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Thromboelastography in obese horses with insulin dysregulation compared to healthy controls

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Abstract

Background: Both obesity and metabolic syndrome are associated with hypercoagulability in people, increasing the risk of cardiovascular disease and thromboembolic events. Whether hypercoagulability exists in obese, insulin-dysregulated horses is unknown.

Hypothesis/Objectives: To determine if coagulation profiles differ between healthy horses and those with obesity and insulin dysregulation.

Animals: Fifteen healthy horses (CON) and 15 obese, insulin-dysregulated horses (OBID). Individuals were university or client owned.

Methods: Case-control study. Obesity was defined as a body condition score (BCS) ≥7.5/9 (modified Henneke scale). Insulin dysregulation status was assessed by an oral sugar test (OST). Kaolin-thromboelastography and traditional coagulation variables were compared between groups. The direction and strength of the association between coagulation variables and BCS and OST results were determined using Spearman's correlation.

Results: Thromboelastography variables MA (OBID: 69.5 ± 4.5 mm; CON: 64.8 ± 4.3 mm; $P = .007$) and G-value (OBID: 11749 ± 2536 dyn/m^2; CON: 9319 ± 1650 dyn/m^2; $P = .004$) were higher in OBID compared to CON. Positive correlations between MA and BCS ($R = 0.45$, $P = .01$) and serum insulin (T_0: $R = 0.45$, $P = .01$; T_{60}: $R = 0.39$, $P = .03$), and G-value and BCS ($R = 0.46$, $P = .01$), and serum insulin (T_0: $R = 0.48$, $P = .007$; T_{60}: $R = 0.43$, $P = .02$; T_{90}: $R = 0.38$, $P = .04$) were present.

Conclusions and Clinical Importance: Obese, insulin-dysregulated horses are hypercoagulable compared to healthy controls.

KEYWORDS
equine metabolic syndrome, hemostasis, hypercoagulability, obesity, oral sugar test, viscoelastic testing

Abbreviations: aPTT, activated partial thromboplastin time; BCS, body condition score; BMI, body mass index; EMS, equine metabolic syndrome; ID, insulin dysregulation; MS, metabolic syndrome; OST, oral sugar test; PT, prothrombin time; TEG, thromboelastography.

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1 | INTRODUCTION

The term equine metabolic syndrome (EMS) was originally derived from the analogous human disease, "metabolic syndrome" (MS). Equine metabolic syndrome is defined as a collection of risk factors for endocrinopathic laminitis that primarily consists of obesity, regional adiposity, and insulin dysregulation (ID) resulting from both genetic and environmental interactions.[1]

Adipose tissue dysregulation and the resulting interplay between proinflammatory and prothrombotic states in people with MS and obesity is a central aspect of the pathophysiology that predisposes these individuals to life-threatening cardiovascular disease and thromboembolic events.[2] Although these systemic alterations have also been speculated to be part of the pathophysiology in horses with EMS,[3] results from previous studies are inconsistent.[4-7] To the authors' knowledge, whether a prothrombotic, hypercoagulable state also exists in obese, insulin-dysregulated horses has not yet been investigated.

Viscoelastic testing is used to demonstrate hypercoagulable tendencies in people with MS and obesity in multiple clinical research studies.[8-14] These studies have identified a correlation between hypercoagulability and increasing fat mass, leptin levels, and inflammatory markers,[13] as well as a relationship with increasing body mass index (BMI)[10] whereby obesity is associated with postinjury hypercoagulability.[9] The advantages of viscoelastic testing such as thromboelastography (TEG) compared to traditional coagulation testing is that it is a global assessment of hemostasis (both cellular and plasma components are represented), it can identify both hypocoagulability and hypercoagulability, it is point-of-care, and it is inexpensive. Thromboelastography yields real-time graphical and numerical results that represent initial clot formation (reaction time [R-time] and kinetic time [K-time]), clot strengthening (α-angle, maximum amplitude [MA], G-value), and clot degradation (LY60). It is extensively used in human research and clinical settings, particularly for hemostatic monitoring in perioperative patient management, guiding anticoagulant treatment and in cases of polytrauma. The utility of TEG in equine research and clinical medicine is also increasing.[15-25]

The primary objective of this study was to investigate whether a sample of obese horses with ID displayed differences in their coagulation profiles consistent with hypercoagulability when compared to healthy horses of normal body condition and insulin regulation. A second objective was to investigate whether any correlations existed among oral sugar test (OST) insulin concentrations over time, body condition score (BCS), and the coagulation variables evaluated (using TEG and traditional methods).

