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Original Research

Comparison of Two Diagnostic Methods to Detect Insulin Dysregulation in Horses Under Field Conditions

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ABSTRACT

Straightforward testing procedures to enable the diagnosis of insulin dysregulation (ID) in horses that are suitable for use in daily veterinary practice are needed because of the risk that ID could result in laminitis. In our study (that included 90 horses), we compared the proportion of horses classified as ID-positive, ID-suspect, and ID−not diagnosed according to the basal insulin concentration (BIC) with the proportion of horses classified as ID-positive or ID-negative according to a practical and feasible version of an oral sugar test (OST). Furthermore, BIC, basal glucose concentration, and insulin and glucose concentration after OST were analyzed and compared. In the total study population, the OST detected significantly more ID-positive cases than the BIC, with cutoffs at equivalent specificities. Receiver operating characteristics analysis showed that at a lower cutoff, the sensitivity of the BIC could be increased, but at the cost of a significantly lower specificity. Taking this into account, we found diagnostic performance of the OST to be considerably better than the BIC and therefore considered it more recommendable for use as a screening test for ID in ambulatory practice. Furthermore, we investigated the relationship between body condition score and breed type with glucose and insulin concentration as determined after our version of the OST. For that purpose, the study group was subdivided into lean, moderate, and obese horses and "easy keeper breeds" versus "non-easy keeper breeds". Results supported the general assumption that obese horses and "easy keeper breeds" are more prone to the development of ID.

1. Introduction

Insulin dysregulation (ID) in horses refers to a clinical picture involving fasting hyperinsulinemia, excessive insulin responses to carbohydrates, and tissue insulin resistance (IR) [1,2]. This endocrine disorder receives special attention because of the association of hyperinsulinemia with laminitis [3–5] which can develop into a serious life-threatening complication [6]. The occurrence of ID has been associated with obesity, which is cause for concern because the prevalence of equine obesity seems to be increasing with associated health risks such as ID possibly becoming more important [7–9]. For use in ambulatory veterinary practice, a feasible way of identifying horses with ID would be very helpful. It would enable equine veterinarians to convince horse owners involved to abide by management recommendations with the intention of diminishing the risk of occurrence of hyperinsulinemia [10], and thus decreasing the risk of resulting laminitis. In the Netherlands, veterinary practitioners still use basal insulin concentration (BIC) extensively to detect ID in horses. However, because the BIC is currently considered insufficiently sensitive at the widely used cutoff of 20 mU/L [10–12], nowadays the oral sugar test (OST) is increasingly mentioned for this purpose [13–15]. The BIC identifies horses with resting hyperinsulinemia [16], whereas the OST serves as a diagnostic tool for an excessive insulin response after oral administration of carbohydrates [13] and provides an indication for tissue insulin resistance to be present [17]. So far, mostly a dose of 0.15 mL/kg body weight (bwt) corn syrup is used with the aim of evaluating an OST [10,13,15,18] but a higher dose could possibly improve diagnostic ability [14,19]. In this study, we determined the BIC and the insulin concentration after an OST using 0.45 mL/kg bwt of corn syrup in a group of 90 client-owned

Animal welfare/ethical statement: The study protocol was ethically weighed and approved by the Animal Welfare Body (IVD) committee of GD Animal Health Service Deventer.

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horses located at their home premises. The main objective was to evaluate the BIC (at different cutoffs) as compared with the OST as being two different, easy to implement techniques to diagnose ID for use in daily veterinary practice. In addition, we compared ID status and glucose and insulin concentration after OST between different categories after dividing the total study group into lean, moderate, and obese horses and in "easy keeper breeds" versus "non-easy keeper breeds". The purpose of this was to find out whether clear differences could be identified in our study group that support the general assumption that obese horses and easy keeper breeds are at greater risk of having ID.

2. Materials and Methods

The study protocol was ethically weighed and approved by the Animal Welfare Body committee of GD Animal Health Service Deventer.

