks yet remained sound. The owner discontinued the compounded paste, changing to the human pill form and on retest, insulin concentration was normal at 120 pmol/l. The horse remained sound throughout this period.

Despite a diet of ESC + starch less than or equal to 10%, 13 of the 20 horses had blood glucose levels >5.5 mmol/l, ranging from 5.7 to 11.7 mmol/l before starting treatment. Glucose samples were non-fasting, with animals on diets as described. When free choice intake was not maintained overnight or throughout the day, all glucose samples were taken at least 4 hours after feeding resumed. There were no documented episodes of hypoglycemia even in horses normoglycemic prior to treatment and no clinical signs suggestive of hypoglycemia.

Box and whisker plots for the pre-intervention baseline (PRE) and the first (R1) and second (R2) retest are shown in Figure (1A–C). After the initiation of SGLT2 inhibitors, mean glucose decreased by 18.8% from the pre-intervention baseline. At the second retest, glucose was essentially stable, with only a 1.5% decrease from the first retest. Insulin decreased by 48.5% between the baseline and first retest. There was a 6.9% decrease between the first and second retest. Triglycerides were low before initiating drug therapy. At the first retest, triglycerides increased by 256.1% over pretreatment

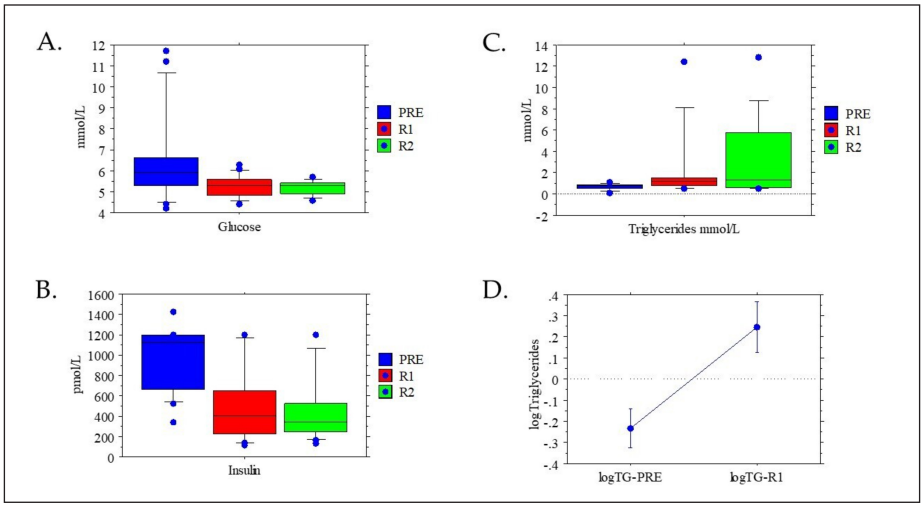

Fig. 1. Box and whisker plots. Raw data for pre-treatment baseline (PRE), the first (R1) and second (R2) retest following SGLT2 inhibitor intervention. (A): Glucose (mmol/l); PRE $n = 17$, R1 $n = 17$, R2 $n = 15$. (B): Insulin (pmol/l); PRE $n = 20$, R1 $n = 19$, R2 $n = 18$. (C): Triglycerides (TG; mmol/l); PRE $n = 10$, R1 $n = 12$, R2 $n = 12$. (D): Log transformed triglycerides at pre-treatment baseline (PRE) and the first retest (R1).

Table 1. Individual animal information.