2 | MATERIALS AND METHODS

This prospective case-control study was approved by the Oklahoma State University's Institutional Animal Care and Use Committee (IACUC). Both university- and client-owned horses were evaluated for study inclusion and informed client consent was obtained before evaluation. Data collection and testing were either performed at university facilities or in the field at clients' properties during the period of February to September 2020. A power calculation to determine sample size was performed using the TEG G-values of healthy horses from a previous study.[22] Based on these calculations, a minimum sample size of 15 horses per group was selected.

2.1 | Horses

All horses included were systemically healthy based on history, physical examination (including normal cardiac auscultation), normal CBC, and plasma fibrinogen concentration. Complete blood count and fibrinogen were determined via commercial bench-top analysis (Antech Diagnostics Inc, Los Angeles, California) on EDTA whole blood samples. Exclusion criteria included horses <3 or >25 years old, miniature horses, drafts, donkeys, horses with a hematocrit <28% or >45% (anemia and polycythemia can affect TEG profile), and horses with evidence of clinically active laminitis.

Body condition score was assigned for each horse using the Kohnke modification of the original Henneke body condition scoring system.[26,27] This modified approach involves individually scoring 6 separate body regions (neck, withers, shoulder, ribs, back, and tailhead) on a scale of 1 to 9; these 6 individual scores are then averaged to determine an overall BCS. The BCS was performed by 2 independent observers (A.L. Lovett and L.L. Gilliam) blinded to each other but not blinded to the study group allocation. The scores of the 2 observers were averaged for a final overall BCS. To be enrolled in the healthy control group (CON), horses were to have a BCS between 4 and 6 out of 9 and evidence of normal insulin regulation on an OST. To be enrolled in the obese, insulin-dysregulated group (OBID), horses were to have a BCS ≥7.5 out of 9[28] and demonstrate evidence of ID on an OST.[29]

2.2 | Oral sugar test

Postprandial insulin response was assessed to evaluate insulin and glucose dynamics using the OST in accordance with the 2020 recommendations provided by the Equine Endocrinology Group.[30] All horses were allowed water access during the testing period but otherwise were strictly withheld from feed for a minimum of 4 hours before testing and no grain was offered for a minimum of 12 hours before the first blood draw. Each horse's body weight was determined either by electronic scale (cases assessed at the university) or by weight-tape (cases assessed in the field). Corn syrup (Karo Light Corn Syrup, ACH Foodservice Inc, Oakbrook Terrace, Illinois) was administered PO at a dosage of 0.15 mL/kg. Time points for blood collection were before corn syrup administration (T_0), and 60 (T_{60}) and 90 (T_{90}) minutes after corn syrup administration. At each time point, blood was collected by jugular venipuncture directly into blood tubes (1 plain serum-separator tube, 1 EDTA tube). The collected blood was analyzed immediately for stall-side glucose measurements using a handheld

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glucometer.[31] Coagulated (serum-separator) and anticoagulated (EDTA) whole blood samples were chilled immediately and centrifuged within 30 to 120 minutes of collection. Separated serum and plasma were pipetted into cryovials for storage at −20°C. Frozen serum samples were batched and shipped frozen to an external laboratory (Animal Health Diagnostic Center Endocrinology Laboratory, Cornell University College of Veterinary Medicine, Ithaca, New York) for measuring insulin via an insulin radioimmunoassay (EMD Millipore Corporation, Billerica, Massachusetts). Horses with T_{60} or T_{90} insulin concentrations >45 μIU/mL were categorized as having postprandial hyperinsulinemia, indicative of ID (OBID group inclusion criteria).[30] Horses were considered negative for ID (CON group inclusion criteria) if insulin concentrations were <45 μIU/mL at all 3 time points.[30]

