Rasayely
International Journal of Equine Science
https://rasayely-journals.com/index.php/ijes
Vol 1(1); 3–10, 2022

Original Article Open Access

Haptoglobin, Magnesium, Adiponectin, and Leptin as Biomarkers for Obesity and Insulin Dysregulation in Horses

G.H.M. Counotte[1,*], A. Durham[2], V. Vandendriessche[3], and L. van den Wollenberg[4]

[1]*CVier, Steenboerweg, The Netherlands*
[2]*Liphook Equine Hospital, Hampshire, United Kingdom*
[3]*PAVO, Heijen, The Netherlands*
[4]*GD Animal Health, Deventer, The Netherlands*

[*]Author to whom any correspondence should be addressed; e-mail: guillaume.counotte@tip.nl

Received: 02 November 2021; Revised: 14 December 2021; Accepted: 13 January 2022; Published: 09 February 2022

Academic Editor: Gigi kay, The American Fondouk Veterinary Clinic, Morocco

Abstract

The aim of this project was to identify blood analytes which are associated with obesity and/or insulin dysregulation (ID) in horses. For this study, 57 horses were selected. At the start, body condition score (BCS) was established, and an oral sugar test (OST) was performed. Furthermore, the following blood analytes were determined: basal glucose, insulin, albumin, high molecular weight (HMW) adiponectin, leptin, haptoglobin, total protein, calcium, magnesium, phosphate, non-esterified fatty acids, and also glucose and insulin 75 min after an OST. Horses were classified according to their body condition as 'lean' (BCS 4/9-6/9) or 'obese' (BCS > 6/9). Horses were classified as insulin dysregulated (ID-positive) when insulin was 40 mU/L or higher than 75 min after OST. Multivariable logistic regression analysis showed a correlation between obesity and HMW adiponectin, leptin, total protein, and magnesium and between ID, haptoglobin, and leptin. Receiver operating characteristic plots (ROC) of obesity with leptin, adiponectin, total protein, and magnesium, which had an area under the curve (AUC) of 0.932, showed that the combination of these four blood analytes has a high association with obesity. The AUC of the ROC analysis of ID with haptoglobin and leptin was 0.805 implicating that this combination has a high association with ID. Although obesity can be observed (eyes and hands), the health risk of obesity can be monitored with a combination of leptin, adiponectin, total protein, and magnesium, while leptin and haptoglobin are good markers for ID (a health risk of obesity).

Keywords

Horse; obesity; adiponectin; leptin; haptoglobin; insulin dysregulation

1. Introduction

Approximately 400,000 horses live in The Netherlands [1]. About 50,000 of these are active competitive athletes, while the remaining 350,000 horses are kept for recreational purposes. It has been estimated that approximately 55% of horses kept for recreational purposes are 'overweight' [2]. Excessive weight has been associated with various health issues such as poor performance, abnormal thermoregulation, reduced fertility, mesenteric lipomata, insulin dysregulation (ID), and laminitis [3,4]. It is obvious that not every health risk is easy to determine. A body condition score (BCS) above 6.8/9 indicates that the total body fat is greater than 20% and thus a risk of adipose-related adverse health effects [5].

Basal insulin concentration is traditionally often used to identify horses suffering from ID and thus risks of disorders in horses due to prolonged or excessive hyperinsulinemia as part of EMS [6,7]. However, other biomarkers measured in blood are also described that could give a better indication of whether adverse health consequences of adiposity are present [6,8]. When obesity or any associated health consequences

are indeed established, an attempt should be made to reduce body weight by dietary measures and/or exercise [9]. In the recent literature, adiponectin [10–14], leptin [6,15], NEFA [16], haptoglobin and IL-6 [17,18], SAA [11], insulin [19–21], and RBP4 (retinol binding protein 4 [22] are all mentioned as potential markers for obesity and associated with health consequences. The aim of this project was to identify blood analytes, which are associated with obesity and/or ID in horses for monitoring health risk.

2. Materials and Methods

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

2.1. Animals

In total, 57 horses were selected as described in the study by van den Wollenberg *et al.* [23]. The breeds eventually included were the following: Belgian Warmblood (6), Belgian Sport Horse (2), Dutch Warmblood (8), Arabian (6), Friesian (2), and mixed breeds of these (9). Also included were Tinkers (2), Haflingers (6), Lusitanos (4), Welsh Ponies (7), New Forest ponies (3), and Fjord horses (2). The mean weight of the horses was 498 kg (SD: 105 kg, min: 199, max: 693 kg). All horses were in good health.

2.2. Study Design

Horses were visited at their home premises, and BCS was determined as described in the research by van den Wollenberg *et al.* [23]. The BCS of the horse was assessed as described by Henneke [24,25], ranging from BCS 1 (very poor) to BCS 9 (extremely fat). A horse with a score of 4/9 to 6/9 was considered 'lean'. A horse with a BCS higher than 6/9 was considered to be 'obese'.

2.3. Blood Samples

Blood samples were taken by two experienced veterinarians and sent to the laboratory (chilled) within 24 hours after sampling. Upon arrival, the blood samples were centrifuged (10 min/3000 g) and stored at -20°C until further analysis was performed. Testing was performed at the laboratory of the GD Animal Health (Deventer, The Netherlands), except for HMW adiponectin and leptin. HMW adiponectin was analyzed at the Liphook Equine Hospital (UK), and leptin was analyzed at the laboratory of the KU-Leuven (Belgium).