2.1. Animals

An online call to recruit participants (= client-owned horses) for a study concerning a field trial with the purpose of evaluating weight-loss management yielded 509 applications. The trial covered a six-month period during which participating horses had to adhere to a tailor-made rations prescription and to a recommended amount of exercise. Instructions regarding rations and exercise were adjusted monthly depending on the desired weight loss. Inclusion criteria for this study focused on the physical ability of the horses to meet the requirements of the weight-loss program. Eligible were clinically healthy horses of all breed types, except for Shetland ponies and multipace breeds (activity was tracked using an Equestic SaddleClip, which was not suitable for use in these breed types). The horses had to be between 3 and 25 years of age without a known incidence of laminitis or previously established diagnosis of pituitary pars intermedia dysfunction. In addition, external characteristics that could fit with pituitary pars intermedia dysfunction (such as hirsutism) also were a reason not to accept a horse as a participant in the trial. Furthermore, the horses should not be lactating, pregnant, or very actively ridden (we aimed for horses intended for recreational use). The first selection of suitable participants was made based on the information submitted online. Subsequently during October and November 2017, 200 horses housed on private premises spread over the Netherlands and Belgium were visited on location. At this point, the visiting veterinarian assessed whether each horse actually met the criteria for participation and discussed with owners if they were sufficiently aware of the efforts required for this project. Blood samples were collected from 90 horses that were eventually selected to participate in the trial. The laboratory results of these samples are used for the analysis discussed in this paper. All horses eventually included were clinically healthy, with a mean age of 10.5 years (age ranged between 3 and 25 years). Represented breed types were Dutch, German, and Belgian Warmbloods (n = 24); Baroque horses (n = 13); Welsh pony breeds or crosses (n = 12); Arab horses or crosses (n = 11); Haflingers (n = 8); New forest ponies (n = 5); Tinkers (n = 5); Thoroughbreds or crosses (n = 3); Fjord horses (n = 3); Icelandic horse and crossbred (n = 2); Irish cob, Appaloosa, Quarter horse and Schwarzwälder fuchs (all n = 1). One stallion, 35 mares, and 54 geldings took part. Mean weight was 492 kg (weight ranged between 199 and 693 kg) and body condition score (BCS) ranged from 4 to 9 on a 9-point scale.

2.2. Study Design

At the intake consultation, horses were visited at their home premises during morning hours (8.00–11.00 AM) by a veterinarian who determined morphometric measurements (i.e., BCS), performed an OST and acquired the blood samples which were used to assess blood glucose and insulin concentrations. All visits (and associated scorings) were performed by one of the two veterinarians who participated in this study. Both veterinarians had ample experience in determining the BCS. Before sampling, the horses were not allowed to be fed any concentrates, nor have had access to grass for 8 hours. Roughage could be fed the evening before according to the way they were used to in the particular stable where the horse was housed. Remains of the roughage, if any, had to be removed 2 hours before the agreed time of the visit. Water access was freely allowed. Body weight was measured with a set of scales designed for horses (Allscales W-1500, Bos), and BCS was determined according to the Henneke scoring system [20]. We considered horses with a BCS of 4–5 to be in a lean condition, horses with a BCS of 6 to be in a moderate condition, and horses with a BCS of 7–9 as being obese [21–23]. Furthermore, it was noted to which breed or cross-breed type the horse belonged according to the registration certificate and/or the visiting veterinarian, so the study group could be divided into horses belonging to a breed type or cross-breed type regarded to be "easy keepers" or "non-easy keepers" [5,24–26]. Heparinized blood samples were collected for determination of insulin concentration and a sodium fluoride blood tube was collected for glucose concentration. The samples were chilled and sent to the laboratory overnight for further processing. After centrifugation, plasma was collected for analysis of insulin. Insulin concentration was measured with a Siemens Immulite 1000 using the insulin kit 6602443, with the determination of glucose being performed using the glucose reagents of Beckman (hexokinase method) on a Synchron D×C 600. Both assays had previously been validated for use in horses and ponies at the GD Animal Health Service following ISO-17025 procedures. According to the instructions of the manufacturer of the insulin test kit, blood serum as well as lithium heparin plasma can be used to measure insulin concentration. During validation it was confirmed that no significant differences were found between the two matrices.

2.3. Oral Sugar Test

After taking blood samples for determining BIC and basal glucose concentration at T = 0, 0.45 mL/kg bwt corn syrup (Karo Light Syrup, ACH Food Companies Inc, Memphis, TN) was administered orally using a dosing syringe. Generally, horses readily accepted the syrup, but care was taken to minimize spillage. After 75 minutes (T = 75), blood sampling for measurement of insulin and glucose concentrations was repeated. In our study no adverse effects resulting from administration of the Karo light syrup were seen in any of the participating horses and ponies.

2.4. Classification With Regard to ID-Status

Cutoff values used in this study to categorize horses with respect to their ID status were based on the most recent recommendations of the EMS working group [14]. Based on these recommendations, the basal insulin concentration (BIC) is considered to be non-diagnostic when the BIC is < 20 mU/L. A horse is considered to be ID-suspect if the BIC ranges from 20 to 50 mU/L, and as ID-positive if the BIC is higher than 50 mU/L. For the OST, a cutoff value for insulin concentration of 40 mU/L at T = 75 was used to categorize horses as ID-negative or ID-positive. The aforementioned recommendations indicate that for the OST, a cutoff value of 40 mU/L is appropriate when 0.45 mL/kg bwt corn syrup is administered [14]. This was confirmed by a personal communication (A. Durham, cutoff value of 40 mU/L corresponded with 95% specificity, as determined with robust statistics).

Table 1
Classification of horses with respect to their ID status based on the BIC and on the insulin concentration after OST.