2.3 | Coagulation evaluation

All horses that met the inclusion criteria for each group had both traditional coagulation assessment and kaolin-TEG performed. Traditional coagulation assessment was performed within 24 hours of blood collection at a commercial veterinary laboratory (Antech Diagnostics Inc) and included a platelet count and fibrinogen measurement (EDTA whole blood), D-dimer measurement, prothrombin time (PT), and activated partial thromboplastin time (aPTT; 3.2% sodium citrate whole blood). D-dimer results below the level of detection (reported as <16 ng/mL) were considered 0 (0.0 ng/mL) for statistical analysis.

Thromboelastography was performed using a TEG 5000 Thromboelastograph Hemostasis System (Haemonetics Corp, Braintree, Massachusetts). All blood samples for TEG were tested within 24 hours of having machine controls run as per manufacturer's recommendations and an E-test was performed every time the analyzer was turned on as per manufacturer's recommendations. Venous blood was collected via jugular venipuncture directly into 2 3.2% sodium citrate blood tubes until tubes were completely full, ensuring blood was collected on the first attempt from a vein that had not had any recent venipunctures (≥2 weeks).[32] Once collected, blood tubes were gently inverted twice and then rested upright and undisturbed in a blood tube holder for 30 minutes. Kaolin-TEG was performed as per manufacturer's directions (Haemonetics Corp). Measured TEG variables (recorded from channel 1) included R-time, K-time, α-angle, maximal amplitude (MA), G-value, and LY60. The TEG testing was considered complete once the LY60 was reached.

2.4 | Statistical analysis

Hypercoagulability was defined as a relative shortening of R-time or K-time, or increased α-angle, MA, or G-value. Hypofibrinolysis was defined as a decrease in LY60. Statistical analysis was performed using commercial software (GraphPad Prism 5.0, San Diego, California). Data were tested for normality via the Shapiro-Wilk test. An unpaired Student's *t* test was performed to compare coagulation variables (TEG and traditional methods) and age between the CON group and OBID group. Fisher exact test was used to compare sex differences (binary data), and Mann-Whitney *U* test was used to compare BCS differences. The strength and direction of correlations between coagulation variables and insulin concentrations, BCS, and age were evaluated via Spearman's correlation (r_s). Multiple linear regressions were performed with coagulation variables as the dependent outcomes and insulin concentration, BCS, and age as the independent outcomes. Significance was defined as a *P*-value <.05.

3 | RESULTS

Thirty horses were included in the final data analysis. The CON group consisted of 10 geldings and 5 mares (n = 15), and the OBID group consisted of 7 geldings and 8 mares (n = 15). Ten horses in the CON group and 1 horse in the OBID group were university owned. The remainders of horses were client owned. No university owned animal had received any medication or nutraceutical supplementation in at least the 4 weeks preceding the study. The specific medication and nutraceutical supplementation history of the client-owned horses was not known, but none were receiving any medication or nutraceutical supplementation at the time of participation. A variety of breeds were represented across the 2 groups. Breeds represented in CON were Quarter Horse (n = 6), Thoroughbred (n = 3), Haflinger (n = 2), Quarter Horse cross (n = 1), Warmblood (n = 1), Warmblood cross Arabian (n = 1), and Arabian cross (n = 1). Breeds represented in OBID were Quarter Horse (n = 3), Welsh Pony (n = 3), Welsh Pony cross (n = 3), Gypsy Cob (n = 2), Warmblood (n = 1), Missouri Fox Trotter (n = 1), Connemara cross (n = 1), and Morgan cross (n = 1). The mean ±SD of age and median (IQR) of BCS and pre- and post-OST serum insulin concentrations values are reported in Table 1.