2.3.1. Blood Parameters

At T = 0, the following analytes were determined in the blood sample: albumin, haptoglobin, total protein, calcium, magnesium, phosphate, and non-esterified fatty acids (NEFA). Tests were carried out using standard operating procedures of the laboratory on a Synchron DxC 600 analyzer. All tests were validated for equine samples: haptoglobin: Tridelta kit; calcium: arsenazo method; magnesium: xylidyl blue method; phosphate: ammonium-molybdate method; albumin: bromocresol green method; total protein: biuret method; NEFA: oxidase/peroxidase method.

High Molecular Weight (HMW) adiponectin was measured (Liphook Equine Hospital, UK) using an ELISA (MyBiosource Inc., P.O. Box 153308, San Diego, CA 92195-3308, USA): 'Horse High Molecular Weight Adiponectin ELISA Kit', Catalog # MBS028647. The adiponectin assay has a detection limit of 0.1 mg/L and a range of 0.25-8 mg/L. The intra-assay and inter-assay variabilities are 5.1% and 12.9%, respectively, with 93.7% recovery from mixed horse samples of varying concentrations.

Leptin was analyzed with RIA (Merck XL-85K) at the laboratory at the KU-Leuven (Belgium) (Dr. Daniel Vermeulen).

2.3.2. Insulin Dysregulation (ID)

All horses were examined for ID using an oral sugar test (OST) that was carried out as described in the study by van den Wollenberg *et al.* [23]. A horse was considered to have ID (ID-positive) when insulin concentration at T = 75 min was 40 mU/L or higher [26].

Insulin was measured in heparinized plasma with a Siemens Immulite 1000 using the insulin kit 6602443. Plasma was used instead of serum because during our validation of insulin in horse blood, we found insulin to be stable during 24 hours in plasma, while in serum insulin it appeared to be 15% lower after 24 hours (≈ the average time needed for field samples to arrive at the laboratory by post). Determination of glucose in NaF-blood was performed using the hexokinase method (Beckman) on a Synchron DxC 600.

2.4. Statistical Analysis

Stata (14.1, Stata Corp US) was used for statistical analysis. All figures were made with SigmaPlot (version 13, Systat Software). Regression analysis was performed, and all residuals of variables were tested for normality (Shapiro Wilk W test for normal data). Residuals of glucose, insulin, HMW adiponectin, leptin, NEFA, and haptoglobin were found to be not normally distributed. Median (P50) and interquartile ranges are reported for not-normally distributed biomarkers. Statistical differences were tested using Kruskal Wallis calculations. All blood analytes were tested for significant differences when using binomial classification of ID (yes or no) and obesity (yes or no). Blood analytes that were significantly different in outcome were combined in logistic regression. Logistic regression was performed for obesity (yes or no) or ID (yes or no) for blood analytes with a p value less than 0.25 combined in one regression. When regression yielded an outcome with a significance of 0.05, the blood analyte was used in a receiver operating characteristic plot, and sensitivity and specificity were calculated for each concentration. Youden's index analysis was used to calculate optimal cut-off values.

3. Results

The median age of the 57 horses used in this study was 10 years (interquartile range / minimum and maximum: Q1 = 7, Q3 = 13, min: 3, max: 20). There was a tendency (R2 = 0.10) that younger horses had a higher BCS compared to older horses.

The number of animals per combination (obese or lean and ID-positive or ID-negative) is shown in **Table 1**. In the 'obese' group, 50% of the horses were considered to be ID-positive, while in the 'lean' group, this was only the case for 17% of the horses.

Data were tested for normality. The outcome of the Shapiro-Wilk W test is shown in **Table 2**. Haptoglobin, NEFA, leptin, and adiponectin were not normally distributed (p > [z] <

0.05), albumin, total protein, calcium, magnesium, and phosphate were normally distributed.

Table 3 lists the mean results of blood analytes of obese horses (BCS > 6/9) and lean horses (BCS < 6/9). HMW adiponectin showed a tendency to be lower in obese horses, as expected. An unexpected finding was that magnesium was significantly lower in obese horses. Leptin and NEFA were significantly higher in obese horses.

Table 1: Number of horses classified according to BSC and ID.

		Obese	Lean	Total
Insulin Dysregulation	Positive	17	4	21
	Negative	17	19	36
Total		34	23	57

Obese horse: BCS > 6/9; lean horses: BCS < 6/9
ID positive: insulin after OST ≥ 40mU/L; ID negative: insulin after OST < 40mU/L

Table 2: Statistical results of Shapiro-Wilk W test for blood analytes.

Variable	Obs	W	Z	$p > \|z\|$
Albumin	57	0.97430	0.630	0.264
Haptoglobin	57	0.92673	2.882	0.002
Total protein	57	0.98248	-0.193	0.577
NEFA	57	0.86407	4.210	0.001
Calcium	57	0.98288	-0.242	0.596
Magnesium	57	0.98772	-0.957	0.831
Phosphate	57	0.96542	1.268	0.102
Leptin	57	0.82630	4.737	0.001
Adiponectin	57	0.61412	6.452	0.001

Table 3: Results of blood analytes in obese horses compared to lean horses.