Case # and signalment				
Time	**Glucose mmol/l**	**Insulin pmol/l**	**Triglycerides mmol/l**	**Owner/attending veterinarian report**
Case #1: 16 years old Welsh pony gelding, laminitic for 7 months, metformin no longer effective.				
Pre	4.4	780	0.4	Start 0.05 mg/kg ertugliflozin. Began free choice hay with added beet pulp.
4 weeks	Not done	Not done	0.8	Laminitis pain and polyphagia resolved.
8 weeks	5.3	>1,200	0.5	Still sound but changed from compounded to human pill form ertugliflozin due to elevated insulin values at 8 weeks.
Case #2: 17 year old Tennessee walking horse gelding, laminitic for 4 years, metformin no longer effective.				
Pre	5.7	>1,200	Not done	Started on 0.61 mg/kg canagliflozin. No diet changes.
1 week	5.6	271.1	Not done	No changes
5 weeks	5.6	167.4	Not done	More comfortable. Losing weight slowly. Completely sound by 20 weeks.
40 weeks	4.8	>1,200	0.8	Drug had been stored improperly in a very cold barn. After test results, started with new drug supply, properly stored.
7 weeks after new supply	5.1	151.1	Not done	7 weeks after starting new drug supply.
14 weeks	5.1	361.6	1.73	Horse has remained sound after the 5 months mark.
Case #3: 22 years old Mini Mule gelding, laminitic for 14 months, metformin no longer effective.				
Pre	Not done	>1,200	Not done	Started canagliflozin 0.24 mg/kg.
1 day	Not done	669	Not done	
7 days	Not done	338	Not done	
8 weeks	Not done	476	Not done	Laminitis resolved after 1 month. Does not need boots for first time in 3 years.
24 weeks	Not done	210.7	1.1	Still sound.
52 weeks	Not done	349.6	1.12	Mule had been spitting out his pill. Still sound.
72 weeks	Not done	410.1	0.88	Dietary indiscretion – getting access to grass. Still sound.
Case #4: 11 year old Paint mare, never laminitic, metformin no longer effective.				
Pre	5.2	564	0.53	Started 0.05 mg/kg ertugliflozin.
6 weeks	5.1	216	1.48	Crest softened within days.
16 weeks	5.1	360	0.52	
32 weeks	5.5	408	0.71	Mild weight loss noted at 28 weeks.
40 weeks	4.8	282	1.67	Ran out of acetyl-L-carnitine before this test (had been feeding it since the beginning).
Case #5: 18 year old Quarterhorse/Arabian cross mare, laminitic for years, metformin no longer effective.				
Pre	4.9	575	Not done	Started 0.28 mg/kg canagliflozin.
6 weeks	5.2	354.5	Not done	Much less tender, crest receding. Increased hay and beet pulp slightly because of 10 kg weight loss.
16 weeks	4.9	316.3	Not done	Sound. Dose increased to 0.44 mg/kg. Hay increased at 24 months due to weight loss
40 weeks	4.7	148.9	0.81	Sound. Beet pulp increased.
60 weeks	4.7	352.7	0.66	Still doing well.