The values for coagulation variables (TEG and traditional methods) of the 2 groups and their coinciding *P*-values are reported in Table 2. A difference was detected for MA with a difference between means (±SD; 95% confidence interval [CI]; *P*-value) of 4.72 (±1.61; 1.43-8.02; $P = .007$) mm and for G-value with a difference between means (±SD; 95% CI; *P*-value) of 2430 (±781.2; 830.4-4030; $P = .004$) dyn/m^2, where the OBID group was higher for each value. There was no difference for R-time ($P = .26$), K-time ($P = .86$), α-angle ($P = .77$), or LY60 ($P = .49$) values, reflecting similar timing between the 2 groups for clot formation and clot degradation. These TEG comparisons are illustrated in Figure 1. When traditional coagulation variables were compared between CON and OBID, no differences were identified for any variable; PT (10.4 ± 0.5 vs 10.3 ± 0.6, $P = .87$), aPTT (40.7 ± 2.5 vs 41.0 ± 2.3, $P = .73$), fibrinogen (163.1 ± 26.9 vs 167.3 ± 27.9, $P = .67$), D-dimer (108.1 ± 173.4 vs 58.35 ± 138.1, $P = .39$), and platelet count (154.6 ± 24.7 vs 162.2 ± 42.1, $P = .55$). Spearman's correlation was performed between all coagulation outcomes, insulin results (T_0, T_{60}, and T_{90}), and BCS. Again, MA and G-value were the only coagulation variables that correlated ($P < .05$) with insulin and BCS (Table 3).

Body condition score (median [IQR]) was greater in OBID compared to CON (8.2 [7.9-8.3] vs 4.8 [4.5-5.3], $P < .001$). There was no difference in sex between the 2 groups (10 geldings and 5 mares in

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TABLE 1 Mean ± SD[a] and median (IQR)[b] values of various characteristics of obese horses with insulin dysregulation (OBID) and healthy control horses (CON)

Characteristic	CON group (n = 15)	OBID group (n = 15)	*P* value
Age (years old)[a]	8.7 ± 4.5	13.8 ± 4.4	**.004**
Sex (gelding, mare)	10 G, 5 M	7 G, 8 M	.46
Body condition score (0-9)[b]	4.8 (4.5-5.3)	8.2 (7.9-8.3)	**<.001**
Insulin T_0 (μIU/mL)[b]	5.5 (5.4-8.5)	21.4 (17.9-30.7)	
Insulin T_{60} (μIU/mL)[b]	17.1 (13.8-20.2)	67.3 (56.0-89.5)	
Insulin T_{90} (μIU/mL)[b]	14.8 (12.6-21.4)	68.6 (38.6-81.9)	

Note: The bold font was to denote *P* values < 0.05 i.e. the values that had a statistical difference between the groups.

TABLE 2 Thromboelastography and traditional coagulation variables of healthy control horses (CON) and obese horses with insulin dysregulation (OBID)

Variable	CON group Mean ± SD (95% CI) (n = 15)	OBID group Mean ± SD (95% CI) (n = 15)	Difference between means Mean ± SD (95% CI)	*P* value
R-time (min)	14.5 ± 3.7 (12.48-16.60)	15.8 ± 1.8 (14.74-16.77)	1.213 ± 1.12 (−0.983 to 3.410)	.27
K-time (min)	5.0 ± 1.3 (3.82-6.178)	4.9 ± 1.3 (4.155-5.618)	−0.113 ± 0.68 (−1.438 to 1.211)	.86
Angle (α)	44.1 ± 9.9 (38.66-49.58)	43.3 ± 5.9 (40.00-46.54)	−0.853 ± 3.10 (−6.934 to 5.227)	.78
MA (mm)	64.8 ± 4.3 (62.41-67.15)	69.5 ± 4.5 (66.99-72.01)	4.72 ± 1.61 (1.43-8.02)	**.007**
G (dyn/m^2)	9319 ± 1650 (8405-10 232)	11 749 ± 2536 (10345-13 153)	2430 ± 781.2 (830.4-4030)	**.004**
LY60 (%)	2.0 ± 1.2 (1.40-2.68)	1.7 ± 1.3 (0.976-2.465)	−0.320 ± 0.479 (−1.258 to 0.618)	.49
PT (s)	10.4 ± 0.5 (10.08-10.67)	10.3 ± 0.6 (10.02-10.66)	−0.033 ± 0.211 (−0.448 to 0.381)	.87
APTT (s)	40.7 ± 2.5 (39.29-42.07)	41.0 ± 2.3 (39.73-42.23)	0.300 ± 3.56 (−1.484 to 2.084)	.73
Fibrinogen (mg/dL)	163.1 ± 26.9 (148.2-178.0)	167.3 ± 27.9 (151.9-182.8)	4.27 ± 10.454 (−16.22 to 24.76)	.67
D-Dimer (ng/mL)	108.1 ± 173.4 (23.72-209.6)	58.35 ± 138.1 (−6.221 to 142.1)	−49.78 ± 59.83 (−167.04 to 67.48)	.39
Platelet count (×10^3/L)	154.6 ± 24.7 (140.9-168.3)	162.2 ± 42.1 (138.9-185.5)	7.60 ± 13.158 (−18.19 to 33.39)	.55