Analyte	Obese (n = 34)		Lean (n = 23)		*p* value
	P50	IQR	P50	IQR	
HMW adiponectin (mg/L)	0.97	0.55	1.46	1.48	0.06
Glucose (resting) (mmol/L)	4.6	0.30	4.6	0.30	0.53
Insulin (resting) (mU/L)	5.4	4.9	3.4	6.5	0.58
Glucose after OST (mmol/L)	6.4	1.2	5.4	1.5	< 0.002
Insulin after OST (mU/L)	38.6	53.8	10.2	16.3	< 0.002
Leptin (µg/L)	14.2	16.4	2.5	4.8	< 0.001
Haptoglobin (g/L)	1.4	1.1	1.3	0.4	0.36
NEFA (mmol/L)	0.16	0.13	0.11	0.08	0.03
	Mean	SE	Mean	SE	*p* value
Total protein (g/L)	68.5	0.76	66.3	1.13	0.12
Albumin (g/L)	35.0	0.35	34.8	0.44	0.49
Calcium (mmol/L)	2.89	0.017	2.92	0.022	0.47
Phosphate (mmol/L)	1.18	0.03	1.11	0.03	0.089
Magnesium (mmol/L)	0.75	0.013	0.81	0.019	< 0.005

Obese horse: BCS > 6/9; lean horses: BCS < 6/9
P50: median, IQR: interquartile range, SE: standard error

The results of analytes measured in the blood of ID-positive horses and ID-negative horses are shown in **Table 4**. Basal glucose and basal insulin were not different between ID-positive and ID-negative horses, as expected. Magnesium in the blood of ID-positive horses was not different from ID-negative horses, but haptoglobin and leptin were both statistically significantly higher in ID-positive horses compared to ID-negative horses.

Blood analytes that showed a difference ($p < 0.25$) between lean and obese horses were used in a logistic regression. The same was done for blood analytes that showed a difference ($p < 0.25$) between ID-positive and ID-negative horses. The outcome of the logistic regressions is shown in **Tables 5** and **6**. The results of these calculations show that HMW adiponectin, leptin, total protein, and magnesium were correlated with BCS when using multivariable logistic regression. The results of the calculations of the multivariable logistic regression of ID with blood analytes showed that leptin and haptoglobin were correlated with ID.

Blood analytes that were significantly different ($p < 0.05$) in the multivariable logistic regression of obesity (HMW adiponectin, leptin, total protein, and magnesium) were used to calculate receiver operating characteristic plots, and sensitivity and specificity were calculated for each concentration. Corresponding plots are shown in **Figure 1** (BCS) and **Figure 2** (ID).

The cut-off values for both sets of plots and the AUC are listed in **Table 7**. The optimal cut-off values for BCS (obese or lean) were calculated using Youden's index: 8.9 µg/L for leptin, 0.77 mmol/L for magnesium, 1.46 mg/L for HMW adiponectin, and 65.1 g/L for total protein. For ID (ID-positive or ID-negative), the optimal cut-off values were 5.66 µg/L for leptin and 1.3 g/L for haptoglobin.

When the significant blood analytes are combined into one ROC-plot, the AUC of the plot for BCS is 0.932 and for ID it is 0.805 (see **Figure 3**).

4. Discussion

The aim of this project was to identify blood analytes, which are associated with obesity and/or ID in horses for monitoring health risk. In our study group, 50% of the obese horses showed an insulin after OST of 40 mU/L or higher, while in the lean group only 17% were diagnosed to be ID-positive. This ratio (50/17 = 2.94) is comparable with that found in humans: in a study performed in 2015 [27], 17.3% of the human population (above 4 years of age) in the Netherlands which were classified as 'obese' were diagnosed with insulin dysregulation, while only 3.3% of people classified as lean were diagnosed with insulin dysregulation (ratio = 5.2) [27]. In the UK, 12.4% of people with obesity aged 18 years or older

are diagnosed with diabetes, five times as much as people having a healthy weight [28].

We found magnesium to be significantly different in lean versus obese horses (0.81 versus 0.75 mmol/L, $p < 0.005$). This finding that magnesium is lower in obese horses is novel. In humans, a relation between obesity and lower magnesium has already been described [29]. Nadler et al. postulated that in humans a lower magnesium concentration is causally associated with ID [30]. Subclinical hypomagnesemia is common in critically ill humans and animals [31], but obesity is not a critically ill situation. A possible explanation could be taken from the fact that that hypomagnesaemia is associated with increased cytokine production as in humans [32]. Reactive white adipose tissue in horses secretes cytokines [33] and therefore could result in magnesium being lower in obese horses.