Continued

Case # and signalment				
Time	**Glucose mmol/l**	**Insulin pmol/l**	**Triglycerides mmol/l**	**Owner/attending veterinarian report**
Case #6: 18 year old Tennessee walking horse mare, not laminitic when started, metformin no longer effective.				
Pre	4.2	680.9	0.82	Start 0.57 mg/kg canagliflozin.
4 weeks	4.4	149.3	1.13	Increased hay to free choice.
8 weeks	4.6	136.6	0.89	Despite low insulin, horse became laminitic early spring with a "mare colic". Resolved.
28 weeks	5.2	279.4	0.95	Stable.
36 weeks	4.5	177.4	1.03	Developed lameness despite controlled insulin. Found to have polycystic ovaries.
Case #7: 18 year old Arabian gelding. History of laminitis off and on when in work. A decrease in work level results in chronic laminitis for 3 months before starting drug.				
Pre	6.1	1,001.4	Not done	Start 0.74 mg/kg canagliflozin.
3 weeks	5.0	145	Not done	Decrease to 0.37 mg/kg canagliflozin.
7 weeks (4 weeks after dose reduced)	5.7	476.	Not done	Dose increased to 0.57 mg/kg. Horse eating 1.7× maintenance digestible energy and still losing weight.
16 weeks (12 weeks after dose increase)	5.8	200	1.08	Lameness greatly improved. Weight stable.
Case #8: 26 year old Shetland pony gelding, laminitic for a year, metformin no longer effective.				
Pre	5.7	>1,200	0.11	Start 0.67 mg/kg canagliflozin.
4 weeks	4.9	>1,200	0.51	Lameness improving.
20 weeks	5.4	654	0.61	Sound.
36 weeks	4.7	362.4	1.33	Sound. Put on free choice hay with the other horses at this time.
Case #9: 18 year old New Forest Pony gelding, laminitic for 4 months, metformin no longer effective.				
Pre	Not done	1,056	Not done	Start 0.05 mg/kg ertugliflozin.
2 weeks	4.6	116.4	0.8	Sound. Dose reduced to 0.038 mg/kg
4 weeks	4.8	231	1.2	Sound.
Case #10: 31 year old grade pony gelding, laminitic for 15 months, metformin no longer effective.				
Pre	Not done	343	0.77	Start 0.41 mg/kg canagliflozin.
1.5 weeks	Not done	1,107.8	1.46	Sound but dose increased to 0.82 mg/kg due to elevated insulin.
4.5 weeks (21 days after dosage increase)	4.8	231	1.2	Sound.
Case#11: 23 year old Arabian gelding, repeated laminitis episodes for 2 years (See index case).				
Case #12: 21 year old Missouri Fox Trotter mare, intermittent laminitis for 3 years then refractory for 1 year, metformin no longer effective.				
Pre	5.9	>1,200	1.05	Start 0.35 mg/kg canagliflozin.
5.5 weeks	5.6	551.9	1.9	Laminitis improved but owner ran out of drug and lameness returned.
Case #13: 17 year old Arabian/Hanovarian mare, laminitic for 2 years, metformin no longer effective.				
Pre	8.6	>1,200	0.72	Start 0.66 mg/kg canagliflozin. On free choice hay.
10 days	5.3	282	Not done	Trotting freely sound after 3 doses. Crest reduced, slight weight loss. Animated.

Continued

Case # and signalment				
Time	**Glucose mmol/l**	**Insulin pmol/l**	**Triglycerides mmol/l**	**Owner/attending veterinarian report**
16 weeks	5.6	324	7.05	Still sound. Supplemental feeding instituted and dose reduced to 0.34 mg/kg.
22 weeks	5.8	312	1.49	Stable.
Case #14: 13 year old Quarter Horse cross mare, intermittent laminitis for 6 years, metformin no longer effective.				
Pre	7.6	852	Not done	Start 0.05 mg/kg ertugliflozin.
4 weeks	6.3	408	0.75	Laminitis improved, more active. No diet changes.
16 weeks	4.7	252	0.54	Steadily improved to sound. Out with another horse and active.
Case #15: 18 year old Westphalian gelding repeated episodes of fall laminitis, no metformin trial.				
Pre	6.1	647	Not done	Start 0.27 mg/kg canagliflozin.
4 weeks	6.1	465	Not done	Dosage increased to 0.54 mg/kg.
8 weeks (2 weeks after dosage increase)	5.5	354	Not done	First year with no fall laminitis.
16 weeks	4.5	119	Not done	Weight loss noted. No laminitis. Hay increased to free choice. Dose reduced to 0.27 mg/kg canagliflozin.
36 weeks	5.2	359	Not done	Stable. Sound. Good weight. Dose increase to 0.54 mg/kg.
44 weeks	5	201	Not done	Dose reduced to 0.41 mg/kg
68 weeks	5.4	195	Not done	Stable. Sound.
80 weeks	5.7	525	1.0	No changes.
84 weeks	5.5	301	1.9	No changes.
Case #16: 20 year old Icelandic mare, laminitic for 6 months, no metformin trial.				
Pre	5.3	>1,200	0.54	Start 0.46 mg/kg canagliflozin.
3 weeks	5.6	>1,200	1.23	Improving rapidly in soundness. Crest decreasing. Beet pulp increased.
6 weeks	5.3	1,200	1.39	Sound.
Case #17: 16 year old Missouri Fox Trotter gelding, laminitic for 1 month, no metformin trial.				
Pre	5.3	>1,200	0.82	Start 0.64 mg/kg canagliflozin.
6 weeks	4.6	657.6	12.38	Pain relieved after 5 days. Stopped drug for 4 days after these results because of high triglycerides
4 days off treatment	9.8	>1,200	5.49	Rapidly became laminitic again. Resumed drug at 0.32 mg/kg then increased to 0.64 mg/kg after 4 days
3 weeks	5.2	1,200	4.16	Pain resolved.
5 weeks	5.9	>1,200	5.73	Sound, alert, active. Beet pulp added to diet.
9 weeks	5.8	>1,200	5.47	No change, doing well.
3 weeks after dosage decrease to 0.32 mg/kg	5.3	1,074	4.16	Condition stable, still sound.
Case #18: 25 year old Kentucky Mountain Horse mare, episodes of laminitis for 10 years, metformin no longer effective.				
Pre	6.2	526	Not done	Start 0.63 mg/kg canagliflozin.
3 weeks—1 week after dosage increase to 0.82 mg/kg	4.6	139.2	Not done	Greatly improved pain. Fat deposits diminishing.