Note: The bold font was to denote *P* values < 0.05 i.e. the values that had a statistical difference between the groups.
Abbreviations: α-angle, the angle in degrees between the baseline and a line tangent to the tracing curve representing the rapidity of clot formation; aPTT, activated partial thromboplastin time in seconds; G, the G-value, which is a calculated value, representing the clot's viscoelastic shear/strength (dyn/m^2); K-time, the kinetic time in minutes from clot initiation until an amplitude of 20 mm is reached; LY60, the percentage of clot lysis present at 60 minutes from the time at which MA is reached; MA, the maximum amplitude of the tracing in millimeters reflecting the maximal clot strength; PT, prothrombin time in seconds; R-time, the reaction time in minutes from the beginning of the test until initiation of clot formation.

CON vs 7 geldings and 8 mares in OBID, $P = .46$). The mean (±SD) age of OBID (13.8 ± 4.4) was higher than CON (8.7 ± 4.5), $P = .004$. Age was positively correlated with G in OBID ($r_s = 0.54$, $P = .04$) but not in CON ($r_s = -0.3$, $P = .28$). Multiple linear regression analysis was performed with group and age as the independent variables and MA or G as the dependent variables. Metabolic group (OBID vs CON) was retained in the model for MA ($P = .04$) and G ($P = .04$), but not age (MA: $P = .44$, G: $P = .30$).

4 | DISCUSSION

This study investigates coagulation profiles, including TEG, in a group of obese horses with ID. When compared to a healthy group of horses (CON), both MA and G-value were greater in the OBID group. Higher MA and G-value are reflective of larger final clot strength and stability and are suggestive of a hypercoagulable tendency. The appearance of 2 TEG tracings from this study (1 CON horse and 1 OBID horse) in Figure 2 illustrates this comparison. None of the other coagulation variables measured (TEG and traditional methods) were different between the 2 groups.

Higher BCS and a greater degree of ID were associated with a hypercoagulable tendency (higher MA and G-value) as demonstrated by positive Spearman's correlation coefficients between the variables tested. Maximum amplitude, the maximal width of a TEG tracing, is determined by platelet number and function (adhesion, activation, and aggregation), fibrinogen activation, and the resulting fibrin cross-linking.[33] The G-value is a calculated value that uses the MA measurement. As such, it is logical that an elevation in MA is also accompanied by an elevation in G-value and why differences in both variables were observed concurrently. The G-value specifically represents the viscoelastic shear/strength of the final clot.[33]

Obesity is a risk factor for hypercoagulability in humans.[2,34] Of measured TEG variables, MA and G-value have consistently attracted the most attention in the literature, particularly in the setting of bariatric surgery and high-level trauma. Two small-scale studies identified elevated MA and G-value in obese patients (BMI ≥30 kg/m^2) undergoing bariatric surgery.[11,12] In another larger study, 53% of obese