We found that the differences between HMW adiponectin in the blood of obese horses and lean horses were less pronounced compared to previous studies in horses [9,11,33]. Menzies-Gow *et al.* had a similar finding in their study [34]. Adiponectin is inversely associated with the quantity of white adipose tissue (WAT) in horses [11,14] and is a protein hormone that regulates a number of metabolic processes in humans: glucose regulation and fatty acid oxidation [35]. Recently, Karikoski *et al.* also found that horses with ID had lower serum adiponectin concentrations [36], longer neck circumference, and larger height than horses in the non-ID group. Menzies-Gow *et al.* found total adiponectin to be significantly lower ($p < 0.01$) in previously laminitic ponies (mean ± S.D. 8.9 ± 2.9 mg/L) compared to non-laminitic ponies (24.2 ± 11.8 mg/L) [37]. An immunoturbidimetric method was used for measuring HMW adiponectin, and it was compared with ELISA methods. They found that none of the ELISA methods could be validated satisfactorily, and therefore, concluded that ELISA methods are not suitable for the measurement of equine adiponectin. The HMW adiponectin concentrations found in this study were comparable to those found in previous studies in horses [11,30] and humans [30,38]. However, leptin was an even better marker for obesity (median 2.5 µg/L for lean horses versus 14.2 µg/L for obese horses, $p < 0.001$). Leptin values can differ markedly depending on the analytical method and specific antibody used, and one must be careful when comparing results from different methods mutually. Therefore, our results cannot be directly compared with older leptin results found in horses [9,14,38,39]. However, our results are comparable with newer publications [34,40]. Leptin results in 'lean' horses (median 2,5 µg/L, interquartile range 4.8 µg/L) in our study are comparable with the results of other studies: for example, Kedzierksi found a basal leptin concentration of 1.4 – 3.1 µg/L for horses with a BCS of 5.0/9 or lower and 1.2 – 8.2 for horses with a BCS of 6.6/9 [40]. Pleasant *et al.* reported an average leptin of 3.5 µg/L (CI: 3.2 – 3.7) in 300 mixed light breed horses in Southwest Virginia [41]. Leptin itself is not considered to be a good indicator for ID [26]; however, in our study, we found leptin to be significantly correlated with ID ($p < 0.05$): higher leptin is higher risk for ID (AUC of ROC-plot is 0.664) because obese horses have a higher chance of having ID.

Table 4: Results of blood analytes in ID-positive horses compared to ID-negative horses.

Analyte	ID positive (n = 21)		ID negative (n = 36)		*p* value
	P50	IQR	P50	IQR	
HMW adiponectin (mg/L)	0.99	0.47	1.17	1.11	0.25
Glucose (resting) (mmol/L)	4.6	0.4	4.6	0.3	0.27
Insulin (resting) (mU/L)	6.9	2.9	2.6	3.6	< 0.001
Glucose after OST (mmol/L)	6.6	1.2	6.0	1.2	< 0.02
Insulin after OST (mU/L)	70.0	57.0	12.2	1.5	< 0.001
Leptin (µg/L)	11.6	15.1	4.6	13.0	< 0.05
Haptoglobin (g/L)	1.8	0.8	1.2	0.7	< 0.002
NEFA (mmol/L)	0.18	0.15	0.14	0.08	0.14
	Mean	SE	Mean	SE	*p* value
Total protein (g/L)	68.7	0.88	66.9	0.89	0.26
Albumin (g/L)	35.2	0.40	34.8	0.35	0.22
Calcium (mmol/L)	2.91	0.018	2.90	0.020	0.61
Phosphate (mmol/L)	1.14	0.05	1.16	0.03	0.65
Magnesium (mmol/L)	0.77	0.015	0.77	0.017	0.88

ID positive: insulin after OST ≥ 40mU/L; ID negative: insulin after OST < 40mU/L
P50: median, IQR: interquartile range, SE: standard error

Table 5: Results of multivariable logistic regression of obesity with blood analytes.

			N	57	
BCS (lean or obese)			LR (Chi^2)	48.64	
			Prob > Chi^2	0.0001	
			Pseudo R^2	0.6326	
Variable	coef.	Z	*p* > |z|	95% CI	
HMW adiponectin	-1.824	1.96	0.050	-3.64	-0.01
Glucose after OST	0.124	0.01	0.988	-1.60	1.63
Insulin after OST	0.527	2.55	0.159	0.066	0.507
Leptin	0.287	2.55	0.011	0.066	0.507
NEFA	10.99	1.62	0.104	-2.27	24.26
Total protein	0.475	2.23	0.026	0.057	0.894
Phosphate	3.07	0.83	0.405	-4.16	10.31
Magnesium	-15.44	-1.98	0.048	-30.743	-0.141
Constant	-26.186	-1.78	0.076	-55.082	2.709

