Thromboelastography in obese horses with insulin dysregulation compared to healthy controls

Amy L. Lovett1 | Lyndi L. Gilliam1 | Benjamin W. Sykes2 | Dianne McFarlane1

1Department of Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma, USA
2School of Veterinary Sciences, Massey University, Stillwater, Palmerston North, New Zealand

Correspondence
Amy L. Lovett, Department of Clinical Sciences, College of Veterinary Medicine, Massey University, Stillwater, OK 74078, USA.
Email: a.lovett@massey.ac.nz

Present address
Amy L. Lovett, School of Veterinary Sciences, Massey University, Palmerston North, New Zealand

Funding information
Oklahoma State University (OSU) Research Advisory Committee (RAC) 2019

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/m²; CON: 9319 ± 1650 dyn/m²; P = .004) were higher in OBID compared to CON. Positive correlations between MA and BCS (R = 0.45, P = .01) and serum insulin (T0: R = 0.48, P = .007; T60: R = 0.43, P = .02; T90: 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.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. Journal of Veterinary Internal Medicine published by Wiley Periodicals LLC on behalf of American College of Veterinary Internal Medicine.
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. 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. Although these systemic alterations have also been speculated to be part of the pathophysiology in horses with EMS, results from previous studies are inconsistent. 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. These studies have identified a correlation between hypercoagulability and increasing fat mass, leptin levels, and inflammatory markers, as well as a relationship with increasing body mass index (BMI). Whereby obesity is associated with postinjury hypercoagulability. 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 hypercoagulability 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.

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).

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. Based on these calculations, a minimum sample size of 15 horses per group was selected.

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. 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 and demonstrate evidence of ID on an OST.

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. 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 (T₀), and 60 (T₆₀) and 90 (T₉₀) 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
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 radioimmunossay (EMD Millipore Corporation, Billerica, Massachusetts). Horses with $T_{\lambda_{0}}$ or $T_{\alpha_{0}}$ insulin concentrations $>45$ μU/mL were categorized as having postprandial hyperinsulinemia, indicative of ID (OBID group inclusion criteria). Horses were considered negative for ID (CON group inclusion criteria) if insulin concentrations were $<45$ μU/mL at all 3 time points.

### 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 $\geq$2 weeks. 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, $\alpha$-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 $\alpha$-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 remainder 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 ($\pm$1.61; 1.43-8.02; $P=0.007$) mm and for G-value with a difference between means (±SD; 95% CI; P-value) of 2430 ($\pm$781.2; 830.4-4030; $P=0.004$) dyn/m², where the OBID group was higher for each value. There was no difference for R-time ($P=0.26$), K-time ($P=0.86$), $\alpha$-angle ($P=0.77$), or LY60 ($P=0.49$) 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 ($10.4 \pm 0.5$ vs $10.3 \pm 0.6$, $P=0.87$), aPTT ($40.7 \pm 2.5$ vs 41.0 $\pm 2.3$, $P=0.73$), fibrinogen ($163.1 \pm 26.9$ vs 167.3 $\pm 27.9$, $P=0.67$), D-dimer ($108.1 \pm 173.4$ vs 58.3 $\pm 138.1$, $P=0.39$), and platelet count ($154.6 \pm 24.7$ vs 162.2 $\pm 42.1$, $P=0.55$). Spearman’s correlation was performed between all coagulation outcomes, insulin results ($T_{\alpha_{0}}$, $T_{\alpha_{90}}$, and $T_{\alpha_{0}}$), and BCS. Again, MA and G-value were the only coagulation variables that correlated ($P<0.05$) 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 $[4.5-5.3]$, $P<0.001$). There was no difference in sex between the 2 groups (10 geldings and 5 mares in...
The G-value is a calculated value that uses the MA measure-

Mean ± SDa and median

In another larger study, 53% of obese

Abbreviations: α

activated partial thromboplastin time in seconds; G, the G-value, which is a calculated value, representing the clot’s viscoelastic shear/strength (dyn/m²); PT, prothrombin time in seconds; R-time, the reaction time in minutes from the beginning of the test until initiation of clot formation.

