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Title: Effect of varying the dose of corn syrup on the insulin and glucose response to the oral sugar test

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Ethical Animal Research

The study was conducted under Home Office Project Licence with ethical approval from Royal Veterinary College Animal Welfare Ethical Review Board.

Authorship:

N. A. Jocelyn, N.J. Menzies-Gow and P.A. Harris contributed to the study design, preparation and final approval of the manuscript. N.A. Jocelyn and N.J. Menzies-Gow contributed to the study execution, data analysis and interpretation.

Competing Interests

Dr Harris is employed by the study funder.

Sources of Funding

WALTHAM Centre for Pet Nutrition.

Acknowledgements

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Summary

Reasons for performing study: The oral sugar test (OST) is used to identify equine insulin dysregulation (ID); however only a dose of 0.15 ml/kg bwt corn syrup (Karo Light Corn Syrup)[a] has been evaluated.

Objectives: To determine the effect of varying the dose of corn syrup syrup on insulin and glucose response to the OST and the test's ability to distinguish between ponies with (PL) and without (NL) a history of laminitis.

Study Design: Randomised crossover experiment.

Methods: After an overnight fast, in a 3-way randomised crossover study with a 7-day washout, 0.15 ml/kg bwt, 0.3 ml/kg bwt or 0.45 ml/kg bwt corn syrup (Karo Light Corn Syrup)[a] was administered orally to eight ponies (5PL, 3NL) and blood obtained between 0 and 120 min. Serum [insulin] and [glucose] were measured using previously validated radioimmunoassay and colorimetric assays respectively. The repeatability of and the effect of continued pasture access on the dose that best distinguished PL and NL ponies was then assessed. The effect of dose, laminitis history and fasting on serum [insulin] and [glucose] responses were assessed using mixed effects models.

Results: The serum [insulin] following 0.15 ml/kg bwt were not significantly different from 0.3 ml/kg bwt at any time point; whilst serum [insulin] following 0.45 ml/kg bwt significantly ($p<0.01$) differed from 0.15 ml/kg bwt and 0.3 ml/kg bwt at all time points apart from 0 min. The serum [insulin] concentration significantly ($p<0.01$) differed between NL (mean 86 [95% CI 59, 113] μiu/ml) and PL (146 [95% CI 124, 167] μiu/ml) only following

0.45 ml/kg bwt at 60 min. Repeatability of serum [insulin] at 60 min following 0.45 ml/kg bwt dose under fasted conditions was 0.51. Using AUC insulin improved repeatability to 0.83. There was no significant difference between the fasted and at pasture results.

Main Limitations: The OST was performed in small numbers of ponies on limited occasions.

Conclusions: A dose of 0.45 ml/kg bwt corn syrup may be preferable to differentiate PL and NL ponies.

Introduction

Insulin dysregulation (ID) in the horse encompasses fasting hyperinsulinaemia, an excessive insulin response to oral carbohydrates and tissue insulin resistance [1]. The importance of ID and its association with an increased risk of laminitis [2] is clinically relevant to practitioners and a practical reliable and repeatable test for ID diagnosis would therefore be advantageous [3]. The oral sugar test (OST) has been advocated as simple field based dynamic test to identify equids with an excessive insulin response following ingestion of carbohydrate [4] and tissue insulin resistance [5]. Ease of administration of the sugar in syrup form and the possibility of obtaining a single blood sample post-administration make the OST an attractive option for use by practitioners.

Initial work suggested a good positive correlation between the OST and the intravenous glucose tolerance test [4] and the OST and the oral glucose test [6]. However recent publications found the OST to have poor sensitivity [7] and no significant relationship when directly compared to other ID testing [8]. Both these studies [7,8] however, compare intravenous tests, which are measures of tissue insulin resistance to the OST. The OST is a test that explores ID characterised by an excessive response to oral carbohydrate and thus evokes the enteroinsular axis [9]. As such, a direct association is not expected. Repeatability has varied from acceptable [10] to poor [11].

A single dose of 0.15ml/kg bwt corn syrup (Karo Light Corn Syrup)[a] for the OST has been used [11] [12], but limited differing doses have been investigated [13]. Higher doses, which provide amounts of sugar more similar to the oral glucose test, may afford improved diagnostic abilities. Current advice is to perform OST after fasting [3], however a study comparing horses at pasture and after fasting found no significant difference in OST outcomes [8]. Another study [11] with ponies at pasture found significant differences between fasting and fed state for area under curve insulin and insulin concentration at 60,75 and 90 minutes, however dichotomous interpretation for ID was similar using study identified cut-off values.