		OST		Total Number of Horses
		ID-Positive	ID-Negative	
BIC	ID-positive	3	0	3
	ID-suspect	4	2	6
	ID-not diagnosed	18	63	81
Total number of horses		25	65	90

Abbreviations: BIC, basal insulin concentration; ID, insulin dysregulation; OST, oral sugar test.

2.5. Data Analysis

The data were analyzed using Stata 15 (StataCorp LP, TX). Residuals were tested for normality: the residuals of insulin and glucose were not normally distributed. Therefore nonparametric statistics were used (Kruskal–Wallis test) to test differences between groups. The significance of differences between percentages was calculated with a two-sample proportion test. Using the OST as a reference, receiver operating characteristics curve and Youden's Index analysis were used to calculate optimal cutoff values for the BIC. Results are also shown in graphs as Box-and-Whisker plots (Sigma Plot, Systat software version 13).

3. Results

3.1. Comparison of the BIC and the OST in the Total Study Group

The number of horses classified as ID–not diagnosed, ID-suspect, and ID-positive according to the BIC, or as ID-negative or ID-positive according to the OST, are shown in Table 1. As shown, the BIC classified 3 horses as ID-positive and 6 horses as ID-suspect (9/90 = 10%), whereas according to the OST, 25 of the 90 horses (25/90 = 28%) were classified as ID-positive. At the BIC cutoff of 20 mU/L (according to the aforementioned recommendations), results of BIC and OST were significantly different in outcome (Kruskal Wallis, $P < .001$).

Fig. 1A shows the BIC as well as the insulin concentration after OST in horses of different ID status as based on the BIC. Median BIC was 4.3 mU/L for horses classified as ID–not diagnosed, 24.6 mU/L