Continued

Case # and signalment				
Time	**Glucose mmol/l**	**Insulin pmol/l**	**Triglycerides mmol/l**	**Owner/attending veterinarian report**
11 weeks	4.9	330	6.38	Dose decreased to 0.41 mg/kg due to triglycerides. Sound, doing well.
3 weeks after first dosage change	5.3	193.2	9.19	Still sound, doing well. Dose decreased to 0.21 mg/kg and hay liberalized to above maintenance digestible energy.
3 weeks after second dosage change	Not done	174	1.57	Continues to do well clinically.
Case #19: 13 year old Missouri Fox Trotter gelding, severely laminitic and recumbent for 1 week, no metformin trial.				
Pre	11.7	>1,200	Not done	Start on 0.6 mg/kg canagliflozin.
2 weeks	5.4	609.8	0.52	On free choice hay. Some improvement in pain and attitude.
15 weeks	5.4	752.4	Not done.	Laminitis resolved by 3 weeks, active, bright.
21 weeks	5.9	524.2	0.75	Still sound, alert, active.
30 weeks	6.2	825	0.72	Horse had access to grass clippings, became laminitic again, resolved quickly with diet. Started losing weight
36 weeks	5.8	459.6	0.72	Sound. Continuing to improve in weight and fat deposits.
56 weeks	5.0	196.2	0.66	Sound. Being ridden.
Case #20: 20 year old Arabian cross gelding, two prior episodes of laminitis but sound when started on the drug, metformin no longer effective.				
Pre	11.2	>1,200	0.94	Start 0.52 mg/kg canagliflozin.
4 weeks	5.9	628	6.27	Diet adjusted to include more beet pulp and free choice hay. Sound. More alert.
12 weeks	Not done	528.3	5.06	Sound, alert, active.

baseline. At the second retest, triglycerides decreased by 38% when compared to the first retest period.

Log-transformed triglycerides were compared at baseline (PRE) and the first retest time point (R1) in 10 animals with complete data. Triglycerides were higher at R1 (M = 0.247, SD = 0.381) than PRE (M = −0.233, SD = 0.285), a statistically significant difference $t(9) = -5.03$, $p = 0.0007$ (Fig. 1D).

Consistent with previous reports, the initiation of SGLT2 inhibitors resulted in a reduction in glucose and insulin in this cohort of privately owned animals. However, expanded monitoring revealed there was a significant increase in triglycerides at the first retest. Only three of our animals were on ertugliflozin but a similar pattern of asymptomatic increased triglycerides has been observed elsewhere in horses on ertugliflozin (Sundra *et al.*, 2022).