CI: confidence interval

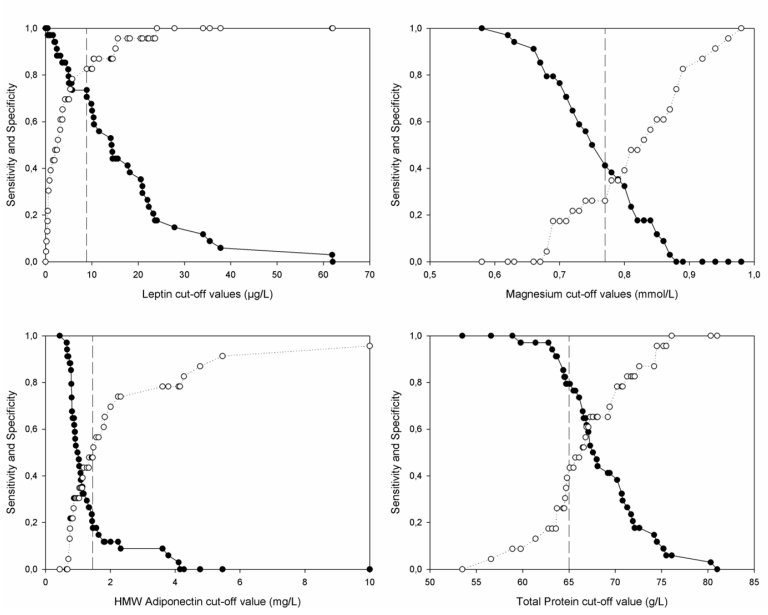

Figure 1(a): ROC-plots showing sensitivity (solid line) and specificity (dotted line) of leptin at a range of cut-off values, with reference to BCS (lean or obese). The black long dashed line indicates the recommended cut-off value (8.90 µg/L). **(b):** ROC-plots showing sensitivity (solid line) and specificity (dotted line) of magnesium at a range of cut-off values, with reference to BCS (lean or obese). The black long dashed line indicates the recommended cut-off value (0.77 mmol/L). **(c):** ROC-plots showing sensitivity (solid line) and specificity (dotted line) of HMW adiponectin at a range of cut-off values, with reference to BCS (lean or obese). The black long dashed line indicates the recommended cut-off value (1.46 mg/L). **(d):** ROC-plots showing sensitivity (solid line) and specificity (dotted line) of total protein at a range of cut-off values, with reference to BCS (lean or obese). The black long dashed line indicates the recommended cut-off value (65.1 g/L).

We found ID-positive horses to have a higher haptoglobin concentration in blood (median 1.8 g/L) compared to ID-negative horses (median 1.2 g/L) ($p < 0.002$). In obese versus lean horses, haptoglobin was not significantly different. In humans, higher haptoglobin serum levels seem to be a strong marker of hyperinsulinemia, independently of body mass index as reported by Pergola *et al.* [42]. Obesity is associated with inflammation of WAT resulting from chronic activation of the innate immune system, which can subsequently lead to insulin resistance, impaired glucose tolerance, and even diabetes mellitus in horses [9]. WAT depots secrete cytokines including IL-6 in horses [33,43] which has been shown to activate transcription of haptoglobin and other acute phase proteins [33]. Haptoglobin is also directly released from adipose tissue, but the amount is quite low in comparison to the concentrations of haptoglobin in the blood of humans [17]. Some previous researchers have also found acute phase proteins to be associated with obesity and ID in humans [17,18,43]. The source of most of these acute phase proteins (such as haptoglobin) is white adipose tissue which upon excessive weight gain becomes infiltrated with macrophages and lymphocytes and undergoes changes in gene expression: IL-6 is excreted when WAT becomes 'reactive' in human [13] and horses [22,44]. As IL-6 activates the production of haptoglobin in the liver, a higher haptoglobin could be expected in horses that are obese and thus at risk for ID. The disadvantage of haptoglobin is the sensitivity for hemolysis. Although in the haptoglobin kit from Tridelta mildly hemolyzed samples may be used, grossly hemolyzed samples (hemoglobin > 2.5 g/l) should be avoided as results may not be reliable.

Table 6: Results of multivariable logistic regression of ID with blood analytes.

ID (positive or negative)			N	57	
			LR (Chi²)	20.2	
			Prob > Chi²	0.0011	
			Pseudo R²	0.2693	
Variable	coef.	Z	$p > \|z\|$	95% CI	
HMW adiponectin	-0.356	-1.17	0.244	-0.954	0.242
Leptin	0.0724	2.35	0.019	0.012	0.133
Haptoglobin	2.194	2.81	0.005	0.666	3.722
NEFA	0.266	0.08	0.940	-6.613	7.145
Albumin	-0.0163	-0.1	0.922	-0.344	0.312
Constant	-3.671	-0.65	0.519	-14.819	7.477

CI: confidence interval

Table 7: AUC and cut-off values calculated with Youden's index.

BCS / blood analyte	AUC	Cut-off value	Youden's index
HMW adiponectin	0.351	1.46 mg/L	-0.35
Leptin	0.831	8.90 µg/L	0.56
Total protein	0.621	65.1 g/L	0.23

Magnesium	0.293	0.77 mmol/L	-0.35
ID / blood analyte	AUC	Cut-off value	Youden's index
Haptoglobin	0.751	1.30 g/L	0.44
Leptin	0.664	5.66 µg/L	0.42

Because leptin and NEFAs were higher in obese horses compared to lean horses and HMW adiponectin and magnesium were lower in obese horses, we combined these findings in a multivariable logistic regression. This analysis showed a significant correlation between obesity and adiponectin, leptin, total protein, and magnesium with an AUC of 0.932 implicating that these four blood analytes have a very good association with obesity.

Furthermore, a significant correlation (multivariable logistic regression analysis) was found between ID and haptoglobin and leptin. The AUC of the combined ROC analysis of insulin dysregulation with haptoglobin and leptin is slightly lower: 0.805, but it still indicates a good association between ID and these two blood analytes.

5. Conclusion

A combination of leptin, adiponectin, total protein, and magnesium was found to have a good association with obesity, and the combination of leptin and haptoglobin had a good association with ID. Therefore, these biomarkers can be used as a monitoring tool for health risk associated with obesity and ID in horses.