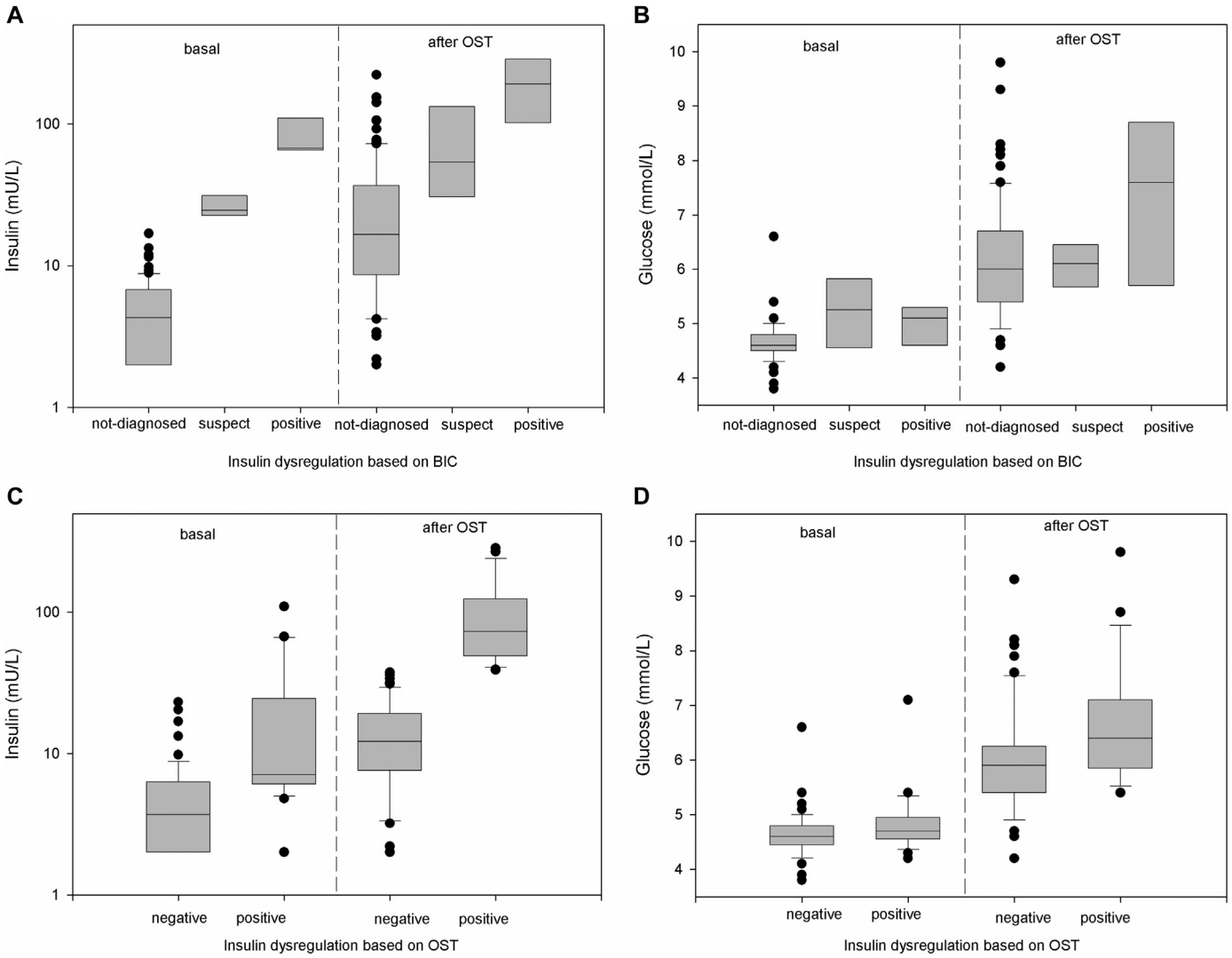

Fig. 1. (A) BIC and insulin concentration after OST (log scale) in horses of different ID-statuses based on the BIC. (B) Basal glucose concentration and glucose concentration after OST in horses of different ID-statuses based on the BIC. (C) BIC and insulin concentration after OST (log scale) in horses of different ID-statuses based on the OST. (D) Basal glucose concentration and glucose concentration after OST in horses of different ID-statuses based on the OST. Abbreviations: BIC, basal insulin concentration; OST, oral sugar test; ID, insulin dysregulation.

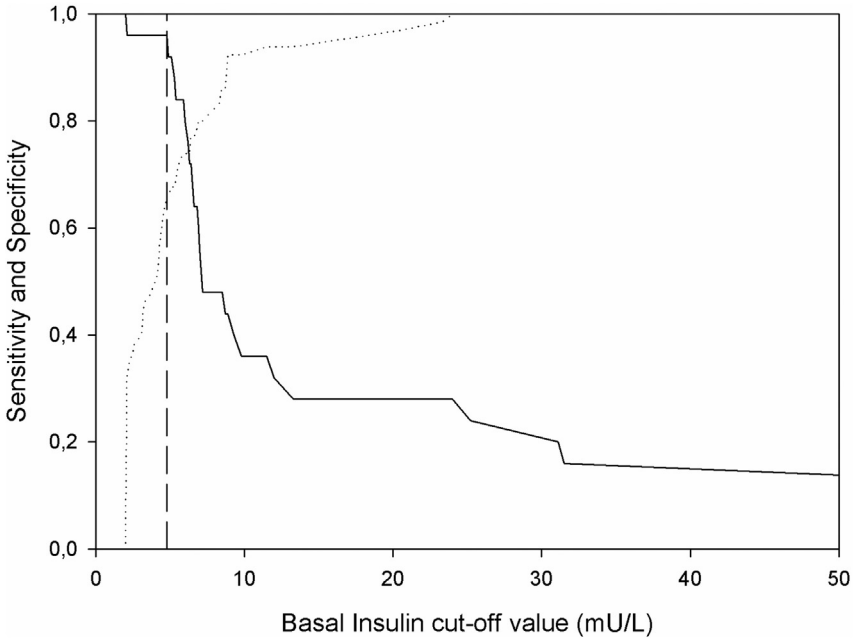

Fig. 2. Plot showing sensitivity (solid line) and specificity (dotted line) of basal insulin at a range of cutoff values, with reference to insulin after OST. The dashed black vertical line indicates the cutoff derived in this study of 5 mU/L. Abbreviation: OST, oral sugar test.

for the horses classified as ID-suspect, and 67.2 mU/L for the horses classified as ID-positive. Median insulin concentration after OST was 16.6 mU/L for the horses classified as ID−not diagnosed, 53.9 mU/L for the horses classified as ID-suspect, and 191 mU/L for the horses classified as ID-positive. Insulin concentration after OST differed significantly between the three categories ($P < .001$).

Fig. 1B shows the glucose concentration in horses of different ID-statuses based on the BIC. Median basal glucose was 4.6 mmol/L for the horses classified as ID−not diagnosed, 5.25 mmol/L for the horses classified as ID-suspect, and 5.10 mmol/L for the horses classified as ID-positive. The glucose concentration of the horses classified as ID-suspect and ID-positive is significantly higher than in the horses classified as ID−not diagnosed ($P < .05$). The glucose concentration after OST was 6.0 mmol/L for the horses classified as ID−not diagnosed, 6.1 mmol/L for the horses classified as ID-suspect, and 7.6 mmol/L for the horses classified as ID-positive (differences between these categories are not statistically significant).

Fig. 1C shows the BIC, as well as the insulin concentration after OST, in horses considered ID-negative or ID-positive based on the OST. Median BIC was 3.7 mU/L in the horses classified as ID-negative and 7.1 mU/L in the horses classified as ID-positive (difference is statistically a trend, $P < .10$). Median insulin concentration after OST was 12.2 mU/L in the horses classified as ID-negative and 72.9 mU/L in the horses classified as ID-positive.

Fig. 1D shows the glucose concentration in horses of different ID-statuses based on the OST. Median basal glucose was 4.6 mmol/L for the horses classified as ID-negative and 4.7 mmol/L for the horses classified as ID-positive. The difference between the two categories was not statistically significant. The glucose concentration after OST was 5.9 mmol/L for the horses classified as ID-negative and 6.4 for the horses classified as ID-positive. The difference between these two categories was statistically significant ($P < .01$).