A review of the case notes in Table 1 demonstrates the importance of controlling the diet and managing PPID with adequate medication during treatment with SGLT2 inhibitors. In cases where diet and PPID were not fully controlled, the result was an increase in insulin concentration. These cases also highlight the dose effect of canagliflozin on insulin and triglycerides. As doses were decreased due to high triglycerides, insulin increased. We found doses below 0.3 mg/kg to be ineffective for insulin control. Canagliflozin administered at 0.6 mg/kg appears to have a more rapid effect, but as dosage increases, so do triglycerides.

Nineteen of 20 treated horses were reported sound, despite variable individual insulin responses to the drug. The time to resolution of laminitis lameness ranged from 24 hours to several weeks, likely due to individual differences in the degree of rotation and/or sinking as well as the adequacy of hoof care.

One horse (Case #6) developed laminitis despite good insulin control. This was a mare observed to have an increased "cresty neck" and fluctuations of pain with her estrus cycles. She was subsequently found to have anovulatory follicles. After prolonged estrus signs resolved she became sound and is scheduled for ovariectomy. Hyperinsulinemia effects on ovarian function or vice versa, have been well described in women with the polycystic ovarian syndrome (Zhao *et al.*, 2023). Potential similar interactions have been reported in mares (Sessions *et al.*, 2004).

Case #15 was stable and sound for 21 months on canagliflozin. The attending veterinarian became concerned about hypertriglyceridemia despite it being asymptomatic. When every third-day dosing

was attempted, laminitis occurred within a month but resolved quickly upon resuming the drug daily.

Insulin responses were highly variable, despite owner/veterinarian reports of laminitis relief. Case #16 had multiple extremely high insulin results (>1,200 pmol/l, the upper limit of the assay) yet was reported to experience relief of laminitis. Case #17 also had multiple insulin readings of 1,200 pmol/l or higher but became and remained sound on canagliflozin. The remaining cases had insulin concentrations vacillating between normal (less than 200 pmol/l) and elevated but, again, remained sound.

Discussion

Members of ECIR were using SGLT2 inhibitors for 2 years before the first case of hypertriglyceridemia was incidentally noted. Hypertriglyceridemia was not an expected side effect from the review of the response of humans to this class of drugs (Euh *et al.*, 2021; Li *et al.*, 2021; Luo *et al.*, 2021; Zhao *et al.*, 2021; Chen *et al.*, 2022; Lee *et al.*, 2022). However, the human diet even with diabetes is considerably higher in simple sugar and starch (35+%) (Gray and Threlkeld, 2019) than the equine on restricted metabolic syndrome diets (less than 10%). The combination of a low carbohydrate diet and SGLT2 inhibitors in humans was reported to induce diabetic ketoacidosis (Paul and Jonklass, 2021) and there is also a published case of hypertriglyceridemia in a human on SGLT2 inhibitor therapy who was on a low carbohydrate diet (Senoo *et al.*, 2021).

Like all species, horses mobilize triglycerides when the demand for serum glucose exceeds dietary levels and gluconeogenic capacity (Brinkmann *et al.*, 2018; Grabner *et al.*, 2021). For example, hypertriglyceridemia is seen after fasting/starvation and extreme exercise (Freestone *et al.*, 1991; Van Weyenberg *et al.*, 2008; Li *et al.*, 2012; Szalat *et al.*, 2018).

Humans with metabolic syndrome are well-documented to have hypertriglyceridemia which is associated with a high risk of nonalcoholic fatty liver disease and is a cardiovascular risk factor (Bajaj and Prajapati, 2022; Podlipskyte *et al.*, 2022). Hypertriglyceridemia is believed to be related to insulin resistance reducing the inhibitory effect of insulin on triglyceride release. Obese insulin-resistant horses have been reported to be dyslipidemic (Frank *et al.*, 2006). However, triglycerides are not necessarily different between normal and hyperinsulinemic horses (Frank *et al.*, 2006). In ponies, a high cresty/fatty neck score is positively associated with triglycerides with no differences between normal weight and obese ponies (Ragno *et al.*, 2021). Triglycerides were not of value in predicting endocrinopathic laminitis (Fitzgerald *et al.*, 2019b). The opinion regarding triglycerides in the 2019 combined ECEIM and ACVIM consensus statement on EMS reads, "Given the relatively mild and inconsistent changes, however, this [triglycerides] has not proved to be a reliable diagnostic test" (Durham *et al.*, 2019).