The aim of this study was to evaluate the effect of varying the dose of corn syrup on 1) the insulin and glucose response and 2) the ability of the OST to distinguish between ponies with a history of laminitis (PL) and non-laminitic ponies (NL). Once an optimal dose was identified, further aims of the study were to further explore this dose with respect to repeatability, season and the effect of fasting.

Materials and Methods

Animals

Eight British native pony mares from a research herd kept at pasture were used in the study. All were clinically healthy, aged between 12 and 23 years and weighing between 245-441kg (Supplementary information 1); 5 had a known clinical history of laminitis but had no active signs of laminitis in the 3 months prior to and during the entire study period; 3 had no history of laminitis. All of the animals had been part of the herd for at least 10 years. None had clinical signs of pituitary pars intermedia dysfunction and basal ACTH concentrations were within the seasonally adjusted reference range.

Study design

Dose Study

The study was undertaken in December 2015. All eight ponies were brought into a bare dirt paddock the night before each study day. Haylage was provided to last until midnight and adlib water was provided throughout. The following morning, a 14 g jugular catheter (Angiocath)[b] was placed under local anaesthesia (Intra-Epicaine)[c] and a baseline blood sample obtained (T_0). In a randomised crossover design, animals were given either 0.15ml/kg bwt, 0.3ml/kg bwt or 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)[a] by oral syringe. Further blood samples were collected at 30, 60, 75, 90 and 120 ($T_{30–120}$) min after oral dosing. A 7-day washout period between doses was undertaken during which times the animals were kept at pasture.

Analysis of the corn syrup (Karo Light Corn Syrup)[a] using the following method (Longland personal communication) found it to contain 356.3 mg/ml of maltose and glucose combined. (Supplementary information 2) The doses used therefore equated to 53.4mg/106.9mg/160.3mg/kg bwt of maltose and glucose combined respectively. Samples were diluted on a weight/volume basis (100mg/ml). Then 50 μL of sample were added to 950 μL of a buffer comprising 5 mMH_2SO_4 with a 5 mM crotonic acid internal standard. Samples were analyzed using via high-performance liquid chromatography[d]. Injection volume was 25 μL. Sugars were separated on a Rezex ROA-Organic acid column[e] and a mobile phase of 5 μM H_2SO_4 at 0.6 ml min^{-1}. Sugars were detected with a refractive index detector and identified by comparison with an internal library of standard compounds.

Blood for serum insulin concentration measurement was collected into plain tubes (vacutainer)[a] and allowed to clot at 37°C for at least 20 min. Blood for glucose concentration measurement was collected into fluoride oxalate tubes (vacutainer)[a]. All samples were centrifuged (3000 x ***g***) for 10 min at 4°C and the serum or plasma stored at -80°C before analysis

Repeatability Study

The study was undertaken in June 2016. The OST was repeated with all eight ponies receiving 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)[c] on 2 occasions with a 7-day washout period between.

Fasting vs Fed Study

Seven days after the repeatability study was completed, the OST was repeated for a third time with all ponies receiving 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)[c], however the ponies were not fasted and instead remained at pasture prior to and during the study.

Sample analysis

Serum insulin concentrations were measured using a radioimmunoassay (Insulin CT)[f] and serum glucose using a colorimetric assay (Glucose Colormetric Assay Kit)[g]. All samples were measured in duplicate and both assays had been previously validated for use in ponies [11].

Data analysis

All analysis was performed using statistical (IBM SPSS Statistics 22)[h] and graphic (Graphpad Prism)[i] software. The area under the curve (AUC) was calculated for the full insulin response (T0-120; AUC_i), insulin response at T0,60 and 90 ($AUC_{insulin\ modified}$; AUC_{iM}) and full glucose response (AUC_g) for each test using the trapezoidal sum method with the x axis (y = 0) as the baseline insulin or glucose concentration. Linear mixed effects models were generated to investigate the differences between NL and PL. Sampling time (for single time points), dose, NL/PL and their interactions were initially included as fixed variables and removed according to statistical significance. Pony was included as a random variable. Insulin concentration/ AUC_i/AUC_{iM}, maximal insulin concentration ($Cmax_i$), time to maximal insulin concentration ($Tmax_{iM}$), maximal insulin concentration T0,60 and 90, glucose concentration/ AUC_g, maximal glucose concentration ($Cmax_g$) and time to maximal glucose concentration, ($Tmax_g$) were the outcome variables examined and an auto- regressive covariance structure (AR1) was used. Estimated marginal means were calculated from the final model and pairwise post-hoc comparisons were performed (without adjustment of confidence intervals for multiple comparisons/least significant difference). The normality of the distribution of the residuals was assessed by histogram to ensure normality. Linear mixed effects model was repeated using the 0.45ml/kg bwt dose and differences between NL and PL investigated. Sampling time (for single time points), NL/PL, pasture, season and their interactions were initially included as fixed variables and removed according to statistical significance. Repeatability was assessed using repeated measures [14], briefly, using estimates of covariance parameters the pony variance was divided by the sum of residual and pony variance combined. Statistical significance was set at $P \leq 0.05$.