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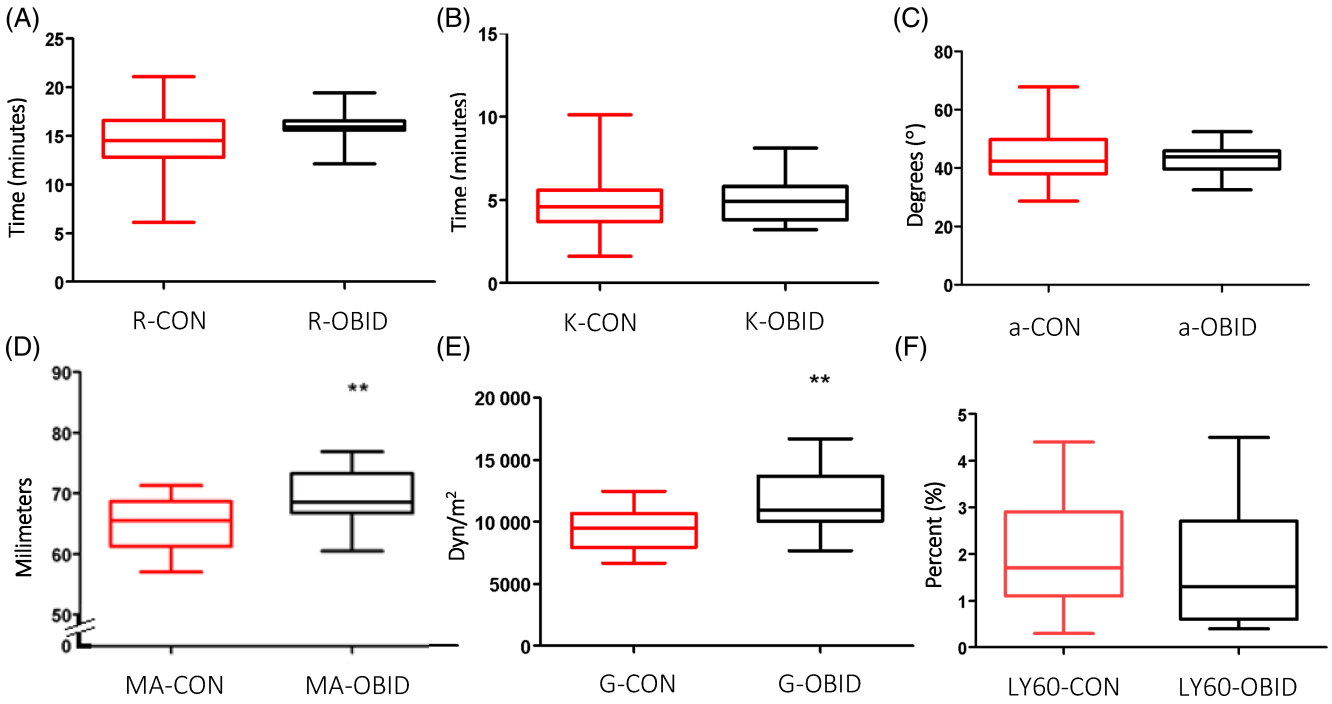

FIGURE 1 Box-and-Whisker plots of the 6 measured thromboelastography variables between the healthy control group (CON, n = 15; red plots) and the obese, insulin-dysregulated group (OBID, n = 15; black plots). A, Plots for R-time (R, minutes); B, plots for K-time (K, minutes); C, plots for α-angle; D, plots for maximum amplitude (MA, mm); E, plots for G-value (dyn/m^2); and F, plots for LY60 (%). Only MA and G-value were different between groups (denoted by asterisks **)

TABLE 3 Spearman's correlations between coagulation variables (thromboelastography and traditional) and oral sugar test serum insulin results (T_0, T_{60}, and T_{90} minutes) and body condition score (BCS) for all study subjects (CON and OBID horses, n = 30). Significances are denoted by bold font

	Insulin - T_0 (μIU/mL)		Insulin - T_{60} (μIU/mL)		Insulin - T_{90} (μIU/mL)		BCS (0-9)	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
R-time (min)	0.23	0.22	0.13	0.49	0.019	0.92	0.25	0.19
K-time (min)	0.075	0.69	0.025	0.90	0.011	0.96	−0.062	0.75
Angle (α)	−0.049	0.80	−0.045	0.81	−0.018	0.92	0.076	0.70
MA (mm)	0.45	**0.012**	0.39	**0.032**	0.35	0.060	0.45	**0.012**
G (dyn/m^2)	0.48	**0.007**	0.43	**0.019**	0.38	**0.037**	0.46	**0.010**
LY60 (%)	−0.15	0.43	−0.17	0.36	−0.17	0.31	−0.12	0.53
PT (s)	−0.13	0.49	−0.0078	0.7	−0.088	0.64	−0.099	0.60
APTT (s)	0.041	0.83	−0.037	0.85	−0.024	0.90	0.098	0.61
Fibrinogen (mg/dL)	0.026	0.89	0.076	0.69	−0.0027	0.99	0.0033	0.99
D-dimer (ng/dL)	−0.11	0.58	0.020	0.92	−0.046	0.81	−0.11	0.56
Platelet count (×10^3/L)	0.19	0.30	0.16	0.40	0.24	0.19	0.081	0.67