For these reasons, combined with the lack of predictive value of pre-treatment triglycerides on post-treatment values, and the lack of a relationship between hypertriglyceridemia and being a high-risk breed for hyperlipemia (miniature horse, pony, donkey), we believe the elevated triglyceride levels during SGLT2 therapy are physiologic, secondary to glucose loss in the urine.

Fasting healthy horses for 120 hours resulted in peak mean triglycerides of 2.23 mmol/l, while triglycerides in inappetent, ill horses have been reported as high as 33.9 mmol/l (Naylor *et al.*, 1980). Inappetence and severe illness can also result in severe hypertriglyceridemia in horses with systemic inflammatory response syndrome (Dunkel and McKenzie, 2003). Hypertriglyceridemia is a recognized component of human metabolic syndrome but, as noted above, is inconsistent in equines. Nevertheless, severe hypertriglyceridemia has been documented in horses with hyperinsulinemia on occasion, but without the clinical picture associated with life-threatening hyperlipemia syndrome in ponies, miniature horses, and donkeys. Hypertriglyceridemia can persist for years with no adverse effects other than some weight loss (Dunkel *et al.*, 2014).

Hypertriglyceridemia in this cohort of SGLT2-treated animals has been asymptomatic even when persisting for a prolonged period, as has been previously reported (Dunkel *et al.*, 2014). Adjustment of the diet from restricted maintenance level hay to free choice with the addition of beet pulp has been of some value in controlling triglycerides. Beet pulp is fermented primarily to acetate which can substitute for glucose in the tricarboxylic acid cycle without triggering an insulin response.

It is interesting to note that the use of SGLT2 inhibitors was reported in all cases to alleviate laminitis (with an exception of the mare with the ovarian complication) despite individual insulin concentrations that would be expected to trigger laminitis. As expected, SGLT2 inhibitors were universally effective in restoring euglycemia.

Because this was a retrospective case study report, there are several limitations. Weights were estimated by weight tape and visual assessment. Diets were all below 10% combined ESC + starch but not otherwise standardized. Complications arose when PPID was not adequately controlled with medication, when liberalization of the simple carbohydrates in the diet was attempted, or dosages were decreased below suggested effective amounts. The drugs used, dose, testing intervals, and lab tests performed at each interval were not identical between the cases. Nonetheless, these results should serve to highlight the unexpected side effect of hypertriglyceridemia when using this class of drugs and should be of value to practitioners in the field. Further, these results provide preliminary evidence necessary for the design of carefully controlled trials of the -flozin class of drugs in equines with refractory hyperinsulinemia

While recognizing the limitations of uncontrolled case reports, we believe the significant increase in triglycerides and reported laminitis relief deserves further investigation. Carefully designed controlled trials will help to determine ideal dosages and optimal diet, develop strategies to control hypertriglyceridemia, and explore potential drug mechanisms behind the relief of laminitis.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: E.M.K.; methodology: E.M.K. and K.M.G.; validation: E.M.K. and K.M.G.; formal analysis: E.M.K. and K.M.G.; investigation: E.M.K.; resources: E.M.K. and K.M.G.; data curation: E.M.K. and K.M.G.; statistical analysis: K.M.G., writing—original draft preparation: E.M.K; writing—review and editing: E.M.K. and K.M.G.; visualization: E.M.K. and K.M.G. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

The authors declare the study exempt as veterinary clinical care of a privately owned animal is not a research activity and thereby does not require institutional oversight.

Informed consent statement

All horse owners are members of the ECIR group and gave permission to use their case histories in this report.

Data availability statement

The index case and all case reports are available in the manuscript.

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