Results

No adverse effects were seen in any of the ponies throughout the two study periods. All ponies tolerated the corn syrup administration very well and received the full dose on all occasions.

Insulin

Dose response

The serum insulin concentration was significantly different for both 0.15 ml/kg bwt and 0.3 ml/kg bwt dose compared to the 0.45 ml/kg bwt at all time points apart from T_0 for all ponies combined (P<0.001); whilst the 0.15 ml/kg bwt and 0.3 ml/kg bwt doses were not significantly different at any time point (Figure 1). The $Cmax_i$ was significantly (P<0.001) greater following 0.45ml/kg bwt dose (mean 174 [95% CI 141, 206] µiu/ml) compared to either 0.15ml/kg bwt (72 [95% CI 39, 104] µiu/ml) or 0.3ml/kg bwt dose (87 [95% CI 54, 119] µiu/ml).

When the serum insulin concentrations were compared between the two groups of ponies (NL and PL) at the 6 time points, the only significant (P=0.04) relationship between group and dose was at 60 minutes for the 0.45ml/kg bwt dose (NL mean 86 [95% CI 59, 113] vs PL 146 [95% CI 124, 167] µiu/ml; Figure 2). The 0.45ml/kg bwt dose with an insulin concentration cut off value of ≥ 110 µiu/ml at 60 min allowed for correct identification of all 5 PL and 3 NL ponies for the current data.

When the results from the two groups of ponies (NL and PL) were compared, there was a significant interaction (P=0.05) between dose and group for AUC_i. There was a significant (p=0.01) difference between NL and PL for AUC_i following 0.45ml/kg bwt, but not following 0.15ml/kg bwt or 0.3 ml/kg bwt (Table 1). The 0.45ml/kg bwt dose with an AUC_i cut off value of ≥ 10,000 µiu/ml/min allowed for correct discrimination of all 5 PL and 3 NL ponies. There was no significant interaction between dose and group for $Cmax_i$ and $Tmax_i$ (Table 1).

Repeatability

When comparing the 0.45ml/kg bwt dose repeated under fasting conditions in the same month (June), the repeatability for AUC_i was 0.83; whereas the repeatability of the serum insulin concentration at the single significant time point of 60 minutes was 0.5. $Tmax_i$ repeatability was low at 0.19 but $Cmax_i$ was 0.64.

Season

When comparing the fasted 0.45ml/kg bwt dose between December (Winter, northern hemisphere) and June (Summer, northern hemisphere), there was a significant interaction between season and group (NL and PL) when comparing both AUC_i (P=0.04) and insulin concentration at 60 minutes (P=0.03) but no significant interaction for $Cmax_i$ and $Tmax_i$. There was a significant difference (P=0.03) between the AUC_i in winter and the summer in PL but not NL (Table 1). Similarly, the serum insulin concentration at 60 minutes was significantly (P=0.01) lower in winter (146, [95% CI 108, 184], μiu/ml) compared to summer (204, [95% CI 172, 236], μiu/ml) in PL but not NL (winter 86 [95% CI 37, 136] μiu/ml and summer 71 [95% CI 30, 112] μiu/ml). There was no significant difference for $Cmax_i$ (P=0.53) or $Tmax_i$ (P =0.9) between winter and summer. (Table 1)

Fed vs Fasting

When comparing the insulin response of all 8 ponies combined when fasted and at pasture during the summer following 0.45ml/kg bwt dose, there was no significant interaction between group (NL and PL) and feeding and no significant differences between fasted and at pasture for the outcomes variables (Table 1).