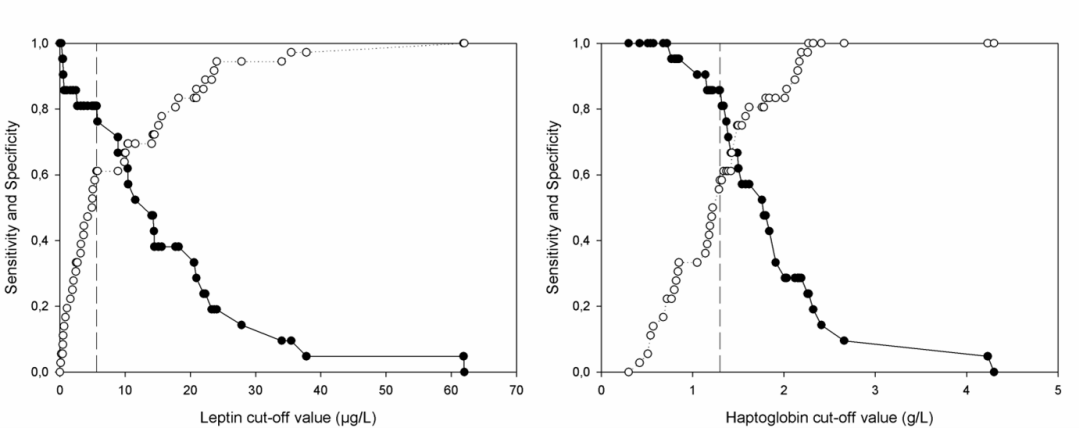

Figure 2(a): ROC-plots showing sensitivity (solid line) and specificity (dotted line) of leptin at a range of cut-off values, with reference to ID (ID-positive or ID-negative). The black long dashed line indicates the recommended cut-off value (5.66 µg/L). **(b):** ROC-plots showing sensitivity (solid line) and specificity (dotted line) of haptoglobin at a range of cut-off values, with reference to ID (ID-positive or ID-negative). The black long dashed line indicates the recommended cut-off value (1.30 g/L).

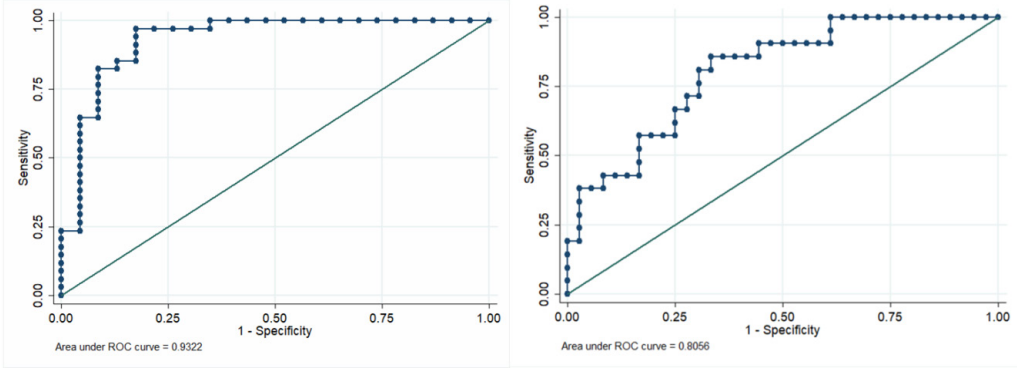

Figure 3(a): Combined ROC-plot: BCS (obese or lean) with significant ($p < 0.05$) blood analytes (leptin, magnesium, HMW adiponectin, and total protein). **(b):** Combined ROC-plot: ID (positive or negative) with significant ($p < 0.05$) blood analytes (leptin and haptoglobin).

Authors' Contributions

G. H. M. Counotte was responsible for laboratory results, statistical analysis, and preparation of the manuscript.

A. Durham was responsible for the adiponectin results (validation and laboratory results), reviewing of the manuscript, and discussing the results.

V. Vandendriessche was responsible for selection of the horses, blood sampling, and contact with horse owners.

L. van den Wollenberg was responsible for the background information, literature, and reviewing of the manuscript.

Acknowledgements

The authors wish to thank C. van Weeghel for analysing the blood samples and Dr. Daniel Vermeulen (KU-Leuven, Belgium) for performing the leptin analysis.

Data Availability Statement

The data supporting the findings of this study are openly available in https://doi.org/10.17026/dans-2b3-f8wj.

Conflicts of Interest

The authors declare that there is no conflicts of interest.

Funding

No funding was received to support this study.

Ethical Approval

The Animal Welfare Body of the Royal GD approved the experiments (according to 2010/63/EU).

References

[1] Gunnink C. [The Netherlands Horse Country: Equestrian Sports and Key Figures] 2016.

[2] Graaf-Roelfsema ED. [Is Being Overweight a Disease?] 2012.

[3] Radin MJ, Sharkey LC, Holycross BJ. Adipokines: A Review of Biological and Analytical Principles and an Update in Dogs, Cats, and Horses. Veterinary Clinical Pathology 2009;38:136–56. https://doi.org/10.1111/j.1939-165X.2009.00133.x.

[4] Suagee JK, Corl BA, Crisman MV, Pleasant RS, Thatcher CD, Geor RJ. Relationships Between Body Condition Score and Plasma Inflammatory Cytokines, Insulin, and Lipids in a Mixed Population of Light-Breed Horses. Journal of Veterinary Internal Medicine 2013;27:157–63. https://doi.org/10.1111/jvim.12021.

[5] Dugdale AHA, Grove-White D, Curtis GC, Harris PA, Argo CM. Body Condition Scoring as a Predictor of Body Fat in Horses and Ponies. Vet J 2012;194:173–8. https://doi.org/10.1016/j.tvjl.2012.03.024.

[6] Bertin FR, de Laat MA. The Diagnosis of Equine Insulin Dysregulation. Equine Veterinary Journal 2017;49:570–6. https://doi.org/10.1111/evj.12703.

[7] Frank N, Tadros EM. Insulin Dysregulation. Equine Veterinary Journal 2014;46:103–12. https://doi.org/10.1111/evj.12169.