3.2. Diagnostic Performance of the BIC Using the OST as a Reference

Using the OST as a reference, a receiver operating characteristics curve was made to calculate the optimal cutoff value for the BIC (as shown in Fig. 2). Using Youden's index to maximize test efficiency, the optimal cutoff value for BIC was 5 mU/L, with an associated sensitivity of 96% and specificity of 66%.

The sensitivity and specificity of the BIC with reference to the OST was calculated to be 25% and 95%, respectively, at a cutoff value of 20 mU/L (ID-suspect according to the recommendations of the EMS working group [14]), whereas at a cutoff value of 50 mU/L (ID-positive according to the recommendations of the EMS working group [14]), sensitivity and specificity were 7% and 100%, respectively.

3.3. Comparison of OST Results After Subdivision of the Total Study Group in Different BCS Categories

To evaluate the outcome of the OST in horses of different body condition, we categorized the study group as follows: horses with a BCS of 4 and 5 (considered to be in a lean condition), horses with a BCS of 6 (moderate condition) and horses with a BCS of 7−9 (obese condition). This resulted in 28 horses being classified as in a lean condition, 15 horses as in a moderate condition, and 47 horses as being obese. In the category of obese horses, the OST classified 38% of the horses as ID-positive. In the category of horses with a moderate BCS, the OST classified 13% of the horses as ID-positive. In the category of lean horses the OST classified 18% of the horses as ID-positive. In the obese group a significantly higher percentage of horses is considered to be ID-positive compared with the lean and moderate group ($P < .05$).

Fig. 3A shows insulin concentration after OST in categories of horses with different BCSs. The OST resulted in a median insulin concentration of 12.1 mU/L in the lean horses, 12.4 mU/L in the moderate horses, and 26.2 mU/L in the obese horses. The insulin concentration after OST in the obese horses was significantly higher ($P < .01$) when compared with the horses in a lean or moderate body condition.

Fig. 3B shows the glucose concentration after OST, in categories of horses with different BCSs. The OST resulted in a median glucose concentration of 5.65 mmol/L in the lean horses, 5.70 mmol/L in the moderate horses, and 6.20 mmol/L in the obese horses. The glucose

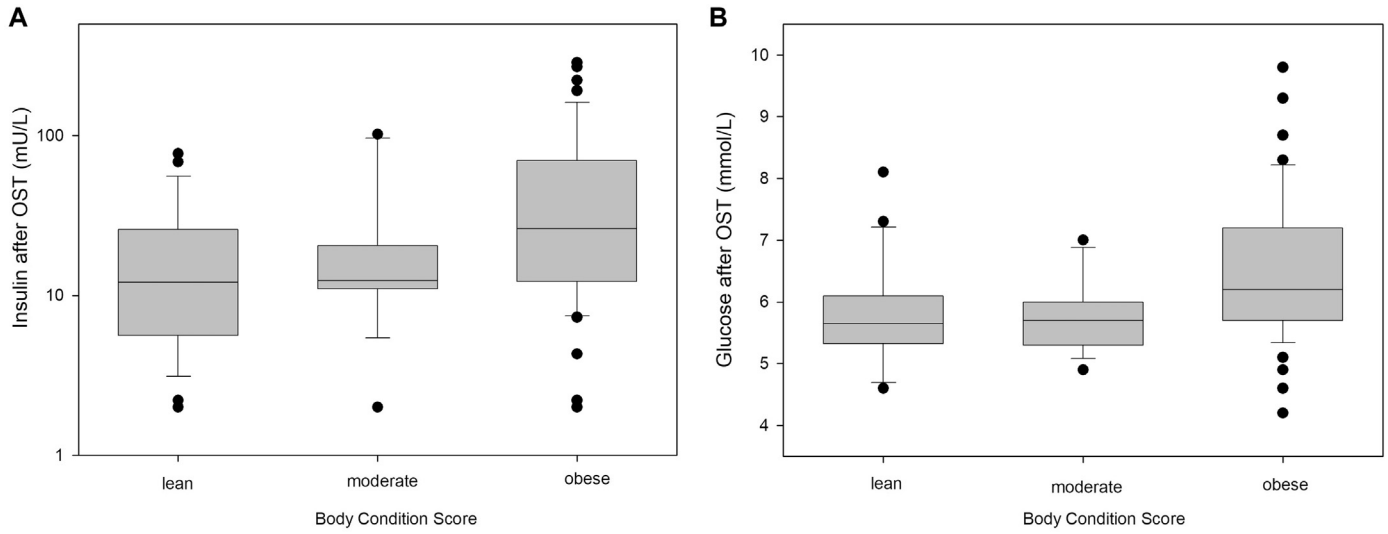

Fig. 3. (A) Insulin concentration (log scale) after OST in horses of different BCS categories. (B) Glucose concentration after OST in horses of different BCS categories. Abbreviations: BCS, body condition score; OST, oral sugar test.

concentration after OST in the obese horses was significantly higher ($P < .01$) than the concentration in the horses with a lean or moderate body condition.