The bold font was to denote

Note:

Values < 0.05 i.e. the values that had a statistical difference

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/m²); 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 = 0.54, P = .04) but not in CON (r = −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. 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.

Obesity is a risk factor for hypercoagulability in humans. 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/m²) undergoing bariatric surgery. In another larger study, 53% of obese

### Table 1

| Variable | CON group (n = 15) | OBID group (n = 15) | Difference between means | P value |
|----------|-------------------|---------------------|--------------------------|--------|
| Angle (°) | 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 |
| 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 |
| 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 |
| MA (mm) | 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 |
| G (dyn/m²) | 9319 ± 1650 (8405–10 232) | 11 749 ± 2536 (10345–13 153) | 2430 ± 781.2 (830.4–4030) | .007 |
| LY60 (%) | 0.2 ± 1.2 (0.76–2.465) | 1.7 ± 1.3 (0.976–2.465) | -0.320 ± 0.479 (–1.258 to 0.618) | .49 |
| D-Dimer (ng/mL) | 108.1 ± 173.4 (23.7–209.6) | 58.35 ± 138.1 (16.221 to 142.1) | -49.78 ± 59.83 (–167.04 to 67.48) | .39 |
| Platelet count (×10³/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.

**TABLE 1** Mean ± SD and median (IQR) values of various characteristics of obese horses with insulin dysregulation (OBID) and healthy control horses (CON).
preoperative bariatric surgery patients had an elevated G-value and 39.1% had an elevated MA. In the trauma setting, 2 studies have demonstrated higher MA in obese people compared to normal and overweight groups. 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.

**TABLE 3** Spearman’s correlations between coagulation variables (thromboelastography and traditional) and oral sugar test serum insulin results (T₀, T₆₀, and T₉₀ minutes) and body condition score (BCS) for all study subjects (CON and OBID horses, n = 30). Significances are denoted by bold font.

|                  | Insulin - T₀ (μIU/mL) | Insulin - T₆₀ (μIU/mL) | Insulin - T₉₀ (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/m²)       | 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 (x10¹³/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/m²); 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.
Some adipose
Horses with disease of
Hyp-
Ideally, each subject within the OBID
enhanced biosynthesis of
adipokine influences on platelet function and fibrin for-
Insulin sensitivity decreases
endothelial-derived and
The results of this study suggest that
In this study, all obese
Two individual thromboelastograms superimposed: 1 healthy control horse of CON group (black line graphical tracing) and
41,42
36
In the context of equine medicine, a common vascular
cardiovascular disease and thromboembolic events (including
atherosclerosis, cardiac remodeling, transient ischemic attack, and
stroke) are not common disease sequelae with EMS and equine obe-
sity (although these conditions might be underdiagnosed). Instead, the
central concern surrounds endocrinopathic laminitis.31,42 Hyp-
eroagulability and endothelial injury might contribute to the progres-
sive homeostatic disturbance of the lamellae in chronic, naturally
occurring endocrinopathic laminitis cases.43
Several limitations are present in the study. These limitations pri-
arily 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 asso-
ciated 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 expla-
nation for the difference that was identified in G-value between the
2 groups, not age. It is not surprising that the OBID group was inher-
ently 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

The hypercoagulable state of obese human patients is a conse-
quence of complicated interactions of adipose tissue dysregulation,
oxidative stress, and chronic, systemic inflammation.7 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 for-
amation, and increased adipose tissue and hepatic expression of plas-
minogen activator inhibitor-1 leading to hypofibrinolysis.7