Modified $AUC_{insulin}$

Further analysis of the AUC_i was explored to attempt to reduce the frequency of the blood sampling requirements. Using data from only 3 time points, namely T0, 60 and 90, a modified insulin AUC (AUC_{iM}) was calculated. There was a significant ($p<0.001$) difference between AUC_{iM} for all the 8 ponies combined following the 0.45ml/kg bwt dose (8526, [95% CI 7060, 9991] µiu/ml/min) and both 0.15ml/kg bwt (4249, [95% CI 2784, 5715] µiu/ml/min) and 0.3ml/kg dose (4481, [95% CI 3016, 5947] µiu/ml/min). However, there was no significant difference between the 0.15ml/kg bwt dose and 0.3ml/kg bwt dose. This relationship also held true for $Cmax_{iM}$ (data not shown).

A significant interaction was found between group (NL and PL) and dose for AUC_{iM} ($P=0.04$) and $Cmax_{iM}$ ($P=0.05$) but not $Tmax_{iM}$ ($P=0.96$). There was a significant difference for AUC_{iM} between NL and PL following 0.45ml/kg bwt ($p=0.001$) but not following 0.15ml/kg bwt or 0.3 ml/kg bwt dose (Table 1). A cut off value of ≥7500 µiu/ml/min AUC_{iM} distinguished between NL and PL ponies in the current data. The repeatability of the modified AUC_i was 0.63.

Glucose

Dose

When the data from all 8 ponies was combined, the AUC_g was significantly greater following the 0.45ml/kg bwt ($P= 0.049$) and 0.3ml/kg bwt ($P=0.005$) doses compared to the 0.15ml/kg bwt dose. $Cmax_g$ was significantly greater following the 0.3ml/kg bwt ($P=0.001$) dose compared to the 0.15ml/kg bwt dose and the 0.45ml/kg bwt ($P=0.04$). Tmax was significantly ($P=0.05$) later following 0.45ml/kg bwt dose compared to 0.15ml/kg bwt dose.(Table 2). There was no significant interaction between dose and group (NL and PL) for AUC_g, Cmax or Tmax (data not shown).

Group, season and pasture

When the data from all 8 ponies was combined, in summer AUC_g (P=0.04) and $Cmax_g$ (P=0.02) were significantly greater whilst Tmax was significantly (p=0.004) shorter compared to winter (Table 2). AUC_g (P= 0.02) and $Cmax_g$ (P=0.01) were significantly greater when the ponies were fasted compared to when they remained at pasture.

Discussion

In this small study population, only a dose of 0.45ml/kg bwt of corn syrup allowed NL and PL ponies to be reliably distinguished compared to lower doses. The lower 0.15ml/kg bwt dose was unable to distinguish between the two populations which contrasts to two previous studies [4,13], but is consistent with a third [7]. Only one single time point in our study, a blood sample at 60-minutes post corn syrup administration allowed for the test to correctly assign all the individual ponies to their respective groups (NL and PL); time points either side of this failed to provide certainty. This is in agreement with previously published data, in which a blood sample obtained 60 minutes after corn syrup administration provided the strongest correlation with the result obtained using the intravenous glucose tolerance test [4].

The poor repeatability of the serum insulin concentration at the 60-minute sampling point is concerning. Individual animal variability has been similarly established in other studies including healthy horses [15]. A previous study found within subject agreement for a single sample to be moderate to fair, with the same animal having varying response at the same time point [11]. A study using larger numbers of animals (n=53) found the agreement to be good at 60 and 75 minutes [10]. AUC_i was more repeatable in the present study than a single time point. Thus, taking multiple samples and calculating area under the curve may help counteract individual variation and provide more repeatable results. The modified AUC_{iM} reduced the number of sampling points to 3 and required only 90 minutes to complete. However, the repeatability was reduced to 0.63, which is better than that of the single time point of 0.5, but less repeatable than full AUC_i of 0.83.

The significant effect of season on the insulin response in only the PL ponies undergoing OST is novel. However, other metabolic hormones have been observed to vary with season [16]. Bailey et al [17] found that basal serum insulin concentrations were increased in summer but not winter in a group of PL ponies compared to a group of NL ponies. There was no effect of season, on serum insulin concentration at T75 following the 0.15ml/kg bwt dose during two seasons in horses considered insulin sensitive [8]. Contrastingly, Borer et al[18] found an increased insulin response in autumn (October –November) only in PL ponies undergoing an oral glucose test compared to Spring (May-June). An exaggerated insulin response to the greater pasture non-structural carbohydrates during growing season in those ponies who may be insulin dysregulated would be consistent with the suggested pathophysiology of endocrinopathic laminitis [9]. This includes alterations such an exaggerated intestinal incretin response to the ingested carbohydrate [9],lower hepatic insulin clearance [19], worse peripheral tissue insulin resistance [20] or altered insulin-like growth factor signalling in lamellar tissue [21,22]. Further repeated testing over a 12-month period would provide better grounding for an understanding of the seasonal changes and the relationship with pasture alterations.

