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## Comparison of the in-feed glucose test and the oral sugar test.

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**Keywords:** horse; insulin; laminitis; metabolic; glucose; sugar

### Summary

**Reason for performing the study:** The in-feed oral glucose test (OGT) and oral sugar test (OST) are advocated as field tests of insulin sensitivity in horses and ponies but have not been directly compared previously.

**Objectives:** To compare the insulin response to OGT and OST in 8 ponies and 5 horses of unknown insulin sensitivity.

**Study design:** Experimental, randomised cross-over study.

**Method:** Animals were fasted for 8 h overnight before and throughout testing. Subjects were fed 1 g/kg glucose powder with chaff (OGT) or 0.15 ml/kg corn syrup (Karo™ Light Syrup, OST) was administered orally in a randomised crossover study with 48 h between tests. Blood samples were obtained at 0, 30, 60, 75, 90, 120 and 180 min. Maximum insulin concentration ( $C_{max_i}$ ), time to maximum insulin concentration ( $T_{max_i}$ ) and area under the curve of insulin concentration over time ( $AUC_i$ ) for the tests were compared using a paired t-test. The effect of individual subject, horse or pony and test were analysed using a linear mixed model.

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**Results:** OGT  $C_{max_i}$  (mean  $\pm$  s.d.;  $154 \pm 116$  mU/l),  $T_{max_i}$  ( $136 \pm 52$  min) and  $AUC_i$  ( $15308 \pm 9886$ ) were significantly ( $p < 0.05$ ) greater compared to OST  $C_{max_i}$  ( $72 \pm 55$  mU/l),  $T_{max_i}$  ( $63 \pm 25$  min) and  $AUC_i$  ( $5980 \pm 4151$ ).  $C_{max_i}$ ,  $T_{max_i}$  and  $AUC_i$  varied significantly between individual subjects.  $T_{max_i}$  was significantly different between horses and ponies during OGT and OST. Using previously defined criteria of insulin dysregulation OGT identified 7/13 animals as insulin resistant whereas OST identified 5/13 animals as insulin resistant.

**Conclusions:** OGT and OST showed agreement in identification of insulin dysregulation in 85% of equine subjects. Results of the OGT and OST are not comparable in all cases. Further work is required to establish which test more accurately diagnoses insulin dysregulation in horses and ponies.

## Introduction

Ponies with insulin dysregulation are predisposed to pasture-associated laminitis [1,2] and identification of equids with insulin dysregulation may aid in the prevention of laminitis. It is therefore important to establish appropriate tests of insulin dysregulation to be used by veterinarians in clinical practice. In a research environment, the minimal modelling of a frequently sampled intravenous glucose tolerance test [3] and the euglycaemic-hyperinsulinaemic clamp methods are considered to be the gold standard techniques [4], however they are not practical in a clinical setting. Basal insulin concentration is not increased in all insulin resistant equids and is affected by age, breed, exercise, stress, disease, feeding and diet [5-13]. Thus dynamic testing has been recommended [20]. Tests providing an oral dose of water-soluble carbohydrate and then measuring the serum insulin concentration after a defined time period has elapsed have been advocated [14] [7]. However, the optimum dose of water-soluble carbohydrate or time to measure the insulinemic response have not been determined. Despite this, 2 tests are currently advocated for clinical use [15, 16]. The in-feed oral glucose test (OGT) provides the carbohydrate bolus in the form of glucose

powder mixed with chaff and the oral sugar test (OST) uses unknown carbohydrates in a commercially available corn syrup. The OGT most commonly requires 1 g/kg glucose powder with the cut-off for insulin resistance set as an insulin concentration of  $>85 \mu\text{IU/ml}$  at 120 min [7, 17].

The OST requires 0.15 ml/kg corn Syrup<sup>1</sup> to be syringed *per os* and defines insulin resistance as an insulin concentration  $>60 \mu\text{IU/ml}$  at 75 min [15, 16, 17]. These 2 tests have not been compared to the gold standard tests and it is not known if the results of the 2 tests are directly comparable. Thus the aim of this study was to compare the OGT and the OST, indirect methods of measuring insulin sensitivity, in 13 horses and ponies of unknown insulin sensitivity.