The clinical relevance of the relationship between ID and hyper-
coagulability 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/m2. In human patients, it has
been suggested that obesity is a more important factor for hyper-
coagulability than MS, and obesity-related hypercoagulability can
occur independently of IR and MS.8,13,34,37 In 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,
obes 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 phar-
macological 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
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 obe-
sity (although these conditions might be underdiagnosed). Instead, the
central concern surrounds endocrinopathic laminitis.31,42 Hyp-
eroagulability and endothelial injury might contribute to the progres-
sive homeostatic disturbance of the lamellae in chronic, naturally
occurring endocrinopathic laminitis cases.43

Several limitations are present in the study. These limitations pri-
arily 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 asso-
ciated 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 expla-
nation for the difference that was identified in G-value between the
2 groups, not age. It is not surprising that the OBID group was inher-
ently 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

The hypercoagulable state of obese human patients is a conse-
quence of complicated interactions of adipose tissue dysregulation,
oxidative stress, and chronic, systemic inflammation.7 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 for-
amation, and increased adipose tissue and hepatic expression of plas-
minogen activator inhibitor-1 leading to hypofibrinolysis.7

The clinical relevance of the relationship between ID and hyper-
coagulability 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/m2. In human patients, it has
been suggested that obesity is a more important factor for hyper-
coagulability than MS, and obesity-related hypercoagulability can
occur independently of IR and MS.8,13,34,37 In 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,
obes 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 phar-
macological 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
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 obe-
sity (although these conditions might be underdiagnosed). Instead, the
central concern surrounds endocrinopathic laminitis.31,42 Hyp-
eroagulability and endothelial injury might contribute to the progres-
sive homeostatic disturbance of the lamellae in chronic, naturally
occurring endocrinopathic laminitis cases.43

Several limitations are present in the study. These limitations pri-
arily 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 asso-
ciated 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 expla-
nation for the difference that was identified in G-value between the
2 groups, not age. It is not surprising that the OBID group was inher-
ently 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
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 clot structure in transient ischemic attack individuals in the presence of metabolic syndrome: a microscopy and thromboelastography study. Cardiovasc Diabetol. 2015;14:86.

ACKNOWLEDGMENT
This study was funded by the Oklahoma State University (OSU) Research Advisory Committee (RAC) grant and the June Jacobs Endowed Chair in Equine Medicine. The authors thank the equine clients and referring veterinarians of OSU for the provision of horses for enrollment in this study, to the OSU Equine Research Park and OSU teaching herd.

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.

ORCID
Amy L. Lovett https://orcid.org/0000-0001-5167-939X
Dianne McFarlane https://orcid.org/0000-0003-4836-8069