This study provides further evidence that allowing ponies to remain at pasture does not significantly alter the diagnostic abilities of the OST compared to fasted animals [8]. A previous study using the lower dose 0.15ml/kg bwt found a significant effect, but that the results still allowed for a comparable diagnostic outcome with correct identification of ID vs insulin sensitive animals. [11].

There was no relationship between previous laminitis and glucose response in these PL and NL ponies. This is in contrast to previous studies [4] which found the glucose concentration to be higher at all time points and AUC_g greater in a group considered to have equine metabolic syndrome (EMS) compared to controls. The EMS group in this prior study [4] all had a history of forelimb lameness, consistent with laminitis and were classified as EMS on body condition score, adiposity and intravenous tests of insulin resistance. A further study [13] using a modified OST with a dose of 0.2ml/kg bwt also found glucose at 120-180 minutes to be significantly different between EMS and healthy animals. Though testing was undertaken up to 120 minutes in this current study, no further blood samples were taken beyond that point, therefore it is not possible to state whether a difference would have been seen at 180 mins. However, a more recently published study [10] with larger numbers, found no significant difference in glucose response between insulin dysregulated and normal animals at time 0-75 minutes on 2 occasions. It is surprising that the dose relationship in this study was not incremental in that 0.45ml/kgBW dose did not lead to a significantly higher glucose response than the 0.3ml/kgBW dose. However, it should be acknowledged that using 0.3ml/kgBW, 2 ponies, both PL, had very large, over double, glucose responses compared to other PL. So the results from these two individual animals are potentially responsible for absence of a dose relationship. The results may also reflect the variable bioavailability of oral glucose seen in other studies [9].

This study used the radioimmunoassay (RIA) to measure insulin concentrations, whereas in the UK a chemiluminescence immunoassay (CL) is widely used by commercial laboratories. Previous studies have reported that the two techniques are inequivalent [23]. When two RIAs were compared to the CL, all differed significantly with values from the CL being significantly lower than those from the two RIAs [20]. When the CL was compared with the now discontinued gold standard RIA[j] , there was a strong positive correlation between results but with fixed and proportional bias[24]. Both of these studies [20][24] found the greatest relative differences to be observed at lower concentrations. Thus, the cut off values suggested in this paper may not be applicable for values obtained with different assays and different populations of animals. Previous studies [6]have found differing insulin responses in ponies verses horses and between breeds [25].

No adverse effects were seen in any of the 4 occasions the highest dose was given and the amount of oral sugar provided is still much lower than that administered in the oral glucose test. Our analysis of the maltose and glucose content of the corn syrup (Karo Light Corn Syrup)[a] found it to contain lower digestible sugars than previously thought [4]. The dose of sugar given using the 0.45mls/kg bwt dose equates to 160.3mg/kg bwt. This is 6 times lower than the dose recommended for the oral glucose test of 1000mg/kg bwt. Further work at the 0.45ml/kg bwt dose should be undertaken in larger numbers but in this limited population it would appear safe and there may be room to use a higher dose still.

The OST is a promising dynamic test, employing the enteroinsular axis for identification of PL ponies, whom show an excessive insulin response to oral carbohydrate. Previous reports of poor sensitivity and repeatability may be improved by the adoption of the higher 0.45ml/kg bwt dose and calculation of the insulin area under the curve from at least three sampling time points. Further studies with larger numbers of both ponies and horses of differing breeds, during all four seasons and using the various insulin assays are needed to provide a frame work for reference ranges and better understanding of intra pony variability.

Tables

TABLE 1: Mean (95% confidence intervals) area under curve insulin (AUC_i), maximal insulin concentration ($Cmax_i$), time to maximal insulin concentration ($Tmax_i$), modified (T0-90) area under curve insulin (AUC_{iM}), modified maximal insulin concentration ($Cmax_{iM}$) and modified time to maximal insulin concentration ($Tmax_{iM}$) for the 3 doses of corn syrup (Karo Light Corn Syrup)[a] in normal (NL; n=3) and previously laminitic ponies (PL; n=5). $^{a–r}$ Significant ($P \leq 0.05$) difference between values with the same letter superscript in both the horizontal and vertical direction.