## **Materials and Methods**

### *Subjects*

Eight ponies and 5 horses were included in the study. All ponies were mixed, native British breeds and were aged mean  $\pm$  s.d.  $18 \pm 3.5$  years. There were 7 mares and one gelding weighing  $286 \pm 54$  kg. The horses were 4 mares and one gelding of mixed breeds, aged  $14 \pm 5$  years weighing  $580 \pm 91$  kg. No animals were related, had history of systemic disease within the previous 3 months or clinical signs of pituitary pars intermedia dysfunction. They were all maintained on average mixed-grass pasture with no supplementary feeding and were in reasonable body condition score (5-6/9) with no marked regional adiposity.

### *Study Design*

The study was performed in October. The animals were brought in to a bare paddock or stable and given ad lib dry hay 16 h prior to testing. Food was withheld from 8 h prior to time 0 h and throughout each test. A blood sample was obtained at time 0 h. In a randomised cross over design animals were given either 1 g/kg glucose powder<sup>a</sup> mixed with a handful of commercially-available chaff-based feed<sup>b</sup> which was eaten entirely within 10 min, or 0.15 ml/kg corn syrup<sup>c</sup> syringed by mouth. Blood samples were obtained 30, 60, 75, 90, 120 and 180 min after carbohydrate

administration. Blood for insulin concentration measurement was collected into plain blood tubes and allowed to clot at 37°C for at least 20 min. Blood for glucose concentration measurement was collected into fluoride oxalate blood tubes and placed on ice. The samples were centrifuged (3000 x g) for 10 min at 4°C and the serum or plasma was stored at -80°C before analysis. The animals were returned to pasture for a 48 h washout period between tests.

### *Glucose and insulin assays*

Serum insulin concentration was measured using a radioimmunoassay kit<sup>d</sup> and serum glucose was measured using a colourimetric assay<sup>e</sup>. All samples were measured in duplicate and both assays had been previously validated for use in horses and ponies [18].

### *Data analysis*

Data was tested for normality using a Shapiro-Wilk normality test. Maximum insulin concentration ( $C_{max_i}$ ), maximum glucose concentration ( $C_{max_g}$ ), time to maximum insulin concentration ( $T_{max_i}$ ) and maximum glucose concentration ( $T_{max_g}$ ) and area under the curve of insulin concentration over time ( $AUC_i$ ) and area under the curve of glucose concentration over time ( $AUC_g$ ) for the 2 tests were compared using a paired t-test. An unpaired t-test was used to assess the effect of performing the OGT or OST first. A linear mixed model was used to assess the effect of individual animals, horse or pony and OGT or OST. The outcome of each test, namely insulin resistance or sensitive, was determined using insulin concentration >85  $\mu$ IU/ml at 120 min for the OGT or >60  $\mu$ IU/ml at 75 min for the OST to define insulin resistance. This was repeated using  $C_{max_i}$  rather than either insulin concentration at 120 or 75 min and the results compared. Significance was set at  $p < 0.05$ .

## **Results**

### *Insulin response in ponies and horses*

There was significant ( $p < 0.0001$ ) variation in  $C_{max_i}$  (Fig 1) and  $AUC_i$  (data not shown) for both the OGT and OST between individual animals. OGT  $C_{max_i}$ ,  $T_{max_i}$  and  $AUC_i$  were significantly ( $p = 0.001$ ) greater than OST values amongst the ponies (Table 1). Amongst the horses only OGT  $C_{max_i}$  was significantly ( $p = 0.05$ ) greater than the OST value. For both tests,  $C_{max_i}$  and  $AUC_i$  were significantly ( $p = 0.05$ ) greater in ponies compared to horses.

When the data for all animals was combined, OGT  $C_{max_i}$ ,  $T_{max_i}$  and  $AUC_i$  were significantly ( $p = 0.001$ ) greater than OST values. Finally, there was no significance effect of test order on values for an individual test.

#### *Identifying insulin resistance*

Using the previously defined criteria of insulin concentration  $>85 \mu\text{IU/ml}$  at 120 min for OGT [16] and  $>60 \mu\text{IU/ml}$  at 75 min for OST [15, 16], the OGT identified 7/8 ponies as insulin resistant whereas the OST identified 5/8 ponies as insulin resistant. All horses were insulin sensitive with both tests. When ranked by  $AUC_i$ , the ponies and horses were not ranked in the same order for the OGT and the OST.