3.4. Comparison of OST Results After Subdivision of the Total Study Group in "Easy Keepers" and "Non-easy Keepers"

Furthermore, we divided the study group into horses belonging to a breed type or cross-breed type regarded to be "easy keepers" (n = 60) and compared them with horses considered to belong to "non-easy keeper" breeds (n = 30). In the group of "non-easy keepers", the OST classified 17% as ID-positive, whereas in the group of "easy keepers", the OST classified 33% of the horses as ID-positive. There is a trend ($P < .10$) for more horses to be identified as ID-positive in the group of "easy keepers" than in the group of "non-easy keepers".

Fig. 4A shows the insulin concentration after OST in these two categories.

After the OST, the "easy keepers" in our study population showed a statistically significantly higher ($P < .05$) median insulin concentration (20.9 mU/L) compared with the "non-easy keepers" (12.1 mU/L).

Fig. 4B shows the glucose concentration after OST in blood of "easy keepers" versus "non-easy keepers".

The glucose concentration after OST was 5.9 mmol/L in the "non-easy keepers" versus 6.3 mmol/L in the "easy keepers". This difference was statistically a trend ($P < .10$).

4. Discussion

Insulin dysregulation in horses bears the risk of triggering laminitis in affected individuals due to the potential occurrence of hyperinsulinemia associated with this condition [3,5]. Testing horses for ID can help to identify individuals at risk and thus play a role in subsequent prevention of laminitis [1,10]. The BIC is widely used in ambulatory practice for diagnosis of ID, mainly because of its practicality. However, nowadays it is known that not all insulin dysregulated horses show an increased BIC, although observations in this area mainly result from few studies [1,10,11]. In addition, according to Bertin and de Laat [1], all of the currently available tests suffer limitations making this an area in desperate need of further research. Although a gold standard has not yet been

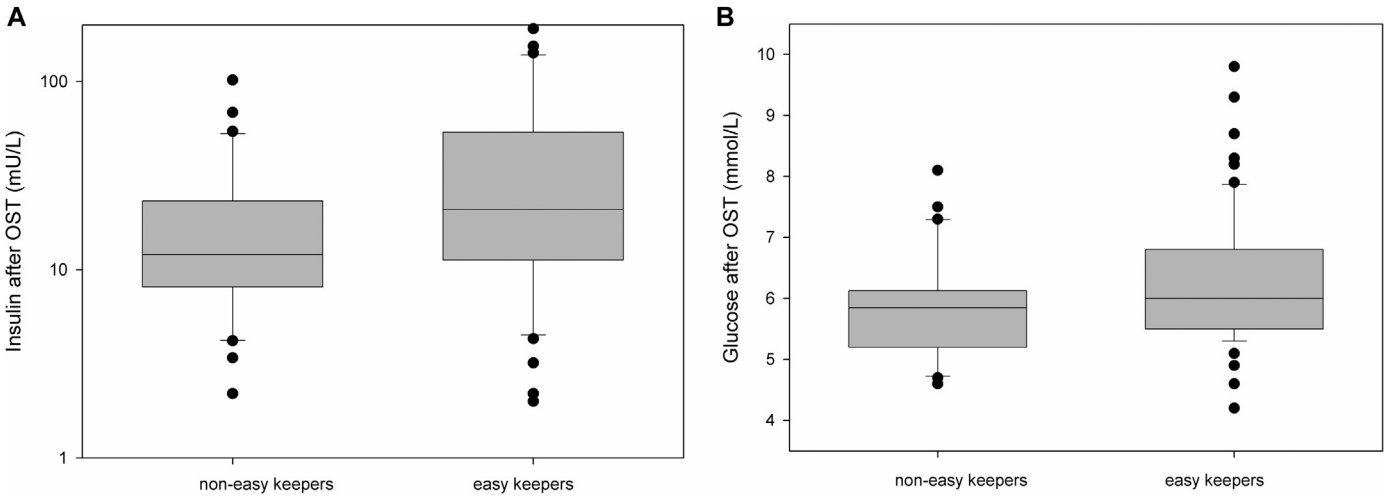

Fig. 4. (A) Insulin concentration after OST (log scale) in easy keeper—and non-easy keeper breeds. (B) Glucose concentration after OST in easy keeper—and non-easy keeper breeds. Abbreviation: OST, oral sugar test.