Dose (ml/kg bwt)	AUC_i (µiu/ml/min)		$Cmax_i$ (µiu/ml)		$Tmax_i$ (min)		AUC_{iM} (µiu/ml/min)		$Cmax_{iM}$ (µiu/ml)		$Tmax_{iM}$ (min)	
	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
0.15	5409^{a} (2282, 8535)	5797^{d} (3375, 8219)	72^{f} (21-124)	80^{g} (40-119)	80 (52-108)	72 (51-94)	3948 (2229-5669)	4430 (3097-5762)	62 (11-113)	61 (10-112)	70 (50-90)	72 (56-88)
0.3	4653^{b} (1526, 7780)	7434^{e} (5012, 9857)	61 (10-112)	111 (71-150)	80 (52-108)	93 (71-115)	3301 (1581-5021)	5189 (3857-6522)	98 (47-149)	78 (39-118)	80 (60-100)	78 (62-94)

0.45	9681[a,b,c] (6554, 12808)	14968[c,d,e] (12546, 17390)	135[f] (83-186)	206[g] (166-246)	75 (47-103)	78 (56-100)	5722[q] (4001-7442)	10208[q] (8876-11541)	98[r] (58-137)	206[r] (167-246)	80 (60-100)	78 (62-94)

TABLE 2: Mean (95% confidence intervals) area under curve insulin (AUC_i), maximal insulin concentration ($Cmax_i$), time to maximal insulin concentration ($Tmax_i$) for season and fasting following 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup)[a]. [a–i] Significant ($P \leq 0.05$) difference between values with the same letter superscript in both the horizontal and vertical direction.

Season	AUC_i (µiu/ml/min)		$Cmax_i$ (µiu/ml)		$Tmax_i$ (min)	
	NL	PL	NL	PL	NL	PL
Summer	6304^{a} (614, 11993)	$19032^{a,c}$ (14625, 23439)	87^{d} (10-164)	262^{d} (202-321)	55 (28-82)	86 (68-109)
Winter	9681^{b} (3551, 15812)	$14968^{b,c}$ (10220, 19717)	135^{e} (48-221)	206^{e} (139-273)	75 (46-104)	78 (55-101)
Fasting						
Fasting	6304^{f} (-211-12819)	19032^{f} (13985-24079)	87^{h} (19-155)	262^{h} (209-315)	55 (32-78)	89 (32-78)

Pasture	7639^{g}	16397^{g}	107^{i}	245^{i}	40	75
	(919-14359)	(11191-21602)	(24-190)	(181-309)	(12-68)	(53-106)

TABLE 3: Mean (95% confidence intervals) area under curve glucose (AUC_g), maximal glucose concentration ($Cmax_g$) and time to maximal glucose concentration ($Tmax_g$) for the 3 doses of corn syrup (Karo Light Corn Syrup)[a] in normal (NL; n=3) and previously laminitic ponies (PL; n=5). Effect of season and fasting on these variables following 0.45ml/kg bwt dose. [a–k] Significant ($P \leq 0.05$) difference between values with the same letter superscript in both the horizontal and vertical direction.

	AUC_g (µiu/ml/min)	$Cmax_g$ (µiu/ml)	$Tmax_g$ (min)
Dose (ml/kg bwt)			
0.15	507[a,b] (297-717)	5.4[c,d] (2.7-8.1)	76[f] (61-90)
0.3	907[a] (697-1117)	12.0[e,d] (9.5-14.9)	89 (74-103)
0.45	759[b] (550-969)	8.6[c,e] (5.9-11.3)	96[f] (82-111)
Disease state			
NL	756 (579-934)	9.0 (7.2-10.8)	55 (38-72)
PL	880 (742-1017)	10.7 (9.4-12.1)	71 (58-84)
Season			
Winter	742[g] (590-894)	8.5[i] (6.7-10.3)	94[k] (77-112)
Summer	889[g] (750-1027)	10.9[i] (9.5-12.4)	61[k] (48-73)
Fasting			
Fasting	874[h] (759-989)	10.8[j] (9.4-12.1)	60 (51-87)
Pasture	706[h] (571-840)	8.1[j] (6.4-9.7)	69 (47-73)

Figure Legends

Figure 1: Estimated marginal mean (±1.96 s.e.) serum insulin concentration at single time points in response to 3 different doses of corn syrup (Karo Light Corn Syrup)[a]. (n=8)
*Values that are significantly different (P<0.05) from the equivalent values from a different dose.