#### *Time to maximum insulin concentration*

There was significant ( $p = 0.005$ ) variation in  $T_{max_i}$  between individuals (Fig 3).  $T_{max_i}$  was compared to the time at which a single blood sample is recommended to be taken in the OGT (120 min) and OST (75 min). The horses had a shorter time difference than the ponies between  $T_{max_i}$  and previously defined time to obtain a single sample; in the OGT, the mean of the  $T_{max_i}$  of horses was 6 min greater than 120 min and for the ponies was 22 min greater than 120 min. For both horses and ponies in the OGT there was a wide standard deviation of the mean of  $T_{max_i}$ . In the OST, the mean  $T_{max_i}$  for the horses was 7 min less than 75 min whereas the mean  $T_{max_i}$  for the ponies was 17 min less than 75 min. The standard deviation of the mean  $T_{max_i}$  was much smaller for the OST than OGT.

## *Glucose response*

When compared either as ponies, horses or with all data combined there was no significant difference in  $C_{max_g}$ ,  $T_{max_g}$  or  $AUC_g$  between the OGT and OST (Table 2).

## **Discussion**

Testing for insulin dysregulation may aid in identification of individual animals at increased risk of pasture associated laminitis [19] and of equine metabolic syndrome [20]. Whilst minimal modelling of the results of a frequently sampled intravenous glucose tolerance test [3] or a hyperinsulinemic euglycemic clamp [4] are considered to be the gold standard techniques for identifying insulin dysregulation, the most appropriate test of insulin dysregulation for use in clinical practice has not been identified. Administering a single dose of oral water-soluble carbohydrate and measuring the insulin response following this has been suggested to be an effective indirect method of estimating the insulin responsiveness of ponies and horses [14]. More recently it has been demonstrated that peak serum insulin concentration following the OGT using a higher dose of 1.5 g/kg glucose powder correlates with insulin dysregulation defined using a frequently sampled intravenous glucose tolerance test (FSIGT), [6], with the FSIGT providing a more direct measure of insulin dysfunction. In addition, the results of the OST and an intravenous glucose tolerance test were found to correlate closely when performed in horses with equine metabolic syndrome and a control group [21] and identification of insulin dysfunction correlated between the OGT and an intravenous insulin tolerance test in horses previously diagnosed with equine metabolic syndrome [22]. However, in a recent study comparing the OST with both the insulin response to dexamethasone test and the hyperinsulinemic euglycemic clamp in horses which all appeared to be insulin sensitive found no correlation between the results of the 3 tests [23]. It is possible that the correlation between the results of these tests is altered by insulin dysregulation; however, the test which most accurately identifies insulin dysregulation has not been confirmed.

The results of the current study show that ponies defined as insulin resistant by the OGT may not be defined as insulin resistant by the OST. When previously established, but not validated, cut off

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values for insulin resistance [15] were applied to each test the OGT identified more ponies as insulin resistant than the OST. It is not possible to say which test more correctly identifies ponies with insulin dysregulation; this requires future studies in which the 2 tests are compared under the same conditions in conjunction with direct tests of insulin sensitivity. In a previous study comparing the OGT with the FSIGT, inter-individual variation made it impossible to find a single cut-off value of insulin concentration in the OGT above which all animals had insulin dysregulation [6]. Recent comparison of the OST with the intravenous insulin tolerance test found that  $AUG_g$  for the OST correlated significantly with the slope of maximal reduction in blood glucose concentration in the intravenous insulin tolerance test [22]. Comparison of the OST with the intravenous glucose tolerance test showed that  $AUC_g$  and  $AUC_i$  were positively correlated for the 2 tests [21]. It has been suggested that measuring the area of under the curve of insulin concentration against time provides a more reliable estimate of insulin sensitivity [24] and OST  $AUC_i$  has been shown to correlate positively with  $AUC_i$  of the intravenous tolerance test [21]. However, in this study, ranking the animals by  $AUC_i$  did not rank them in the same order for the OST and OGT and no cut off values for insulin resistance have been determined for the  $AUC_i$  of the OST and OGT.