[8] EMS Working Group. Recommendations for the Diagnosis and Treatment of Equine Metabolic Syndrome (EMS) 2016.

[9] Gordon ME, McKeever KH, Betros CL, Manso Filho HC. Exercise-Induced Alterations in Plasma Concentrations of Ghrelin, Adiponectin, Leptin, Glucose, Insulin, and Cortisol in Horses. The Veterinary Journal 2007;173:532–40. https://doi.org/10.1016/j.tvjl.2006.01.003.

[10] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, *et al*. Paradoxical Decrease of an Adipose-Specific Protein, Adiponectin, in Obesity. Biochem Biophys Res Commun 1999;257:79–83. https://doi.org/10.1006/bbrc.1999.0255.

[11] Bamford NJ, Potter SJ, Harris PA, Bailey SR. Effect of Increased Adiposity on Insulin Sensitivity and Adipokine Concentrations in Horses and Ponies Fed a High Fat Diet, With or Without a Once Daily High Glycaemic Meal. Equine Vet J 2016;48:368–73. https://doi.org/10.1111/evj.12434.

[12] Bastard JP, Vidal H, Jardel C, Bruckert E, Robin D, Vallier P, *et al*. Subcutaneous Adipose Tissue Expression of Plasminogen Activator Inhibitor-1 Gene During Very Low Calorie Diet in Obese Subjects. Int J Obes Relat Metab Disord 2000;24:70–4. https://doi.org/10.1038/sj.ijo.0801087.

[13] Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, *et al*. Regulation of Adiponectin by Adipose Tissue-Derived Cytokines: In Vivo and In Vitro Investigations in Humans. American Journal of Physiology-Endocrinology and Metabolism 2003;285:E527–33. https://doi.org/10.1152/ajpendo.00110.2003.

[14] Kearns CF, McKeever KH, Roegner V, Brady SM, Malinowski K. Adiponectin and Leptin Are Related to Fat Mass in Horses. The Veterinary Journal 2006;172:460–5. https://doi.org/10.1016/j.tvjl.2005.05.002.

[15] Kearns CF, Mckeever KH, Kumagai K, Abe T. Fat-Free Mass Is Related to One-Mile Race Performance in Elite Standardbred Horses. The Veterinary Journal 2002;163:260–6. https://doi.org/10.1053/tvjl.2001.0656.

[16] Chameroy K. Diagnosis and Management of Horses with Equine Metabolic Syndrome (EMS). Doctoral Dissertations 2010.

[17] Fain JN, Bahouth SW, Madan AK. Haptoglobin Release by Human Adipose Tissue in Primary Culture. Journal of Lipid Research 2004;45:536–42. https://doi.org/10.1194/jlr.M300406-JLR200.

[18] Maffei M, Barone I, Scabia G, Santini F. The Multifaceted Haptoglobin in the Context of Adipose Tissue and Metabolism. Endocrine Reviews 2016;37:403–16. https://doi.org/10.1210/er.2016-1009.

[19] Geor RJ. Metabolic Predispositions to Laminitis in Horses and Ponies: Obesity, Insulin Resistance and Metabolic Syndromes. Journal of Equine Veterinary Science 2008;28:753–9. https://doi.org/10.1016/j.jevs.2008.10.016.

[20] Johnson PJ, Wiedmeyer CE, LaCarrubba A, Ganjam VKS, Messer NT. Diabetes, Insulin Resistance, and Metabolic Syndrome in Horses. J Diabetes Sci Technol 2012;6:534–40. https://doi.org/10.1177/193229681200600307.

[21] Selim S, Elo K, Jaakkola S, Karikoski N, Boston R, Reilas T, *et al*. Relationships Among Body Condition, Insulin Resistance and Subcutaneous Adipose Tissue Gene Expression During the Grazing Season in Mares. PLOS ONE 2015;10:e0125968. https://doi.org/10.1371/journal.pone.0125968.

[22] Ungru J, Coenen M, Vervuert I, Blüher M, Raila J, Boston R. Effects of Body Weight Reduction on Blood Adipokines and Subcutaneous Adipose Tissue Adipokine mRNA Expression

Profiles in Obese Ponies. Veterinary Record 2012;171:528–528. https://doi.org/10.1136/vr.100911.

[23] Van Den Wollenberg L, Vandendriessche V, van Maanen K, Counotte GHM. Comparison of Two Diagnostic Methods To Detect Insulin Dysregulation in Horses Under Field Conditions. Journal of Equine Veterinary Science 2020;88:102954. https://doi.org/10.1016/j.jevs.2020.102954.

[24] Carroll CL, Huntington PJ. Body Condition Scoring and Weight Estimation of Horses. Equine Vet J 1988;20:41–5. https://doi.org/10.1111/j.2042-3306.1988.tb01451.x.

[25] Carter RA, Geor RJ, Burton Staniar W, Cubitt TA, Harris PA. Apparent Adiposity Assessed by Standardised Scoring Systems and Morphometric Measurements in Horses and Ponies. Vet J 2009;179:204–10. https://doi.org/10.1016/j.tvjl.2008.02.029.

[26] Durham AE, Frank N, McGowan CM, Menzies-Gow NJ, Roelfsema E, Vervuert I, *et al.* ECEIM Consensus Statement on Equine Metabolic Syndrome. Journal of Veterinary Internal Medicine 2019;33:335–49. https://doi.org/10.1111/jvim.15423.