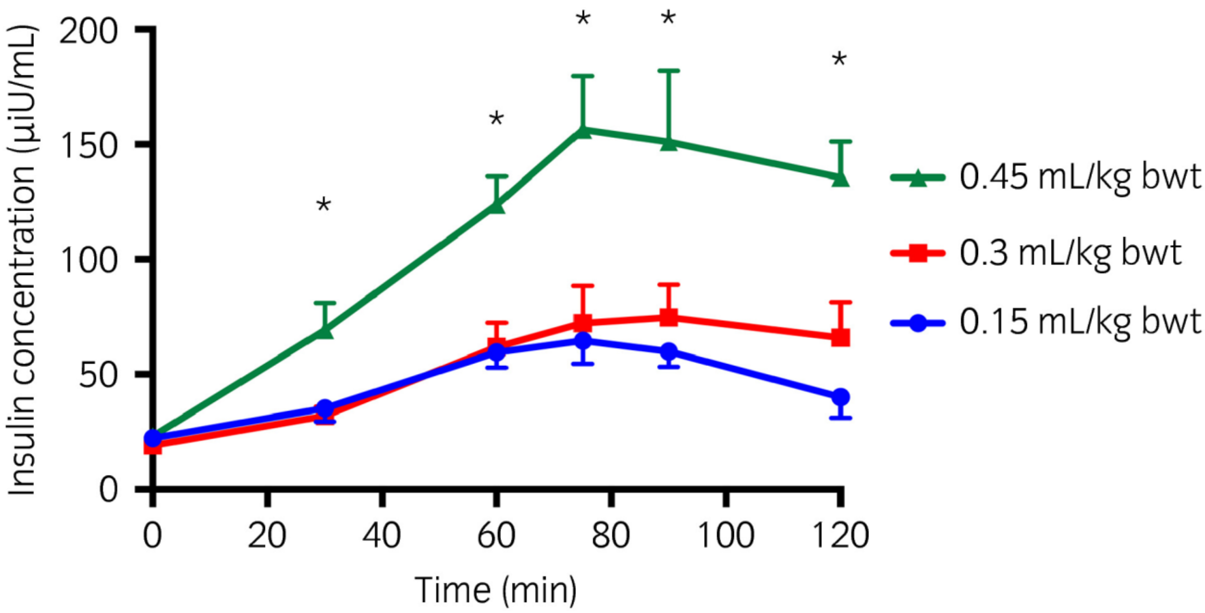


Figure 2: Estimated marginal mean (±1.96 s.e.) insulin concentration at single time points for NL (n=3) and PL (n=5) ponies when given a dose of 0.45ml/kg bwt corn syrup (Karo Light Corn Syrup). *Values that are significantly different (P<0.05) between groups (NL and PL).