Variation in  $T_{max_i}$  between individuals underlines the difficulty of using a single insulin concentration at a defined time point to identify insulin dysregulation in the OGT and OST. The wide standard deviation of  $T_{max_i}$  in the OGT suggests that the OST may be a more reliable test to diagnose insulin resistance when using a one-time blood sample. The variation in  $T_{max_i}$  between the 2 tests may be explained by the difference in administration techniques; the corn syrup<sup>c</sup> was given as an oral bolus whereas the glucose powder<sup>a</sup> was given in chaff. It has previously been shown that both meal size and starch content alter the rate of gastric emptying with higher starch, larger meals emptying most slowly [26]. The larger excursion of mean  $T_{max_i}$  from the identified test-time for the ponies than the horses also suggests that it may be necessary to have separate



protocols for horses and ponies, for example obtaining single blood samples from ponies at 60 min rather than 75 min for the OST.

The overall and maximum insulin responses to the OGT were significantly greater than to the OST but it took longer to reach a maximum insulin concentration following the OGT. The carbohydrate composition of corn syrup<sup>c</sup> is unknown. It is stated to contain 5 g of sugar per 15 ml and to be 15-20% glucose. Previous studies have shown a lower insulinaemic response in ponies to both fructose and inulin when given at the same dose as a bolus of glucose [7]. Thus it is likely that corn syrup<sup>c</sup> contains sources of carbohydrate other than glucose such as fructose and inulin.

The insulin response to an oral carbohydrate bolus has been shown to vary with many factors including age, diet in the preceding weeks [11, 12], breed [14] and disease [5, 25, 26]. The animals in this study were kept in a constant environment and each animal in the study acted as its own control, limiting the effect of variation in individual insulin sensitivity. However subjectively the response curves of insulin concentration over time (Fig 1) appear to be monophasic in some animals and biphasic in other animals. Despite the fact that all animals were kept at pasture immediately prior and during the study and received no supplementary feeding, individual animal variation in dietary intake prior to the study as well as gastric emptying time or intestinal motility may have led to this variation [26].

The small study group will have reduced the ability to identify differences between the groups, particularly the small number of horses may have reduced the apparent differences within the horse group and between the horse and pony groups. In this study of animals in moderate body condition the majority of ponies were defined as insulin resistant and all horses as insulin sensitive by both the OST and OGT. This finding is in agreement with previous studies showing breed-related

differences in insulin sensitivity [13, 14]. It may be necessary to develop breed specific reference ranges for these tests but this data is not available at the present time.

In conclusion, when the OGT and OST are used to identify insulin dysregulation in horses or ponies, the results are not equivalent. The OGT identified a larger number of ponies as insulin resistant than the OST. There is wide variation in the insulin response to the 2 tests between each subject tested.  $T_{max_i}$  has a wider deviation from the mean for the OGT than the OST. Further work is required to establish which test more appropriately identifies insulin dysregulation in equine subjects including a direct comparison of all of the tests used to identify insulin dysregulation in the research and clinical setting in the same animals and under identical conditions.

#### **Authors' declaration of interests**

Dr Harris is employed by the study funders.

#### **Ethical Animal Research**

The protocol was approved by the Royal Veterinary College Ethics and Welfare Committee and was carried out under UK Home Office license.

#### **Source of funding**

WALTHAM Centre for Pet Nutrition.

#### **Authorship**

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

## Manufacturers' addresses

<sup>a</sup>Glucose Powder (Dextrose monohydrate), W. and J. Dunlop Ltd, Dumfries, DG2 0NU, UK.

<sup>b</sup>Happy Hoof, Spillers Effem Equine, Ltd., Mars HorseCare UK Ltd., Milton Keynes, Buckinghamshire, UK.

<sup>c</sup>Karo Light Syrup, ACH Food Companies Inc, Memphis, Tennessee, USA.

<sup>d</sup>Insulin RIA, Coat-A-Count, Siemens, Camberley, Surrey, UK.

<sup>e</sup>Glucose Colormetric Assay Kit, Cayman Chemical Company, Michigan, USA.

## Figure Legends

**Fig 1:** Serum insulin concentration (uIU/ml) during the oral glucose test (left) and oral sugar test (right) in all animals (ponies A-H, horses I-M).

**Fig 2:** The mean  $\pm$  standard deviation of  $C_{max_i}$  for horses and ponies during the OST and OGT. Mean  $C_{max_i}$  was significantly different between all groups ( $P < 0.05$ ).