established for oral glucose tests [1,15,26,27], for use in daily veterinary field practice, an OST could serve as a practical alternative to the BIC [13,19]. The OST is a dynamic test that may reflect a naturally occurring course of events that pose a risk for laminitis more closely than the BIC, by measuring the insulin response to an oral dose of carbohydrates (commercially available corn syrup), thus mimicking ingestion of concentrates or nutritious grass [12–14,28]. It is hypothesized that the OST might detect postprandial hyperinsulinemia before fasting hyperinsulinemia develops [27]. Recently, however, Dunbar et al [10] stated that although they found the OST, as well as the BIC, to be highly specific, the OST as they performed it was also poorly sensitive. Therefore they concluded it had no added diagnostic value compared with the BIC. In our study, we used a higher amount of corn syrup to perform the OST (0.45 mL/kg bwt instead of 0.15 mL/kg) with a matching adjusted cutoff value which should improve test performance (increased sensitivity combined with a high specificity) [14,19] (A. Durham, personal communication). Repeatability of the OST is reported to be acceptable at T = 75 [27], although taking multiple samples and calculating an AUC would provide more repeatable results [19]. However, single blood sampling is considered much more convenient for use in ambulatory practice, thus making it more likely that an OST will actually be carried out for use as a screening test. For this reason, in our study, we have opted for a protocol using a single time point for blood sampling at T = 75.

When we compared the BIC and the OST in our overall study population (section 3.1), the OST identified significantly more horses as ID-positive than the BIC identified horses to be ID-suspect or ID-positive. For horses that were ID-positive according to the BIC, the ID status was in all cases confirmed by the OST. Although horses that are ID-positive based on the OST show a trend of having a higher BIC than horses considered ID-negative (Fig. 1C), 18 of the 25 ID-positive horses stay below the cutoff value of 20 mU/L resulting in a low sensitivity (25%) and therefore a limited use for clinical interpretation. Olley et al [11] found an optimal cutoff value for basal insulin of 5.2 mU/L with reference to the combined glucose-insulin test result. At this cutoff value, the sensitivity was 63% and the specificity 87%, whereas we found a sensitivity of 96% and specificity of 66% at a similar cutoff value using the OST as a reference. However, as discussed before, at a specificity of >95% the sensitivity is much lower. A good test sensitivity to identify horses with an aberrant insulin metabolism should be considered very important because a false-negative test result may place a horse at greater risk of acquiring laminitis, and is therefore detrimental to the horse in question [10]. On the other hand, a good specificity is also deemed desirable to avoid too many false-positive test results. Taking these considerations into account, in our hands, the OST showed superior diagnostic performance as compared with the BIC.

As mentioned before, in addition to (postprandial) hyperinsulinemia, IR is part of what is nowadays referred to as ID. It has been reported that horses and ponies affected with IR tend to be characterized by a high normal or slightly elevated basal glucose concentration [8,29]. In addition, Schuver et al [13] reported ID-positive horses to have significant higher basal glucose values compared to ID-negative horses. Although we found the median basal glucose concentrations in the ID-suspect and ID-positive horses (based on the BIC) to be significantly higher than in the horses in which ID was not diagnosed, only a limited number of horses were included in the ID-suspect and ID-positive group with much variation between individuals. Moreover, as shown in Fig. 1D, in horses considered ID-positive according to the OST, the mean basal glucose did not differ significantly from that in horses considered ID-negative. Therefore, basal glucose concentration was not useful for clinical interpretation in our study group.

After OST, all horses but one showed a higher blood glucose concentration compared with their basal state. An increase was logically to be expected after administration of corn syrup, so the reason why one specific horse showed a slight decrease (of 0.3 mmol/L) remains unknown. Despite the fact that intake of the corn syrup was closely monitored by the visiting veterinarian, incidental loss due to leakage out of the mouth cannot be completely excluded. Another possible explanation is that an aberrant gastric emptying time or intestinal motility may have led to this variation [30]. We found a significantly higher glucose concentration at T = 75 in the horses considered to be ID-positive (based on the OST) compared with the ID-negative horses (Fig. 1D). Furthermore, median glucose concentration after administration of 0.45 mL/kg bwt corn syrup increased with 1.30 mmol/L in horses diagnosed as ID-negative, whereas the ID-positive horses showed an increase of 1.82 mmol/L. This difference in increase is statistically significant ($P < .05$). A study that compared median glucose concentrations during an OST using 0.15 mL/kg corn syrup orally reported a comparable finding: the glucose concentrations were significantly higher in EMS horses compared with a control group at the several different time points measured [13]. This could possibly be explained by the fact that ID can be the result of tissue insulin resistance and therefore a (partial) failure of insulin to elicit a fast and effective response in lowering blood glucose when it is increased [1,31]. Although in our study, we found the OST to result in a significantly higher glucose concentration at T = 75 minutes in ID-positive horses compared with ID-negative horses, we agree with Manfredi [26] who states that glucose concentration during an OST (using 0.25 mL/kg bwt corn syrup) was not useful as an indicator of ID. Variation in increase of glucose concentration between individual horses in our study group appeared too large to consider it useful for clinical interpretation.