[27] CBS. More Diabetics in Obese Population. Statistics Netherlands 2016. https://www.cbs.nl/en-gb/news/2016/45/more-diabetics-in-obese-population (accessed January 18, 2022).

[28] Gatineau M, Hancock C, Holman N, Outhwaite H. Adult Obesity and Type 2 Diabetes 2014.

[29] Burns TA, Geor RJ, Mudge MC, McCutcheon LJ, Hinchcliff KW, Belknap JK. Proinflammatory Cytokine and Chemokine Gene Expression Profiles in Subcutaneous and Visceral Adipose Tissue Depots of Insulin-Resistant and Insulin-Sensitive Light Breed Horses. Journal of Veterinary Internal Medicine 2010;24:932–9. https://doi.org/10.1111/j.1939-1676.2010.0551.x.

[30] Nadler JL, Buchanan T, Natarajan R, Antonipillai I, Bergman R, Rude R. Magnesium Deficiency Produces Insulin Resistance and Increased Thromboxane Synthesis. Hypertension 1993;21:1024–9. https://doi.org/10.1161/01.hyp.21.6.1024.

[31] Stewart AJ. Magnesium Disorders in Horses. Veterinary Clinics of North America: Equine Practice 2011;27:149–63. https://doi.org/10.1016/j.cveq.2010.12.009.

[32] Díez JJ, Iglesias P. The Role of the Novel Adipocyte-Derived Hormone Adiponectin in Human Disease. Eur J Endocrinol 2003;148:293–300. https://doi.org/10.1530/eje.0.1480293.

[33] Pratt SE, Geor RJ, McCutcheon LJ. Relationship of Insulin Sensitivity to Aerobic Capacity in Standardbred Mares and Geldings. Equine and Comparative Exercise Physiology 2005;2:185–93. https://doi.org/10.1079/ECP200550.

[34] Menzies-Gow NJ, Harris PA, Elliott J. Prospective Cohort Study Evaluating Risk Factors for the Development of Pasture-Associated Laminitis in the United Kingdom. Equine Veterinary Journal 2017;49:300–6. https://doi.org/10.1111/evj.12606.

[35] Buff PR, Dodds AC, Morrison CD, Whitley NC, McFadin EL, Daniel JA, *et al.* Leptin in Horses: Tissue Localization and Relationship Between Peripheral Concentrations of Leptin and Body Condition. Journal of Animal Science 2002;80:2942–8. https://doi.org/10.2527/2002.80112942x.

[36] Karikoski NP, Box JR, Mykkänen AK, Kotiranta VV, Raekallio MR. Variation in Insulin Response to Oral Sugar Test in a Cohort of Horses Throughout the Year and Evaluation of Risk Factors for Insulin Dysregulation. Equine Vet J 2021. https://doi.org/10.1111/evj.13529.

[37] Menzies-Gow NJ, Knowles EJ, Rogers I, Rendle DI. Validity and Application of Immunoturbidimetric and Enzyme-Linked Immunosorbent Assays for the Measurement of Adiponectin Concentration in Ponies. Equine Vet J 2019;51:33–7. https://doi.org/10.1111/evj.12960.

[38] Chutia H, Lynrah KG. Association of Serum Magnesium Deficiency with Insulin Resistance in Type 2 Diabetes Mellitus. J Lab Physicians 2015;7:075–8. https://doi.org/10.4103/0974-2727.163131.

[39] Fried SK, Bunkin DA, Greenberg AS. Omental and Subcutaneous Adipose Tissues of Obese Subjects Release Interleukin-6: Depot Difference and Regulation by Glucocorticoid1. The Journal of Clinical Endocrinology & Metabolism 1998;83:847–50. https://doi.org/10.1210/jcem.83.3.4660.

[40] Kędzierski W. Changes in Plasma Leptin Concentration During Different Types of Exercises Performed by Horses. Animal 2014;8:1456–61. https://doi.org/10.1017/S1751731114001220.

[41] Pleasant R s., Suagee J k., Thatcher C d., Elvinger F, Geor R j. Adiposity, Plasma Insulin, Leptin, Lipids, and Oxidative Stress in Mature Light Breed Horses. Journal of Veterinary Internal Medicine 2013;27:576–82. https://doi.org/10.1111/jvim.12056.

[42] De Pergola G, Di Roma P, Paoli G, Guida P, Pannacciulli N, Giorgino R. Haptoglobin Serum Levels Are Independently Associated With Insulinemia in Overweight and Obese Women. J Endocrinol Invest 2007;30:399–403. https://doi.org/10.1007/BF03346317.

[43] Kosolofski HR, Gow SP, Robinson KA. Prevalence of Obesity in the Equine Population of Saskatoon and Surrounding Area. Can Vet J 2017;58:967–70.

[44] Wooldridge AA, Edwards HG, Plaisance EP, Applegate R, Taylor DR, Taintor J, *et al.* Evaluation of High–Molecular Weight Adiponectin in Horses. American Journal of Veterinary Research 2012;73:1230–40. https://doi.org/10.2460/ajvr.73.8.1230.

How to Cite
Counotte, G.; Durham, A.; Vandendriessche, V.; van den Wollenberg, L. Haptoglobin, Magnesium, Adiponectin, and Leptin As Biomarkers for Obesity and Insulin Dysregulation in Horses. Int J Equine Sci 2022, 1, 3–10.