**Fig 3:** The mean  $\pm$  standard deviation of  $T_{max_i}$  for horses and ponies during the OST and OGT. \*denotes a significant difference in mean  $T_{max_i}$  between groups ( $p < 0.05$ ).

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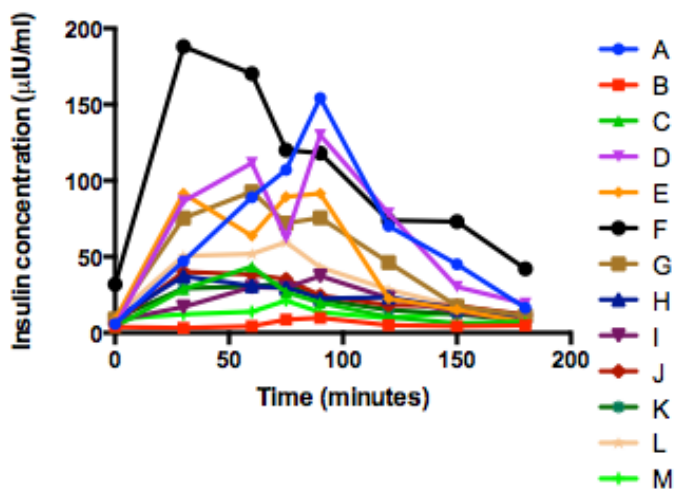
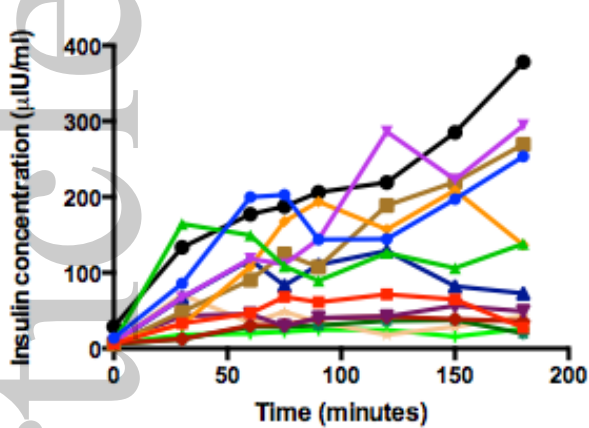


Figure 1: Serum insulin concentration (uIU/ml) during the oral glucose test (left) and oral sugar test (right) in all animals (ponies A-H, horses I-M).

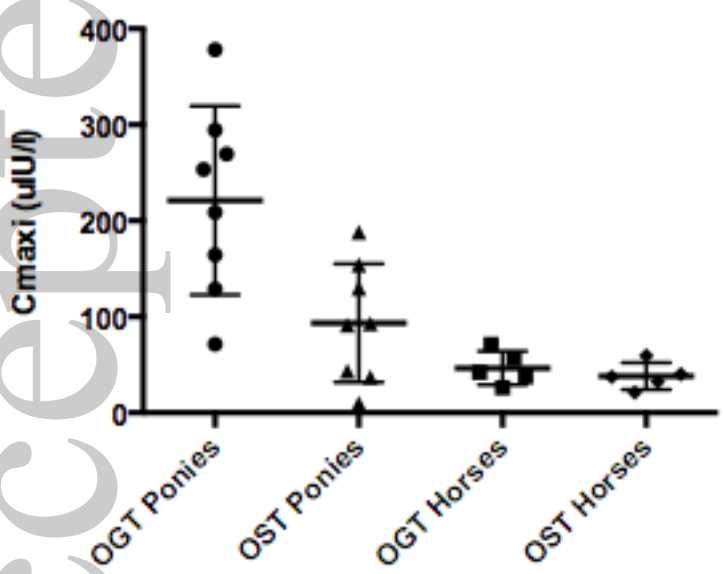


Figure 2. The mean  $\pm$  standard deviation of  $C_{max_i}$  for horses and ponies during the OST and OGT. Mean  $C_{max_i}$  was significantly different between all groups ( $P < 0.05$ ).