Abbreviations: α-angle, the angle in degrees between the baseline and a line tangent to the tracing curve representing the rapidity of clot formation; aPTT, activated partial thromboplastin time in seconds; G, the G-value, which is a calculated value, representing the clot's viscoelastic shear/strength (dyn/m^2); K-time, the kinetic time in minutes from clot initiation until an amplitude of 20 mm is reached; LY60, the percentage of clot lysis present at 60 minutes from the time at which MA is reached; MA, the maximum amplitude of the tracing in millimeters reflecting the maximal clot strength; PT, prothrombin time in seconds; R-time, the reaction time in minutes from the beginning of the test until initiation of clot formation.

preoperative bariatric surgery patients had an elevated G-value and 39.1% had an elevated MA.[35] In the trauma setting, 2 studies have demonstrated higher MA in obese people compared to normal and overweight groups.[9,10] The findings of this study suggest that a relationship between obesity and hypercoagulability similar to that which is observed in human medicine might be present in the horse.

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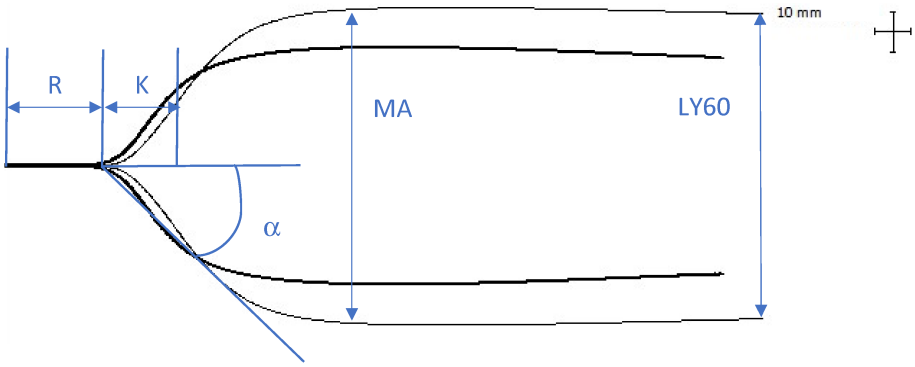

FIGURE 2 Two individual thromboelastograms superimposed: 1 healthy control horse of CON group (black line graphical tracing) and 1 obese, insulin-dysregulated horse of OBID group (gray line graphical tracing), whose relative hypercoagulability (wider maximum amplitude [MA]) is demonstrated. The tracing develops in a direction starting from left to right over time until LY60 is reached (60 minutes after the time at which MA is achieved). Measurements represented are: R-time (R, minutes), K-time (K, minutes), α-angle, MA (mm), and LY60 (%)

The hypercoagulable state of obese human patients is a consequence of complicated interactions of adipose tissue dysregulation, oxidative stress, and chronic, systemic inflammation.[2] Some adipose tissue-related pathophysiological mechanisms that have been described include: increased tissue factor release from adipocytes and thus increased thrombin generation,[36] enhanced biosynthesis of fibrinogen and clotting factors by the liver,[36] endothelial-derived and leukocyte-derived circulating microparticles thus promoting platelet activation,[34] adipokine influences on platelet function and fibrin formation, and increased adipose tissue and hepatic expression of plasminogen activator inhibitor-1 leading to hypofibrinolysis.[2]

The clinical relevance of the relationship between ID and hypercoagulability observed in this study is less clear. As with horses, people can have obesity without MS or have MS with truncal obesity but not necessarily a total BMI >30 kg/m^2. In human patients, it has been suggested that obesity is a more important factor for hypercoagulability than MS, and obesity-related hypercoagulability can occur independently of IR and MS.[8,13,34,37] In this this study, all obese horses were ID as per the inclusion criteria. Separating the influences of obesity and ID on TEG and hypercoagulability would have been of interest but would have required additional case recruitment (eg, obese non-ID, ID nonobese, obese, and ID, vs healthy controls) beyond this study's scope.