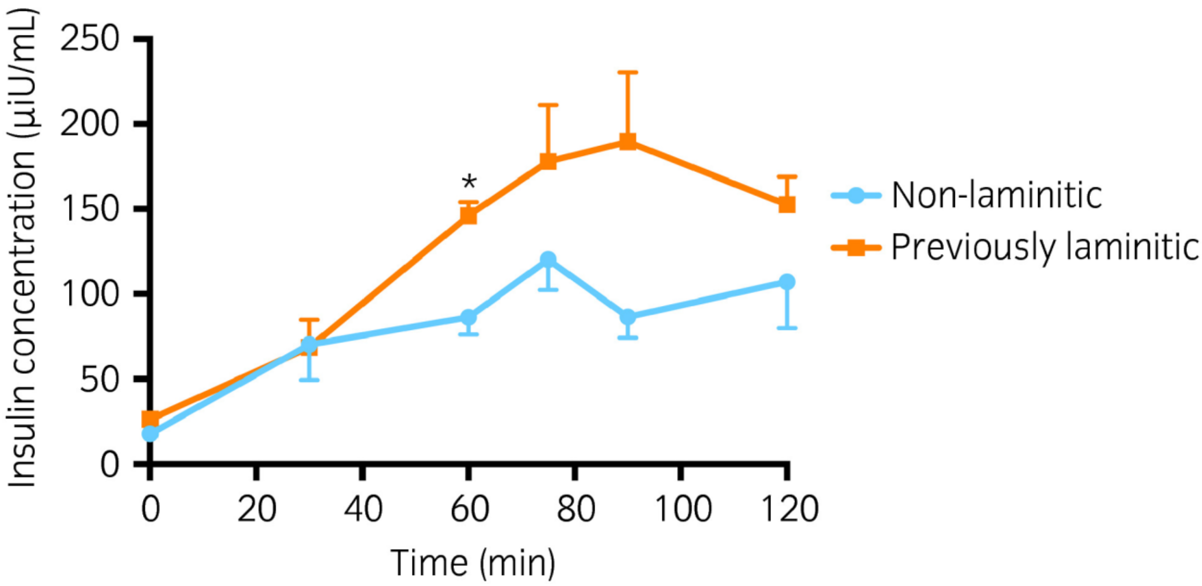
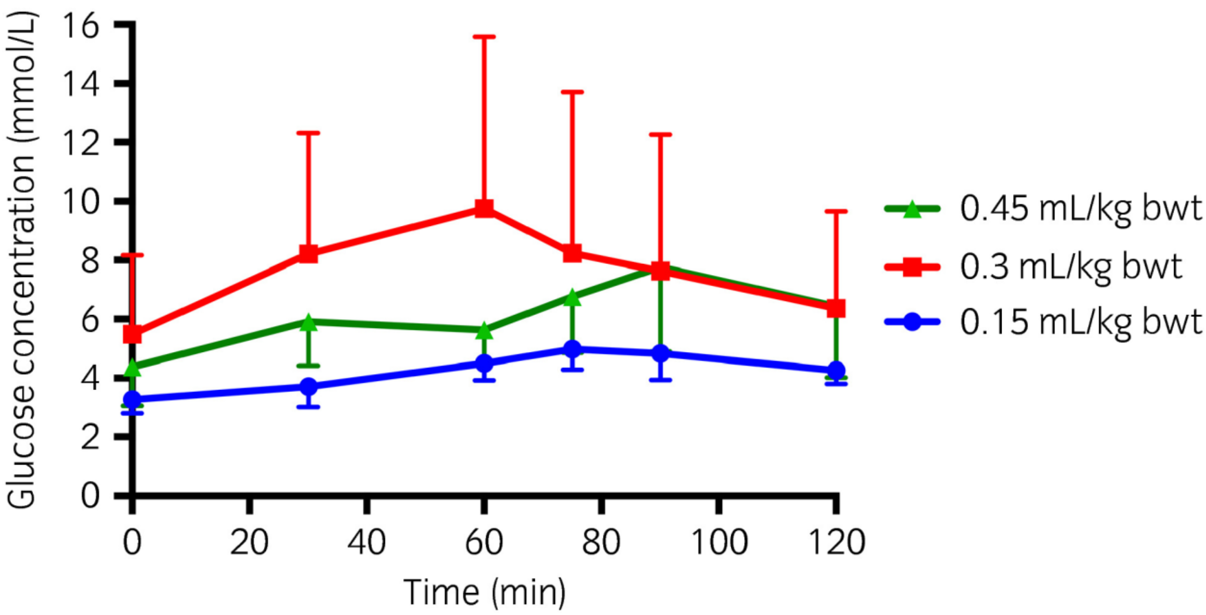


Figure 3. Estimated marginal mean (±1.96 s.e.) serum glucose concentration at single time points in response to 3 different doses of corn syrup (Karo Light Corn Syrup)[a]. (n=8)
*Values that are significantly different (P<0.05) from the equivalent values from a different dose.



Manufacturers' addresses

[a]ACH Food Companies Inc, Cordova, Tennessee, USA.

[b] Becton Dickinson, Sandy, Utah, USA.

[c] Dechra Veterinary Products, Shrewsbury, Shropshire, UK.

[d]Jasco Ltd., Essex, UK

[e] Phenomenex, Torrance, California, USA

[f]MP Biomedical, Ilkirch, France.

[g]Cayman chemical company, Michigan, USA

[h]IBM UK, Portsmouth, Hampshire, UK.

[i]Graphpad Software, La Jolla, California, USA.

[j]Coat-A-Count, Siemens, Camberley, Surrey, UK.

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Supplementary Information Items

1. Table – Signalment, laminitis history and weight from the pony subjects.
2. Table- Corn Syrup analysis