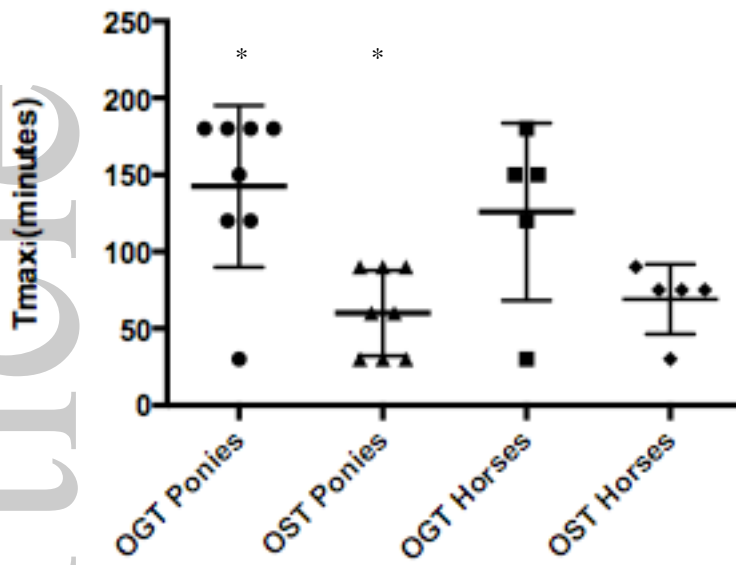


Figure 3. The mean  $\pm$  standard deviation of T<sub>max<sub>i</sub></sub> for horses and ponies during the OST and OGT. \*denotes a significant difference in mean T<sub>max<sub>i</sub></sub> between groups ( $p < 0.05$ ).



**Table 1:** AUC<sub>i</sub>, Cmax<sub>i</sub>, Tmax<sub>i</sub> for OGT and OST in ponies (n = 8), horses (n = 5) and all (n = 13).  
<sup>a-j</sup> denotes a significant (p≤0.05) difference between values with the same letter superscript

		<b>OGT</b>	<b>OST</b>
<b>AUC<sub>i</sub></b> ( $\mu\text{IU/ml}\cdot\text{min}^{-1}$ )	Ponies	21397 $\pm$ 7489 <sup>a,b</sup>	7153 $\pm$ 4926 <sup>a,c</sup>
	Horses	5567 $\pm$ 1525 <sup>b</sup>	4105 $\pm$ 1443 <sup>c</sup>
	All	15308 $\pm$ 9886 <sup>d</sup>	5980 $\pm$ 4151 <sup>d</sup>
<b>Cmax<sub>i</sub></b> ( $\mu\text{IU/ml}$ )	Ponies	221 $\pm$ 9 <sup>e,f</sup>	93 $\pm$ 62 <sup>e,g</sup>
	Horses	46.65 $\pm$ 17 <sup>f,h</sup>	38.19 $\pm$ 14 <sup>g,h</sup>
	All	154 $\pm$ 116 <sup>i</sup>	72 $\pm$ 55 <sup>i</sup>
<b>Tmax<sub>i</sub></b> (min)	Ponies	142 $\pm$ 53 <sup>j</sup>	60 $\pm$ 10 <sup>j</sup>
	Horses	126 $\pm$ 58	69 $\pm$ 23
	All	136 $\pm$ 52	63 $\pm$ 25

**Table 2:** AUC<sub>g</sub>, Cmax<sub>g</sub>, Tmax<sub>g</sub> for OGT and OST in ponies (n = 8), horses (n = 5) and all (n = 13). There were no significant differences (p<0.05) in AUC<sub>g</sub>, Cmax<sub>g</sub>, Tmax<sub>g</sub> between OGT and OST.

		<b>OGT</b>	<b>OST</b>
<b>AUC<sub>g</sub></b> (mmol/l.min <sup>-1</sup> )	Ponies	309.1 ± 157.1	184.7 ± 83.93
	Horses	841.6 ± 258.9	744.9 ± 132.5
	All	513.9 ± 330.8	400.2 ± 300.7
<b>Cmax<sub>g</sub></b> (mmol/l)	Ponies	7.2 ± 1.7	5.9 ± 1.1
	Horses	6.5±2.0	6.2±1.6
	All	7.0 ± 1.8	6.0 ± 1.3
<b>Tmax<sub>g</sub></b> (min)	Ponies	111 ± 40	69 ± 57
	Horses	117 ± 50	96 ± 49
	All	113 ± 42	85 ± 48