Human patients with hypercoagulability have a heightened risk for thromboembolic events and a need for individually tailored pharmacological prophylaxis. Thus, the clinical utility of perioperative and postinjury TEG monitoring is increasing, to predict which obese patients might be at higher risk for pulmonary thromboembolism, deep vein thrombosis and portal/mesenteric vein thrombosis, and therefore which patients might benefit from extended anticoagulant treatment.[35] In the context of equine medicine, a common vascular complication in hospitalized horses is catheter-associated jugular thrombosis or thrombophlebitis, a condition for which anticoagulant treatment is often recommended and for which clinical application of TEG monitoring could be advantageous.[38,39] Horses with disease of the large intestine, salmonellosis, or endotoxemia are at increased risk for developing this condition.[40] The results of this study suggest that critically ill obese or insulin-dysregulated horses might be at further increased risk due to an increased likelihood of having a pre-existing hypercoagulable state. Accordingly, critically ill obese or insulin-dysregulated horses might benefit from closer hemostatic monitoring to guide therapeutic intervention.

Cardiovascular disease and thromboembolic events (including atherosclerosis, cardiac remodeling, transient ischemic attack, and stroke) are not common disease sequelae with EMS and equine obesity (although these conditions might be underdiagnosed). Instead, the central concern surrounds endocrinopathic laminitis.[41,42] Hypercoagulability and endothelial injury might contribute to the progressive homeostatic disturbance of the lamellae in chronic, naturally occurring endocrinopathic laminitis cases.[43]

Several limitations are present in the study. These limitations primarily surround the inconsistencies of signalment and husbandry between the 2 groups. The influence of age and sex on TEG variables reported in the human literature have been inconsistent, with some studies reporting no difference, while others report differences associated with age and sex.[44] Ideally, each subject within the OBID group would have been age-, breed-, and sex-matched in order to minimize confounding variables. Case recruitment for the OBID group was challenging and thus limited the ability to align signalment between the groups. The influence of age, breed, and sex on equine TEG profiling is unknown currently but in this study's analysis, there was no influence of sex. There was a difference in age between the 2 groups and age positively correlated with G-value in the OBID group; however, multiple linear regression analysis demonstrated that metabolic and body condition status was the more likely explanation for the difference that was identified in G-value between the 2 groups, not age. It is not surprising that the OBID group was inherently older. Age is a risk factor associated with EMS in native ponies in the United Kingdom, with odds of EMS diagnosis increasing by 1.38 with each year of increasing age.[45] Insulin sensitivity decreases and insulin response to non–structural-carbohydrates increases in aged, healthy horses (>19 years) compared to younger adults

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(5-12 years).[46] A further weakness is that husbandry management conditions (housing, feeding, and exercise) were not standardized and thus differed within and between university- and client-owned horses. The influence of diet and fitness on equine TEG profiling is currently unknown, and thus, these differing management factors might be a source of potential bias within the study. Lastly, the exact weight of the client-owned horses was not known as these were estimated using a weight tape. Given that the majority (14/15) of OBID horses were client owned vs a minority (5/15) of CON horses, this might have influenced the accuracy dosage of corn syrup administered between the 2 groups. However, the authors consider that the impact of this is likely to be minimal considering that an elevated BCS was an additional criterion required for inclusion into the OBID group.

In conclusion, this study provides preliminary evidence that, similar to people with obesity, obese horses with ID also have TEG profiles supportive of a hypercoagulable tendency compared to healthy controls. However, the clinical importance of these findings, particularly in regard to laminitis pathophysiology, requires further research.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approval by the Oklahoma State University IACUC.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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