REFERENCES
1. Durham AE, Frank N, McGowan CM, et al. ECEIM consensus statement on equine metabolic syndrome. J Vet Intern Med. 2019;33: 335-349.
2. Morange P-E, Alessi M-C. Thrombosis in central obesity and metabolic syndrome: mechanisms and epidemiology. Thromb Haemost. 2013;110:669-680.
3. Frank N, Geor RJ, Bailey SR, Durham AE, Johnson PJ. Equine metabolic syndrome. J Vet Intern Med. 2010;24:467-475.
4. Wray H, Elliott J, Bailey S, et al. Plasma concentrations of inflammatory markers in previously laminitic ponies. Equine Vet J. 2013;45: 546-551.
5. Holbrook TC, Tipton T, McFarlane D. Neutrophil and cytokine dysregulation in hyperinsulinemic obese horses. Vet Immunol Immunopathol. 2012;145:283-289.
6. Basinska K, Marycz K, Śmieszek A, Nipcjo P. The production and distribution of IL-6 and TNF-α in subcutaneous adipose tissue and their correlation with serum concentrations in Welsh ponies with equine metabolic syndrome. J Vet Sci. 2015;16:113-120.
7. Vick M, Adams A, Murphy B, et al. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse. J Anim Sci. 2007;85:1144-1155.
8. Van Rooy MJ, Duim W, Ehlers R, et al. Platelet hyperactivity and fibrin clot structure in transient ischemic attack individuals in the presence of metabolic syndrome: a microscopy and thromboelastography study. Cardiovasc Diabetol. 2015;14:86.
9. Samuels JM, Moore EE, Coleman JR, et al. Obesity is associated with postinjury hypercoagulability. J Trauma Acute Care Surg. 2019;87: 876-882.
10. Kombliht L, Howard B, Kuintake R, et al. Obesity and clotting: BMI independently contributes to hypercoagulability after injury. J Trauma Acute Care Surg. 2015;78:30-38.
11. Tuovila M, Erkinaro T, Koivukangas V, et al. Thromboelastography values remain hypercoagulative 6 months after obesity surgery: a pilot study. Obes Surg. 2018;28:3943-3949.
12. Kupcinskiene K, Trepanenitis D, Petherit R, et al. Monitoring of hypercoagulability by thromboelastography in bariatric surgery. Med Sci Monit. 2017;23:1819-1826.
13. Campello E, Spiezia L, Zabeo E, Maggiolo S, Vettor R, Simioni P. Hypercoagulability detected by whole blood thromboelastometry (ROTEM(R)) and impedance aggregometry (MULTIPLATE(R)) in obese patients. Thromb Res. 2015;135:548-553.
14. Pivalizza EG, Pivalizza PJ, Weavind LM. Perioperative thromboelastography and sonoclot analysis in morbidly obese patients. Can J Anaesth. 1997;44:942-945.
15. Hyldahl Laursen S, Andersen PH, Kjeldgaard-Hansen M, Wünnberg B. Comparison of components of biological variation between 3 equine thromboelastography assays. Vet Clin Pathol. 2013;42:443-450.
16. Machackova K, Boselova M, Vanova I, Drabkova Z, Doubek J. Evaluation of kaolin-activated thromboelastography and sample stability in healthy horses. Vet Med. 2018;63:203-209.
17. Scruggs JL, Flatland B, McCormick KA, et al. Biological variation of thromboelastography variables in 10 clinically healthy horses. J Vet Emerg Crit Care (San Antonio). 2016;26:80-84.
18. Thane K, Bedenice D, Pacheco A. Operator-based variability of equine thromboelastography. J Vet Emerg Crit Care. 2017;27: 419-424.
19. Epstein KL, Brainard BM, Lopes MA, et al. Thrombolastography in 26 healthy horses with and without activation by recombinant human tissue factor. J Vet Emerg Crit Care (San Antonio). 2009;19:96-101.
20. Lemon AV, Goddard A, Hooijberg EH. Effects of storage time and temperature on thromboelastographic analysis in dogs and horses. Vet Clin Pathol. 2021;50:9-19.
21. Dunkel B, Chan D, Boston R, et al. Association between hypercoagulability and decreased survival in horses with ischemic or inflammatory gastrointestinal disease. J Vet Intern Med. 2010;24: 1467-1474.
22. Mendez-Angulo JL, Mudge MC, Vilar-Saavedra P, Stingl N, Couto CG. Thromboelastography in healthy horses and horses with inflammatory gastrointestinal disorders and suspected coagulopathies. J Vet Emerg Crit Care (San Antonio). 2010;20:488-493.
23. Mendez-Angulo JL, Mudge M, Zaldívar-Lopez S, Vilar-Saavedra P, Couto G. Thromboelastography in healthy, sick non-septic and septic neonatal foals. Aust Vet J. 2011;89:500-505.
24. Epstein KL, Brainard BM, Giguere S, Vrono Z, Moore JN. Serial viscoelastic and traditional coagulation testing in horses with gastrointestinal disease. *J Vet Emerg Crit Care (San Antonio)*. 2013;23:504-516.

25. Dallap Schaer BL, Wilkins PA, Boston R, Palmer J. Preliminary evaluation of hemostasis in neonatal foals using a viscoelastic coagulation and platelet function analyzer. *J Vet Emerg Crit Care*. 2009;19:81-87.