After subdivision of the group into lean, moderate, and obese horses (section 3.3), it turns out that in the group of obese horses the OST identifies significantly more horses as ID-positive compared with the group of horses in a lean and moderate body condition. Furthermore, the obese horses showed a significantly higher median insulin level after OST (26.2 mU/L) compared with the horses in a moderate (12.4 mU/L) and lean (12.1 mU/L) condition. This is consistent with the view that obesity is correlated with an increasing incidence of ID [32,33] and corresponds with literature which reports that insulin sensitivity is lower in obese horses as compared with lean horses [7,23,34]. Another interesting observation was that five of 28 horses with a lean body condition were found to be ID-positive according to the result of the OST. This supports the conclusion of Manfredi [26] that a lack of obesity should not preclude a diagnosis of ID in a horse.

Hoffman et al [23] estimated obese horses in his study to have an approximately 80% lower insulin sensitivity compared with nonobese horses. This might explain the significantly higher glucose concentration we found after OST in the obese horses versus the lean or moderate conditioned horses: in obese horses, the glucose is not transported into the cells as quickly as normal. It also matches findings of Frank et al [29] who demonstrated, using a combined glucose-insulin test, that plasma glucose concentrations returned significantly slower to the baseline values in obese-IR horses than in nonobese horses.

Breed-related differences in insulin dynamics have been observed in several studies [25,26,31]. It is tempting to attribute this to the tendency of a breed to get obese, but a study in a group of ponies and horses with a mean BCS of 5.0 ± 0.3 belonging to three different breed types found clear differences in insulin responses to oral and intravenous glucose administration. This indicates that breed-related differences in insulin dynamics also occur

independent of obesity [24]. It is hypothesized that insulin resistance may have been a positive adaptation in the past in situations where nutrition for horses was limited, with decreased nutrient availability maybe having promoted selection of an insulin-resistant genotype (so-called "thrifty genotype" or "easy keepers") [5,8,35].

If we make a comparison in our study group based on the result of the OST, indeed a higher proportion of "easy keeper" horses were classified as ID-positive compared with the "non-easy keepers", although statistically this was only a trend. Furthermore, after OST, a significant difference in insulin concentration and modest difference in glucose concentration was observed between the two groups of breeds. Currently little is known about the contribution of the genome to the development of abnormalities in the insulin metabolism of horses. However, there are some studies available that report a different insulin response after OST in different breed types [24,26]. A recent study, performed in two breeds commonly regarded as "easy keepers" (Welsh ponies and Morgan horses), found insulin after OST to be moderately to highly heritable [36]. Hopefully in the future, more data regarding identification of genetic risk factors will become available, thus enabling better prevention of ID-related laminitis in specific horse breeds.

A limitation of our study was that we did not conduct a technique to assess specifically for tissue insulin resistance (i.e., the euglycemic hyperinsulinemic clamp = EHC method or the frequently sampled intravenous glucose tolerance test = FSIGTT) in addition to the contribution of the enteroinsular axis that the OST focuses on. This would have generated a more complete picture of the alterations in insulin metabolism [1,28]. However, we considered these procedures too complex and time-consuming to perform under the field conditions in which our study group was sampled [12]. Even though the two-step insulin response test described by Bertin is described as a safe, rapid, and low-cost method to diagnose IR [37], we have not applied this either because it could incite many of the horse owners not to participate in this study, as this procedure involves intravenous administration of insulin. Furthermore, it would have required another visit for all of the selected horses because it cannot be combined with an OST at the same time. Something else that deserves attention is the fact that the outcome of the OST may vary due to many factors, including age, diet during the period before the test, whether the horse is experiencing any stress, disease and pasture composition and season [18,30,38]. In addition, in our study, owner compliance in following instructions concerning the test protocol is a factor to consider. The animals in the present study may indeed have differed somewhat in these respects. However, they were all tested in October/November during the stable period without having access to pasture and they stayed in their own trusted environment. Moreover, the relatively large number of horses included should ensure that individual differences are leveled as much as possible.

5. Conclusions

Our study confirms that the sensitivity of the BIC as compared with the OST (as we performed it), is low (25% and 7% respectively) at the cutoffs of 20 and 50 mU/L defined in the EEG recommendations. At a lower cutoff, for example, the maximum Youden cutoff of 5 mU/L, sensitivity increases to 96%, but the specificity decreases from 95 % to 66 %. Therefore, to our opinion, under field conditions, the OST is preferred over the BIC for use as a screening test for ID.

In addition, our results support the assumption that obese horses and "easy keeper" breeds have a higher tendency of having ID. Further studies are required to assess the repeatability of the OST as we performed it, under different circumstances and within an individual. Another recommendation for future studies could be to combine the OST, which focuses on the enteroinsular axis, with a method that is more applicable for estimating tissue insulin resistance to optimize the diagnosis of ID with the aim of improving the detection of horses at risk of laminitis.

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