26. Kohnke JR. *Feeding and Nutrition: The Making of a Champion*. Melbourne, Australia: Birubi Pacific; 1992:163-166.

27. Henneke D, Potter G, Kreider J, et al. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J*. 1983;15:371-372.

28. Dugdale AH, Grove-White D, Curtis GC, et al. Body condition scoring as a predictor of body fat in horses and ponies. *Vet J*. 2012;194:173-178.

29. Schuver A, Frank N, Chameroy KA, Elliott SB. Assessment of insulin and glucose dynamics by using an oral sugar test in horses. *J Equine Vet*. 2014;34:465-470.

30. Equine Endocrinology Group [Internet]. Tufts University; 2020. https://sites.tufts.edu/equineendogroup/

31. Hackett E, McCue P. Evaluation of a veterinary glucometer for use in horses. *J Vet Intern Med*. 2010;24:617-621.

32. Garcia-Pereira BL, Scott MA, Koenigshof AM, Brown AJ. Effect of venipuncture quality on thromboelastography. *J Vet Emerg Crit Care*. 2012;22:225-229.

33. Mendez-Angulo J, Mudge M, Couto C. Thromboelastography in equine medicine: technique and use in clinical research. *Equine Vet Educ*. 2012;24:639-649.

34. Campello E, Zabeo E, Radu CM, et al. Hypercoagulability in overweight and obese subjects who are asymptomatic for thrombotic events. *Thromb Haemost*. 2015;113:85-96.

35. Cowlings JC, Zhang X, Bajwa KS, et al. Thromboelastography-based profiling of coagulation status in patients undergoing bariatric surgery: analysis of 422 patients. *Obes Surg*. 2021;31:8:3590-3597.

36. Faber D, De Groot PG, Visseren F. Role of adipose tissue in haemostasis, coagulation and fibrinolysis. *Obes Rev*. 2009;10:554-563.

37. Stepanian A, Bourguignon L, Hennou S, et al. Microparticle increase in severe obesity: not related to metabolic syndrome and unchanged after massive weight loss. *Obesity*. 2013;21:2236-2243.

38. Feige K, Schwarzwal CC, Bombeli T. Comparison of unfractioned and low molecular weight heparin for prophylaxis of coagulopathies in 52 horses with colic: a randomised double-blind clinical trial. *Equine Vet J*. 2003;35:506-513.

39. Reef VB. Diseases of the cardiovascular system. In: Smith BP, Van Metre DC, Pusterla N, eds. *Large Animal Internal Medicine*. 6th ed. Maryland Heights, MO: Elsevier; 2020:507.

40. Dolente BA, Beech J, Lindborg S, Smith G. Evaluation of risk factors for development of catheter-associated jugular thrombophlebitis in horses: 50 cases (1993–1998). *J Am Vet Med Assoc*. 2005;227:1134-1141.

41. Ertelt A, Barton AK, Schmitz RR, Gehlen H. Metabolic syndrome: is equine disease comparable to what we know in humans? *Endocr Connect*. 2014;3:R81-R93.

42. Ragno VM, Zello GA, Klein CD, Montgomery JB. From table to stable: a comparative review of selected aspects of human and equine metabolic syndrome. *J Equine Vet*. 2019;79:131-138.

43. van Eps AW, Burns TA. Are there shared mechanisms in the pathophysiology of different clinical forms of laminitis and what are the implications for prevention and treatment? *Vet Clin*. 2019;35:379-398.

44. Ahammad J, Kurien A, Shastry S, et al. Age-and gender-related reference ranges for thromboelastography from a healthy Indian population. *Int J Lab Hematol*. 2020;42:180-189.

45. Carslake HB, Pinchbeck GL, McGowan CM. Equine metabolic syndrome in UK native ponies and cobs is highly prevalent with modifiable risk factors. *Equine Vet J*. 2021;53:923-934.

46. Raspoll JD, Schott H, Nielsen B, et al. Effects of age and diet on glucose and insulin dynamics in the horse. *Equine Vet J*. 2018;50:690-696.